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National Workshop on Fluorescence and Raman Spectroscopy

International Conference on Optics Within Life Sciences (OWLS-17)

FCS XV

Be creative with light

November 16-21, 2024

International Society on Optics Within Life Sciences

OWLS

Victor Menezes Convention Centre (VMCC)

Indian Institute of Technology Bombay Powai, Mumbai 400 076, India

Convenors

Ruchi Anand
Dipanshu Bansal
Arindam Chowdhury
Shobhna Kapoor
Ambarish Kunwar
Samir Maji
Roop Mallik
Ishita Sengupta

Local Organizing Committee

Debanjan Bhowmik
Jyotishman Dasgupta
Anindya Datta
Sudipta Maiti
Shivaprasad Patil
Sobhan Sen

IITB Student Volunteers

We welcome you all into a week full of Light & Life

Welcome Address

Dear OWLS-17 and FCS XV participants,

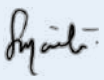
Welcome to six days of total immersion in the science and technology of light, as applied to biology! The first three days (FCS XV) is a deep dive into methodologies. It offers one of the best opportunities available anywhere in the world to train yourself in the most advanced techniques, absolutely hands on, right from the masters. In the last three days (OWLS-17) you get to discuss the new science these techniques make possible.

International Society for Optics Within Life Sciences was founded in 1991 with its headquarters in Germany. It is affiliated with the International Commission for Optics (ICO). Since then, this biennial conference has taken place in many parts of the world, notably in Mumbai in 2016. Now it is back to India again.

FCS (the name of this workshop is derived from Fluorescence Correlations Spectroscopy, though nowadays it contains much beyond that) is an annual hand-on workshop that started in 2009, in TIFR, Mumbai. It is a one-of-a-kind workshop that encourages students to understand, modify and build their own scientific instruments. It is usually followed by a scientific meeting, which is subsumed into OWLS-17 this year.

The FCS series is run by the Fluorescence Society, but made possible by a dedicated set of friends coming from all corners of the earth, every year. I should at least mention the trio whose passion makes it possible every year: Jyotishman Dasgupta, Sobhan Sen, and Anindya Datta.

The FCS series has acquired an iconic status in the Indian scientific calendar. The workshop is intense, but informal and egalitarian, as science should always be. So whether you are here for the first time, or an 'FCS regular', buckle up and enjoy the ride. There are bound to be some wrinkles in the arrangement of such a large endeavor, and we apologize in advance for those. But focus on the science, and you will be mesmerized!



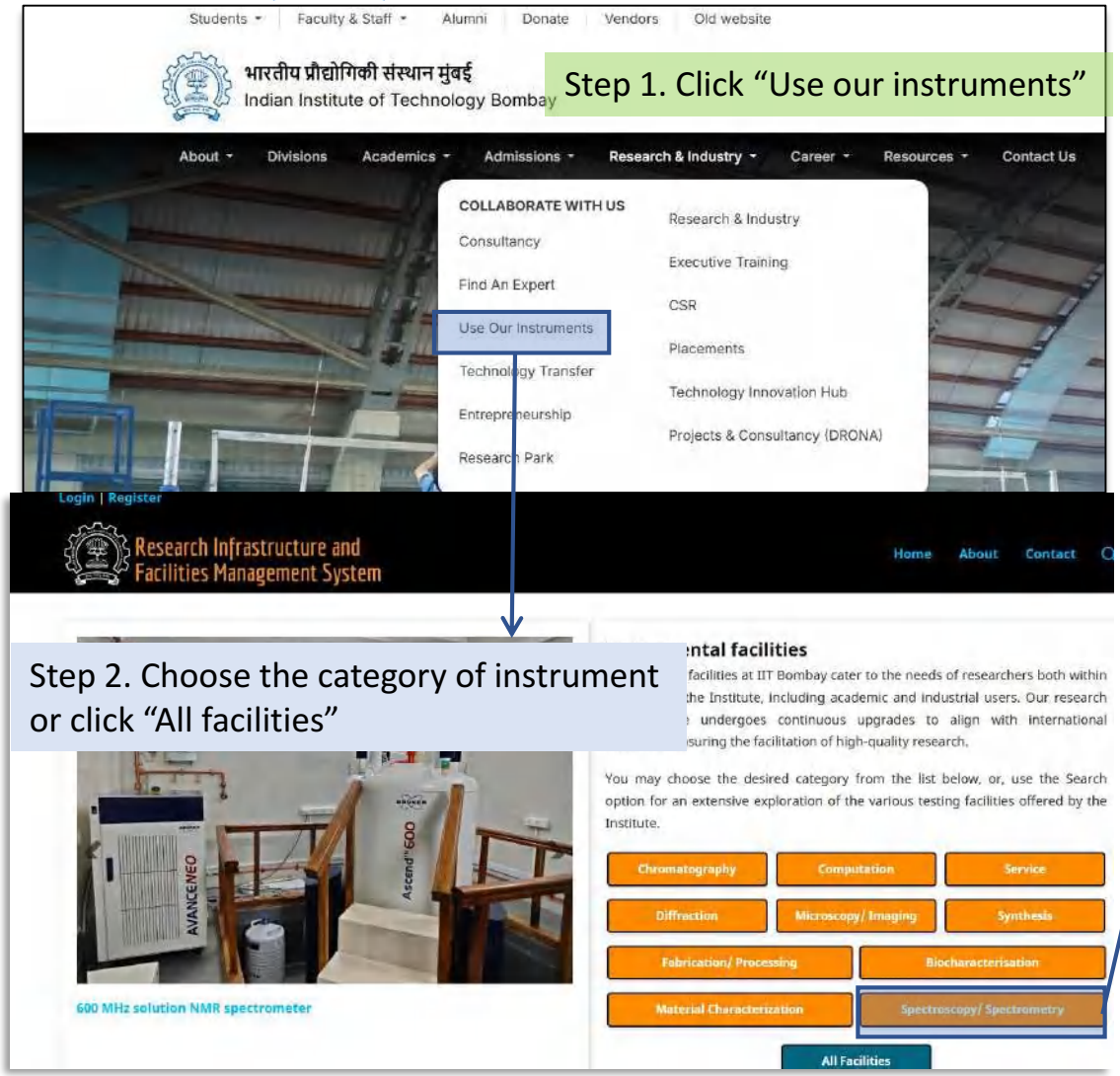
Sudipta Maiti

President, International Society for Optics Within Life Sciences

Centre for Sophisticated Instruments and Facilities

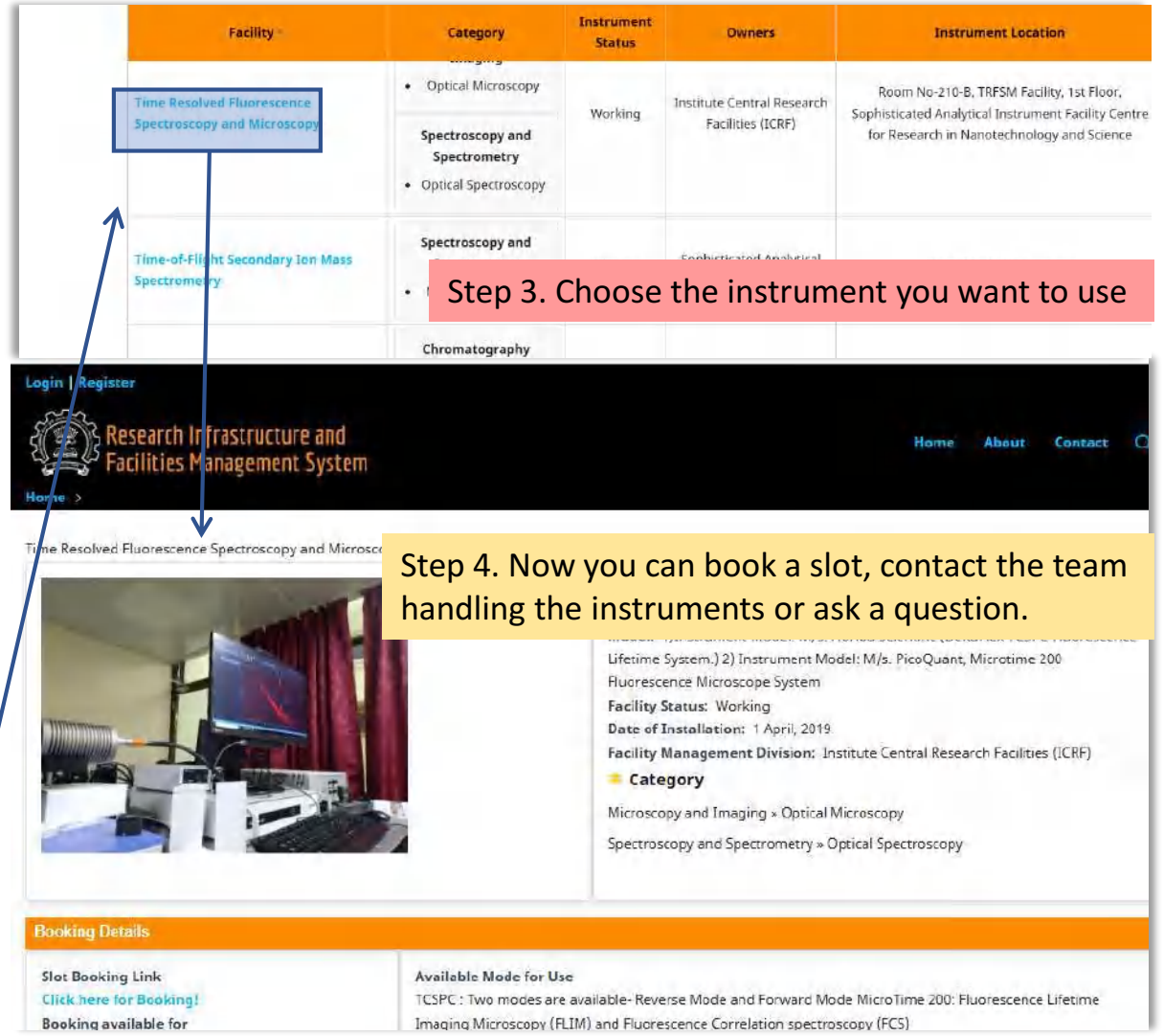
Central Facilities of IIT Bombay are open to users from all parts of India

The instruments are accessible from our institute home page, www.iitb.ac.in



Step 1. Click “Use our instruments”

Step 2. Choose the category of instrument or click “All facilities”



Step 3. Choose the instrument you want to use

Step 4. Now you can book a slot, contact the team handling the instruments or ask a question.

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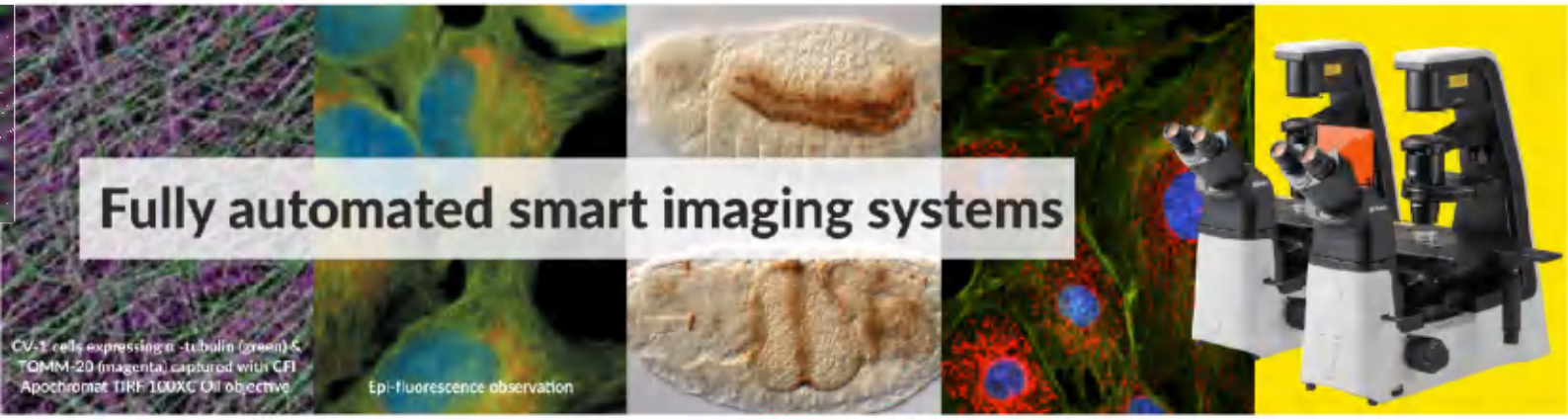

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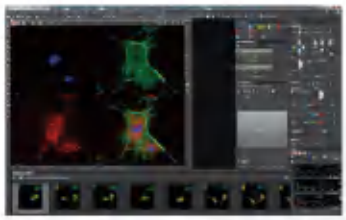

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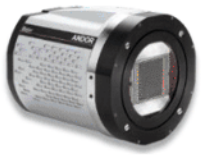

Ultrafast ICCD detectors, Imaging CCD, High resolution spectrographs, Raman, Fluorescence, Micro spectroscopy (UV-VIS-NIR), Plasma spectroscopy

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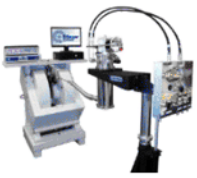
Flow Cryostats for 3K and 77K measurements with ultra low vibration system. Cryogen free cryostats for 4K measurements for optical & transport experiments

Stinger Cold Edge



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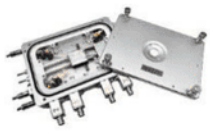
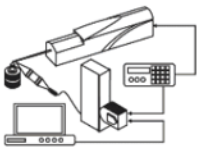
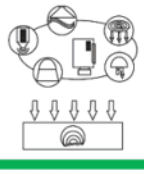
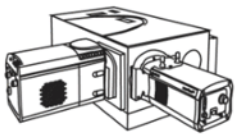


Thermal stages and thermal probe stations for optical and electrical experiments. Under controller environment and L2 setup, -190 to 1000 degree Celsius







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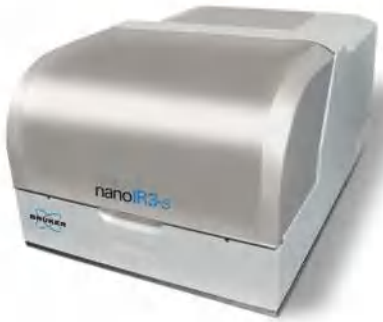

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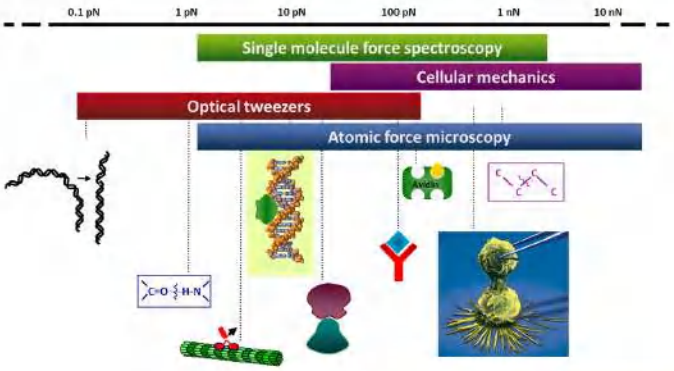
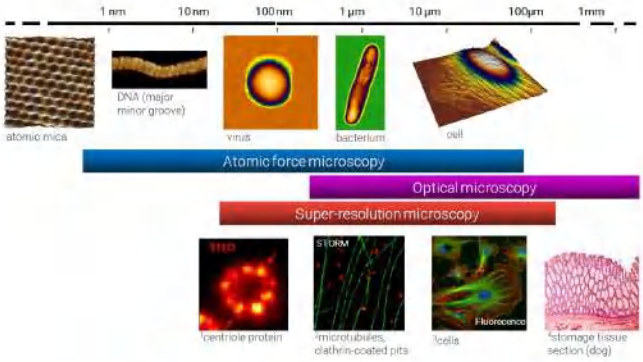
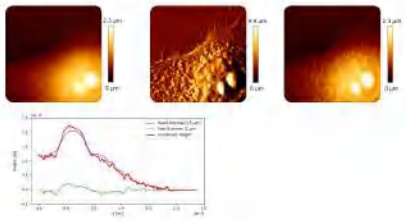
Bruker BIOAFM Products with their Applications in biological research





JPK Nanowizard IV & V BioAFM Platform

NestedScanner technology



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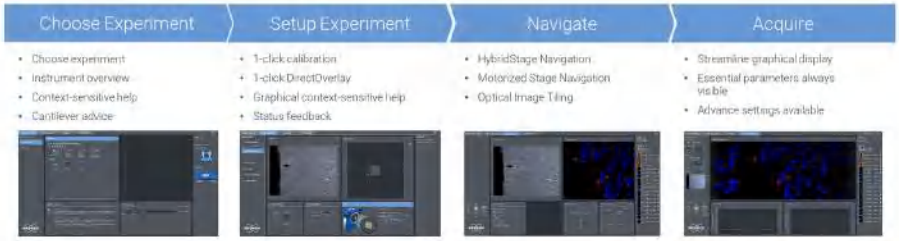
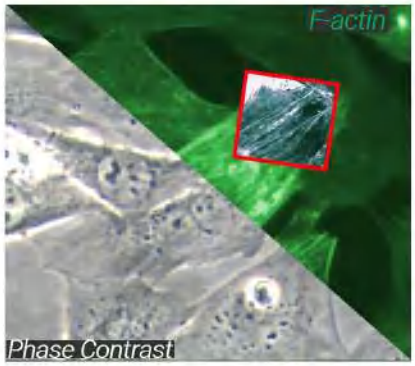
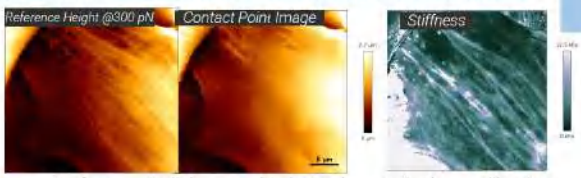


Correlative Multiparametric Characterization of NIH-3T3 Murine Fibroblasts





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Sample courtesy of Dr. S. Vedepohl, Freie Universität Berlin, DE

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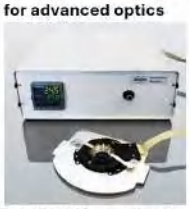

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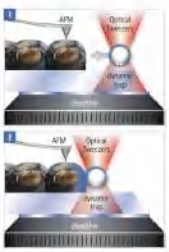
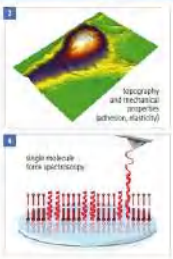


Biochemical stimulation

- Temporal and spatial control
- Multi-component design

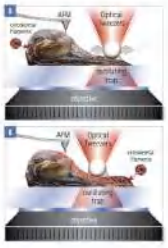
OT-AFM Applications



AFM data delivers

Height, stiffness, adhesion

- Molecular recognition
- Nanoscale imaging



Mechanical stimulation

- External/internal
- Cytoskeletal response
- Mechanical changes

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Specifications :

- *Wavelength : 1030 nm / 920 nm / 780 nm*
- *Power : upto 3.5 W*
- *pulsewidth : < 150 fs*

APPLICATIONS :

- Two Photon Polymerization
- Spectroscopy

CHAMELEON VISION

Specifications :

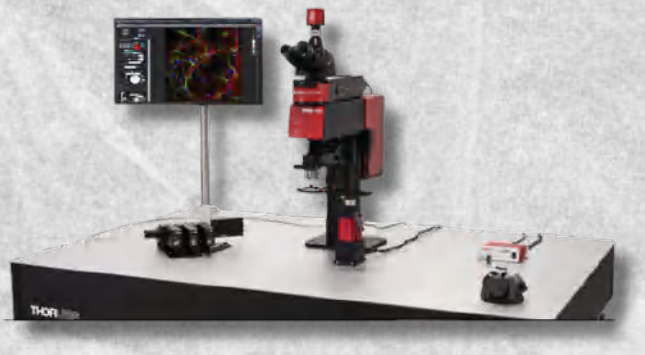
- *Wavelength : 680 nm to 1080 nm*
- *Power : upto 3 W*
- *pulsewidth : < 140 fs / 75 fs*

APPLICATIONS :

- Time Resolved Spectroscopy
- Optogenetic Photo Activation

THORLABS

Thorlabs' Confocal Microscopy System



APPLICATIONS :

- Multi-Channel Fluorescence and Spectral Imaging
- Background Fluorescence Reduction

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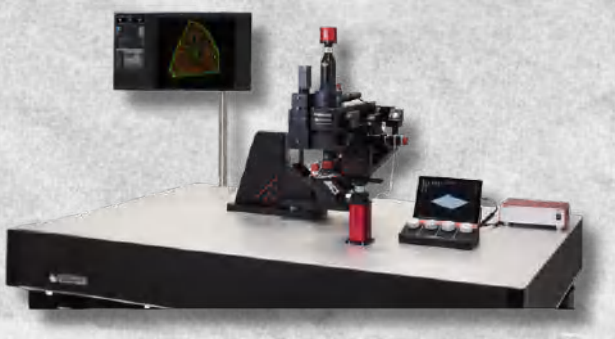

APPLICATIONS :

- Structural Neurobiology
- Neurological Disorders
- Synapses and Circuits

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OWLS 17 Schedule

Day 1. 18th November (Monday)				
Time	**Room A** (LH 21)	**Room B** (LH 22)	**Room C** (LH 32)	**Room D** (LH 33)
11:00-13:00	**Registration**, Ground Floor, Victor Menezes Convention Centre (VMCC), IIT Bombay			
13:00-14:30	Lunch, Second Floor Foyer & Canopy Area			
Session 1.1. 14:30 - 15:45	***Chair***: Sudipta Maiti, *BITS Pilani, Hyderabad campus* **Inauguration** **Plenary Talk 1:** Catherine Royer, *Rensselaer Polytechnic Institute:* When Quantity Counts Prof. B. Nag Auditorium			
15:45 - 16:00	Tea Break, Ground Floor Cafeteria			
	Chair: Pradeepkumar P. I. *IIT Bombay*	**Chair**: Anirban Banerjee *IIT Bombay*	**Chair**: Dipak K. Palit *UM-DAE CEBS*	**Chair**: Roop Mallik *IIT Bombay*
Session 1.2 16:00-18:00	Hagen Hofmann *Weizmann Institute of Science, Israel* Model-free Photon Analysis of Diffusion-based Single-molecule FRET Experiments	Yitzhak Tor *University of California* Fluorescent Nucleosides, Nucleotides and Oligonucleotides	Martin T. Zanni *University of Wisconsin, Madison* Hyperspectral 2D IR Imaging: Application to Amyloid in Cataracts and Pancreas Tissues	Arne Gennerich *Albert Einstein College of Medicine* Kinesin-14 HSET and KlpA are Non-processive Microtubule Motors with Load-dependent Power Strokes
	Amitabha Chattopadhyay *CSIR-CCMB, Hyderabad* Exploring Cholesterol Sensitivity of G Protein-Coupled Receptors: Excitements and Challenges	Ankona Datta *TIFR Mumbai* Lighting up Lipids	Sayan Bagchi *CSIR-NCL, Pune* Water-Enhanced DES Electrolytes: A Path to Stable Zinc-Ion Batteries	Arindam Chowdhury *IIT Bombay* Probing Surface Polarity of Amyloid Fibrils using Sensitized Emission Imaging
	Ruchi Anand *IIT Bombay* Combating Target Modification based Antimicrobial Resistance: A War at the Microscopic Level	Tobias Baumgart *University of Pennsylvania* Mechanisms of Cellular Membrane Shape Transitions: From Lipids to Fast Endophilin mediated Endocytosis	Mahesh Hariharan *IISER Thiruvananthapuram* A Bottom-Up Approach to Explore Structure and Property in Eumelanin	Kanchan Garai *TIFR Mumbai* Single molecule Investigation of Liquid-Liquid and Liquid-Solid Phase Separation of Amyloid Proteins
	Jaydeep Kumar Basu *IISc. Bangalore* Unravelling Direct Correlations between Membrane Nanodomain Reorganization and Antimicrobial Resistance Evolution in Bacterial Cells	Dhiraj Devidas Bhatia *IIT Gandhinagar* DNA based Programmable Nanodevices to Program Biological Membranes	Ajay Jha *Rosalind Franklin Institute* EVV 2DIR Spectroscopy for Protein-Inhibitor Interaction Analysis	Krishnananda Chattopadhyay *CSIR-IICB, Kolkata* Conformational Fluctuations of Proteins: From Test Tubes to Neurodegenerative Disease
18:00 - 18:10	Break			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

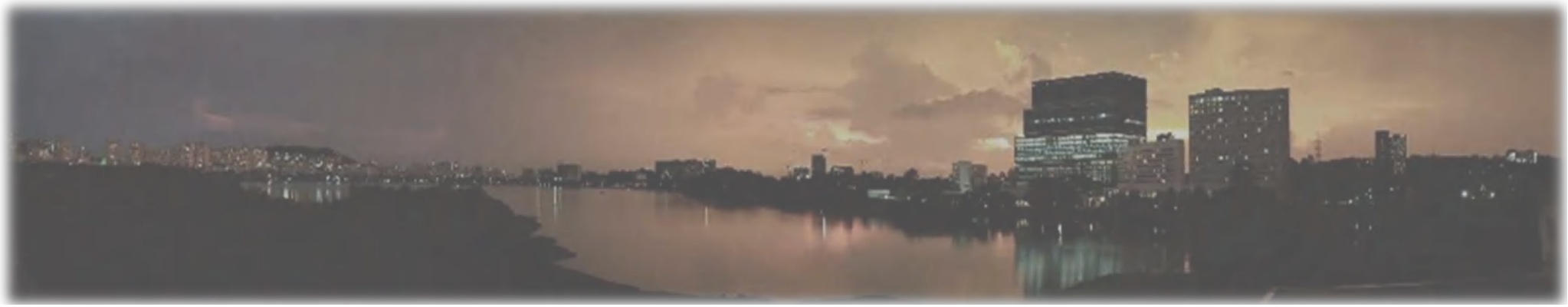
FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

Day 1. 18th November (Monday)

Time	Room A (LH 21)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
	Chair: Arindam Chowdhury *IIT Bombay*	**Chair**: Ruchi Anand *IIT Bombay*	**Chair**: Pradipta Purkayastha *IISER Kolkata*	**Chair**: Nibedita Pal *IISER Tirupati*
Session 1.3 18:10 -19:50	Yale E Goldman *University of Pennsylvania and University of California at Davis* IDR-driven Nanometer-scale RNA Helicase Protein Clusters (RPCS) are Dynamic Assemblies	Hari Shroff *Howard Hughes Medical Institute* Data-Driven Image Restoration	Katsumasa Fujita *Osaka University* Low-temperature Raman Imaging of Cryofixed Biological Samples	Gunjan Mehta *IIT Hyderabad* Single-molecule Imaging and Tracking in Live Yeast S. cerevisiae for Quantifying the Dynamics and Target-search Mechanism of Aurora kinase B (Ipl1)
	Michael Schlierf *TU Dresden, Germany* Farewell to Single well: Automated Multiwell Single-molecule FRET to Screen Molecular Interactions and Dynamics	Shobhna Kapoor *IIT Bombay* Chasing the Functions of Mycobacterium Tuberculosis Glycolipids during Infection	Debanjan Bhowmik *Rajiv Gandhi Centre for Biotechnology, Kerala* Designing of HER2-targeting Metal Nanoconstructs for Therapeutic and Diagnostic Applications	Steve Presse *Arizona State University, USA* Single molecule-Single Photon Analysis: From One Confocal Spot to SPAD Array Tracking
	Saumya Saurabh *New York University* Advances in Super-Resolution Microscopy and Label-Free Condensate Characterization for Cellular Biophysics	Senthil Arumugam *Monash University, Australia* A Matter of Time: Catching Organelles in Action in vitro and in vivo	Nakul Chandra Maiti *CSIR-Indian Institute of Chemical Biology* Raman Parameters to Monitor Toxic Protein Oligomers Linked to Parkinson Diseases	Amartya Bose *TIFR Mumbai* Exciton Transport in Molecular and Polaritonic Aggregates
	Technical Talk (virtual): Ali Raja, ONI, Prof. B. Nag Auditorium			
20:00 - 22:00	Dinner, Second Floor Foyer & Canopy Area			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)



FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

टामूअसं tifr

Day 2. 19th November (Tuesday) ATOS Day

Time	Room A (LH 21)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
Session 2.1 09:00 - 10:30	***Chair*:** Jyotishman Dasgupta, *TIFR Mumbai* **Plenary Talk 2:** Vahid Sandoghdar, *Max Planck Institute for the Science of Light, Erlangen, Germany* iSCAT Microscopy: Label-free 3D Imaging of Live Cells, Viruses and Proteins **Mihir Chowdhury Student Fellowship Talk 1:** Pritam Saha, *IISER Mohali* *Inverse Adaptation to Force Modalities in Multidomain Proteins: The role of Interdomain Linkers* **Invited Short Talk:** Gabriel Moya, *Technische Universität, Dortmund, Germany* Single-molecule Spectroscopy & Super-resolution Microscopy at the Biochemistry Lab Bench **Technical Talk 1.** Folusho Helen, *ATOS:* Advances in Detectors in Fluorescence Spectroscopy Prof. B. Nag Auditorium			
10:30 - 11:00	Tea Break, Ground Floor Cafeteria			
	Chair: Devang Khakhar *IIT Bombay*	**Chair:** Kasturi Saha *IIT Bombay*	**Chair:** A. Q. Contractor *IIT Bombay*	**Chair:** Sabyasachi Rakshit *IISER Mohali*
Session 2.2 11:00 -13:00	Jayant B. Udgaonkar *IISER Pune* Induction of Structure in the Intrinsically Disordered Region of the Mammalian Prion Protein in Prion Protein Condensates	Christoph J. Fahrni *Georgia Institute of Technology, Atlanta, GA, USA* Zn II-Responsive Ratiometric Fluorescent Probes for Two-Photon Excitation Microscopy	Martina Havenith-Newen *Ruhr University Bochum, Germany* Probing Free Energy in Reactions by THz spectroscopy – Ask the Water!	Julie Biteen *University of Michigan* Measuring Interactions and Biomolecular Condensates in Microbes
	Sudipta Maiti *BITS-Pilani, Hyderabad* Reducing Membrane Cholesterol inhibits Neuronal Exocytosis	David Margulies *Weizmann Institute of Science* Fluorescence Labeling of Cancer Cells with Chemically Modified Bacteria	Rajib Kumar Mitra *S.N. Bose National Centre for Basic Sciences* Impact of Ion Solvation on Biomolecular Condensation: The Hofmeister Series and Beyond	Juergen Czarske *TUD Dresden University of Technology, Germany* Human induced Pluripotent Stem Cell-derived Neurons and Cardiomyocytes Investigated by Holographic Closed-loop Optogenetics
	Samir Maji *IIT Bombay* Amyloid Nucleation through Protein Phase Separation	Sarit S. Agasti *JNCASR, Bengaluru* Expanding Imaging Capabilities Beyond Traditional Microscopy through Dynamic Host-Guest Molecular Interactions	Amartya Sengupta *IIT Delhi* THz Sensing for Biochemical and Environmental Applications	Thorsten Wohland *National University of Singapore* The Cortical Actin Cytoskeleton Regulates Membrane Protein Organization and Dynamics
	Samrat Mukhopadhyay *IISER Mohali* Prying into Biological Condensates Using Single-Molecule and HomoFRET	Sebastian Kruss *Ruhr University Bochum* Near Infrared Fluorescence Imaging for Biomedical Applications	Anindya Datta *IIT Bombay* Fluorogenic Probes for Biomolecular Interactions	Bidyut Sarkar *Shiv Nadar Institution of Eminence* Pulsed-interleaved-excitation Two-Dimensional Fluorescence Lifetime Correlation Spectroscopy (PIE 2D FLCS): Development and Applications to Study Biomolecular Structural Dynamics
13:00 - 14:00	Lunch, Second Floor Foyer & Canopy Area			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

OWLS

टामूअसं tifr

Day 2. 19th November (Tuesday) ATOS Day

Time	Room A (LH 21)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
	Chair: Debjani Paul *IIT Bombay*	**Chair**: A. S. R. Koti *TIFR Mumbai*	**Chair**: Sachin Dev Verma *IISER Bhopal*	**Chair**: G. Naresh Patwari *IIT Bombay*
Session 2.3 14:00-16:00	Elvis Pandzic *UNSW Sydney* Biophysical Tools for Characterizing Ciliary Dynamics in Primary Cells: Towards Personalized Respiratory Disease Diagnostics and Treatment	Mily Bhattacharya *Thapar University* Regulation of Protein Aggregation and Disaggregation Using Salts	Shinsuke Shigeto *Kwansei Gakuin University* Nondestructive Single-Cell Identification of Microbial Species and Domains Using Raman Microspectroscopy and Machine Learning	Hugo Sanabria *Clemson University* Refining Single-Molecule FRET Analysis: New Models for Biomolecular Dynamics
	Pramit K Chowdhury *IIT Delhi* Ultrafast Energy Flow of Heme Proteins In Crowded Milieu	Tamal Das *TIFR Hyderabad* Imaging Cellular Organelles during Collective Cell Migration	Yoosaf Karuvath *Cochin University of Science and Technology, Kochi* Raman Spectroscopy for Quantitative Estimation of Food Adulterations and Disease Biomarkers	Jatish Kumar *IISER Tirupati* Circularly Polarized Light Emission in Chiral Nanomaterials
	Chayan Kanti Nandi *IIT Mandi* Fluorescent Nano Probes for in Vivo Long-Term Tracking and Super-Resolution Imaging of Lysosomal Dynamics	Sabyasachi Rakshit *IISER Mohali* Replicating Active Transport of Micro-organisms in Synthetic Systems	Soumik Siddhanta *IIT Delhi* Machine Learning-driven High-resolution Raman Spectral Generation for Accurate Molecular Feature Recognition	Trevor Smith *University of Melbourne, Australia* Time-resolved, Polarised Fluorescence Microscopy – Influence of Alignment
	Sivaprasad Mitra *North-Eastern Hill University, Shillong* Therapeutic Advantages of Drug-Composites: Development and Repurposing of Acetylcholinesterase Inhibitors	Bibhu Ranjan Sarangi *IIT Palakkad* Stiffness Gradient Substrate for Cellular Mechano-sensing	Santhosh Chidangil *Manipal Academy of Higher Education* Human Platelet Activation Dynamics Probed by Optical Techniques	Hema Chandra Kotamarthi *IIT Madras* Fate of Knotted Proteins during Direction Degradation and Constrained Folding Conditions
16:00 - 18:00	Poster Session-1 FCS + OWLS with Tea/Coffee, Ground Floor			
Session 2.4 18:00 - 19:15	***Chair:*** Anindya Datta, *IIT Bombay* **Plenary Talk 3:** Tahei Tahara, *RIKEN Japan* Perpendicular Phantom State in cis-trans Photoisomerization Captured by Ultraviolet Femtosecond Time-resolved Raman Spectroscopy **Kankan Bhattacharyya Student Fellowship Talk:** Arup Kundu, *MIT, USA* Singlet Fission Induced TT Pair Generation in Chiral Diketopyrrolopyrrole Aggregates **Technical Talk 2:** Aum Shethia, *ATOS*: Introduction to New Scientific Instruments Prof. B. Nag Auditorium			
19:30 - 23:00	Banquet Dinner at Athena, Supreme Business Park (Hosted by ATOS)			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

Day 3. 20th November (Wednesday) SIMCO day

Time	Room A (LH 21)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
Session 3.1 09:00 - 10:30	***Chair***: Sobhan Sen, *JNU, New Delhi* **Plenary Talk 4:** Taekjip Ha, *Boston Children's Hospital and Harvard Medical School,* Probing Mechanical Selection through Accurate Prediction of 3D DNA Mechanics **Mihir Chowdhury Student Fellowship Talk 2:** Aisworika Mohanty, *TIFR, Mumbai*, Modulating Singlet Fission in NDI Dimers by Tuning Inter-chromophoric Electronic Coupling **R. S. Daryan Student Fellowship Talk:** Vivek Nagendra Bhat, *IISC Bangalore*: Rapid Scan White Light Two-Dimensional Electronic Spectroscopy with 100kHz Shot-to-Shot Detection **Technical talk 3:** *SIMCO*: Thomas Klos, *TOPTICA Photonics*: Fiber Lasers for FLIM Applications Prof. B. Nag Auditorium			
10:30 - 11:00	Tea Break, Ground Floor Cafeteria			
	Chair: Shobhna Kapoor *IIT Bombay*	**Chair:** Sandip Kar *IIT Bombay*	**Chair:** Tapanendu Kundu *IIT Bombay*	**Chair:** Kanchan Garai *TIFR Hyderabad*
Session 3.2 11:00 -13:00	Yraima Cordeiro *Federal University of Rio de Janeiro, Brazil* Heterotypic Phase Transitions of the Prion Protein Modulated by Copper ions: A Biophysical Approach	Christopher Xu *Cornell University* Pushing the Limits of Multiphoton Imaging in Living Systems	Debabrata Goswami *Indian Institute of Technology Kanpur* Decoding Structure, Dynamics, and Synergism in Solutions Through Femtosecond Laser Spectroscopy	Jelle Hendrix *Biomedical Research Institute, BIOMED, Belgium* Structure and Dynamics of Tau Protein Across Liquid Phases
	Nibedita Pal *IISER Tirupati* Interaction of Human BRAC1 Protein with Holliday Junction: Preference for an Open X-like conformation	Nirmalya Ghosh *IISER Kolkata* Polarized optical pathways towards next generation optical techniques for biomedical imaging and diagnosis	Jahur A. Mondal *BARC, Mumbai* Structural and Orientational Transformations of Water at Air/Water-Polyethylene Glycol Polymer Interface	Oleg Krichevsky *Ben-Gurion University of the Negev, Israel* T cell communication through cytokines: simple physics in a complex system
	Umakanta Tripathy *IIT ISM Dhanbad* Introducing a Novel Optical Device to Study Protein Aggregation in Real-time	Maithreyi Narasimha *TIFR Mumbai* Shaping and Moving Tissues during Morphogenesis: Cytoskeletal Organisation Entrains the Morphodynamic, Mechanical and Material Properties of Cell Cohorts	Rahul Gera *RIE Mysore, NCERT* Understanding Aqueous Interfaces Using Vibrational Sum-Frequency Generation Spectroscopy	Rahul Roy *IISc. Bangalore* Design Principles of Pore Forming Toxins: Learnings from Cytolysin A
	Sobhan Sen *JNU, New Delhi* Probing Ligand Binding Kinetics with G-Quadruplex DNA using Fluorescence Correlation Spectroscopy and MD Simulations	Anjana Badrinarayanan *NCBS-TIFR* Searching for Homology	Luuk van Wilderen *Johann Wolfgang Goethe-Universität* External Manipulation of the Absorption Spectrum of a Photoreceptor	Dibyendu Kumar Das *IIT Kanpur* Single Molecule Imaging of Coronavirus Spike Trimers Conformational Dynamics during Membrane Fusion for Entry
13:00 - 14:00	Lunch, Second Floor Foyer & Canopy Area			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

टामूअसं tifr

Day 3. 20th November (Wednesday) SIMCO Day

Time	Room A (LH 21)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
	Chair: Ashutosh Kumar *IIT Bombay*	**Chair:** Rajarshi Chakrabarti *IIT Bombay*	**Chair:** E. Siva Subramaiam Iyer *IIT Goa*	**Chair:** Padmaja Prasad Mishra *Saha Institute of Nuclear Physics*
Session 3.3 14:00 - 16:00	Tatini Rakshit *Shiv Nadar University* Probing Cancer Biomarkers with Biophysical Tools	Roop Mallik *IIT Bombay* Selective Targeting of Kinesin on Lipid Droplets Reduces Serum Triglycerides	Pratik Sen *IIT Kanpur* Critical Role of Associated Water in Protein Stability and Activity	Nils G. Walter *University of Michigan* Single Molecules Come into Focus: From Bacterial Riboswitches to Mammalian Cellular Phase Separation
	Elizabeth Hinde *University of Melbourne* Pair Correlation Microscopy of Heterotrimeric Transcription Factor Transport	A. Sri Rama Koti *TIFR, Mumbai* Liquid-Liquid Phase Separation (LLPS) of Small Ubiquitin-related Modifier (SUMO1) and its Multimers	Manik Pradhan *S.N. Bose National Centre for Basic Sciences* Breath Analysis in Disease Detection through Spectroscopic Signatures	Abhishek Mazumder *CSIR-IICB* Investigating Mechanism of Coupled Molecular Machines in Live Cells using Single Particle Tracking FRET (spt-FRET)
	Ammasi Periasamy *University of Virginia* Investigation of Mitochondrial Dysfunction Related to Cancer and Alzheimer Disease: Two-photon FLIRR Microscopy	Ayan Banerjee IISER Kolkata Microbubble Lithography: Writing Mesoscopic Architectures of 'Everything Mesoscopic' using Laser-nucleated and Manipulated Microbubbles	Sharmishta Sinha *INST Mohali* Mutants, Molecules, and Mayhem: Bile Acids Fuel p53 R273 Aggregation and Chemoresistance	Don C. Lamb *Ludwigs-Maximillians-Universität München, Germany* Deep-Learning and the Tunability of DNA Origamis
	Takuhiro Otosu *Saitama University* Fluorescence Lifetime Correlation Analyses Reveal Leaflet-specific Lipid Diffusion in a Lipid Bilayer	Subhashis Haldar *SNBNCBS, Kolkata* Force-Driven Transformation of Tunnel-Associated Chaperones: Unveiling a Strain-Energy-Based Mechanism for Enhanced Protein Folding	Neha Jain *IIT Jodhpur* Deciphering the Modulation of α-Synuclein Amyloid Assembly by β_2-Microglobulin Conformers	Dibyendu Kumar Sasmal *IIT Jodhpur* Single Molecule Conformational Dynamics of DNA Holliday Junction and T-cell Receptor
16:00 - 18:00	Poster Session-2 FCS + OWLS with Tea/Coffee, Ground Floor			
Session 3.4 18:00 - 19:15	***Chair:*** Debanjan Bhowmik, *Rajiv Gandhi Centre for Biotechnology, Kerala* **Plenary Talk 5:** Jörg Enderlein, *Georg August University Göttingen, Germany* Image-Scanning Single-Molecule Localization Microscopy **Watt Webb Student Fellowship Talk 1:** Deepika Sardana, *JNU New Delhi,* and *EPFL Switzerland:* DNA's Temporal Voyage Through the Currents of Biological Water **Technical Talk 4:** SIMCO, Mathias Bayer, *Picoquant, Germany*: Single Molecule Microscopy applications accessible as never before. **Technical Talk 5:** SIMCO, Andrea Giudice, *MD-Micro Photon Devices*: SPAD detectors for Single-photon Counting Prof. B. Nag Auditorium			
19:15 - 21:30	Evaluation of Shortlisted Posters, Ground Floor			
20:00 - 22:00	Gala Dinner, Second Floor Foyer & Canopy Area (Hosted by SIMCO)			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

टामूअसं tifr

Day 4. 21st November (Thursday)

Time	Room A (LH 31)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
	Chair: M. Ravikanth *IIT Bombay*	**Chair:** Sreelaja Nair *IIT Bombay*	**Chair:** Anindya Datta *IIT Bombay*	**Chair:** R. B. Sunoj *IIT Bombay*
Session 4.1 09:00 - 10:40	Soumit Sankar Mandal *IISER Tirupati* Cren7: Insights into an Extremophile Protein's Structural and Functional Attributes	Padmaja Prasad Mishra *Saha Institute of Nuclear Physics, Kolkata* Emergence of Dynamic G-Tetraplex Scaffold: Uncovering Low Salt-Induced Conformational Heterogeneity	R. J. Dwayne Miller *University of Toronto* Mapping Atomic Motions with Ultrabright Electrons: Fundamental Space-Time Limits to Imaging Chemistry and Biology	Maria Andrea Mroginski *Technische Universität Berlin* Hydrogen Bonding and Non-covalent Electric Field Effects in the Photoconversion of Phytochrome
	Saptarshi Mukherjee *IISER Bhopal* Designing an Artificial Light Harvesting System and Monitoring Conformational Dynamics of i-motif DNA Using Förster Resonance Energy Transfer	Laura C Zanetti-Domingues *UKRI-STFC* (Cryo-)vEM and (cryo-) CLEM at the CLF Octopus Facility	Donatas Zigmantas Lund University, Sweden Mapping Energy Transfer in Photosynthetic Bacteria in vivo	Biswarup Pathak *IIT Indore* Artificially Intelligent Nanopores for High-Throughput DNA Sequencing
	Tushar Kanti Mukherjee *IIT Indore* Biomolecular Condensation of Trypsin Prevents Autolysis and Promotes Ca^{2+}-Mediated Activation of Esterase Activity	Marisa Martin Fernandez UKRI-STFC Rutherford Appleton Laboratory Drug-resistant EGFR Mutations Promote Lung Cancer by Stabilizing Interfaces in Ligand-free Kinase-active EGFR Oligomers	David M. Jonas *University of Colorado Boulder* Generalized Einstein Relations between Absorption and Emission: A Theory of Fluorescence, Excited State Thermodynamics, and Extreme Stokes Shifts	Ravindra Venkatramani *TIFR Mumbai* The Optical Properties of Charged Amino Acids: New Avenues for Label-Free UV-Visible Spectroscopy of Biomolecules
	Shashi Thutupalli *NCBS, Bangalore* Using FCS to Uncover a Two-Component Molecular Motor driven by a GTPase Cycle	Kaushik Pal *IIT Tirupati* Role of pN Level Molecular Tension in Immune Cell Pathogen Interactions	Jyotishman Dasgupta *TIFR Mumbai* Ultrafast Charge Transfer Chemistry in Metalloproteins and Biomimetic Nanocages	Amber Jain *IIT Bombay* Energy Transfer in Molecular Wires: New Insights
10:40 - 11:10	Tea Break, Ground Floor Cafeteria			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

टामूअसं tifr

Day 4. 21st November (Thursday)

Time	Room A (LH 31)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
	Chair: Haridas Pal *HBNI*	**Chair:** Ishita Sengupta *IIT Bombay*	**Chair:** Nandita Madhavan *IIT Bombay*	**Chair:** Saptarshi Mukherjee *IISER Bhopal*
Session 4.2 11:10 - 13:00	Rajaram Swaminathan *IIT Guwahati* UV-Visible Spectra in Proteins arising from Charge Transfer: A useful Intrinsic Probe to investigate Changes in Protein Structure	Sukhendu Nath *BARC Mumbai* Molecular Probes for protein Oligomers and Fibrils	Achillefs Kapanidis *University of Oxford* Unlocking Gene Expression Mechanisms via Next-generation Single-molecule Imaging	Abhijit Patra *IISER Bhopal* Thermally Activated Delayed Fluorescent Probes for Elucidating Interorganelle Interactions and Time-resolved Imaging of Lysosomal Polarity
	Pradipta Purkayastha *IISER Kolkata* Exploring the Interface between DNA Structures and Fluorescent Nanomaterials	Amrita Chatterjee *BITS Pilani, Goa* Fancying 10,12-Pentacosadiynoic Acids as Multipurpose Dual-output Chemosensors	Kedar Khare *IIT Delhi* Bright-field Imaging Techniques inspired by Super-resolution Microscopy	Hirak Chakraborty *Sambalpur university* Developing Peptide-based Broad-Spectrum Fusion Inhibitors as an Antiviral Strategy
	Manab Chakravarty *BITS Pilani Hyderabad* Suitably Decorated 2,4,6-tristyrylpyrillium Salts in Detecting Crucial Biological Amines through Diverse Optical Responses	Dimpy Kalia *IISER Bhopal* Location-agnostic Site-specific Protein Bioconjugation via BHoPAL	Barun K Maity *Saha Institute of Nuclear Physics, Kolkata* Peptide-PAINT: A Transfected Docker Simplifies Live and Fixed Cell Super-resolution Imaging.	Nirmalya Bag *IIT Kharagpur* Functional Transbilayer Coupling of Plasma membrane Leaflets in Live Cells Revealed by Imaging Fluorescence Correlation Spectroscopy
	Ravikrishnan Elangovan *IIT Delhi* High-precision Myosin II Step Size Measurement with Single Quantum Dot Tracking in Motility Assays	Joshy Joseph *CSIR-NIIST* Design of Fluorescent Probes for Cellular Imaging and Theranostic Applications	Koushambi Mitra *IIT Jodhpur* MitRatiNa: A Fluorescent Reporter for Measuring Mitochondrial Sodium	Kunihiko Ishii *RIKEN* Two-dimensional Fluorescence Lifetime Correlation Spectroscopy: Recent Development and Applications
13:00 - 14:00	Lunch, Second Floor Foyer & Canopy Area			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
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FLUORESCENCE SOCIETY

International Society on Optics Within Life Sciences

टामूअसं tifr

Day 4- 21st November (Thursday)

Time	Room A (LH 31)	Room B (LH 22)	Room C (LH 32)	Room D (LH 33)
	Chair: Sukhendu Nath *BARC Mumbai*	**Chair:** Samir Kumar Maji *IIT Bombay*	**Chair:** Rajaram Swaminathan *IIT Guwahati*	**Chair:** Abhijit Patra *IISER Bhopal*
Session 4.3 14:00 - 16:00	Prabhat Kumar Singh *BARC Mumbai* Fluorescence Sensors for Clinically Relevant Analytes: Harnessing Aggregation-Induced Emission for Enhanced Detection	Takakazu Nakabayashi *Tohoku University* Highly Sensitive Raman Measurements of Biomolecules in a Liquid Droplet formed by Liquid–Liquid Phase Separation	Soumyo Mukherji *BITS Pilani, Hyderabad* Bend it, Functionalise it and Sense the World Around	Cecile Fradin *McMaster University* Tracking, Photobleaching & Correlating: How to Catch Small Mobile Molecular Condensates
	Soumit Chatterjee *IIT ISM Dhanbad* Elucidation of the Role of Electronic Effect on Doubly Locked GFP Chromophore Analogues to Help Design Improved Fluorophores	Sua Myong *Johns Hopkins University* DNA Supercoiling-mediated G4/R-Loop Formation Tunes Transcription by Controlling the Access of RNA Polymerase	Aarat P Kalra *IIT Delhi* Triplet Energy Migration in the Cytoskeleton	Manoj Kumbhakar *BARC Mumbai* Probing Molecular Interaction with Single Molecule Sensitivity
	Sourav Kumar Dey *IIT ISM Dhanbad* Plug-and-play Fluorophores for Squash RNA Aptamer allow mRNA Imaging in Multiple Colors	Subhabrata Maiti *IISER Mohali* Imaging and Analysis of Biocolloidal Taxis and Catalysis in Gradient of Oilgo nucleotides	Sundar Ram Naganathan *TIFR Mumbai* Material Properties Determine the Dynamics of Tissue Shape Transitions	Debasis Das *TIFR Mumbai* Defining a Nascent Protein Conformation on the Ribosome
	Sriram Kanvah Gundimeda *IIT Gandhinagar* Small molecule Fluorescent Probes for imaging Subcellular Organelles	Bappaditya Chandra *St. Jude Children's Research Hospital, Memphis* Phase Separation by the HEY1:NCOA2 Fusion Oncoprotein Drives Transcriptional Rewiring in Mesenchymal Chondrosarcoma		
16:00 - 16:30	Tea Break, Ground Floor Cafeteria			
16:30 - 17:30	Closing Session: Awards, Planning and Feedback, Prof. B. Nag Auditorium			

Fluorescence Methods (FM)	Probes in Biology (PB)	Ultrafast Spectroscopy (UFS)	Single Molecule Spectroscopy (SMS)
Super Resolution Methods (SR)	Bio-imaging in Cells (BiC)	SERS & Raman (S&R)	Theory & Modelling (T&M)

Poster Session-1 FCS + OWLS
(Date-19th November 2024)

Name	Poster No.	Name	Poster No.
Aarcha Radhakrishnan	P1	Dipin Kumar Tomar	P47
Abhirami Ajith	P3	Ganesh Agam	P49
Akhil Pathania	P5	Geetanjali Negi	P51
Anisha Bandyopadhyay	P7	Himanshi M.Devi	P53
Ankit Kumar	P9	Irfan Shafi Malik	P55
Ankita Das	P11	Jyoti Vishwakarma	P57
Anoop Philip	P13	Karpagavalli P	P59
Anuja Anand Walimbe	P15	Koustav Saha	P61
Apurva Mishra	P17	Kunika Gupta	P63
Arkaprava Chowdhury	P19	Lipika Mirdha	P65
Arti Sharma	P21	Madhuri Thorve	P67
Arun Kumar Upadhyaya	P23	Manbit Subhadarsi Panda	P69
Athira B	P25	Mansi	P71
Avijit Maity	P27	Mirza Nasib Begg	P73
Ayush Bose	P29	Mohd Mehkoom	P75
Bhawna Mishra	P31	Naini Bajaj	P77
Chanchal Sharma	P33	Nandha Kumar E	P79
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Plenary Talks

PT 1

When Quantity Counts

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Fluorescence fluctuation microscopy (FFM) approaches have been widely used to interrogate the stoichiometry, interactions and diffusion of proteins in live cells, providing deep insight into regulatory mechanisms that impact cell states. FFM yields another parameter characteristic of proteins in cells, namely their absolute concentration. This parameter has been much less widely reported in the literature. One reason may be that the general interest in the field may have been focused more on stoichiometry and diffusion than on quantity. Alternatively, for these concentration measurements to be relevant, the proteins of interest fused to either fluorescent proteins, must be expressed from their natural genetic loci. This requires more complex genetic manipulation of cell lines. Further challenges to FFM measurements of endogenous protein quantities include de-convolving autofluorescence contributions from low signal levels of weakly expressed proteins, low amplitude fluctuations from strongly expressed proteins and the intrinsic noisiness of fluctuation analysis, itself. I will present a calibration-based approach that minimizes these drawbacks. Moreover, I will discuss the importance of determining absolute concentration and copy number for revealing the mechanisms underlying biological transcriptional regulatory networks in bacteria and yeast.

PT 2

iSCAT microscopy: label-free 3D imaging of live cells, viruses and proteins

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Fundamental limitations of fluorescence imaging have motivated many groups to develop fluorescence-free methods. Among various contrast mechanisms, scattering offers unique opportunities. About two decades ago, we showed that single gold nanoparticles as small as 5 nm could be detected via interferometric detection of their scattering, coined iSCAT [1,2]. Since then, it has been shown that unlabeled nano-objects such as extracellular vesicles [3,4] and proteins as small as 10kDa can be detected, weighed, counted and tracked [5].

iSCAT is a homodyne technique based on the interference of the light scattered from a nano-object with a reference light, e.g., reflected from the sample substrate. Recently, we demonstrated that confocal iSCAT not only addresses the coherent background challenge that arises in live cell imaging, but it also offers label-free 3D images of intracellular organelles at the nanoscale. In this presentation, we show how confocal iSCAT microscopy exploits information about the material, size, shape and axial position of a nano-object to image a range of organelles and subcellular features, including mitochondria, focal adhesion points, endoplasmic reticulum networks, lipid droplets, lysosomes and microtubules [6]. The technique presents a new powerful addition to the microscopy toolbox, can be easily implemented on existing commercial instruments and be carried out simultaneously with fluorescence microscopy. We combine C-iSCAT, fluorescence microscopy and controlled delivery [7] to identify the interplay of cellular organelles and virions during the infection process [8].

References:

1. K. Lindfors, et. al. *Phys. Rev. Lett*. **2004**, 93, 037401.
2. R. Taylor, et. al. *NanoLett.* **2019**, 19, 4827.
3. A. Kashkanova, et. al. *Nat Methods* **2022,** 19, 586.
4. A. Kashkanova, et. al., *J. Extracellular Vesicles* **2023**, 12, 12348.
5. M. Dahmardeh, et. al. *Nat Methods* **2023,** 20, 442.
6. M. Küppers, et. al. *Nat Comm* **2023**, 14, 1962.
7. C. Holler, et. al. *Nat Methods* **2024**, 21, 512.
8. D Albrecht, et. al. *In prepration.*

PT 3

Perpendicular phantom state in cis-trans photoisomerization captured by ultraviolet femtosecond time-resolved Raman spectroscopy

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Trans-cis photoisomerization is one of the most fundamental photochemical reactions and plays essential roles in nature to drive various biological functions as well as in designing artificial light-energy conversion systems and molecular switches. This prototypical photochemical reaction has generally been described by a model in which the reaction proceeds via a common intermediate having a perpendicular conformation around the rotating CC bond, irrespective of from which isomer the reaction starts. However, such an intermediate was not identified unambiguously, so it was often called the "phantom" state. In this study, we succeeded in the structural identification of the common, perpendicular intermediate of stilbene photoisomerization based on its Raman spectrum. To capture the phantom state with clear structural evidence, we introduced two methyl groups to the central ethylenic part of stilbene, which significantly accelerates the photoisomerization from the trans side. Consequently, the photoisomerization processes from both cis and trans proceed on the ultrafast time scale, which can populate a sufficient amount of the perpendicular state to be observed. Second, we employed ultraviolet FSRS [1] to realize resonance with the transient absorption that had been proposed due to the perpendicular state [2]. The results revealed ultrafast birth and decay of an identical, short-lived transient that exhibits a vibrational signature characteristic of the perpendicular state upon photoexcitation of the trans and cis forms. Complementary ab initio molecular dynamics simulations provided a consistent view that the photoexcited trans and cis forms are relaxed to the ground state through the conical intersection near the perpendicular intermediate. This study fills the last piece of this textbook photochemical reaction [3].

Figure 1. Perpendicular phantom state in cis-trans photoisomerization

References:

1. H. Kuramochi et. al. *J. Phys. Chem. Lett.* **2012**, *3*, 2025.
2. F. Berndt et. al. *Chem. Phys. Lett.* **2012**, 544, 39.
3. H. Kuramochi et. al. *Nat. Chem.* **2024**, 16, 22.

PT 4

Probing mechanical selection through accurate prediction of 3D DNA mechanics

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Connections between the mechanical properties of DNA and biological functions have been speculative due to the lack of methods to measure or predict DNA mechanics at scale. Recently, a proxy for DNA mechanics, cyclizability, was measured by loop-seq and enabled genome-scale investigation of DNA mechanics. Here, we use this dataset to build a computational model predicting strand orientation-independent intrinsic cyclizability, at a near-perfect accuracy, solely based on DNA sequence. Further, the model predicts intrinsic bending direction. Using this technique, we aim to understand 'mechanical selection' - that is, the selection of DNA sequence based on its mechanical properties - in diverse circumstances. First, we found that the intrinsic preferred bend direction of DNA sequences in experimentally determined protein-DNA complex structures correlated with the direction observed in the actual structure. Further, we applied our model to the large-scale yeast population genetics data and identified that centromere DNA element II, for which the consensus sequence is unknown and thus the role of DNA sequence in its function remains unclear, is under mechanical selection to increase the stability of inner-kinetochore structure. Finally, in silico evolution discovered hallucinated sequences of extreme mechanics not found in the training set, yet, found in nature in the densely packed mitochondrial DNA of Namystynia karyoxenos, showing our in-silico method can predict extreme natural biology. Our methods offer a way to study the biological functions of DNA mechanics in any genome and open a possibility for engineering DNA sequences with desired mechanical properties.

PT 5

Image-Scanning Single-Molecule Localization Microscopy

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We introduce a significant advancement in super-resolution microscopy by integrating a single-photon detector array into a Confocal Laser-Scanning Microscope (CLSM), enabling the combination of Fluorescence Lifetime Single-Molecule Localization Microscopy (FL-SMLM) with Image Scanning Microscopy (ISM). This unique combination delivers a two-fold improvement in lateral localization accuracy for SMLM while maintaining its simplicity. Moreover, the addition of lifetime information from our CLSM eliminates chromatic aberration, particularly crucial for achieving few-nanometer resolution in SMLM. Our novel approach, named Fluorescence Lifetime ISM-SMLM (FL-iSMLM), is demonstrated through direct Stochastic Optical Reconstruction Microscopy (dSTORM) and DNA Point Accumulation for Imaging in Nanoscale Topography (DNA-PAINT) experiments on fluorescently labeled cells, showcasing both resolution enhancement and fluorescence lifetime multiplexing capabilities.

References:

1. Niels Radmacher et al., *Nature Photonics*, **2024**, *18*, 105

Invited Talks

IT 1

Model-free photon analysis of diffusion-based single-molecule FRET experiments

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Photon-by-photon analysis tools for diffusion-based single-molecule Förster resonance energy transfer (smFRET) experiments often describe protein dynamics with Markov models. However, FRET efficiencies are only projections of the conformational space such that the measured dynamics can appear non-Markovian. Model-free methods to quantify FRET efficiency fluctuations would be desirable in this case. Here, we present such an approach. We determine FRET efficiency correlation functions free of artifacts from the finite length of photon trajectories or the diffusion of molecules through the confocal volume. We show that these functions capture the dynamics of proteins from micro- to milliseconds both in simulation and experiment, which provides a rigorous validation of current model-based analysis approaches.

IT 2

Fluorescent nucleosides, nucleotides and oligonucleotides

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The photophysics of RNA and DNA is unique among biomolecules that possess aromatic building blocks. Unlike proteins, which contain intrinsically fluorescent amino acids, the canonical nucleosides, and hence nucleotides and oligonucleotides, are practically non- emissive. We develop emissive nucleoside surrogates that facilitate the monitoring of nucleoside-, nucleotide- and nucleic acid-based transformations at a “nucleobase- resolution” in real time. The lecture will articulate the fundamental challenges and will present the design, synthesis and photophysical features of emissive nucleosides as well as selected examples for their utilization in biophysical and discovery assays.

References:

1. Yitzhak Tor, *Acc. Chem. Res.* **2024**, *57*, 1325

IT 3

Hyperspectral 2D IR imaging: Application to amyloid in cataracts and pancreas tissues

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Hyperspectral 2D IR imaging is a new microscopy method for the lifesciences. It is based on ultrafast 2D IR spectroscopy, which probes biomolecular structures through their vibrational modes. This talk will present technological advances that turn this spectroscopy into a hyperspectral imaging technique. The technology behind the technique will be explained, along with confocal and wide-field imaging. Spectral signatures of amyloid will show, including reduced anharmonicities and strong oscillator strengths, created by vibrational delocalization along the amyloid fibril backbone. With those signatures, it was determined that cataracts are an amyloid disease and that fixed pancreas tissues degrade on the molecular level into amyloid deposits.

IT 4

Kinesin-14 HSET and KlpA are non-processive microtubule motors with load-dependent power strokes

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Accurate chromosome segregation during cell division relies on coordinated actions of microtubule (MT)-based motor proteins in the mitotic spindle. Kinesin-14 motors play vital roles in spindle assembly and maintenance by crosslinking antiparallel MTs at the spindle midzone and anchoring spindle MTs' minus ends at the poles. In this study, we investigate the force generation and motility of the Kinesin-14 motors HSET and KlpA. Our findings reveal that both motors are non-processive, producing single load-dependent power strokes per MT encounter, with estimated load-free power strokes of ~30 and ~35 nm, respectively. Each homodimeric motor generates forces of ~0.5 pN, but when assembled in teams, they cooperate to generate forces of 1 pN or more. Notably, the cooperative activity among multiple motors leads to increased MT-sliding velocities. These results quantitatively elucidate the structure-function relationship of Kinesin-14 motors and underscore the significance of cooperative behavior in their cellular functions.

IT 5

Exploring Cholesterol Sensitivity of G Protein-Coupled Receptors: Excitements and Challenges

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G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major drug targets. Serotonin$_{1A}$ receptors are important neurotransmitter receptors of the GPCR superfamily and are implicated in generation and modulation of cognitive, behavioral and developmental functions. We previously demonstrated that membrane cholesterol is necessary for ligand binding, G-protein coupling and signaling of serotonin$_{1A}$ receptors. In our recent work, we explored the molecular basis of cholesterol sensitivity exhibited by the serotonin$_{1A}$ receptor by site-specific mutations and MD simulations. We show that a lysine residue (K101) in one of the transmembrane helices is crucial for sensing altered membrane cholesterol levels (Kumar *et al.* (2021) *Science Advances* 7: eabh2922 (recommended in Faculty Opinions (F1000Prime)). Our results constitute one of the first reports comprehensively demonstrating that cholesterol sensitivity could be knocked out by a single point mutation at a cholesterol binding site. I will end my talk by presenting our recent observations on the role of cholesterol in GPCR endocytosis and trafficking.

References:

1. Kumar, G.A. *et al*. *Science Advances* **2021**, *7*, eabh2922 (Recommended in **Faculty Opinions** (**F1000Prime**))
2. Kumar, G.A. *et al. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **2021**, *1866*, 158882
3. Kumar, G.A. et al. *Biophysical Reviews* **2021**, *13*, 1007
4. Sharma, A. et al. (manuscript in preparation).

IT 6

Lighting up Lipids

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Dynamic changes in molecular levels and distribution are associated with all life processes and importantly regulate key decision-making events in biology. Thus, spatiotemporal dynamics of small molecules and ions are central to biological function ranging all the way from the essential, encompassing cell-signaling, transport, immunity, and recycling, to most pathophysiological conditions including cancers, inflammation, and neurodegeneration. In this backdrop, the ability to catch molecules of life in action using optical imaging modalities is extremely powerful in that it can reveal the spatial organization and temporal dynamics of molecules within living systems. Central to scientific endeavors in visualizing molecules in action toward deciphering life processes, are chemical probes that can non-invasively enter living systems and report on molecular localization in an optical imaging set-up. In this backdrop, the spatiotemporal dynamics of lipids, structural constituents of cell-membranes, lies at the crux of cell-signaling and regulation of membrane-transport, and we have developed rapid-response, cell-permeable, fluorescent probes that report on s-min timescale dynamics of these essential molecules. The novel probes reveal temporally-diverse lipid dynamics in the context of ligand-binding induced GPCR signaling,[1] track growth-factor induced signaling in cancers, and visualize lipid organization in membrane-less intranuclear structures like the nucleolus.[2] In this talk, I will present our recent endeavors and design principles underlying the development of reversible lipid probes and highlight examples of imaging lipid dynamics in living systems.

References:

1. R. Kundu et al. *bioRxiv* **2024**, 06.17.599302.
2. R. Kundu et al. *JACS Au* **2024**, *4*, 1004.

IT 7

Water-Enhanced DES Electrolytes: A Path to Stable Zinc-Ion Batteries

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Aqueous Zn-ion batteries (ZIBs) are gaining attention as promising, safer alternatives to traditional Li-ion batteries. As a core component of ZIBs, the electrolyte significantly influences the electrochemical performance and overall battery efficiency. However, Zn anodes in conventional aqueous electrolytes often suffer from poor reversibility and cycling stability due to dendrite formation on the Zn surface. "Water-in-Deep Eutectic Solvent (DES)" electrolytes have emerged as environmentally friendly and economically viable candidates for ZIBs. Yet, fundamental questions remain unresolved, including optimal water content in DES, changes in electrolyte dynamics at this concentration, disruption of the DES hydrogen bonding network by water, and alterations in the Zn^{2+} solvation layer in water-in-DES systems.

Through ultrafast 2D IR and 2D NMR spectroscopy, we explore structural rearrangements, solvent fluctuation dynamics, and hydrogen bond behavior across varying water concentrations, both with and without Zn salt. Additionally, MD simulations provide a molecular-level understanding of these processes. Our results indicate that adding 10 moles of water per mole of DES yields optimal conditions, as evidenced by peak conductivity in impedance spectroscopy. The implications of these findings for ZIB development will be discussed.

IT 8

Probing Surface Polarity of Amyloid Fibrils using Sensitized Emission Imaging

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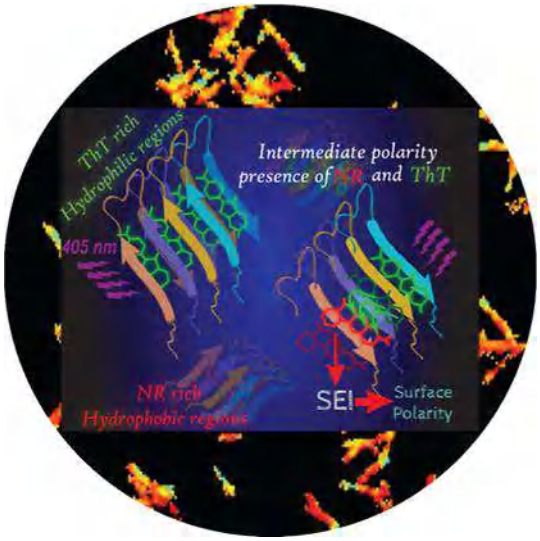
Forster resonance energy transfer (FRET) has been extensively used in the condensed phase to retrieve information on nanoscale proximity and interaction of bio-molecules or higher assemblies in solution. While FRET microscopy is routinely employed to detect interactions and conformational changes in biomolecules at the nanometers length scales, estimate of distances using this method becomes sketchy when the exact number of donor-acceptor (tagged) molecules are unknown, such as in for self-assembled systems. I will present a spectrally-resolved sensitized emission imaging (SEI) method which allowed us to authenticate (bio) molecular interactions of donor-/acceptor molecules in assemblies, given the sensitization of acceptor(s) mediated via excitation energy transfer from donor(s) becomes negligible beyond several nanometers. SEI, which utilizes two-color excitation coupled with spectrally-resolved imaging is quite versatile and can be utilized to study excitation energy transfer in various self-assembled systems thereby providing insights on nanoscale heterogeneity in molecular interactions. I will discuss an application this method to map out nanoscale surface polarity of polymorphic aggregates (amyloids) of a-Synuclein, an inherently disordered protein responsible for Parkinson's disease. The spatial diversity of local surface polarity within each fibril as well as amongst various fibrils observed in our measurements likely owes to polymorphic species which are known to exist during fibrillary growth.

This work is in collaboration with Samir Maji of Biosciences and Bioengineering at IIT Bombay



IT 9

Combatting target modification based antimicrobial resistance: A war at the microscopic level

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Drug resistance is often a concerted interplay of myriad mechanisms in order to circumvent the effect of antibiotics. Target modification is one such highly efficient and straightforward strategy utilized by pathogenic systems to render themselves resistant to antibiotics. The ribosome has always served as a hot target for antibiotics owing to the integral role it plays in the central dogma of life. Erythromycin-resistant methyltransferases (Erms) serve as an example of pathogenic target modifying enzymes that site specifically methylate a ribosomal base in the exit tunnel of the 50S ribosomal subunit. This simple modification leads to resistance against the macrolides, lincosamides, and streptogramin B class of antibiotics. Our work primarily aims to develop a mechanistic basis for this resistance via undertaking two strategies; first, via unravelling the general mechanism of rRNA methylation, and second, by curbing expression of the pathogenic enzyme thereby eliminating the root cause of resistance. Using structural biology approaches and extensive biochemical techniques we have highlighted subtle recognition elements in both the protein as well as the *in vitro* and *in vivo* enzyme substrate that govern their interaction and lead to effective catalysis. Our work has helped uncover a unique universal dual base flipping mechanism employed by Erms. These findings lead to identification of allosteric sites in the protein, distal from the catalytic site. It has edged us towards successful development of novel drug scaffolds that show promising results against Erm-containing microbes. Overall, we draw a holistic picture towards curbing methylation-based resistance in pathogens and provide avenues towards develop strategies to reverse resistance.

IT 10

Mechanisms of Cellular Membrane Shape Transitions: From Lipids to Fast Endophilin mediated Endocytosis

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The process of endocytosis is of tremendous biomedical interest since it enables numerous pathogens to enter the cell, including bacteria, viruses, and bacterial toxins. Despite having been the subject of an extraordinary amount of published research, key questions remain regarding mechanisms of initiation and regulation. Focusing on a recently discovered clathrin-independent pathway that enables fast response to receptor stimulation, we aim to improve the understanding how membrane shape transitions are regulated in healthy and pathological conditions. We will discuss membrane shape regulation in several different layers of complexity, beginning with the lipid bilayer itself and all the way to the question of how cytoskeletal interactions help shape biological membranes.

References:

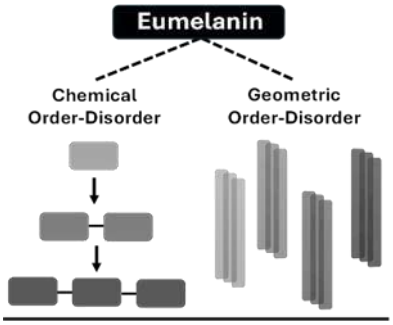
1. E.Boucrot, et.al.Nature. **2015**;517(7535):460-5.
2. S.Shukla,et.al. Biophys J. **2019**;117(5):962-74.
3. S.Mondal,et.al. Nature communications. **2022**;13(1):5017.

IT 11

A Bottom-Up Approach to Explore Structure and Property in Eumelanin

Mahesh Hariharan

IISER Thiruvananthapuram



Eumelanin, a biopolymer providing photoprotection in the animal kingdom, consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) building blocks.[1] Despite its ubiquity in nature, the origins of eumelanin's broadband electronic absorption and ultrafast non-radiative decay mechanisms remain largely unknown. Oligomers of varying lengths and redox states, along with exciton interactions within these moieties, are believed to play a critical role in eumelanin's unique electronic absorption characteristics. To unravel the origin of eumelanin's optical properties, we undertake a synthetic, bottom-up approach, investigating the structure–property relationship of eumelanin's monomers, dimers and trimers in monomerc as well as aggregate/crystalline forms. In DHI crystals, hydrogen bonding is identified as the dominant interaction, facilitating Frenkel exciton delocalization along the DHI units.[2] In DHICA crystals, both hydrogen bonding and extensive π–π stacking drive the architecture, enabling charge-transfer exciton delocalization as the main energy transfer mechanism within the crystal network.[3] Spectroscopic investigations reveal broad, red-shifted absorption bands in DHI and DHICA crystals, with crystalline samples exhibiting substantial spectral differences from their monomeric counterparts, indicating strong electronic coupling. To further explore the role of structure and exciton coupling in eumelanin's optical properties, we examine eumelanin dimers and trimers, where extensive Coulombic and charge-transfer coupling progressively red-shifts the electronic absorption of the multimers relative to the monomer.[4] Functionalization of eumelanin monomers, particularly through halogenation, induces distinct photophysical pathways, including intersystem crossing and room-temperature phosphorescence, paving the way for developing eumelanin-inspired materials.[5,6]

References:

1. E.Kaxiras et al. *Phys. Rev. Lett.* **2006**, *97*, 218102.
2. D Sasikumar et al. *Chem. Sci.* **2022**, *13*, 2331-2338.
3. K.Vinod, et al. *Chem. Sci.* **2024**, 10.1039/D4SC05453A.
4. K.Vinod, et al. *(Manuscript Submitted)*.
5. K.Vinod et al. Monomer. *Chem. Eur. J.* **2024**, *30*.
6. K.Vinod et al. *Org. Chem. Front*. **2024**, *(Just Accepted)*.

IT 12

Single molecule investigation of liquid-liquid and liquid-solid phase separation of amyloid proteins

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While liquid liquid phase separation (LLPS) has emerged as a fundamental principle in biological functions, and liquid to solid transition (LST) of the protein rich liquid phase is implicated in the pathology of a spectrum of neurodegenerative disorders. However, molecular mechanisms underlying LST, characterized by conversion of the soluble liquid phase into insoluble amyloid fibrils, is poorly understood. We have used total internal reflection fluorescence microscopy (TIRFM), single molecule tracking, fluorescence recovery after photobleaching, fluorescence correlation spectroscopy, confocal microscopy and electron microscopy to monitor LLPS and the subsequent LST of the intrinsically disordered proteins tau and α-synuclein in the presence of polyU RNA and polyethylene glycol respectively. High resolution and/or high sensitivity of these techniques enable us to visualize conversion of the homogeneous solution phase to the liquid and subsequently to the solid phase. Our results indicate that LST is characterized by gradual growth of a porous network of solid amyloid phase within a homogeneous liquid phase. Notably, our study accentuates the role of factors such as droplet interface dynamics, protein concentration, and viscosity in dictating the transition from liquid to solid phases, offering valuable insights into these complex phenomena.

IT 13

Unravelling Direct Correlations between Membrane Nanodomain Reorganization and Antimicrobial Resistance Evolution in bacterial Cells

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Bacterial drug resistance is a major global health emergency which needs newer approaches for its detection especially those which are rapid and sensitive at single cell level. One of the major limitation of existing antimicrobial resistance (AMR) screening is that it relies on culturing bacterial samples, which is time and resource intensive or on detection of known mutations which impart resistance. Here we provide the first evidence for existence of direct correlations between nanoscale dynamical reorganization in bacterial cell membranes in cells undergoing evolution of phenotypic resistance under sub-lethal dosage of last-line antibiotic Colistin. While super-resolution fluorescence microscopy in combination with fluorescence correlation spectroscopy enables probing dynamical lipid nanodomains on single E. coli cells undergoing AMR evolution, high-resolution atomic force microscopy provides information on nanoscale morphological changes in the same cell population. Interestingly our study also reveals intricate correlations between nanoscale bacterial membrane organization and biochemical signalling responses that eventually drive evolution of antimicrobial resistance. In addition, we detect signatures of cooperative lipid motion and dynamic heterogeneity as quantified through the non-Gaussian parameter, $\alpha 2$, for lipid number fluctuations in the illumination volume. Further, this parameter is also correlated with the evolution of resistance in the strains. Our study suggests subtle feedback mechanism for emergence of antimicrobial resistance which is initiated by membrane nanoscale organization and lipid dynamics leading to biochemical signalling that leads to membrane compositional changes. These compositional changes alter these membrane nanoscale parameters to mitigate the antibiotic mediated stress and increases survival probability of the cell population which thus becomes more resistant.

IT 14

DNA based programmable nanodevices to program biological membranes

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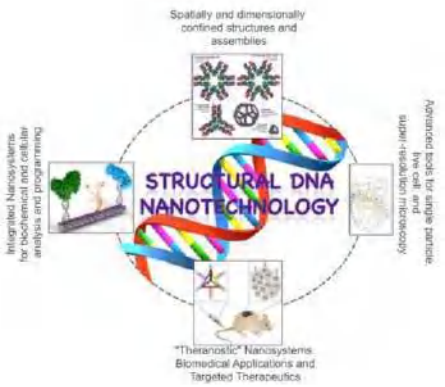
My laboratory ask how nanometer-sized biomolecules transmit and integrate information across much larger length scales of the orders of cells and tissues. We seek to explore how collections of macromolecules work together to establish a common functional system like cellular pathways, organelles, living cells and further into tissues, organs and entire organisms. Different biomolecules establish long-range orders in living systems by self-assembling into much larger structures, such as molecular complexes, membranes, and cytoskeletal organelles, intra- and inter-cellular contacts, and long range contacts. The main theme of our lab will be understand the assembly principles of biological systems and the roles they play in living cells, tissues and full organisms...and further developing technologies to modulate the same.

To address these problems, we adapt multidisciplinary, bottom-up approach using DNA nanotechnology. DNA has immense potential to arrange the matter at nanoscale with extreme robustness and spatial specificity. The compatibility of DNA to interface with other biomolecules like proteins, carbohydrates, lipids, small molecules make DNA a natural choice of material for bottom-up self-assembly. Thus, we will merge the complex programmability of DNA nanotechnology with the structural and functional diversity of other biomolecules. Our interdisciplinary research, along with national and international collaborations with experts, will leverage expertise from chemistry, nanotechnology, biophysics, biology, engineering, and medicine. The overarching goal of the my team would be to translate laboratory findings into the development of new therapeutic strategies.



References:

1. Anjali Rajwar et.al. ACS Nano **2022**, 16, 7, 10496–10508
2. Pravin Hivare. et.al., Nanoscale, **2022**, 14, 8611-8620
3. Dhiraj Bhatia,et.al. Nature Nanotechnology, **2016**, 11, 1112-1119.

IT 15

EVV 2DIR Spectroscopy for Protein-Inhibitor Interaction Analysis

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Optical spectroscopic techniques provide valuable, complementary insights into the structural analysis of biomolecular interactions. Recently, electron-vibration-vibration two-dimensional infrared spectroscopy (EVV 2DIR) has shown promising potential for probing these interactions.[1,2] As a hybrid IR/Raman technique, EVV 2DIR captures a third-order nonlinear signal by combining two IR beams that stimulate molecular vibrations with a visible beam that probes the resulting polarization.[3] In this study, we apply EVV 2DIR to investigate the binding interactions of FatA and FGFR1 proteins with a range of potent inhibitors. By analysing difference EVV 2DIR spectra (protein+inhibitor minus protein only), we identify distinct spectral peaks associated with protein-inhibitor interactions, which are further interpreted using density functional theory-based quantum mechanical calculations. Through comparisons across multiple ligand bindings, we uncover novel insights into protein-ligand interactions, advancing both the methodology and interpretive depth of EVV 2DIR spectroscopy in biomolecular research.

References:

1. Fournier, F. et al. *Acc. Chem. Res.* **2009**, 42, 1322.
2. Soenarjo, A. L. et al. *J. Am. Chem. Soc.* **2023**, 145, 19632.
3. Zhao, W. et al. *J. Am. Chem. Soc.* **1999**, 121, 10994.

IT 16

Conformational fluctuations of proteins: from test tubes to neurodegenerative diseases

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While protein conformational disorder and aggregation have serious implications in a number of neurodegenerative diseases, these processes are difficult to study. This is because; they often share common conformational landscapes, which are inherently heterogeneous, consisting of multiple pathways and intermediates of varying toxicities. Our lab has been developing and using sensitive fluorescence methods-both at ensemble and single molecule resolution-and complementing these using traditional biochemical assays, to address the heterogeneity and toxicity of a number of neurodegenerative disorders, including Parkinson's Disease (PD) and ALS. In this talk, we will discuss conformational fluctuations of alpha Synuclein and Superoxide Dismutase (SOD1) and their implications in the early events of their aggregation, with specific reference to protein phase separation.

IT 17

IDR-driven nanometer-scale RNA helicase protein clusters (RPCS) are dynamic assemblies

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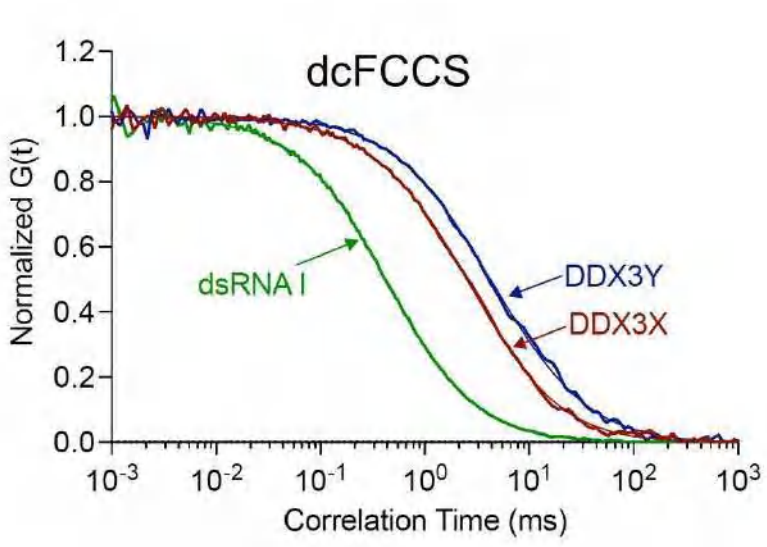
Many DEAD-box helicases, crucial for many aspects of RNA metabolism, contain intrinsically disordered regions (IDRs), known for specifying interaction partners, acting as "sensors" for the cellular environment, and facilitating cellular condensation. Using multi-parameter confocal spectroscopy, we previously showed that the sex chromosome-encoded RNA helicases, DDX3X and DDX3Y, form small RPCs containing dozens of protein subunits in the nanomolar range well below the critical μM concentrations that lead to liquid-liquid phase separation *in vitro* and in cells. We further investigated the requirements for RPC formation and the activity of the helicases within RPCs. Truncation of both, N- and C-terminal IDRs from either protein suppressed cluster formation, ATPase rate and duplex (ds) RNA unwinding, indicating a strong correlation between cluster formation and catalytic activity. Mixing DDX3 RPCs containing Cy3-labeled dsRNA with those containing Alexa647-labeled RPCs did not show evidence of exchange of the strands between clusters until ATP was added. Fluorescence cross-correlation spectroscopy then showed gradual presence of dually labeled RPCs indicating ATP-dependent helicase activity in the RPCs and escape and re-capture of the ssRNA strands. The strand exchange process is slow, as expected from the very low concentration of RNA free in the medium. To test whether IDRs drive cluster formation in another system, we used eukaryotic initiation factor 4A (eIF4A), a related DEAD-box helicase with much shorter IDRs. Fluorescence correlation spectroscopy spectra of eIF4A bound to labeled dsRNA showed much faster diffusion corresponding to an apparent complex of RNA and only two eIF4A subunits. Activity of eIF4A is very low in the absence of its cofactors, eIF4B, eIF4G and others. These data support the role of IDRs in nano-RPC formation and helicase activity and they indicate that the protein subunits within clusters are dynamic rather than inactive or denatured.



IT 18

Data-Driven Image Restoration

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I will describe two data-driven methods for improving fluorescence imaging in aberrating samples. In the first, we intentionally degrade data acquired on the near side of thick fluorescent samples, synthetically aberrating them so that they resemble data acquired deeper into the specimen. We then train neural networks to reverse this image degradation, and apply the trained networks to 'de-aberrate' data unseen by the network[1]. In the second method, we modify phase diversity-based wavefront sensing techniques originally developed in astronomy for applications in fluorescence microscopy. Our phase diversity method enables rapid calibration of deformable mirrors as well as rapid correction of optical aberrations. We demonstrate the power of these approaches on a variety of fixed and live fluorescent samples[2].

References:

1. Guo et al., *bioRxiv*, **2024**, https://doi.org/10.1101/2023.10.15.562439.
2. Johnson, Guo et al., *Optica,* **2024**, *11*, 806.

IT 19

Low-temperature Raman imaging of cryofixed biological samples

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Raman microscopy offers valuable insights into the molecular composition, chemical states, and environmental conditions of biological samples. However, its application in biological imaging is often hindered by a low signal-to-noise ratio due to the inherently low scattering efficiency of Raman processes. To address this, we developed a cryo-Raman microscope that incorporates a cryostat capable of rapid freezing and low-temperature Raman imaging. This approach enables spatiotemporal cryofixation of biological samples, allowing for extended exposure times to enhance signal accumulation without causing photodamage. The improved signal-to-noise ratio achieved through low-temperature imaging significantly enhances both the spatial and spectral resolution in the imaging of biological samples. We applied this technique to multimodal imaging to gain complementary information across different imaging modes. In addition, we observed reduced photobleaching in the resonant Raman signals of cytochromes in cryofixed HeLa cells. Furthermore, cryofixation allowed us to preserve the redox states of cytochromes in rat heart tissue and observe the chemical state during heartbeat.

References:

1. Mizushima et al. *Sci. Adv.* (in press).

IT 20

Single-molecule imaging and tracking in live yeast *S. cerevisiae* for quantifying the dynamics and target-search mechanism of aurora kinase B (Ipl1).

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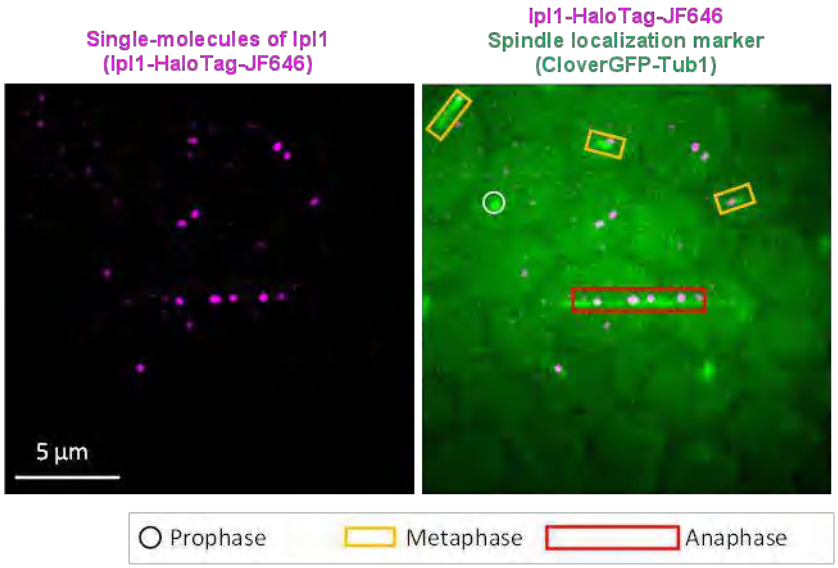
Single-molecule Imaging (SMI) has revolutionized the way we understand biological processes, as it provides direct real-time measurements of various cellular activities under their physiological environment with high spatiotemporal resolution [1]. Aurora kinase B, Ipl1 in *Saccharomyces cerevisiae*, is the master regulator of cell division required for checkpoint regulation, spindle assembly and disassembly, chromosome segregation, and cytokinesis. Decades of research employed ensemble averaging methods to understand its dynamics and function; however, the dynamic information was lost due to population-based averaging. We developed single-molecule imaging and tracking (SMIT) to quantify the recruitment dynamics of Ipl1 at the kinetochores and spindles in live cells of *S. cerevisiae* [2]. Our data suggest that Ipl1 is recruited to these locations with different dynamics [3]. We have demonstrated how the recruitment dynamics of Ipl1 at the kinetochores during metaphase changes in the presence and absence of tension across the kinetochore, in the absence of protein phosphatase 1 (Glc7), and the absence of its known recruiters (Ctf19 and Bub1) [3]. The SMIT of other chromosome passenger complex (CPC) members (Bir1, Nbl1, Sli15) suggests the hierarchical assembly of CPC at the kinetochore [3]. Hence, SMIT provides a dynamic view of the Ipl1 trafficking at the kinetochores and spindles in live yeast cells. The method (SMIT) that we developed here is versatile as it can be applied to quantify the dynamics of any DNA-interacting proteins or protein-protein interactions in live cells of bacteria, yeast, and cell lines.



References:

1. Podh, et al., *Journal of Molecular Biology*, **2021**, *433*, 167250.
2. Podh et al., *STAR Protocols (Cell Press)*, **2022,** *3*, 101900.
3. Podh et al., *bioRxiv*, **2024**.

IT 21

Farewell to single well: Automated multiwell single-molecule FRET to screen molecular interactions and dynamics

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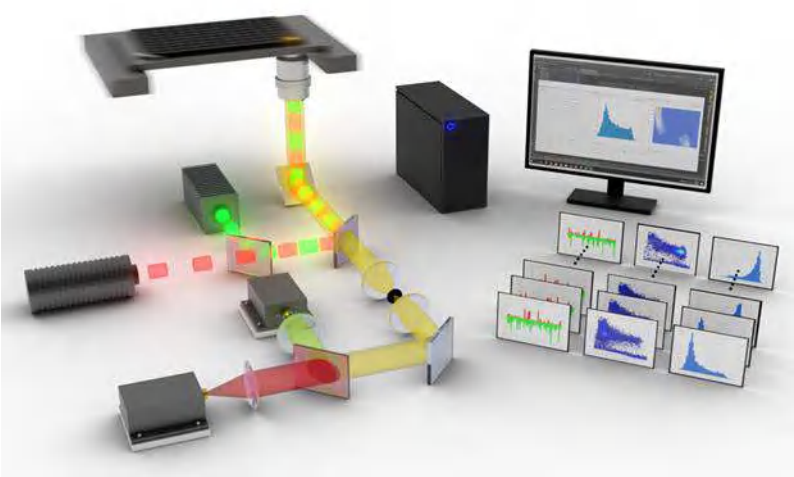
Single-molecule FRET (smFRET) has become a versatile tool for probing the structure and functional dynamics of biomolecular systems, and is extensively used to address questions ranging from biomolecular folding to drug discovery. Confocal smFRET measurements are amongst the widely used smFRET assays and are typically performed in a single-well format. Thus, sampling of many experimental parameters is laborious and time consuming. To address this challenge, we extend the capabilities of confocal smFRET beyond single-well measurements by integrating a multiwell plate functionality to allow for continuous and automated smFRET measurements [1]. We demonstrate the broad applicability of the multiwell plate assay towards protein folding, competitive and cooperative protein–DNA interactions, and drug-discovery and miRNA sensing.



References:

1. Hartmann et al. *Nature Communications* **2023**, *14*, 6511.

IT 22

Chasing The Functions Of Mycobacterium Tuberculosis Glycolipids During Infection

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Microbial lipids play a critical role in the pathogenesis of infectious diseases.[1] Mycobacterium tuberculosis (Mtb)—causative agent of Tuberculosis—synthesizes chemically distinct glycolipids that are exposed on its outer membrane and interact with the membranes of host macrophages.[2-3] However, the effects of the structurally diverse Mtb glycolipids on the host cell membrane properties to fine-tune the host cellular response is unknown. In this work, we combined membrane biophysics, cell biology and microscopy to assess the effects of different Mtb lipids on cell membrane mechanics, lipid diffusion, and cytoskeleton of THP-1 macrophages. [4-6] We found that Mtb lipids are transferred to macrophage membranes in a lab infection model, followed by modulation of macrophage membrane biophysical properties, and actin cytoskeleton. Extensive cellular FRET analysis revealed that the modulation of actin cytoskeleton is mediated by alteration in the localization and interactions between PIP2 lipid, actin and actin regulatory proteins. These observations provide a novel lipid-centric paradigm of Mtb pathogenesis that is amenable to pharmacological inhibition and could promote the development of robust biomarkers of Mtb infection and pathogenesis.

References:

1. van der Meer-Janssen, Y.P., et al. *Prog Lipid Res*, **2010**, *49*, 1-26.
2. Astarie-Dequeker, C., et al., *PLoS Pathog*, **2009**, *5*, e1000289.
3. Hayakawa, E., et al., *Biophys J*, **2007**, *93*, 4018.
4. Mishra, M., et al., *Sci Rep*, **2019**, *9*, 12844.
5. Dadhich, R., et al., *ACS Chem Biol*, **2020**, *15*, 740-750.
6. Mishra, M., Dadhich, R., Mogha, P., Kapoor, S. *ACS Infectious Diseases*, **2020**, *6*, 2386.

IT 23

Designing of HER2-targeting metal nanoconstructs for therapeutic and diagnostic applications

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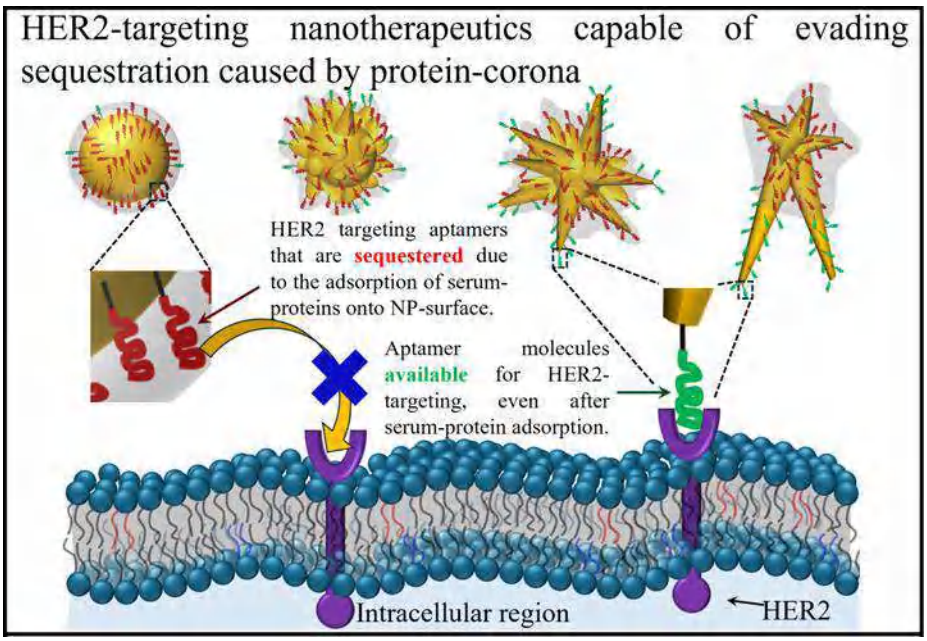

Surface-functionalized metal nanoconstructs are evolving as excellent drug delivery platforms, imaging agents, and diagnostic tools. Different properties of these nanoconstructs strongly depend upon their metal-core (e.g, Au, Ag etc.), their shape, sizes, and their stability under experimental conditions. Earlier our work indicated that HER2-receptor targeting ability of aptamer functionalized anisotropic gold nanostars (AuNS)—a heterogeneous mixture of star shaped Au-particles—is better than that of spherical Au-nanoparticles (AuNPs).*(1-3)* We hypothesized that the AuNS having branches with sharp-tips (2-3 nm radius), were able to evade the detrimental effect of protein corona formation and were projecting the aptamers form their surface more effectively, resulting in better HER2-targeting. To test this hypothesis, we first develop synthetic producers that yield AuNS with similar sizes but with controllable branch length distribution. This allowed us to test the effect of branch-lengths of AuNS on their ability to result-in targeted killing of HER2 overexpressing cancer cells. We have found that AuNS with longer branches were able to forestall the effect of protein-corona more efficiently and resulted in enhanced cytotoxicity against HER2 overexpressing SKOV3 cells.

References:

1. Bhowmik, D.et.al. *ACS Nano*. **2019**, *13*, 13637-13644.
2. Choo, P.et.al. *J Am Chem Soc*. **2021**, *143*, 4550-4555.
3. Diloknawarit, B.et.al. *ACS Nano*. **2024**, *18*, 12537-12546.

IT 24

Single molecule-single photon analysis: from one confocal spot to SPAD array tracking

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FCS has been around since the 70's and has provided a means by which to estimate diffusion coefficients and reaction rates of molecules as these freely traverse a single illuminated confocal volume. However, implicit to the analysis, is the fact that very many photons must be collected for very many molecules traversing the volume in order to obtain any reasonable estimate of diffusion coefficients or reaction rates. A such FCS is fundamentally a bulk method. The reason for this is simple: we must assume a fixed average number of molecules within the volume in order for the analysis to move forward. By contrast, if we were to analyze the data, photon-by-photon, as it arrives at the detector, we would need to know the instantaneous number of molecules within the volume contributing those photons and their locations within that volume. Only then could we estimate from mere thousands of photons the individual properties of each molecule as they traverse the volume. To achieve this, we need new Mathematics. This is what I will discuss and illustrate our method on the interaction of intrinsically disordered proteins, the positively charged linker histone H1.0 as well as its negatively charged chaperone prothymosin alpha where we anticipate interaction heterogeneity of amongst complexes. As time allows, we will move to full 512x512 pixel SPAD array tracking from single photon arrivals. This will require we abandon the localization paradigm altogether in tracking as molecules can no longer be localized from binary 0–1-pixel output.

References:

1. Xu et. al., "Single Molecule Reaction Diffusion" bioRxiv 2024.
2. Xu et. al., "Single Photon Single Molecule Tracking", in prep.
3. Sina Jazani et. al. *Nat. Commun*. **2019**, *10*, 3662.

IT 25

Advances in Super-Resolution Microscopy and Label-Free Condensate Characterization for Cellular Biophysics

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Cellular biophysics benefits from innovations in microscopy that allow for precise visualization of molecular interactions and structural organization. Here, I introduce two complementary methods: PEPCy, a fluoromodule that enhances Cyanine dye photostability for super-resolution imaging of cell surface proteins, and Total Holographic Characterization (THC), a label-free approach to analyze biomolecular condensates.

PEPCy leverages single-chain variable fragments (scFvs) that bind their cognate Cyanine dyes with nanomolar affinity and enhance their photophysical properties, enabling single-molecule imaging and high-resolution mapping of protein distributions on the cell surface. This approach provides deep insights into protein clustering and interaction dynamics critical to cellular signaling and regulation.

In parallel, THC is employed for real-time, label-free 3D characterization of condensates, focusing on size, morphology, and phase behavior without perturbing the native environment. This technique elucidates condensate assembly and dissolution, enhancing our understanding of condensate functionality in bacterial differentiation and adaptive responses. Together, PEPCy and THC offer a robust framework for analyzing cell surface architecture and intracellular condensate dynamics, paving the way for comprehensive studies in cellular structure and function.

References:

1. Sasazawa, Moeka, et al. *bioRxiv* (**2024**): 2024-07.

IT 26

A Matter Of Time: Catching Organelles In Action In Vitro And In Vivo

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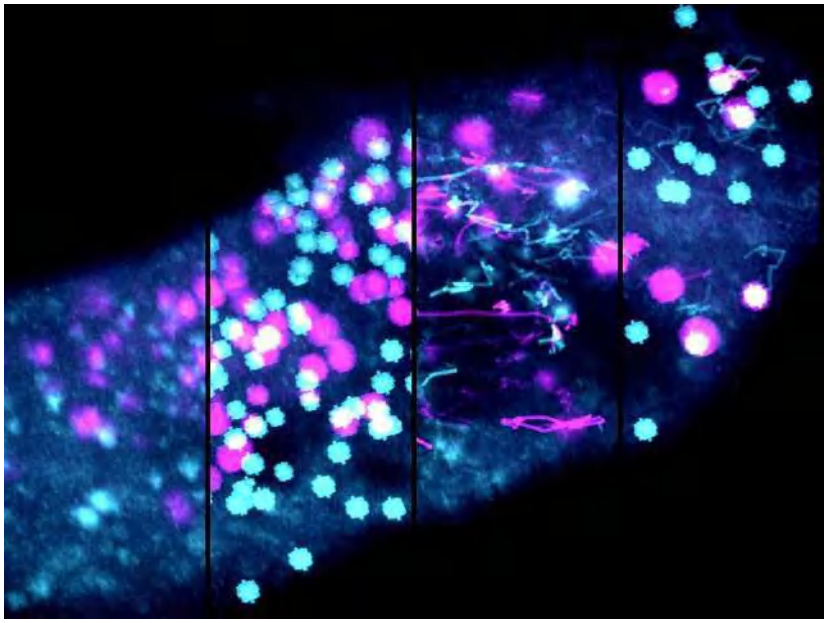
The dynamics and fate of endosomal vesicles following endocytosis at the plasma membrane are central to the understanding of transport phenomena of various cargoes including signaling receptors in living cells. The complex dynamics of endosomes and their stochastic motility characteristics in the 3-dimensional milieu demands rapid volumetric imaging to decipher their organization within single cells on a coverslip *(in vitro)* as well as single cells within living organisms *(in vivo)*. Light-sheet microscopy based on diffraction free beams such as the Bessel and Lattice light-sheet approaches are suitable to capture ensemble endosomal dynamics at whole cell volumes. Using Lattice light-sheet microscopy along with tailored image analysis routines, examples of 'timekeeping' in the endosomal network and will be described in the context of endosomal conversions. Further stochastic and rare events such as endosomal escape of lipid nanoparticles relevant to therapeutic delivery will be described in cell culture models. Lastly, can we capture organelle dynamics within cells in their native environment of growing tissues? The use of Airy beams, that offer an increased flexibility between resolution, field-of-view, and insensitivity to optical obstacles in a light-sheet geometry for subcellular imaging in tissues will be discussed. Examples from our newly built microscope with data from model organisms such as Zebrafish and drosophila will be described where sub-cellular resolution with a step-change in temporal resolution has been achieved.



Imaging and analysis of endosomal dynamics in cells.

IT 27

Raman Parameters to Monitor Toxic Protein Oligomers linked to Parkinson Diseases

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Parkinsons disease (PD) is a neurological disorder and it primarily affects individuals as they age. The formation of different type of α-synuclein (aS) aggregates is linked to pathology of the diseases formation and progression. We established several Raman signatures of oligomers produced from different proteins including α-synuclein. The C-terminal region of aS is highly dynamic in nature and possesses no defined stable conformation. This region, however, plays a critical role in maintaining the protein's solubility and stability in an ambient solution conditions. In our recent study we showed structural intricacies and aggregation propensities of two single-point mutated α-synuclein (mutations located at position 129). The mutation of the protein was made by substitution of serine residue at position 129 with alanine (S129A) or with tryptophan (S129W). The structural changes were monitored by circular dichroism (CD) and Raman spectroscopic analysis. The aggregation kinetics and morphological features of the protein aggregates were investigated using Thioflavin T fluorescence assay and atomic force microscopy imaging. It was observed that compared to wild type aS, the S129A and S129W mutants gained structural stability and a high tendency to adopt α-helical secondary structures. CD analysis also showed a preference for α-helical conformation in the mutant proteins. These changes resulted in prolonged lag phases for fibril formation and reduced growth rates of β-sheet-rich fibrils. Cytotoxicity tests conducted on SH-SY5Y neuronal cell lines demonstrated that the aggregates formed by the mutant proteins were potentially less harmful than those from wt aS. It was noticed that cells treated with oligomers derived from mutant proteins exhibited an approximate 80% survival rate. On contrary, the cells treated with wt aS-derived oligomers showed around 40% survival. The imposed structural stability of the mutants, coupled with their increased propensity for α-helical structure, may contribute to their slower rates of oligomerization and subsequent fibrillation.

IT 28

Exciton Transport in Molecular and Polaritonic Aggregates

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Exciton transport forms the first step in the multistep process of photosynthesis, where the molecular excitation generated by solar photons is transported to the so-called special pair of chlorophyll molecules following which charge separation and further chemical reactions happen. The efficiency of this transport far exceeds our expectations. Thus it becomes interesting to probe it further. However owing to the high dimensionality and the non-adiabatic nature of the problem, it is challenging to simulate it efficiently. It is also interesting to think about the routes of transport that are used by the excitation. All this should, ideally, be done taking care of the quantum nature of the excitations and the vibronic coupling to the molecular vibrations and the protein motions. Path integral using tensor network decomposition[1,2] provides an elegant method to simulate such processes. I would discuss recent developments that make these simulations increasingly feasible. How the route of excitonic transport[3,4] is affected by the environment will be demonstrated. We shall also discuss how talking about these routes in a time-independent manner is a gross simplification and the relative importance of various routes change with time. Finally, I will show how the loss of the excitation to the special pair can be incorporated in a simple and elegant manner using a combination of path integrals and Lindblad master equation without having to explicitly describe the special pair itself[5].

References:

1. A. Bose et al. *The Journal of Chemical Physics* **2022**, 156, 024101.
2. A. Bose et al. *Journal of Chemical Theory and Computation* **2022**, 18, 4095.
3. A. Bose et al. *Journal of Chemical Theory and Computation* **2023**, 19, 4828.
4. A. Bose et al. *Journal of Physical Chemistry B*, **2023**, 127, 7663.
5. A. Bose. *The Journal of Physical Chemistry Letters*, **2024**, 15, 3363.

IT 29

Induction of structure in the intrinsically disordered region of the mammalian prion protein in prion protein condensates

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Indian Institute of Science Education and Research Pune

The mouse prion protein can undergo different types of phase transitions. In one type of phase transition, the protein forms misfolded aggregates, either oligomers or fibrils. In the first part of my talk, I will briefly describe the multiple structural mechanisms by which the prion protein can begin to misfold.

In another type of phase transition, the mouse prion protein can undergo liquid-liquid phase separation under physiological conditions to form protein condensates. Upon aging, the liquid-like condensates become more solid-like. Structural changes in the protein during the aging process have been studied by fluorescence methods as well as hydrogen-exchange mass spectrometry. In the structured C-terminal domain of the protein, some segments become more stable, segments remain unaltered in their stability while one segment becomes less stable. Quite strikingly, the N-terminal domain, which remains unstructured in other phase transitions of the protein such as fibril formation, attains significant structure during the aging of the liquid condensates. The N-terminal domain becomes structured with about the same rate constant at which helix 3 becomes more stable, significantly slower than the rate constant at which helix 2 becomes more stable.

IT 30

Zn(II)-Responsive Ratiometric Fluorescent Probes for Two-Photon Excitation Microscopy

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The spatiotemporal detection of transition metal ions within the complex chemical environment of live cells requires sensitive analytical tools and reagents. Two-photon excitation microscopy (TPEM) has emerged as a powerful technique in biological research for deep-tissue and intravital imaging. Based on the simultaneous absorption of two low-energy photons in the near-infrared region, TPEM offers increased penetration depth and minimizes tissue scattering, background autofluorescence, and phototoxicity. Although traditional fluorescent probes and tags can be employed in TPEM, their brightness is often compromised due to a less favorable absorption cross-section compared to one-photon excitation. Expanding on a push-pull fluorophore design, we developed a series of Zn(II)-selective fluorescent probes that respond with a chromatic emission shift upon Zn(II)-binding suitable for ratiometric image analysis. In addition to achieving a balanced two-photon absorption cross section between the free and Zn(II)-saturated probes, significant efforts were devoted towards minimizing undesired non-radiative deactivation pathways, including the suppression of a twisted intramolecular charge transfer state and an excited state proton transfer process. With Zn(II) dissociation constants in the low nanomolar range, the probes are well suited for visualizing physiologically relevant changes of mobile zinc levels in cells and tissues.

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IT 31

Probing free energy in reactions by THz spectroscopy – Ask the water!

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Exploring the unique role of water for life is one of the top future challenges in chemistry. Whether fast protein motion and solvent dynamics are correlated at the very heart of enzymatic reactions is still under heated debate. The underlying molecular mechanism of enthalpy-entropy (H/S) compensation in protein-ligand binding remains controversial. Systematic studies under steady state conditions revealed that differences in the structure and thermodynamic properties of the waters surrounding the bound ligands are an important contributor to the observed H/S compensation. These hydration free energies are dictated by a subtle balance of hydrophobic and hydrophilic interactions.

Calorimetry served as a powerful biophysical tool by measuring changes in thermodynamic state variables, but it is restricted to measurements in equilibrium and in macroscopic samples. THz calorimetry is a novel spectroscopic approach, which allows to deduce hydration free entropy and enthalpy based on spectroscopic observables. Thereby, we can probe reach time resolutions of up to picoseconds and probe inhomogeneous samples. By probing the low frequency range which is most sensitive to the intermolecular interactions (100–600 cm^{-1}), we established a direct correlation between spectroscopic observables and local contributions to the solvation free energy [1]. Following are two applications developed in our group.

Liquid-liquid phase separation (LLPS) describes the reversible compartmentalization of protein solutions into a protein-rich and a dilute phase; the former one being a local hotspot for neurotoxic aggregation in case of Alzheimer or Huntington disease. Using THz calorimetry, we observe how LLPS can be tuned by changes in hydration entropy and enthalpy [2]. This allows to unravel two underlying molecular mechanism, which drive this process: The release of “Cavity-wrap” water hydrating hydrophobic patches during LLPS yielding an increase in entropy. In contrast, “Bound” water hydrating hydrophilic patches is retained. This process is enthalpically favorable. Both contributions favor protein aggregation in liquid droplets. A fundamental understanding of these processes is a prerequisite for tuning of LLPS.

The solvated electron is a fundamental reaction intermediate in physical, chemical, and biological processes. Using time resolved THz calorimetry we were able to follow the birth and time evolution of the solvated electron and compare these results directly to molecular simulations [3]. We were able to observe in real time the evolution from a spectroscopic signature attributed to the delocalized electron (restricted to 500 fs) up to the spectroscopic signature of the localized electron, which is long lasting (>250 ps).

References:

1. S. Pezzotti et al. *Angew. Chem. Int. Ed.* **2022**, *61*, e202203893.
2. S. Pezzotti et al. *J. Phys. Chem. Lett.* **2023**, *14*, 1556.
3. F. Novelli et al. *PNAS* **2023**, *120*, e2216480120.

IT 32

Measuring interactions and biomolecular condensates in microbes

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The Biteen Lab is developing new single-molecule methods to answer fundamental, unanswered questions in microbiology with applications including elucidating cell regulation and mis-regulation, understanding epigenetic inheritance, and visualizing nutrient utilization in the microbiome. These direct, quantitative, and high-resolution approaches have consequences in understanding subcellular biochemistry and biophysics. I will focus on our recently developed approaches to quantifying how cellular components interact and organize in microbiology. We have developed a generalizable, accessible, and rigorous framework to probe the nature of biomolecular condensates on the sub-micron scale in bacterial cells [Hoang et al., *Nature Communications* 2024]. I will show how we probe the formation, reversibility, protein dynamics, and material state of biomolecular condensates in *Escherichia coli* to achieve a general model of bacterial cell organization, and I will describe other examples of organization based on phase separation in bacterial cell biology.

Reference:

1. Hoang et al. *Nature Communications* **2024**, *15*, 3222.

IT 33

Reducing membrane cholesterol inhibits neuronal exocytosis

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Exocytosis is essential for neuronal communication and involves significant membrane remodelling. Cholesterol is a strong modulator of membrane properties and can in principle affect exocytosis. However, there are conflicting reports about its effect on exocytosis. This study investigates this puzzle using multiple techniques on both well-characterized artificial bilayers and on living cells.

We measure various physical properties of a lipid membrane (whose composition mimics the synaptic vesicle membrane) as a function of its cholesterol content (from 0% to 50%). As expected, fluorescence lifetime and spectral shift measurements of indicator dyes (e.g., Flip-tR, Nile Red, and Prodan) show that membrane order increases with cholesterol. Surprisingly, the membrane indentation force, as measured by Atomic Force Microscopy, decreases with increasing cholesterol. Importantly, the indentation force correlates with enhanced fusion kinetics involving artificial vesicles and bilayers, as assessed by Total Internal Reflection Fluorescence Microscopy. This indicates that indentation force is a reliable measure of vesicle fusion propensity, while dye-based membrane order measurements may be misleading.

To explore the biological significance, we examined the effect of reducing cholesterol in live cells using mβCD. We found that vesicular exocytosis slows down with decreasing cholesterol, as observed with artificial bilayers. We also investigated whether serotonin-derivatives, such as N-acetyl serotonin (NAS) and 5-hydroxytryptophan (5HTP), can rescue the effect of reducing cholesterol, since serotonin is known to do so [1,2]. Notably, 10 mM NAS, but not 5HTP, increased the rate of endocytosis in a neuronal cell line by 36%. These insights may aid in identifying small molecules that modulate membrane properties and influence exocytosis without receptor interactions. Though these results are obtained from in vitro cellular systems, they raise potential clinical concerns given that some widely used cholesterol lowering drugs cross the blood brain barrier.

References:

1. Saha Roy et. al., *J. Phys. Chem. B*, **2024**, *128*, 20, 4975
2. Saha Roy et. al., *J. Phys. Chem. Lett.*, **2024**, *15*, 1711

IT 34

Fluorescence Labeling of Cancer Cells with Chemically Modified Bacteria

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Our group has developed a method for decorating bacteria with artificial, self-assembled receptors based on oligonucleotide-small molecule (SM) conjugates. With this approach, bacteria can be 'programmed' to glow, adhere to surfaces, or interact with specific proteins or mammalian cells (Figure 1a).[1] In this talk, I will describe several innovations that were introduced to this technology, which enabled the creation of fluorescent bacterial probes (B-probes) that can selectively bind and label different types of cancer cells (Figures 1b and 1c), as well as methods for creating B-probes that emit at different colors with enhanced brightness.[2] Finally, potential applications of this technology,[3] with an emphasis on the design and operating principles of 'turn-on' B-probes for screening synthetic inhibitors of membrane proteins (unpublished results), will be discussed.

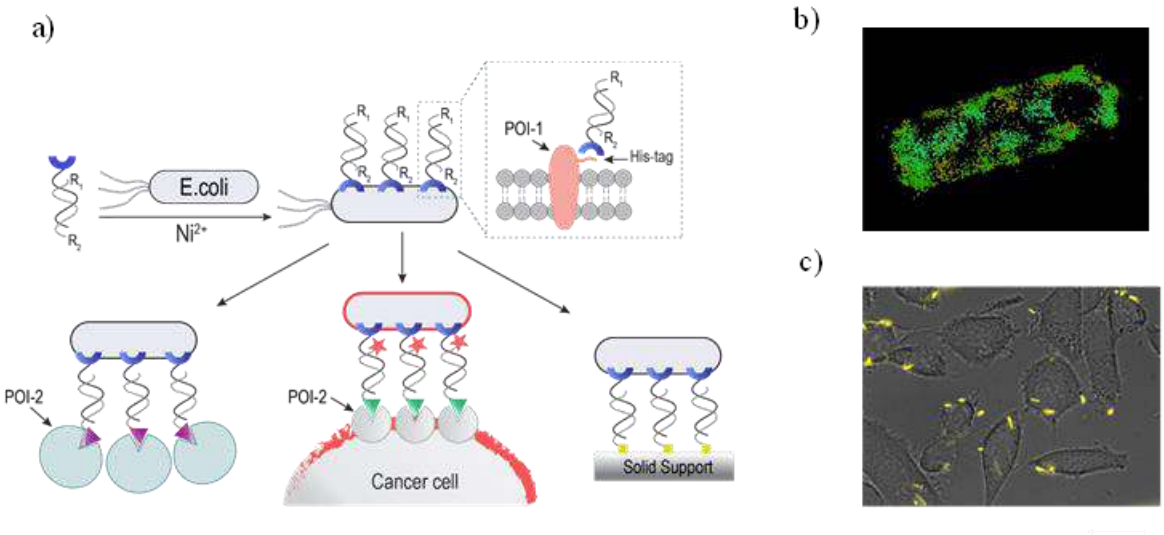

Figure 1. (a) Schematic illustration showing how B-probes are created and utilized. This technology enables bacteria to be 'programmed' to interact with proteins (i), target cancer cells (ii), or adhere to a solid support (iii). (b) STORM images of bacteria decorated with oligonucleotide-SM conjugates. (c) Images of KB cancer cells treated with a folate-bearing B-probe.

References:

1. Lahav-Mankovski et al. D. *Nat. Commun.* **2020**, *11*, 1299.
2. Kishore Prasad et al. *Mater. Today Bio.* **2023**, *20*, 100669.
3. Kishore Prasad et al. *Small* **2023**, *19*, 2206136.

IT 35

Impact of Ion Solvation on Biomolecular Condensation: The Hofmeister Series and Beyond

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Ions play a distinct role in determining the stability of colloids, especially if the surface of the colloids is charged. Historically protein precipitation by ions has been systematically studied and ions have been classified in the form of Hofmeister series. However, the list is not exhaustive, and several complex ions have been observed to offer unexpected (de)stabilization of proteins. Conventionally, biomolecular condensation has been looked upon from the protein's perspective shunning the pivotal role of the associated solvation. This makes the molecular mechanism of ion induced protein conformation change incomplete; it is not exclusively known whether these ions offer electrostatic interactions as they get absorbed at the protein surface or they modulate the hydration structure around the proteins. We will discuss the effect of a few ions, for example, lanthanides, guanidiniums, thiocyanates and phosphates on condensation of some model globular proteins. We put forward a plausible explanation against their behaviors in the light of their hydration structure and dynamics, measured using time- and frequency-domain THz spectroscopy technique.

IT 36

Human induced pluripotent stem cell-derived neurons and cardiomyocytes investigated by holographic closed-loop optogenetics

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Pluripotent stem cells hold promise in the field of regenerative medicine. Optogenetics is an elegant approach of precisely controlling and monitoring the biological functions, which is promising for the investigation of organoids such as neurons or cardiomyocytes. We have investigated holographic optogenetics for both, the monitoring with fluorescence and the actuation with optogenetics towards an closed loop approach. Cardiomyocytes form an electrically coupled syncytium which is the basis for the spatiotemporally synchronized propagation of macroscopic action potential wavefronts. Dysfunctional signal propagation patterns like self-replicating reentrant spiral waves are a main cause of deadly tachycardia. This problem often stems from myocardial fibrosis and is not yet fully understood.

Sample observation was done using high-speed label-free video microscopy to measure of local contractions in the sample and fluorescence imaging. We investigated human stem-cell-derived cardiomyocytes expressing the fast red-shifted excitatory ion channel f-ChRimson-YFP. By cross correlating spatially resolved stimulus responses, we aim to trace action potential wavefronts in in vitro cardiac tissue. We present investigations on the influence of different spatial and temporal stimulation patterns on the shape and propagation of optically invoked action potential wavefronts, paving the way to applications like patient-specific disease modeling in the future.

IT 37

Amyloid nucleation through protein phase separation

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Liquid-liquid phase separation (LLPS) of biological macromolecules has been a prominent field of interest in biology due to its increasing proteome-wide occurrence from bacteria to humans. Protein phase separation is proven to play a significant role with both physiological and pathological consequences. In the disease context, LLPS is often associated with aberrant functions and changes in its physical state, including liquid-to-arrested state transitions that affect the pathology inside cells. On the other hand, LLPS is also involved in several functional processes, including cell signalling, vesicular trafficking, and many others. We show that several regulatory proteins that undergo phase separation may serve as storage depots inside the cells with different functionalities. For example, transcription factor p53 forms condensates that show high spatiotemporal regulation based on its nucleo-cytoplasmic shuttling inside the cell. While nuclear condensates act as a ready supply pool and remain liquid-like, the cytoplasmic condensates undergo a liquid-to-arrested state transition. The formation of p53 condensates is highly regulated by genetic and environmental factors like mutations and the presence of DNA and RNA. Thus, p53 and several other proteins undergo LLPS and liquid-to-solid transition for their storage and functional regulation inside the cells.

IT 38

Expanding Imaging Capabilities Beyond Traditional Microscopy through Dynamic Host-Guest Molecular Interactions

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Fluorescence microscopy provides the most powerful tool for investigating biomolecules, specifically with the invention of diffraction-barrier-breaking super-resolution imaging techniques. Although existing super-resolution imaging techniques offer improved resolution, they require either complex and expensive instrumentation (STED) or specialized buffer conditions (buffer with reducing/oxidizing agents) with high-power laser illumination (STORM) to get optimal results. These limitations hinder the wide adoption of these techniques in standard biological laboratories. In addition, the use of high laser power illumination and specialized buffer conditions to obtain fluorescence blinking, often-time, limit the potential application of these techniques in live-cell imaging experiments. To address these challenges, we recently developed a simple, robust, and easy-to-implement super-resolution imaging method using highly selective, strong, yet dynamic supramolecular interaction between synthetic host-guest pairs. Unlike existing techniques that rely on externally controlled ON- and OFF-blinking of fluorophores, our technique strategically exploits transient binding between host-guest pairs to obtain autonomous blinking. By specifically targeting biomolecules in the living system, we showed that this programmable and autonomous blinking with prescribed brightness and frequency enables two-dimensional (2D) and 3D super-resolution imaging of proteins in cells and tissues. Overall, our method offered an unprecedented level of resolution in a biological system without using complex instrumentation or specialized experimental conditions. We subsequently extended this host-guest-based imaging approach to barcode cells via photochemical programming of their molecular recognition event. In this study, we created a distinct approach to selectively barcode cells by spatially controlling the positioning of fluorescent labels using light. Through this design, we readily achieved multiplexed barcoding of many cells in parallel through the cycling of the photoactivation and fluorophore anchoring steps at different cellular coordinates. This study paves the way for advanced single-cell studies by learning from their individual spatiotemporal dynamics.

References:

1. Das Saha et al. *J. Am. Chem. Soc.* **2022**, *144*, 14363.
2. Sasmal et al. *ACS Cent. Sci.* **2024**, *10*, 1945.

IT 39

THz Sensing for biochemical and environmental applications

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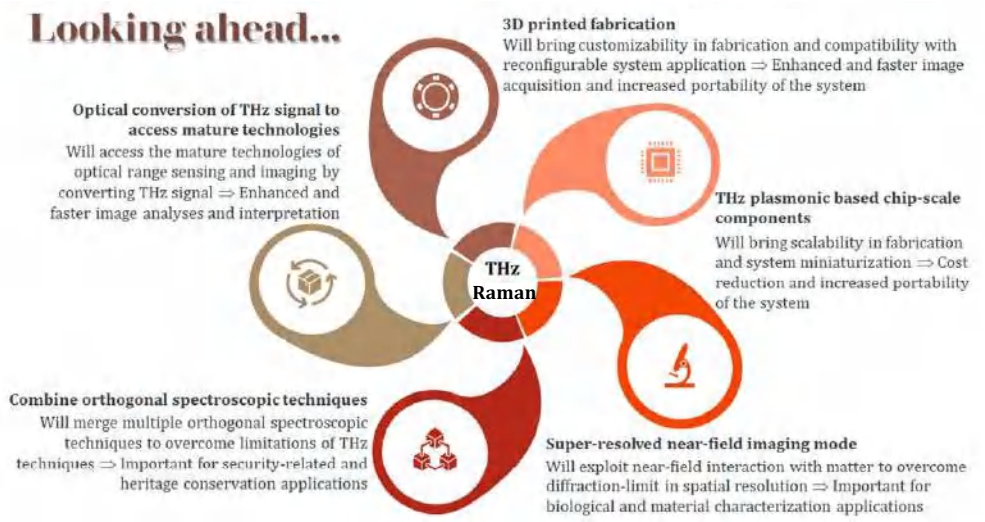
Multi-parameter sensing using non-destructive optical spectroscopic techniques, specifically Terahertz (THz) spectroscopy and THz-Raman spectroscopy have significantly reshaped the landscape of analytical methodologies in various industries; specifically in the biochemical and environmental domain. As technology continues to evolve, the ability to concurrently analyze multiple parameters at the molecular level has become a cornerstone for precision and efficiency in these critical sectors. In the pharmaceutical industry, THz spectroscopy has emerged as a key tool in ensuring the quality and stability of drug formulations, while THz-Raman spectroscopy complements by offering insight into chemical structure as well as composition. The agricultural sector benefits from the capabilities of these spectroscopic techniques in optimizing crop management, soil analysis, and resource utilization. With THz-Raman spectroscopy, one can essentially probe not only the fingerprint of a biomolecule or inorganics, but also the corresponding structural and symmetry properties. On the other hand, time-domain THz spectral imaging could provide critical information regarding molecular degrees of freedom or alternatively, the electrical conductivity of materials, and that too, from within the 'volume' of the substance.

In this talk, I will therefore highlight, how advanced THz based photonic systems with customized handheld scanners could revolutionize the sensing field even outside the laboratory by providing fast, reliable, non-contact, non-invasive spectroscopic and imaging possibilities, especially in the domains of environmental sensing and biochemical diagnostics. I will provide an understanding of how multi-parameter sensing through THz and THz-Raman spectroscopy is driving innovation, enhancing quality control, and fostering sustainable practices across diverse industrial landscapes.



References:

1. Khushboo Singh et al. *Optics & Laser Technology*, **2024**, *177*, 111020.
2. Aparajita Bandyopadhyay et al. *IETE Technical Review*, **2021**, *39*, 471.

IT 40

The Cortical Actin Cytoskeleton Regulates Membrane Protein Organization and Dynamics

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The Epidermal Growth Factor Receptor (EGFR) is a trans-membrane glycoprotein that belongs to the family of receptor tyrosine kinases (RTKs) and is a key player in regulating cell proliferation, differentiation, survival, function, and motility. EGFR is a highly regulated molecule, with its regulation influenced by its inherent structural features, the composition of the plasma membrane, and the adjacent cortical actin (CA). CA - the isotropic actin network bound and located proximally to the plasma membrane - significantly influences membrane organization. Acting as a dynamic scaffold, CA provides structural support and mechanical force for PM remodeling and receptor organization and dynamics. EGFR contains a putative actin-binding domain but to date it has not been determined whether EGFR binds directly to the CA.

Here, we combine Super-Resolution Radial Fluctuations and Imaging Total Internal Reflection – Fluorescence Correlation Spectroscopy (SRRF'n'TIRF) to investigate if and how the CA influences EGFR's organization and dynamics [1]. For this purpose, we created a CA-specific probe, PMT-F-tractin, that binds to both the plasma membrane and the cytoskeleton, and used EGFR, its actin-binding domain deletion mutant ($EGFR^{\Delta ABD}$), and an EGFR-F-tractin fusion mutant to investigate the relation between EGFR and CA. As controls for the influence of CA on membrane proteins at the inner and outer leaflets, we used PMT-EGFP and GPI-EGFP, respectively.

While PMT-F-tractin diffusion is directly dependent on CA density, we find that EGFR-mEGFP and $EGFR^{\Delta ABD}$-mEGFP show similar membrane dynamics independent of CA. EGFR dynamics, however, show CA dependence when F-tractin is anchored to the cytoplasmic tail. Overall, our findings indicate that EGFR-mEGFP does not directly bind, or at the very least not strongly bind, to CA in either the resting or activated state, and that thus any influence of CA on EGFR dynamics is very likely indirect.

References:

1. S. Pandey and T Wohland. *Biophys. J,* **2024**, *123, 3736*

IT 41

Prying into Biological Condensates Using Single-Molecule and HomoFRET

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The field of condensate biology is currently undergoing an exciting transition due to the rapidly unfolding role of highly dynamic, permeable, regulable, viscoelastic, membraneless, phase-separated biomolecular condensates in a wide range of cellular functions and deadly neurodegenerative diseases. A fundamental question in the field is how sequence-encoded molecular grammar is translated into the emergent mesoscale material property that governs the functional and pathological roles of biomolecular condensates. My laboratory has been actively involved in developing, adapting, and applying new methodologies that permit us to delineate the key molecular principles dictating the course of biomolecular phase separation (1-3). Using site-specific intramolecular single-molecule FRET (Förster resonance energy transfer), we were able to dissect the key molecular events associated with phase separation of the prion-like low-complexity domain of FUS (Fused in Sarcoma) that is associated with the formation of cytoplasmic and nuclear membraneless organelles (2). Our results showed that a symphony of structural unwinding events turns intramolecular interactions into dynamic crosslinks of multivalent intermolecular contacts resulting in condensate formation. We recently demonstrated a unique application of intermolecular homoFRET that is recorded by the rapid depolarization of fluorescence anisotropy occurring due to the excitation energy migration to the proximal fluorescently-tagged protein molecules within an assembly (3). Our results demonstrated that homoFRET can capture the modulation in the emergent properties of FUS condensates in the presence of RNA and ATP as well as upon the post-translational arginine methylation that alters the phase behavior of FUS. We further showed that homoFRET imaging can detect and characterize the in-situ formation of cytoplasmic stress granules within mammalian cells. Our findings provide mechanistic underpinnings of the modulation of condensate properties via an intriguing interplay of homotypic and heterotypic interactions that crucially govern the supramolecular packing and internal architecture of phase-separated biological condensates. Additionally, homoFRET imaging can serve as a potent, versatile, and generic tool for studying intracellular phase transitions of a wide range of proteins involved in function and disease.

References:

1. A. Avni et al. *Nature Communications* **2022** *13*, 4378.
2. A. Avni, et al. *Nature Communications* **2023** *14*, 7331.
3. A. Joshi, et al. *Nature Communications* **2024**, *15*, 9215.

IT 42

Near infrared fluorescence imaging for biomedical applications

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Near infrared (NIR, 800 nm -1700 nm) fluorescence imaging promises ultra-low background and scattering (tissue transparency window). We use nanomaterials such as NIR fluorescent single-walled carbon nanotubes (SWCNTs) as building blocks for advanced fluorescent sensors/probes in this spectral range.

Here, I will present novel approaches to extract more information from their fluorescent signals. Fluorescence lifetime imaging microscopy (FLIM) of SWCNT-based sensors is introduced as absolute and calibration-free NIR imaging method for biomolecules. This technique is enabled by laser scanning confocal microscopy (LSCM) optimized for NIR signals (>800 nm) and time correlated single photon counting (TCSPC). Moreover, the potential of spectral phasor approaches is discussed, and how it enables fast hyperspectral NIR imaging and multiplexing.

Finally, such fluorescent biosensors are able to detect (bio)molecules over multiple length and time scales: I will show examples from single proteins, signaling molecules such as neurotransmitters released from networks of cells, molecular profiles of pathogens to stress (reactive oxygen species) in plants. These nanosensors are therefore powerful tools for biomedical diagnostics, imaging, environmental monitoring or precision agriculture. In summary, I present advances in NIR imaging as well as applications of NIR fluorescent biosensors in complex biological systems.

References:

1. Elizarova et al. *PNAS.* **2022**.
2. Sistemich et al. *Angew. Chem. Int. Ed.* **2023**.
3. Metternich et al. *JACS* **2023**.
4. Ma et al. *Nano Letters* **2024**.
5. Settele et al. *Nature Communications* **2024**.
6. Stegemann et al. *arXiv* **2024**.

IT 43

Fluorogenic probes for biomolecular interactions

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Fluorogenic molecules have diverse potential applications, e.g. in fluorescence sensing and as solid state emitters. The key to control their light emitting properties is in the design of conditions that suppress nonradiative deactivation pathways of their excited states. Dimethyl-2,5-bis(4-(methoxyphenyl) amino) terephthalate (DBMPT) is a fluorogen which exhibits Aggregation Induced Enhancement in Emission (AIEE). This molecule exhibits strong fluorescence in its aggregates with surfactants and triblock copolymers [1]. These aggregates form either nanorods or microspeheres and exhibit shape dependent cellular uptake [2]. Their mixtures with appropriate blue emitters result in white light emission. Curiously, such white light emission with thioflavin T-containing amyloid fibils involves a disruption of the fibrils by the nanorods [3]. More recently, the role of polarity and aggregation on the fluorescence of a lipophilic derivative of DBMPT has been explored. It has been shown to have promise in tracking lipid droplets in live cells. Hence, FLIM has been used to monitor ferroptosis in mammalian cells (Figure 1).

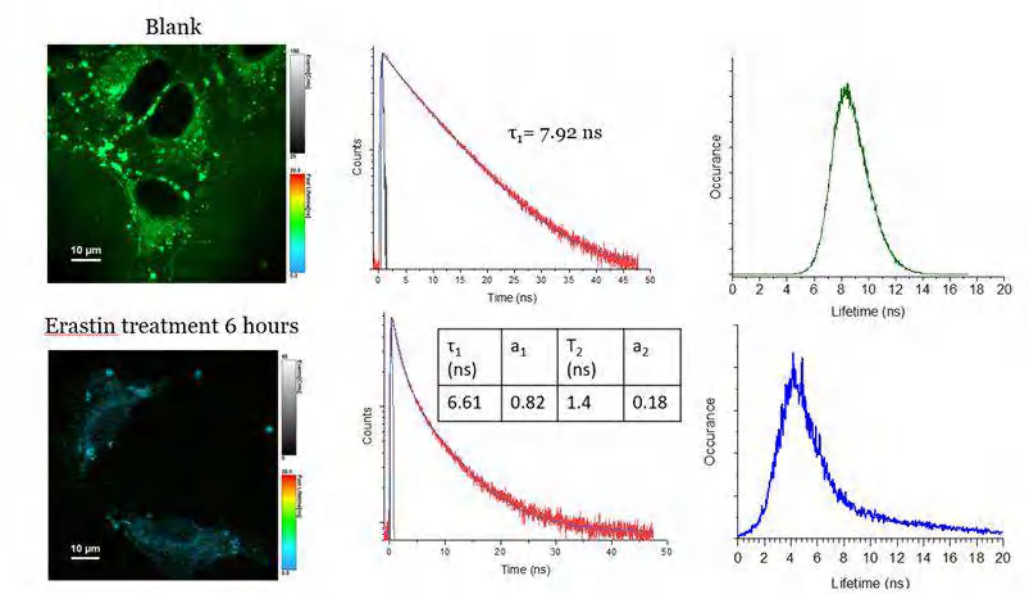

Figure 1. Fluorescence lifetime images of HeLa cells stained with the fluorophore before (upper panel) and after (lower panel) erastin treatment, inducing ferroptosis. The decrease in lifetime is in line with the increased polarity of lipid membranes during the process

References:

1. S.Rakshit et al. *J. Phys. Chem. B* **2020**, *124*, 7484.
2. S. Rakshit et al. *J. Phys. Chem. B* **2020**, *124*, 10282.
3. T.Kistwal et al. *J. Phys. Chem. Lett.* **2022**, *13*, 7355.

IT 44

Pulsed-interleaved-excitation two-dimensional fluorescence lifetime correlation spectroscopy (PIE 2D FLCS): development and applications to study biomolecular structural dynamics

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Single-molecule Förster resonance energy transfer (smFRET) method is widely used for studying biomolecular structural dynamics at the single-molecule level[1]. However, most smFRET methods usually provide only a millisecond or slower time resolution. Two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS) is a novel single-molecule fluorescence method that utilizes fluorescence lifetime information to distinguish different fluorescence species in equilibrium and resolves their interconversion dynamics with a submicrosecond time resolution. However, because 2D FLCS has used only a single-color excitation so far, it was difficult to distinguish a very low-FRET (or zero-FRET) species from only donor-labeled species[2-4]. Recently, we overcame this difficulty by implementing the pulsed-interleaved-excitation (PIE) scheme, i.e., alternate excitation of the donor and acceptor dyes using two temporally interleaved excitations with different colors, to 2D FLCS. This two-color excitation and two-color fluorescence detection in 2D FLCS, realized with PIE 2D FLCS, enables the study of biomolecular structural dynamics with high sensitivity and high time resolution using FRET[5]. In this presentation, I will discuss the framework of PIE 2D FLCS, followed by proof-of-principle PIE 2D FLCS analysis on photon data synthesized with Monte Carlo simulation, and its application to a DNA-hairpin sample. I will show that this method can readily distinguish four fluorescent species, i.e., high-FRET, low-FRET, and two single-dye-labeled species. In addition, I will also show that PIE 2D FLCS can quantitatively evaluate the contributions of the donor–acceptor spectral crosstalk, arising from the leak of donor fluorescence into the acceptor detector and direct excitation of the acceptor dye by the donor excitation pulse, which often appear as artifacts in FRET studies and complicate the analysis [5].

References:

1. E. Lerner et al. *Science*, **2018,** *359*, eaan1133.
2. K. Ishii et. Al. T. *J. Phys. Chem. B*, **2013**, *117*, 11414 & 11423.
3. T. Otosu et. al. *Nat. Commun.* 2015, *6*, 7685.
4. B. Sarkar et. al. *J. Am. Chem. Soc*. **2021**, *143*, 7968.
5. B. Sarkar et. al. *J. Phys. Chem. B*, **2024,** *128*, 4685.

IT 45

Biophysical Tools for Characterizing Ciliary Dynamics in Primary Cells: Towards Personalized Respiratory Disease Diagnostics and Treatment

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The measurement of ciliary beating frequency (CBF) in primary airway cells has emerged as a widely accepted diagnostic tool for respiratory conditions, including cystic fibrosis (CF) and ciliary dyskinesia (CD). The conventional clinical approach involves recording videos of cilia's rhythmic motion in nasal epithelial cells and subsequently processing these image sequences using the Fast Fourier Transform (FFT) technique to derive spectra from which CBF can be determined.

Recent advancements in biophysical tools, specifically Differential Dynamic Microscopy (DDM), have been applied to investigate ciliary spatiotemporal coordination, known as metachronal waves, as demonstrated in previous studies. Additionally, over the past two decades, Fourier space image analysis techniques like k-space Image Correlation Spectroscopy (kICS) and its real-space counterpart, Spatio-Temporal Image Correlation Spectroscopy (STICS) have been employed to study sub-cellular dynamics such as molecular diffusion and flow.

This research aims to explore the interplay between DDM, kICS, and STICS and their utility in elucidating ciliary dynamics at the air-liquid interface (ALI) of primary airway cells. We aim to define the optimal utilization of these tools and determine the minimum spatiotemporal sampling requirements necessary to employ them effectively in high-throughput microscopy of ALI cultures. Subsequently, by measuring CBF and metachronal waves, which are indicative of disease states in CF and CD, both before and after pharmaceutical interventions, we aim to facilitate more personalized diagnostic and therapeutic approaches for patients.

IT 46

Regulation of Protein Aggregation and Disaggregation Using Salts

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Protein misfolding and aggregation are not only associated with a variety of neurodegenerative diseases but also pose a challenge in therapeutic biotechnological processes, food industry, etc. Therefore, there is a pressing need to devise effective disaggregation and inhibitory strategies to combat undesirable self-assemblies. However, deciphering the mechanistic aspects of protein self-assembly remains a formidable task due to the inherent complexity, stochasticity, and hierarchy associated with protein aggregation. In this talk, I will discuss our recent results demonstrating the unique roles of a monovalent salt in effectively regulating the assembly and disassembly processes. Using a host of biophysical tools, we also show that salt-induced intrinsic conformational preference of single sequence monomeric polypeptide chains, prior to aggregation, plays a crucial role in governing the aggregation kinetics ensuing amyloid polymorphism.

References:

1. D. Singla et al. *J. Phys. Chem. B* **2024**, *128*, 10870.
2. A. Giri et al. *Phys. Chem. Chem. Phys.* **2024**, *26*, 25222.
3. D. Singla et al. *J. Phys. Chem. B* **2022**, *126*, 8760.

IT 47

Nondestructive Single-Cell Identification of Microbial Species and Domains Using Raman Microspectroscopy and Machine Learning

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Microorganisms are widely distributed in all three domains of life (Bacteria, Archaea, and Eukarya) and exhibit a great diversity of characteristics. Accurate and rapid identification of microorganisms leads to the discovery of new species, screening for beneficial microorganisms, and detection of pathogens, but conventional methods, such as genome sequencing, are inherently destructive. Recently, Raman spectroscopy has emerged as a potential approach for microbial identification. The Raman spectra of microbial cells encode information on their identity, physiological state, and metabolic function as 'microbial fingerprints', providing a nondestructive means to interrogate individual microbial cells. The major challenge in using these microbial Raman fingerprints is that cells of different microbial species typically exhibit very similar Raman spectra and the data can be very large. Therefore, it is critical to develop a method that can analyze nearly indistinguishable Raman spectral data from different microorganisms with high accuracy.

Here, we present the identification of microbial species and domains by leveraging a combination of single-cell Raman microspectroscopy and decision-tree machine learning techniques, including random forest (RF) and LightGBM. These algorithms are simple and fast compared to deep learning techniques (e.g., convolutional neural networks), yet can be sufficiently accurate. They also allow us to easily visualize the importance of the features that contribute to the identification.

First, we applied RF classification models to identify six microbial species (three bacterial species *Escherichia coli*, *Bacillus subtilis*, *Thermus thermophilus* and three archaeal species *Thermococcus kodakarensis*, *Sulfolobus acidocaldarius*, *Nitrososphaera viennensis*).[1,2] RF classification models trained on single-cell Raman spectra acquired in the stationary phase achieved an overall identification accuracy of 98.8±1.9% for the six species (10-fold cross validation) and 98.4% for three species (*B. subtilis*, *T. thermophilus*, and *N. viennensis*) in an artificially mixed population. We show that, in addition to protein and DNA/RNA abundances, the presence of carotenoids and the structure of membrane lipids play a key role in the identification.

We then investigated how different growth phases (lag/early exponential, mid exponential, late exponential/early stationary, early stationary, and late stationary phases) affect bacterial species identification using six model bacteria (*E. coli*, *B. subtilis*, *Pseudomonas putida*, *Paracoccus denitrificans*, *Herbaspirillum seropedicae*, and *Lactobacillus plantarum*). The average identification accuracy increased from 50–60% (models trained on mid-exponential or late-stationary phase data only) to 83.3% (both mid-exponential and late-stationary phases) and 96.3% (all growth phases). This result indicates that learning Raman spectral data from two physiological states with distinct characteristics significantly improves identification accuracy.

To specifically detect archaea in environmental samples, we developed a bacteria–archaea domain classification model using LightGBM with single-cell Raman spectra of 12 bacterial and 13 archaeal species. A LightGBM model was trained on the data of 24 species and tested on the data of the remaining one species, which was not used in the training process. The resulting binary classification accuracy was found to be 84.4% on average. Our results demonstrate that machine learning-assisted Raman microspectroscopy could provide a novel, efficient method for archaeal identification.

References:

1. N. Kanno et al. *iScience* **2021**, *24*, 102975.
2. N. Kanno et al. *STAR Protoc.* **2022**, *3*, 101812.

IT 48

Refining Single-Molecule FRET Analysis: New Models for Biomolecular Dynamics

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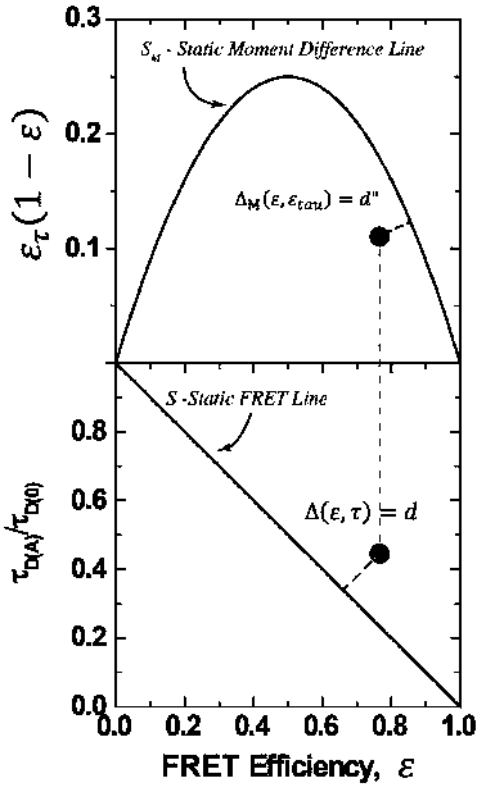
Conformational dynamics are crucial to biomolecular function, and Förster Resonance Energy Transfer (FRET) is a powerful tool for studying these motions at the single-molecule level. To capture dynamics on sub-millisecond timescales, multiparameter fluorescence detection measures both fluorescence lifetimes and intensities, yielding two critical observables—the donor lifetime ($\tau_{D(A)}$) and FRET efficiency (ε)—as unique fingerprints of molecular motion. We have used the "FRET-lines" framework to correlate these measures, enabling us to identify conformational states, resolve connectivity, and evaluate kinetic models[1]. Recently, we introduced a stochastic model of dye rotation and translation, revealing that deviations from the expected FRET relationship, or "dynamic shifts," stem primarily from dye orientation, not spatial distribution as was first introduced[2]. This refined approach enhances FRET accuracy, highlighting the importance of biomolecular behavior across timescales.



References:

1. Barth et al. *Journal of Chemical Physics*, **2022**, *156*, 141501.
2. Frost et al. *arXiv:2404.09883*, **2024**.

IT 49

Ultrafast Energy Flow of Heme Proteins In Crowded Milieu

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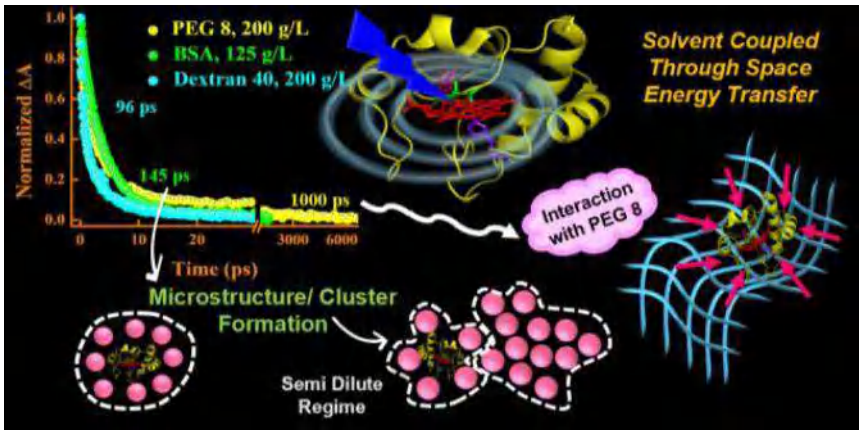
Energy flow in biomolecules is a dynamic process vital for understanding health, disease, and applications in biotechnology and medicine. In crowded environments, where biomolecular functions are modulated, comprehending energy flow becomes crucial for accurately understanding cellular processes like signaling and subsequent functions. Using ultrafast transient absorption spectroscopy we have addressed the various nuances of energy funneling from the photoexcited heme of bovine heart Cytochrome c (Cyt c)to the protein exterior, employing common synthetic (Dextran 40, Ficoll 70, PEG 8 and Dextran 70) and protein-based (BSA and β-LG) crowders. The through-space energy transfer mode for ferric and the methionine rebinding mode for ferrous Cytochrome c show the strongest solvent coupling. The heterogeneous behaviour of crowders, influenced by crowder-protein interactions and caging effects at certain higher concentrations, reveal diverse trends. Notably, protein crowders perturb all transport routes of vibrational energy transfer, causing delays in energy transfer processes. Comparison with the other heme protein myoglobin (Mb) reveals that the crowded scenario has almost no effect on the energy dissipation in Mb. The disparate behaviors of the heme proteins arises from the extent of heme exposure to the surrounding solvent bath, with the heme cofactor in case of Cyt c remaining appreciably buried. These findings provide significant insights into the basic tenets of energy flow, one of the most fundamental processes, in crowded cellular environments.



IT 50

Imaging Cellular Organelles during Collective Cell Migration

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Collective cell migration, essential to wound healing, morphogenesis, and cancer metastasis, depends on dynamic organelle reorganization. Leveraging live cell imaging along with advanced imaging techniques such as Structured Illumination Microscopy (SIM), expansion microscopy, and SoRa super-resolution imaging, we reveal key roles of the Golgi apparatus, lysosomes, and endoplasmic reticulum (ER) in guiding epithelial cell migration. At migration onset, the Golgi disperses around the nucleus, facilitating polarized trafficking. Lysosomes accumulate at the cell periphery in leader cells, regulating Rac1 activity and promoting lamellipodium formation. Additionally, the ER undergoes curvature-dependent reorganization, adopting tubular forms at positive curvature to support dynamic focal adhesions, while sheet-like ER at negative curvature stabilizes adhesions, coordinating distinct migration modes. These insights underscore how super-resolution techniques reveal organelle-driven cellular adaptations to mechanical cues, advancing understanding of collective migration in complex physiological contexts.

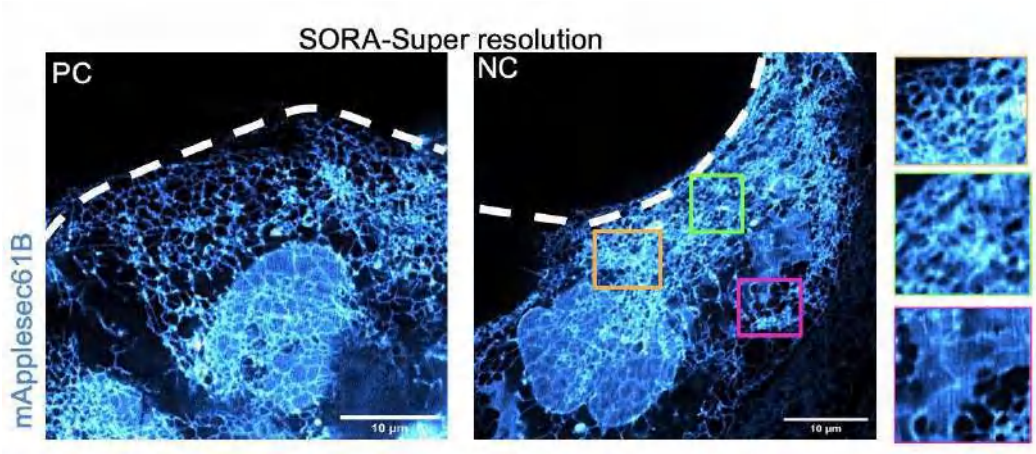

Figure: Structure of ER at positive (convex) and negative (concave) wound interface.

References:

1. Khuntia et al. *PNAS* **2022**, *119*, e2204808119.
2. Marwaha et al. *bioRxiv* **2023**, *doi: 10.1101/2022.08.03.502740.*
3. Rawal et al., *under review* **2024**.

IT 51

Raman Spectroscopy for Quantitative Estimation of Food Adulterations and Disease Biomarkers

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Raman spectroscopy works based on the Raman effect, invented by C. V. Raman, probes the bond vibrations in molecules and materials [1]. The recent developments in optics and electronics have enabled us to overcome the initial instrumentation limitations. Being a scattering-based technique, it offers instrumental design flexibility as per the analysis requirement. Other added advantages include its ability to probe samples in their native state providing fingerprint information about materials with minimum interference from water. As a result, Raman spectroscopy has become a popular analytical tool finding numerous applications across various fields [2]. However, the inherently weak scattering cross-section limits the practical utility of Raman spectroscopy. The interference from various high and low-frequency noises cannot be neglected while considering for quantitative anlysis. Surface Enhanced Raman Spectroscopy (SERS) is one of the developments that enhanced the potential for sensitive and trace-level analysis [3]. However, the major challenges are reproducibility, uniformity and irregularities in sample drying. Food quality assurance is one of the prospectus fields where Raman spectroscopy can play a revolutionary role compared to conventional analytical tools such as GC and LC-MS. Each problem requires a specific analysis methodology, and automating the process of data acquisition, spectral processing, analysis, and results prediction is the key to point-of-care applications. Herein, we investigated a few problem-centered solutions using Raman spectroscopy. Iodine value and saponification value are two bench parameters used to assess the quality of edible oils and are accepted by regulatory authorities like the American Oil Chemical Society (AOCS), Food Safety, and Standard Authority of India (FSSAI) [4]. We investigated how specific analysis methodology can be developed for quick and reagent-free prediction of these parameters using portable Raman spectroscopy. For the disease biomarkers, we attempted easy fabrication of SERS substrates for reliable detection and quantification.

References:

1. C. V. Raman et al., *Nature*, **1928**, *121*, 501-502
2. N. Emmanuel et al., *J. Chem. Educ.*, **2021**, *98*, 2109−2116
3. B. Abraham et al., *ChemNanoMat*, **2023**, *9*, e202300378
4. N. Ajikumar et al., *Food Chemistry*, **2025**, *464* 141580

IT 52

Circularly Polarized Light Emission in Chiral Nanomaterials

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Chirality is a unique geometric property observed in nature at different hierarchical scales ranging from subatomic particles to molecules to galaxies. The field of research is relevant to scientific communities across various disciplines ranging from chemistry, biology and pharmacology.[1] Optical activity in molecules and materials have been investigated for decades using circular dichroism (CD) spectroscopy, a technique that probes the ground state chirality [2,3]. A relatively new technique, that investigates the excited state chiral properties is circularly polarised luminescence (CPL).[4] CPL in chiral molecular systems is gaining vast attention due to their application in fields ranging from optical displays, data encryption, bioimaging and security tags.[5] The field of chirality has seen a rejuvenation after the observation of excited state optical activity in different classes of nanomaterials. We have been able to fabricate chiral light emitting materials adopting three different strategies. These include (i) synthesis of intrinsically chiral nanoparticles,[6] (ii) chirality induced through interaction with chiral organic molecules [7,8] and (ii) the template assisted generation of chirality using a host guest approach.[9,10] Our recent attempts towards synthesizing chiral emissive nanomaterials and understanding the mechanistic details of excited state chirality at the nanoscale will be discussed briefly.

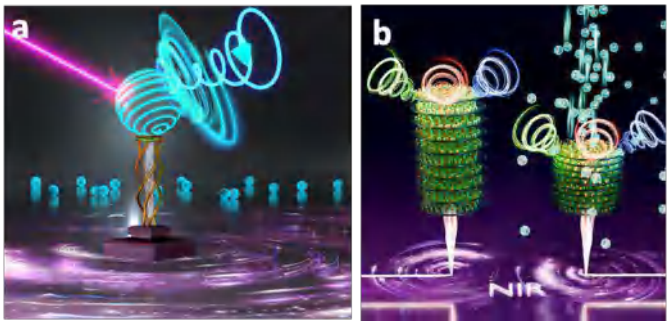

Figure: (a) Scheme illustrating circularly polarized luminescence in (a) downconversion and (b) upconversion luminescent nanophosphors.

References:

1. Jatish Kumar et al., *Bull. Chem. Soc. Jpn*., **2019**, *92*, 30.
2. Sonia Maniappan, et al., *Angew. Chem. Int. Ed.*, **2023**, e202300461.
3. Sonia Maniappan, et al., *Chem. Sci.*, **2024**, *15*, 7121.
4. Jatish Kumar et al., *Chem*, **2024**, *10*, 1628.
5. Ashok Jadhav et al., *J. Phys. Chem. Lett.*, **2024**, *15*, 8125.
6. Sonia Maniappan, et al., *Chem. Sci.*, **2023**, *14*, 491.
7. Camelia Dutta et al., *Chem. Sci.*, **2023**, *14*, 5593.
8. Camelia Dutta et al., *Chem. Commun.*, **2023**, *59*, 13735.
9. Sreelakshmi T. N. et al., *Nanoscale*, **2024**, *16*, 13571.
10. Akshaya K. et al., *ACS Appl. Opt. Mater.*, **2024**, 10.1021/acsaom.4c00057.

IT 53

Fluorescent Nano Probes for *in Vivo* Long-Term Tracking and Super-Resolution Imaging of Lysosomal Dynamics

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Lysosomes are membrane-enclosed small spherical cytoplasmic organelles. Malfunctioning and abnormalities in lysosomes can cause a plethora of neurodegenerative diseases. As a result, understanding the structural functional relationship of lysosomes down to a subnanometer level is essential. Recently, super-resolution imaging enabled us to visualise dynamical processes in living cells down to subnanometer accuracy by breaking the diffraction limit. Herein, the advancement of various fluorescent metal nanoprobes that have been utilised in our laboratory recently for super-resolution imaging of lysosomal dynamics will be discussed. An NIR emissive highly photostable Zn metal complex as a multifaceted fluorescent probe for the long-term dynamical distribution of lysosomes in various cancerous and non-cancerous cells in live condition and *in vivo* embryogenic evolution in *Caenorhabditis elegans (C. elegans)* will be presented. I will also discuss about the potential usage of coinage metal nanoclusters for in the super-resolution imaging of lysosomes. Finally, dual responsive fluorescent superparamagnetic iron oxide nanoparticles (SPIONs) to enhance their photon budget as well as the magnetic resonance imaging contrast will be discussed.

References:

1. Abdul Salam *et.al. Chemical Science*, **2024**, 15, 15659
2. Shagun Sharma *et.al. Nanoscale*, **2024**, 16, 16958
3. Shagun Sharma *et.al. J. Phys. Chem. Lett.* **2023**, 14, 8979
4. Aditya Yadav *et.al. ACS Materials Letters*. **2022**, 4, 1565
5. Aditya Yadav *et.al. J. Phys. Chem. Lett. **2020**, 11, 5741*

IT 54

Replicating Active Transport of Micro-organisms in Synthetic Systems

Sabyasachi Rakshit

IISER Mohali

Abstract.
Enzymes capable of reversible conformational shifts during catalysis exhibit functional similarities to motor proteins, enabling the conversion of chemical energy into mechanical work. In this presentation, I will explore the ability of these enzymes to generate autonomous motility, both as individual entities and collectively within semi-solid and solid-like aggregates. Further, I will demonstrate the application of these enzyme systems on giant unilamellar vesicles (GUVs), showing how they work coherently to replicate the active transport mechanisms of biological microorganisms in low-Reynolds number fluids. This research presentation outlines the design principles of artificial self-propelling swimmers that undergo directed translation and rotation across extended spatial and temporal scales, providing insight into the development of synthetic systems that mimic natural active transport.

IT 55

Machine learning driven high-resolution Raman spectral generation for accurate molecular feature recognition

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Raman spectroscopy, a technique employed to investigate the vibrational energy levels of molecules through inelastic light scattering, offers valuable insights into the molecular composition, structural arrangement, and dynamic behavior of materials. The accessibility of portable, handheld spectrometers has democratized the acquisition of Raman spectra, enabling their application in diverse fields ranging from chemical analysis to biomedical research. We propose a novel approach that leverages the generative capabilities of a Generative Adversarial Network (GAN) to enhance the quality of low-resolution Raman spectra obtained from handheld spectrometers. By training a GAN to generate high-resolution spectra from low-resolution inputs, we can effectively mitigate the limitations imposed by the inherent noise and resolution constraints of these devices. This enhancement enables more accurate and precise spectral analysis and compound identification. The enhanced spectra, characterized by their improved resolution and reduced noise, can be subsequently employed to train an Artificial Neural Network (ANN) for the classification of organic and pharmaceutical drug molecules. Furthermore, the high-quality Raman spectra generated by the GAN can be utilized to create spectral barcodes, a unique identifier for each pharmaceutical drug. These barcodes, analogous to molecular fingerprints, enable the rapid and accurate identification of unknown substances by comparing their spectral signatures to a reference database.

References:

1. V Yadav, AK Tiwari, S Siddhanta - arXiv preprint arXiv:2407.01597, **2024**

IT 56

Time-resolved, polarised fluorescence microscopy – influence of alignment

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Fluorescence anisotropy microscopy can be used to map variations, as a function of location in samples, in the degree of emission polarisation. Emission can become depolarised due to molecular rotation and/or excitation energy migration. Time-resolving the polarisation-selected emission as a function of location adds much additional, and necessary information. In this presentation I will discuss the need for the simultaneous measurement of emission decay kinetics and anisotropy, along with polarised transmission images, to fully interpret fluorescence anisotropy data, particularly in systems which exhibit significant levels of molecular alignment.

IT 57

Therapeutic advantages of drug-composites: Development and repurposing of acetylcholinesterase inhibitors

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The cholinergic hypothesis relies on the disintegration of acetylcholine (ACh) neurotransmitter by acetylcholinesterase (AChE) in the forebrain region as the root cause of Alzheimer's disease (AD). Therefore, acetylcholinesterase inhibitors (AChEIs) which hinder the activity of AChE resulting an increase in ACh level in the brain, have been utilized as therapeutic avenue for AD.

Typically, the AChEIs bind at the peripheral and/or catalytic active sites (PAS and CAS, respectively) of AChE binding domain which consists of a narrow but deep (about 20 Å) gorge. The activity of AChE can be finely tuned in presence of several bio-mimicking environments leading to the development of designed composite systems with greater solubility and inhibition activity potential. Some recent examples from our laboratory in this area will be provided in this presentation. Further, the development of nanocomposites with FDA approved AChEIs and potential repurposing of these AD drugs in different synergistic bio-nano platforms will be analysed critically

IT 58

Stiffness gradient substrate for cellular mechanosensing

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Many important biological processes like neurogenesis, angiogenesis and cancer metastasis are thought to be regulated by extracellular matrix (ECM) stiffness. ECM stiffness varies by multiple magnitudes between different tissues. The cells have the ability to sense and respond to the spatial variation of the rigidity in their microenvironment. Such sensing can lead to directed movement of the cell in response to the rigidity gradient of the ECM. In order to understand the process of rigidity sensing there have been several attempts to create stiffness gradient substrate. However there remains major challenge to create a high stiffness gradient substrate with cell scale variation of rigidity. In this talk we will discuss a relatively simple method developed in our lab to produce high stiffness gradient. Using topographical variation, we have designed a substrate with high stiffness gradient. The rigidity variation has been characterized with atomic force microscopy. We will also discuss several aspects of cellular mechanosensing on such high stiffness gradient substrate.

IT 59

Human Platelet Activation Dynamics Probed by Optical Techniques.

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It is well known that platelet disorders can result from various causes and can lead to different disease conditions such as cardiovascular diseases (CVDs), thrombocythemia, thrombocytopenia, Autoimmune diseases, Alzheimer's disease (AD), and even cancer, to name a few. The diagnosis of many of these diseases mainly depends on imaging examinations, clinical analysis and neuropsychological tests, these techniques are time taking and have high chances of false positive/false negative results. The micro-Raman spectroscopy of live platelets were performed optically trap the cells in their physiological medium using an optical tweezer which can provide trust worthy results without much time delay.

The present study focused on the activation dynamics of platelets. It is a known fact that platelets are usually stored maximum up to 7 days in blood banks, but effects of storage on platelets and their viability are not clearly understood. We observed that the activation leads to biochemical and morphological changes, such as the formation of filopodia on the platelet surface, transformation in the shape from discoid to spherical, and translocation of aminophospholipids from inner leaflet to the outer leaflet of the plasma membrane. The Raman bands corresponding to phospholipids shows remarkable intensity variations during activation. A detailed understanding on the activation dynamics of platelets would be useful in monitoring CVDs, ADs, etc. This study will be the first of its kind to report the trapping of platelets in its live form to study their activation dynamics using an in-house assembled optical tweezers micro-Raman spectrometer.

IT 60

Fate of knotted proteins during direction degradation and constrained folding conditions

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Knotted proteins are a special class of proteins that challenge our understanding of protein folding and degradation processes. The backbone of these proteins cross over themselves forming a knot, plausibly providing additional mechanical stability against proteasomal degradation and improved ligand binding abilities. In addition, it is not well understood if these proteins need chaperone assistance to fold back into their knotted structure upon unfolding. In this talk, I will present our recent results on understanding the folding/unfolding process as well as the degradation process of two different knotted proteins with 4_1 and 5_2 topologies. Intrinsic tryptophan as well as the near IR fluorescence studies on mi-RFP, a 4_1 knotted protein show that an unfolded knotted protein doesn't regain its native knotted structure but folds back to a different compact state. In addition, studies using proteases ClpXP and ClpAP show that the knots do not provide any considerable mechanical stability against mechanical degradation. I will also discuss how SM-AFM could provide additional insights into the folding process of these proteins under constrained conditions.

IT 61

Heterotypic phase transitions of the prion protein modulated by copper ions: a biophysical approach

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Prion diseases involve the aggregation and toxicity of an altered conformation of the prion protein (PrP), the scrapie PrP, in the brain. PrP's physiological role is thought to include binding redox-active Cu^{2+}, with copper imbalances observed in the brains affected by prion diseases. Cu^{2+} contributes to PrP aggregation, recently shown to be mediated by PrP condensation. Herein we explored how Cu^{2+} and oxidative stress affect PrP condensation and aggregation, using various biophysical and biochemical approaches both *in vitro* (recombinant PrP) and in live cells (expressing YFP-fused PrP). Our results [1] show that Cu^{2+} facilitates PrP condensation both at the cell surface and *in vitro* through co-partitioning mechanisms. Additionally, oxidative stress (induced by H_2O_2) led to a liquid-to-solid transition in PrP-Cu^{2+} condensates, promoting amyloid-like aggregation of PrP. We were able to follow the dynamics of heterotypic PrP-Cu^{2+} condensates by fluorescence recovery after photobleaching (FRAP) and by X-ray photon correlation spectroscopy (XPCS) experiments. In cells overexpressing PrP^C-YFP there was an initial protection from Cu^{2+} toxicity but resulted in PrP^C amyloid-aggregation upon extended copper exposure. Overall, our findings suggest that PrP condensates may act as copper buffers to mitigate copper toxicity, yet under sustained oxidative stress, these condensates may shift toward PrP aggregation.

References:

1. Do Amaral et al. *Science Advances* **2023**, *9*, eadi7347.

IT 62

Pushing the limits of multiphoton imaging in living systems

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Multiphoton microscopy is an indispensible tool for high-resolution, non-invasive imaging of structure and function deep within intact tissues and organs. In this talk, we will focus on technology development for imaging deep, wide and fast. We will review the state-of-the-art multiphoton microscopes for in vivo imaging of living tissues and animal models, and describe a number of directions for continued development, including new laser sources, new spectral windows and optimum illumination schemes. We will further discuss the limits of the imaging depth, volume, and speed in multiphoton imaging of living systems.

IT 63

Decoding Structure, Dynamics, and Synergism in Solutions Through Femtosecond Laser Spectroscopy

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In solutions, the structure and dynamics at the molecular level dictate their intermolecular interactions that could result in synergism in some cases. Typically, synergism in solutions refers to the interaction between different substances that leads to an effect greater than the sum of their individual effects or creates a new, emergent property. We demonstrate how femtosecond laser-induced spectroscopic techniques are essential in revealing these interactions, while also allowing control over the outcome depending on the chosen experimental parameters. This is possible because interaction parameters can be modulated based on whether the interactions occur under linear or nonlinear conditions. A comparison between linear and nonlinear fluorescence and absorption in binary solution mixtures at varying concentrations, and introducing a chromophore, whenever needed, establishes the significance of intermolecular interactions. This, in turn, serves as a key control parameter for harnessing synergism in solutions.

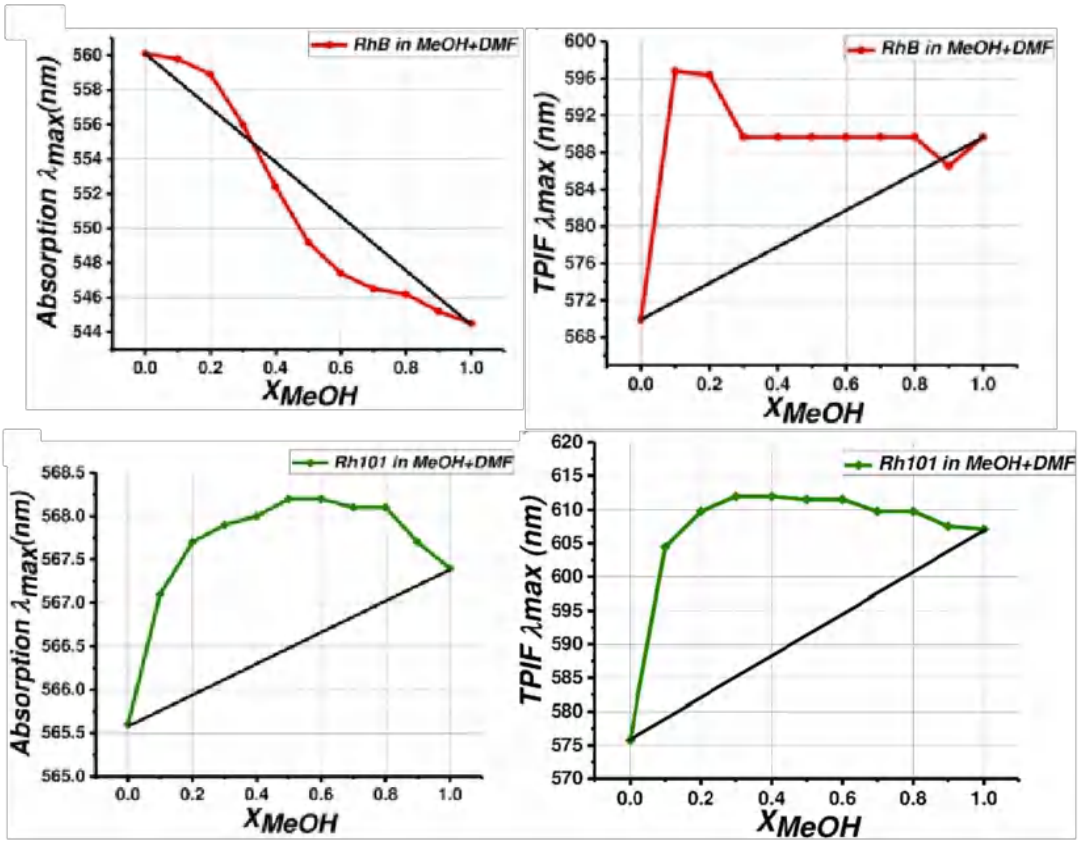

Figure: illustrating variation in linear absorption of two dyes Rhodamine-B (RhB) and Rhodamine-101 (Rh101) in the left panel versus the two-photon induced fluorescence (TPIF) for the same dyes in the binary solutions of Methanol (MeOH) and Dimethyl formamide (DMF) with different mole fractions of MeOH (X_{MeOH}).

IT 64

Structure and dynamics of Tau protein across liquid phases

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Biocondensates formed through liquid-liquid phase separation have been recently linked to a plethora of biological processes, from molecular storage to pathological pathways, and mostly observed with intrinsically disordered proteins. Tau is one example of a protein observed to phase separate in a both solution and intracellular environment. Furthermore, this protein is heavily associated with development and progress of several dementia, such as Alzheimer's, Pick's disease, among others. Interestingly, in healthy conditions tau is intrinsically disordered, but adopts different conformations across different pathologies, opening the possibility for specific drug targeting. Here, we demonstrate the use of different optical microscopy techniques to access the conformation and microenvironment the protein senses when incorporated in the condensed phase. We use a combination of Raster image correlation spectroscopy, single-molecule Förster resonance energy transfer and Fluorescence lifetime imaging microscopy to study Tau in the dilute and condensed phase. We take a first step towards single-molecule research on condensates of Tau protein under flow in a microfluidic channel of an in-house developed microfluidic chip. We employ Fluorescence correlation spectroscopy, a well-known technique to collect molecular characteristics within a sample, using an array detector (AD-FCS), providing detailed diffusion and flow information [Dilissen et al., 2024, BBA]. We foresee that the use and optimization of the mentioned fluorescence techniques will provide a more comprehensive knowledge of biocondensates.

References:

1. Dilissen et al. *BBA General Subjects* **2024**, *1868*, 130673.

IT 65

Interaction of human BRAC1 protein with Holliday Junction: Preference for an open X-like conformation

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BRCA1 is a complex tumor suppressor protein involved in multiple critical cellular processes, e.g., DNA double-strand break repair, cell cycle checkpoint, etc [1]. BRCA1-depleted cells are reported to have decreased homologous recombination (HR) and promote error-prone non-homologous end joining for DNA damage repair [2]. Holliday junction (HJ) is an important intermediate of HR. BRCA1 is shown to have a very high affinity for HJ and recruits several proteins at the DNA damage site. However, the questions remain: what is the binding mode of BRCA1 protein with an HJ? Do certain amino acids are necessary for the binding? What is the role of those amino acids in cellular function? Using single-molecule Fluorescence Correlation Spectroscopy (FCS), we have shown that BRCA1 prefers an open X-like conformation of HJ and has a significantly lower affinity for stacked HJ. Further, through molecular docking and all-atom molecular dynamics simulation, we have demonstrated that predominantly charged and polar amino acids within the DNA binding region (DBR) of BRCA1 are involved in forming the complex with the HJ. Interestingly, most of those amino acids are reported to be places for missense changes [3]. Unravelling the binding mechanism of BRCA1 with HJ and the associated amino acids' role is crucial for understanding BRCA1's contribution to genome stability and its implication in cancer.

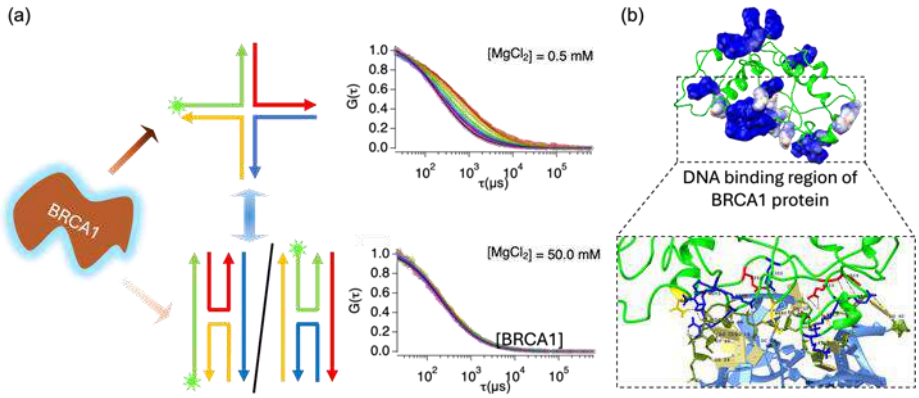

Fig 1: (a) Schematic representation of DBR of BRCA1 protein's preference for an open X-like conformation of HJ. (b) upper panel: Modeled DBR of BRAC1 protein. Polar uncharged and positively charged amino acids are in blue, lower panel: Hydrogen bonds between HJ and DBR of BRCA1 protein. Polar uncharged residues are in yellow, polar positively charged residues are in blue, and polar negatively charged residues are in red color.

References:

1. Li X et al. *Cell Res* **2008**; **18**, 99.
2. Venkitaraman A. R. *Cell* **2002**, *108*, 171.
3. brcaexchange.org

IT 66

Polarized optical pathways towards next generation optical techniques for biomedical imaging and diagnosis

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In this talk I shall introduce some new experimental concepts of polarization microscopy for probing structural anisotropy of biological tissues and other complex materials at the nanometer (sub-wavelength) length scales, and for using polarization anisotropy as intrinsic mechanism of image contrast. Specifically, *a* custom designed state-of-the-art *dark-field spectroscopic Mueller matrix microscopy system will be discussed, which has* the ability to extract complete polarization information and to quantify the intrinsic polarimetry characteristics from even a single isolated nanoparticle / nanostructure. An illustrative example of the exceptional ability of this system will be presented, where this polarization microscopic system in combination with a suitable polarization analysis model enabled quantitative assessment and understanding of the self-healing behavior of a bio-inspired piezoelectric organic crystal by sensing changes in structural anisotropy in the nanometer length scale. A spectral Mueller matrix based inverse light scattering polarimetry method for the quantification of *nanometer scale multifractal (multi-scale self-similar) anisotropy of tissue will be presented and its initial application for pre-cancer detection will be discussed. I shall also present* a novel Signum phase mask differential (SPMD) microscopy scheme that enables simultaneous differential phase and quantitative differential polarization contrast imaging in a single and remarkably simple experimental framework.

References:

1. JK Nayak et al, *Physical Review Letters* 131 (19), 193803 (2023).
2. S. Bhunia et al, *Science*, 373 (6552), 321-327, (2021).
3. S.K. Ray et al, *ACS Nano*, 11, 1641-1648 (2017).
4. SK Ray et al, *Physical Review A* 100 (3), 033805 (2019).
5. N. Das et al, *Laser Phys. Lett. 15 (3), 035601 (*2018).

IT 67

Structural and Orientational Transformations of Water at Air/Water-Polyethylene Glycol Polymer Interface

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Polyethylene glycol (PEG) is a versatile water-soluble non-ionic polymer which plays crucial roles in a wide array of applications, ranging from Li-ion batteries and water-splitting technologies to protein precipitation, anti-biofouling coatings, and drug delivery systems. The performance of PEG in these varied contexts, which depends on the molar mass of the PEG and the ionic strength of the solution, relies on the on the interaction of PEG with the water at its surface and interface. However, the molecular mechanisms by which molar mass and ionic strength govern the structure and dynamics of interfacial water remain largely unexplored. Here, we have investigated the structural and orientational changes of water at air/water-PEG interfaces using heterodyne-detected vibrational sum frequency generation spectroscopy (HD-VSFG),[1] with an emphasis on how the polymer's molar mass and ionic strength modulate the structure and orientation of the interfacial water. Moreover, the H-bonding of water in the immediate vicinity of PEG (i.e. the hydration shell) is measured using Raman difference spectroscopy with simultaneous curve fitting (DS-SCF)[2] analysis. It is observed that low molar mass PEG (e.g., MW = 200u) hardly alters the interfacial water structure at air/water interface (Figure 1a). However, the higher molar mass PEG (MW= 4000u), leads to dual orientations and distinct H-bonding of the interfacial water. Specifically, the interfacial water simultaneously adopts hydrogen-up (H-up i.e., water hydrogens are pointed away from the aqueous phase) and hydrogen-down (H-down i.e., water hydrogens are pointed towards the aqueous phase) orientations (Figure 1b). In the presence of NaCl (1.0 M), the response of the strongly H-bonded and H-up oriented water (positive band around 3200 cm^{-1}) decreases while that of the weakly H-bonded and H-down oriented water (negative band around 3450 cm^{-1}) remains unchanged for the air/water-PEG4000 interface (Figure 1b). At the air/water-PEG200 interface, however no appreciable change is observed in the presence of NaCl (1.0M). Raman-DS-SCF extracted OH stretch spectra of water pertaining to the hydration shells of PEG200 and PEG4000 are positively correlated with the corresponding HD-VSFG results, which suggests that the distinct structure of the interfacial water originates from the hydration of the surface adsorbed PEG at the air/water interface, instead of long range orientational order via the formation of an electric double layer.

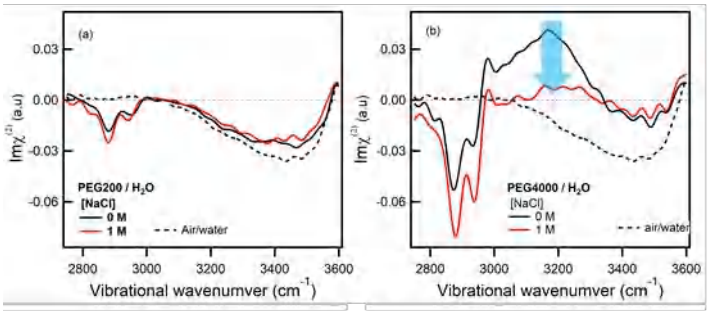

Figure 1. *Im$\chi^{(2)}$ spectra of the air/water interface in the presence of (a) PEG200 and (b) PEG4000. The ionic strength is increased using 1m NaCl.*

References

1. Ahmed, M. et al, *J. Phys. Chem. C*, **2016**, *120*, 10252.
2. Patra, A. et al., *J. Phys. Chem. Lett.*, **2023**, *14*, 11359.

IT 68

T cell communication through cytokines: simple physics in a complex system

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The proper course of the immune response requires a tight coordination in the activity of multiple immune cells of different types. Such coordination happens through the exchange of small signaling molecules - cytokines - between the cells. While this has been known for decades, the physical parameters of this communication: its distance dependence, and the characteristic spatial extent have not been addressed previously. We used theoretical considerations and experimental models of immune responses in vitro and in vivo to quantify the spatial extent of cytokine communications in dense tissues. Using T cell exchange of interleukin-2 as a model system, we demonstrated that the cytokine concentration around the secreting cell follows the predictions of a simple diffusion-consumption model. More recently, same processes were shown to control the spread of interferon g in tumors.

The characteristic size of the diffusion field is set by the competition between the cytokine diffusion and consumption rates, and was measured to be of several cell diameters. Furthermore, as the immune response proceeds, the consumption parameters change as cells respond to the cytokine, which leads to co-evolution of the cytokine and cytokine receptor fields.

References:

1. Oyler-Yaniv A et al *Immunity* **2017**, *46*, 609.
2. Centofanti E et al. *Proc. Natl. Acad. Sci USA* **2023**, *120*, e2304190120.

IT 69

Introducing a novel optical device to study protein aggregation in real-time

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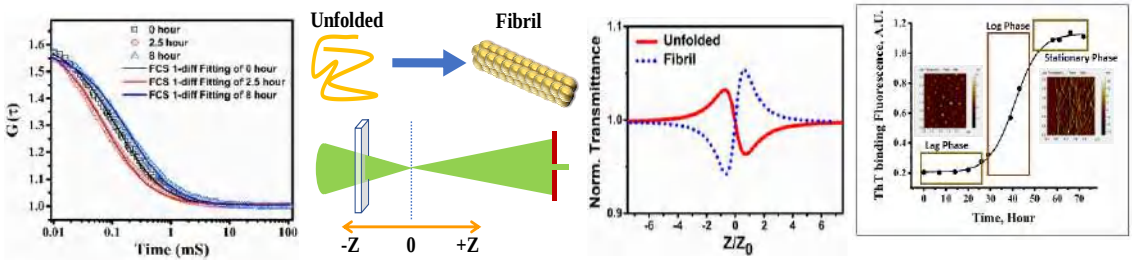
Protein aggregation and its associated diseases are widespread in neurodegeneration, such as Parkinson's disease (PD), Alzheimer's disease (AD), Prion disease, Schizophrenia, Huntington's disease, etc. The accumulation of intrinsically-disorder protein (IDP) aggregates in brain tissue plays a pivotal role in the pathology and etiology of neurodegenerative diseases. The IDP aggregation is a multistep process, progressing from early-stage soluble intermediates to late-stage mature insoluble amyloid fibrils. In this process, the late-stage soluble oligomers are the most toxic and harmful, playing a crucial role in neurodegenerative diseases [1]. The transient nature, inherent heterogeneity, inherent complexity, and wide dynamic range of this aggregation process make it challenging for conventional biochemical and biophysical techniques to capture the entire time window (between a few μs to several days) of protein aggregation in real-time. Therefore, novel approaches that are cost-effective, prototype, sensitive, and user-friendly are in high demand. In the present work, we have introduced a light-guided optical device based on the principle of nonlinear optics [2]. Here, specifically, we have demonstrated that the proposed optical device can be used to study protein aggregation in real-time with the help of two IDPs, *i.e.*, alpha-synuclein (α-syn) [3] and amyloid beta (Aβ-40). The aggregation of α-syn has a prime role in the PD, whereas AD is associated with the aggregation of Aβ-40. In both cases, our proposed device successfully monitored different conformations formed in the early and late stages of temperature and mechanical stress-induced protein aggregation, in which different species showed their characteristic nonlinear features. The perceived nonlinearity is governed by the saturated atomic absorption mechanism. A switch in the sign of the refractive nonlinearity has been observed for the first time as a signature of the late oligomeric conformation, a prime suspect that triggers cell death associated with neurodegeneration, and this will act as a marker for the diagnosis of both PD and AD. We validated our results using a combination of different techniques, like ThT fluorescence assay, FCS, FTIR, and AFM. We strongly believe that this simple, economical, and sensitive method can have potential future applications in detecting/monitoring conformations in other essential peptides/proteins related to different human diseases.



References:

1. S. W. Chen et al., *PNAS USA*, **2015**, *112*, E1994.
2. Sheik-Bahae, M. et al., *IEEE J. Quantum Electron*., **1990**, *26*, 760.
3. S. Ghosh et al., *ACS Chemical Neuroscience*, **2020,** *11*, 1610.

IT 70

Shaping and moving tissues during morphogenesis: cytoskeletal organisation entrains the morphodynamic, mechanical and material properties of cell cohorts.

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Epithelia, tissues that form outer barriers or inner linings of organs and organisms, are deformable cell sheets that can stretch, bend, invaginate and tubulate to generate new layers and branched tubes. Epithelial deformation sculpts tissues, organs and organismal body plans to their desired shapes relies and relies on rich heterogeneities in fate and behaviour of their constituent cells. What guides the stereotypical epithelial sheet deformations that sculpt the tissues and body plans of organisms? What guides cells within epithelial sheets to adopt the fates and behaviours they exhibit? How are these behaviours are ordered in space and time to enable tissues to be sculpted to their final shapes and positioned at their final destinations during their formation? How much are these decisions influenced by genetic prepatterns (top-down control by gene regulatory networks) and the mechanical microenvironment (bottom up control by cell interactions with neighbours and substrates). Do genetic prepatterns (gene expression, protein distribution, molecular interactions) and mechanical properties (tension, compression, shear, stiffness, fluidity) feedback regulate each other? How do they do so? These questions, fundamental to our understanding of the generation of tissue form (morphogenesis) and function, are at the interface between developmental biology, cell biology and soft matter physics. They are also of outstanding clinical importance and while shed light on pathological conditions. In my talk, I will show how a combination of genetics (to perturb gene expression and function) and imaging (to visualise molecular subcellular and cellular dynamics, and to perturb molecules and mechanics) has allowed us to answer some of these questions by examining two morphogenetic transformations in the fruitfly, *Drosophila melanogaster*.

IT 71

Understanding Aqueous Interfaces Using Vibrational Sum-Frequency Generation Spectroscopy

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Aqueous interfaces are crucial for driving processes from molecular to cellular to organismal level. The structure of water and other bio-molecular species is consequential in understanding these processes. Understanding the structure of water can improve understanding while developing artificial systems mimicking biological functions. In this talk, I will present how we can understand the structure of water at aqueous interfaces in the presence of non-ionic surfactants and how it can vary with small perturbations.[1-3] I will discuss how heterodyne-detected vibrational sum-frequency generation spectroscopy can be a powerful technique for extracting structural information from aqueous interfaces.

References:

1. Rahul Gera, et al., *Journal of the American Chemical Society*, **2021**, *143*, 15103.
2. Sanghamitra Sengupta†, Rahul Gera†, Colin Egan†, et al., *The Journal of Physical Chemistry Letters*, **2022**, *13*, 11391.(† - Equal Contributions)
3. Rahul Gera*, et al., *The Journal of Physical Chemistry Letters*, **2022**, *13*, 634.

IT 72

Design principles of pore forming toxins: Learnings from Cytolysin A

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Unlike most membrane proteins, pore-forming toxins (PFTs) are stable in aqueous solution as monomers. Upon exposure to lipid membranes, PFTs bind tightly to bilayers and assemble into nanopores by lateral diffusion and assembly leading to cell lysis. I will discuss how Cytolysin A (ClyA), a representative bacterial alpha-PFT achieves some of the crucial steps necessary for its activity. Using a combination of single-molecule spectroscopy, cell lysis kinetics assisted by MD simulations and cryoEM reconstruction, we have been able to generate mechanistic insight into the workings of ClyA in terms of determinants of membrane binding, conformational change to membrane-embedded protomer form, oligomerization to form the dodecameric ring and finally induction of membrane rupture. Our findings reveal how the protein can switch promptly to the membrane-bound state, selectively targets mammalian membranes, assembles efficiently in the cell membranes and membrane rupture is fine-tuned for mammalian cells.

References:

1. P Sathyanarayana et al. *PNAS* **2018**, *115*, E7323.
2. A Kulshrestha et al. *JPC B* **2022**, 127, 69.
3. A Kulshrestha et al. *Biophysical J* **2023**, 122, 4068.

IT 73

Probing Ligand Binding Kinetics with G-Quadruplex DNA using Fluorescence Correlation Spectroscopy and MD Simulations

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G-quadruplex DNA (GqDNA) structures are formed by guanine-rich DNA sequence in the presence of monovalent cations (mainly, K^+ or Na^+). They are found in the telomere and promoter regions. These structures play vital roles in various biological processes [1,2], which contain specific binding sites for small molecules (ligands), enabling anti-cancer activities and gene regulation upon interacting with GqDNA within the cell. Thus, understanding the kinetics of ligand interaction with GqDNA in cell-like crowded environments is paramount in biology and pharmacology, as it elucidates molecular crowders' influence on the reaction rates governing these interactions. In this talk, I will discuss how using fluorescence correlation spectroscopy (FCS) and molecular dynamics (MD) simulations (both equilibrium and metadynamics simulations), can track the kinetic steps of ligands' binding/unbinding with GqDNA structures in the absence [3,4] and presence of saccharide and EG/PEG crowders [5,6]. Experimental results indicate that saccharide and PEG crowders control the ligand binding kinetics with the GqDNA differently: The saccharide crowders affect the ligand binding affinity to GqDNA through viscosity-induced reduction of association rate (k_+) only, while EG/PEG crowders affect both association and dissociation rates (k_-) of the ligand. Atomistic MD simulations on GqDNA/ligand in absence and presence of EG/PEG crowders reveal the critical role of electrostatic forces and long-lived water-mediated hydrogen-bond-bridges in stabilizing the ligand/GqDNA complex, which is significantly disrupted by the EG/PEG crowders, leading to the destabilization of the complex. Unlike polysaccharide crowders, the EG/PEG crowders affect both the association and dissociation rates. This indicates that special attention is needed while choosing EG/PEG as crowding agents for cell-like *in vitro* experimental conditions. Metadynamics simulations uncovered the rate limiting steps that govern such kinetics of ligand/GqDNA interactions, matching well with the FCS results.

References:

1. Spiegel, J. et al. *Trends Chem.* **2020**, *2*, 123.
2. Rhodes, D. et al. *Nucleic Acids Res.* **2015**, *43*, 8627.
3. Clovis, N. S. and Sen, S. *J. Phys. Chem. B* **2022**, *126*, 6007.
4. Verma, S. D. et al. *Anal. Chem*, **2012**, *84*, 7218.
5. Alam, P. et al. *Methods Appl. Fluoresc.* **2024**, *12*, 045002.
6. Clovis, N.S. et al. *J. Photochem. Photobiol. A Chem.* **2023**, *437*, 114432.

IT 74

Searching for homology

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Double-strand break repair via homologous recombination is an important and conserved process. A key step in this pathway is homology search, where a RecA/ Rad51 nucleoprotein filament associates with the break ends and scans the genome to identify a template for repair. While this appears to be a challenging task given large amount of sequence to be sampled across the cell (or nuclear) volume, homology search is remarkably fast and accurate. Our recent studies in Caulobacter have unveiled dynamic movements of the RecA filament within the cell following a double-strand break, facilitated by the Structural Maintenance of Chromosome (SMC)-like protein RecN. This rapid back-and-forth filament traversal is essential for successful recombination between distantly located homologous sequences. In this talk, I will discuss our latest insights on the mechanistic underpinnings of RecN-driven RecA filament mobility. The observed dynamics of RecN association with the RecA filament, and the function of its ATPase cycle in regulating RecN-RecA interaction and RecA mobility are consistent with possibility that RecN acts as a DNA motor to propel the RecA filament during homology search.

IT 75

External manipulation of the absorption spectrum of a photoreceptor

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The local embedding of a chromophore inside a protein matrix can significantly impact the chromphore's optical properties. We aim to externally alter the visible absorption spectrum of photoactive yellow protein, a photoreceptor, by exciting vibrations of the chromophore as well as its local environment. To that end, we use ultrafast mode-selective IR excitation in combination with white-light probing that can measure the impact of vibrational excitation on the visible spectrum. The resulting two-dimensional vibrational-electronic (2D-VE) spectra show that changes in the visible spectrum occur as a function of time, both for excitation of chromophore modes as well as for excitation of environmental modes. In addition, the local hydrogen bonding network surrounding the chromophore is investigated. The approach presented here provides the starting point for mode-selective energy transfer studies, sub-ensemble selective excitation for photocycle studies as well as the detection of modes that play a role in the crucial visible photon-initiated isomerization of the chromophore.

IT 76

Single molecule imaging of Coronavirus spike trimers conformational dynamics during membrane fusion for entry

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Middle east respiratory syndrome (MERS) coronavirus entry into host cells is mediated by the spike (S) protein trimer. Pre-fusion and post-fusion structures of S have suggested large-scale conformational rearrangement in the S2 domain during entry. What triggers such conformational change and the nature of the conformational refolding intermediates along the fusion pathway have not been identified. To enable the direct imaging of conformational dynamics within S, we introduced fluorophores into variable regions of S2 subunit and measured single-molecule fluorescence resonance energy transfer (smFRET) within the context of native trimers on the surface of MERS CoV virions. Our observations revealed unliganded MERS CoV S to be intrinsically dynamic, transitioning between four distinct prefusion conformations, whose relative occupancies were remodelled by receptor DPP4, protease TMPRSS2 and antibody binding. Acidic pH remodels the conformational equilibrium of S2 in favour of an fusion competent intermediate conformation. In the presence of a target membrane, low pH stimulates irreversible transition to the post-fusion state of S and promotes membrane fusion reaction. The distinct conformational intermediates identified in S protein, support a dynamics-based fusion mechanism and provide mechanistic insights that may guide the design of MERS CoV entry inhibitors.

References:

1.S.Dey et.al. Structure.**2024,** (In Press)

2. P.Singhl et.al. mBio.**2024**,e0107724

3. P.Singh et.al Cell Reports.**2022** 39, 110694

IT 77

Probing Cancer Biomarkers with Biophysical Tools

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Extracellular vesicles (EV) are naturally occurring lipid bilayer-enclosed nanoparticles with great potential in biomedical applications [1]. We used AFM to show differences between EVs derived from colon cancer (HCT-116) and normal colon epithelial cells (CCD-Co18). Hyaluronan (HA), a potential colon cancer biomarker, is a non-sulfated glycosaminoglycan. Cancer EVs exhibited significantly increased HA surface densities at the single-vesicle level compared to normal EVs [2]. We measured the contour lengths of HA on a cancer cell-derived EV surface using force spectroscopy, which revealed the presence of low molecular weight HA (LMW-HA < 200 kDa). These LMW-HA-EVs were significantly more elastic than the normal EVs [3]. This intrinsic elasticity of cancer EVs could be directly allied to the LMW-HA abundance and associated labile water network on the EV surface. We recently elucidated the surface electrical properties of HA-EVs with AFM nanoelectrical modes for sensing HA [4]. These strategies could be realized as a non-invasive colon cancer diagnostic in the future.

References:

1. Rakshit et. al. *JACS Au*, **2024**, *4*, 318.
2. Paul et. al. *J. Phys. Chem. Lett*, **2020**, *11*, 5569.
3. Paul et. al. *J. Phys. Chem. Lett*, **2022**, *13*, 8564.
4. Paul et. al. (unpublished)

IT 78

Selective Targeting of Kinesin on Lipid Droplets Reduces Serum Triglycerides

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Hypertriglyceridemia, characterized by elevated serum triglyceride in the bloodstream, poses a risk for cardiovascular disease, fatty liver and insulin resistance. Conventional therapies against triglyceride (TG) reduction yield inadequate results with side-effects, prompting us to explore an alternative approach. The liver controls serum-TG levels by secreting TG-rich very low-density lipoproteins (VLDL) into blood in homeostatically controlled manner. We recently discovered that Lipid droplets (LDs) recruit the Kinesin-1 motor protein, causing LDs to be transported to the smooth endoplasmic reticulum inside hepatocytes, where TG from LDs is re-packaged into VLDL particles [1,2]. We show here that the binding of kinesin-1 to LDs can be targeted with remarkable selectivity, thus cutting down TG supply for VLDL formation. Targeting kinesin in this manner reduces TG secretion from hepatoma cells, and also reduced circulating TG in Zebrafish larvae by ~50%. Most importantly, it causes no detectable accumulation of TG in cells or in Zebrafish liver.

References:

1. Mukesh Kumar et al. *The Journal of Cell Biology.* **2019**, *218*, 3697
2. Priyanka Rai et al. *PNAS.* **2017**, *114*, 12958

IT 79

Critical Role of Associated Water in Protein Stability and Activity

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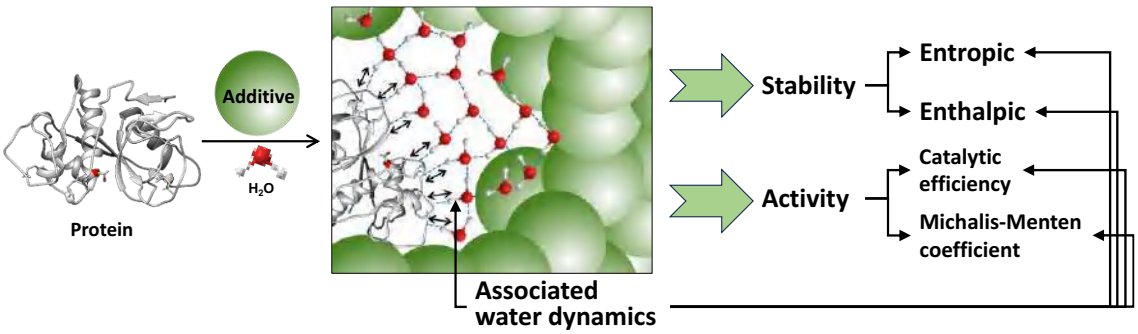
The water we drink and the water surrounding biomacromolecules differ significantly in behavior and properties. Water molecules near biomacromolecules, influenced by specific interactions such as hydrogen bonding with the macromolecule's surface, exhibit restricted dynamics compared to bulk water. This unique layer of water, termed biological water or associated water, directly interacts with macromolecules and impacts key protein characteristics, including stability and activity.

We examined various proteins, including human serum albumin (HSA) and bromelain, in the presence of additives such as osmolytes, macromolecular crowders, and deep eutectic solvents. Our findings revealed the pivotal role of associated water in regulating the stability and activity of proteins. By altering the structure and dynamics of this associated water layer, these additives indirectly influence protein behavior, a process crucial to understanding protein stability in complex environments.

Our results underscore the importance of considering both entropic and enthalpic contributions when analyzing protein stability under altered conditions. In particular, we provide insights into phenomena such as (i) the crowder-induced negative entropic effect and (ii) entropy-enthalpy compensation within biological systems. Additionally, by dissecting the individual components of enzymatic activity, such as enzyme-substrate complex formation and the product formation from the complex, we identified a strong correlation between the dynamics of associated water and enzymatic efficiency.

Notably, our hypothesis was validated across various additives (osmolytes, macromolecular crowders, and deep eutectic solvents) and for different proteins (HSA, bromelain, and papain), adding robustness to our findings. This generalization highlights a potential role of associated water in protein stability and activity modulation.



References:

1. Nilimesh Das et al. *J. Phys. Chem. B* **2023**, *127*, 3151.
2. Tanmoy Khan et al. *Int. J. Biol. Macromol.* **2023**, *253*, 127100.
3. Kuldeep Singh Negi et al. *Phys. Chem. Chem. Phys.* **2023**, *25*, 32602.
4. Tanmoy Khan et al. *J. Phys. Chem. B* **2024**, *128*, 8672.
5. Tanmoy Khan et al. *Manuscript in revision*.

IT 80

Single Molecules Come into Focus: From Bacterial Riboswitches to Mammalian Cellular Phase Separation

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At least 75% of the 3 billion base pairs of the human genome are transcribed into RNA, but the vast majority of these transcripts do not code for proteins but rather for "non-coding" RNAs (ncRNAs), many of which remain uncharacterized in terms of their structure and function [1]. Currently, more than 80,000 unique ncRNAs have been identified in human cells alone, suggesting that for a long time we have underestimated the intricacies involved in human genome maintenance, processing, and regulation by neglecting this far-reaching "RNA World." Nature and modern nanotechnology likewise employ nanoscale RNA machines that self-assemble into structures of complex architecture and functionality. Fluorescence microscopy offers a non-invasive tool to probe, dissect and ultimately control these nanoassemblies in real-time. In particular, single molecule fluorescence resonance energy transfer (smFRET) allows us to measure distances at the 2-8 nm scale, whereas complementary super-resolution localization techniques based on Gaussian fitting of imaged point spread functions (PSFs) measure distances in the 10 nm and longer range [2, 3]. Encapsulating the power of these recent technical advances, we have combined single-molecule and biochemical approaches to show that **a** central, adaptable RNA helix in the widespread manganese-sensing riboswitch functions analogous to a molecular fulcrum to integrate disparate signals for finely balanced bacterial gene expression control [4]. We posit that many more examples of such intimate structural and kinetic coupling between RNA folding and gene expression remain to be discovered, leading to the exquisite regulatory control and kinetic proofreading enabling all life processes. On the more applied side, we are developing tools to study the liquid-liquid phase separation of RNA-protein granules involved in human pathologies [5-7].

References:

1. N. G. Walter *RNA* **2015***, 21*, 756.
2. A. P. Jalihal et al. *Cold Spring Harb. Perspect. Biol.* **2019***, 11*, a032441.
3. S. Ray et al. *Chem. Rev.* **2018***, 118*, 4120.
4. A. Chauvier and N. G. Walter *Cell Chem. Biol.* **2024***, 31*, 71.
5. S. Pitchiaya *et al. Mol. Cell* **2019***, 74*, 521.
6. A. P. Jalihal *et al. Mol. Cell* **2020***, 79*, 978.
7. G. Gao et al. *Nat. Rev. Nephrol.* **2023***, 19*, 756.

IT 81

Pair correlation microscopy of heterotrimeric transcription factor transport

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Transcription factors are core players in the control of gene expression. How transcription factors assemble and move in the nucleus to locate and bind their DNA targets to cause a transcriptional response remains unclear. Here we present a new approach to pair correlation microscopy that enables the transport dynamics of heterotrimeric transcription factors to be tracked throughout a live cell nucleus. This approach is based on spatial cross correlation of the fluorescence fluctuations originating from the three sub-units underpinning a heterotrimeric transcription factor of interest. From application of this method to the highly conserved NF-Y transcription factor that has important roles in both housekeeping and lineage-specific gene expression, we provide quantitative measurement of NF-Y subunit association and diffusion kinetics in the nucleus that demonstrate NF-Y moves and binds to chromatin as a trimeric complex.

IT 82

Liquid-Liquid Phase Separation (LLPS) of Small Ubiquitin-related Modifier (SUMO1) and its Multimers

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Intracellular phase separation of biomolecules into membraneless compartments serves a multitude of biological functions. It has been shown that protein-based post-translational modifications such as ubiquitylation and sumoylation participate in several intracellular biomolecular condensates. Such phase separation can be achieved *in vitro* by using molecular crowders, for example, PEG and dextran. We earlier reported the impact of these inert crowders on ubiquitin-family proteins (ubiquitin, small ubiquitin-like modifiers (SUMO1 and SUMO2)). Ubiquitin is destabilized significantly in the presence of PEG, whereas dextran has a stabilizing effect. Moreover, the extent of stabilization/destabilization depends on the size and concentration of crowders. While there are no substantial changes in the stability of the soluble SUMO1 protein under crowding conditions, it undergoes rapid LLPS, forming micrometer-sized condensates [1]. Conformational changes in SUMO1 lead to the maturation of droplets into amorphous aggregates. SUMO2, however, shows only slight increase in scattering under similar conditions, and no notable change in thioflavin-T fluorescence, suggesting lower LLPS and aggregation propensity. We now studied the chain length dependent LLPS behaviour using head-to-tail-linked multimer chains of SUMO1: monomer, dimer, tetramer, heaxamer and octamer. Interestingly, the threshold concentration required for LLPS is inversely related to the chain length. Moreover, the condensates are devoid of the crowder underscoring the role of volume exclusion. Furthermore, salt-dependent and small organic molecule-based experiments established that the major driving force of the LLPS of SUMO1 is coulombic interactions whereas hydrophobic interactions have little influence. In total, the SUMO1 multimers establish the paradigm of 'multi-valency' of folded protein molecules in dramatically lowering the threshold concentration required for LLPS and in efficiently nucleating the phase separation.

Reference:

1. Arora *et al*, *J. Phys. Chem. Lett.* **2023**, *14*, 9060.

IT 83

Breath Analysis in Disease Detection through Spectroscopic Signatures

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Human exhaled breath contains a large number of different molecular species. Elevated concentrations of these molecules, or of specific isotopologues, can be markers for particular medical conditions. Appropriately devised breath analysis protocol may provide a non-invasive and rapid diagnostic method. Such non-intrusive methods can be developed via coupling sensitive and accurate spectroscopic detection techniques and molecular interactions. Considering that diseases alter the essential chemical reactions among biologically relevant molecules, and affect the outcomes, spectroscopic measurements can provide a unique pathway. This talk will provide a nuanced understanding of how exhaled breath can be used to facilitate early diagnosis for a spectrum of diseases by probing various molecular signatures of exhaled breath species.

IT 84

Investigating mechanism of coupled molecular machines in live cells using single particle tracking FRET (spt-FRET)

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NusG is a universally conserved transcription factor which binds to an elongating RNA polymerase and forms an anti-termination complex during ribosomal (rRNA) synthesis while promoting the formation of a coupled transcription–translation complex during messenger RNA (mRNA) synthesis—both processes critical to gene regulation in bacteria. We have developed methods to internalise and track in real-time single FRET-pair labelled NusG molecules in live E. Coli cells. This enables us to separately study the conformation and dynamics of NusG molecules freely diffusing through the cytoplasm and those bound to elongating RNA polymerases. Using biochemical tools to enhance or decrease levels of rRNA and mRNA synthesis we have been able to identify crucial differences in the conformational dynamics and the bound lifetimes of NusG molecules involved in these two processes.

IT 85

Investigation of Mitochondrial Dysfunction Related to Cancer and Alzheimer Disease: Two-photon FLIRR Microscopy

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We are investigating the intricate balance between mitochondrial biogenesis, dynamics and bioenergetics in cancer; and how this relationship is selectively modified to the advantage of more aggressive phenotypes of cancer. We are using Leica tauSTED super resolution microscopy to assess the morphological changes in mitochondria in different types of cancer (prostate and cervical). Most biological functions are in the nanosecond time domain. Investigation of any small sensitive changes can be detectable or monitored using two-photon fluorescence lifetime imaging microscopy (2p-FLIM) techniques. FLIM assays are of particular interest in measuring responses to treatment in various cancer pathologies, cancer being a metabolically heterogeneous pathology, which generates energy by oxidative phosphorylation (OXPHOS) and (often preferentially) by glycolysis. Two-photon fluorescence lifetime imaging microscopy (FLIM) is widely used to capture auto-fluorescence signals from cellular components to investigate dynamic physiological changes in live cells and tissues. 2-photon FLIM to track changes in mitochondrial metabolism via NAD(P)H/FAD lifetimes and relative abundances. Single wavelength 2p excitation simplifies FLIM imaging, data analysis using artificial intelligence (machine learning), decreasing the total imaging time, avoids motion artifacts and increases temporal resolution. 2p-FLIM and super resolution microscopy will precisely help us to investigate the role of mitochondrial structure -function relationship in cancer and how this relationship gets modified in aggressive forms of cancer for metastasis.

References:

1. A. Periasamy and R.M. Clegg, FLIM Microscopy in Biology and Medicine. 2010. CRC Press; NY.
2. Alam, S.R., Wallrabe, H., ……..and Periasamy, A.. Sci. Rep. 2016. 7: 10451.
3. Wallrabe, H., Svindrych, Z., ……and Periasamy, A. Sci. Rep., 2018. 8: 79.
4. Cao, R., Wallrabe, H. and Periasamy, A. Multiphoton FLIM imaging of NAD(P)H and FAD with one excitation wavelength. J. Biomed. Opt. 2020. 25(1), 014510.
5. Norambuena, A., Wallrabe, H., Cao, R., Wang, D.B., Silva, A., Svindrych, Z., Periasamy, A., Hu, S., Tanzi, R.E., Kim, D.Y. and Bloom, G.S. (2018) The EMBO Journal 37: e100241.

IT 86

Microbubble Lithography: writing mesoscopic architectures of 'everything mesoscopic' using laser-nucleated and manipulated microbubbles

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Lithography at mesoscopic scales has facilitated the revolution in micro-electronics, primarily by top down lithography processes such as e-beam and photolithography. On the other hand, bottom-up lithography techniques such as self-assembly, has also led to the patterning of mesoscopic materials – especially those responsive to external stimuli – at much lower cost. However, both top-down and bottom-up lithographic techniques fall short when it comes to patterning living matter (cells, proteins, etc) into controlled architectures, while also retaining their bio-activity. We have developed a technique called microbubble lithography [1,2], where we deploy self-assembly mediated by optothermally induced fluid flows around a microbubble nucleated by a laser, that is tightly focused (optical tweezers) into the fluid which contains the material to be patterned. We have been able to pattern diverse materials including conducting polymers, also increasing their conductivity in the patterning process itself [3,4], metal nano-particles [1], and recently - bacteria and virus [5] (Fig. 1) – thus laying the foundations for the development of a novel lab-on-a-chip, where multiple disease-causing pathogens may be detected on a single device. Microbubble lithography, thus promises to develop into a ubiquitous technology that can indeed realise the challenge of patterning 'everything' mesoscopic.

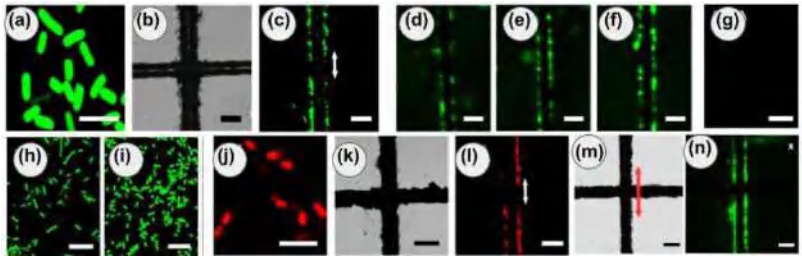

Fig. 1: Using the previous mentioned protocol for self-assembling Bacteria (E. coli and L. lactis) and Virus (H1N1) using MBL (a) Confocal image of the E. coli cells used for patterning (scale bar 5 um) (b) brightfield image of the ATTM pattern (scale bar 15 μm) where rE. coli is immobilized along the vertical direction only (c) the corresponding fluorescence image where the green fluorescence signal emitted by rE. coli cells is detectable only along the vertical direction (scale bar 15 μm) proving the immobilization along vertical direction only (d-f) patterning of E. coli at different laser powers of 3.5, 5, & 12 mW respectively (scale bar 25 μm here and henceforth) (g-i) corresponding confocal image of the cultured bacteria from the patterned samples at respective laser powers (scale bar 10 μm) (j) confocal image of the recombinant L. lactis (rL. lactis) cells used for patterning (scale bar 5 μm) (k) brightfield image of the ATTM pattern where the rL. lactis cells are immoblisised only along the vertical direction (scale bar 25 μm here and henceforth) (l) the corresponding fluorescence image where the red fluorescence signal emitted by rL. lactis cells is visible only along the vertical direction (m) b) the brightfield image of the ATTM pattern where the crosslinker and virus infected MDCK cell lysate is immobilized along the vertical direction as shown by the red arrow (current and subsequent scale bar represents 20 μm) (n) the corresponding fluorescence image of the virus sample where vertical direction shows higher fluorescence intensity compared to the horizontal field suggesting effective immobilization of HINI virus protein along the vertical direction.

References:

1. B. Roy et al., Langmuir **2013**, *29*, 14733.
2. S. Ghosh et al., Nano Letters **2021,** *21*, 10-25
3. S. Ghosh et al., Journal of Material Chemistry C **2017**, *5*, 6718
4. A. D Ranjan et al., Journal of Material Chemistry C **2023**, *11*, 17061
5. A. D Ranjan et al., Small **2024**, *20*, 2401127.

IT 87

Mutants, Molecules, and Mayhem: Bile Acids Fuel p53 R273 Aggregation and Chemoresistance

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Mutations in the p53 protein, especially within its DNA-binding domain, are a major hallmark of cancer, with the R273 position representing a critical DNA-contact site associated with several oncogenic variants. Notably, cancer patients carrying different R273 mutations in p53 exhibit varied survival rates, suggesting that DNA-binding inhibition alone may not fully explain the adverse outcomes linked to these variants. Here, we investigate three prominent oncogenic variants of wild-type (WT) p53—[R273H]p53, [R273C]p53, and [R273L]p53—using a combination of biophysical, biochemical, and theoretical simulation approaches. Our findings reveal that these variants not only suffer from impaired DNA binding but also exhibit distinct profiles of structural stability, aggregation propensity, and cellular toxicity. While WT p53 and [R273H]p53 exhibit the least destabilization and aggregation tendency, [R273C]p53 aggregation is driven by disulfide bonding, resulting in cross-β, thioflavin-T-positive structures, and [R273L]p53 aggregation is dominated by hydrophobic interactions, producing both amyloid and amorphous aggregates. Molecular dynamics simulations highlight variant-specific differences in contact maps and secondary structures. Further investigation of the R273H variant in the context of colorectal cancer (CRC) revealed its unique interactions with secondary bile acids, specifically lithocholic acid (LCA) and deoxycholic acid (DCA), and the chemotherapeutic agent doxorubicin. These bile acids, particularly LCA, promoted the aggregation of [R273H]p53 without affecting WT p53's DNA binding or self-assembly. This effect was amplified in the presence of doxorubicin, with LCA binding more strongly to the [R273H]p53 mutant, intensifying aggregation and driving morphological changes in CRC cells. In cell culture, LCA exhibited greater cytotoxicity towards HT29 cells (harboring [R273H]p53) compared to U87MG cells (WT p53), with significant aggregation observed upon combined treatment with LCA and doxorubicin. Our findings suggest that LCA promotes biomolecular condensate formation in [R273H]p53, sequestering doxorubicin within these condensates and potentially driving chemoresistance. This study uncovers distinct stability and aggregation pathways among [R273X]p53 mutants and provides insights for the development of therapies targeting residue-specific or process-specific aggregation, addressing chemoresistance in p53-mutant CRC.

References:

1. Garg et al. *Biophys. J* **2020**, *118*, 720.
2. Garg et al. *Curr. Res. Str. Biol*, **2024,** *7*, 100133
3. Kaur et al. *ACS Chem.Neurosci.* **2024,** https://doi.org/10.1021/acschemneuro.4c00459

IT 88

Deep-Learning and The Tunability of DNA Origamis

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DNA origamis are well known for their programmability, not only for precisely positioning molecules on the DNA origami structure but also with respect to kinetics. Using a recently developed L-Shaped Origami structure with a flexible linker that fluctuates between two binding sites [1], we investigated the precision with which the kinetic behavior of a DNA origami structure can be programmed using single molecule Förster Resonance Energy Transfer (FRET). For this structure, we noticed that there is a difference in the dwell times for binding to the same sequence at different locations on the DNA origami. Structures were constructed with a different number and positioning of the fluorophores as well as the number of states to investigate what factors influence the dynamics. Dyes in the vicinity of the docking strand influence the hybridization kinetics of the single stranded DNAs, but there is also an inherent asymmetry in the kinetics coming from local environmental effects of the DNA origami in the vicinity of the docking strands. To aid with the analysis, we implemented a recently developed deep-learning approach [2] for sorting the traces and extracting the kinetics, which I will also describe.

References:

1. Schroder T et al. *Proc Natl Acad Sci U S A* **2023** *120*, e2211896120.
2. Wanninger S et al. *Nat Commun* **2023** *14*, 6564.

IT 89

Fluorescence lifetime correlation analyses reveal leaflet-specific lipid diffusion in a lipid bilayer

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A lipid bilayer is composed of two lipid monolayers facing their hydrophobic alkyl tails with each other. Elucidating the strength of interactions between the monolayers, interleaflet coupling, and its lipid-composition dependence is crucial to understand the structural properties of a lipid bilayer and infer the biological function of lipids in a cellular membrane, e.g., cooperative formation of “lipid raft” and interleaflet signal transduction.

In this regard, we developed a methodology of leaflet-specific lipid diffusion analysis [1,2] by means of two lifetime-based fluorescence correlation spectroscopies (FCSs), two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS) [3,4] and fluorescence lifetime correlation spectroscopy (FLCS) [5,6]. In this analysis, potassium iodide is added to bulk solution facing one of the leaflets in a lipid bilayer. Because an iodide ion works as fluorescence quencher, fluorescence of head-labelled fluorescent lipids in the leaflet facing iodide-containing bulk solution is quenched and the fluorescence lifetime is shorter than that of the opposite leaflet. It means that one can discriminate the fluorescence signals based on their excitation-emission delay times. To achieve this, 2D FLCS is applied to extract leaflet-specific fluorescence decay curves of the probe lipids in the lipid bilayer. Then, by utilizing the leaflet-specific decay curves, one can calculate the leaflet-specific autocorrelation curves by means of FLCS. The decay times of the autocorrelation curves give information of diffusion coefficients of the lipids in each leaflet.

We have applied this technique to various systems [7-9]. Especially, we analysed lipid-composition and pH dependence of leaflet-specific lipid diffusion in glass-supported lipid bilayers (SLBs) and revealed that a leaflet-specific lipid diffusion is highly dependent on these factors. In this presentation, I have a summary talk about the application of leaflet-specific lipid diffusion analysis to SLBs.

References:

1. T. Otosu and S. Yamaguchi, *J. Phys. Chem. B*, **2018**, *122*, 10315.
2. T. Otosu and S. Yamaguchi, *Chem. Lett.*, **2020**, *49*, 1473.
3. K. Ishii and T. Tahara, *J. Phys. Chem. B*, **2013**, *117*, 11414 & 11423.
4. T. Otosu and S. Yamaguchi, *Molecules*, **2018**, *23*, 2972.
5. J. Enderlein and I. Gregor, *Rev. Sci. Instrum.*, **2005**, *76*, 033102.
6. A. Ghosh et. al., *Methods*, **2018**, *140-141*, 32.
7. T. Otosu and S. Yamaguchi, *J. Chem. Phys.*, **2019**, *151*, 025102.
8. T. Otosu and S. Yamaguchi, *Phys. Chem. Chem. Phys.*, **2020**, *22*, 1242.
9. K. Shimizu et. al., *Phys. Chem. Chem. Phys.*, **2024**, *26*, 8873.

IT 90

Force-Driven Transformation of Tunnel-Associated Chaperones: Unveiling a Strain-Energy-Based Mechanism for Enhanced Protein Folding

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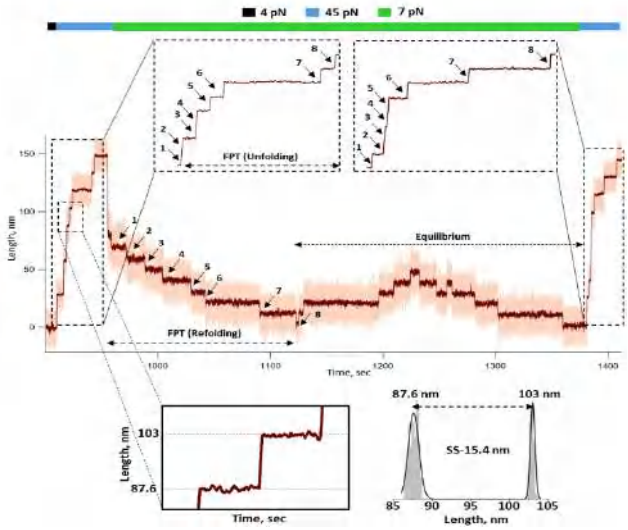
Chaperones play a vital role in cellular proteostasis, traditionally functioning as holdases or foldases to assist protein folding. Our study uncovers a remarkable shift in the role of chaperones under mechanical constraints within the cell. We found that tunnel-associated chaperones like Trigger Factor (TF) and DsbA, previously known as holdases in the absence of force, significantly accelerate protein folding when mechanical force is applied. To further investigate, we studied the tunnel-associated chaperones BiP and ERdj3 and compared them to their cytoplasmic counterparts DnaK and DnaJ. Remarkably, while both BiP and ERdj3 act as holdases without force, they transform into potent foldases under force—a function their cytoplasmic homologs lack. This transition to foldase activity enables BiP/ERdj3 to deliver 54 zJ of mechanical energy, enhancing force transmission through the Sec61 translocon tunnel.

We explain this phenomenon using strain theory, proposing that the unique mechanical behavior of these chaperones arises from internal strain energy, which is modulated based on substrate conformation. Whereas canonical unfoldases like DnaK/DnaJ exhibit higher strain energy with unfolded substrates, BiP/ERdj3 show elevated strain energy differences with folded substrates, stabilizing these proteins in a force-dependent manner. Our findings reveal a novel, feedback-based mechanism in which mechanical force transforms chaperone behavior, suggesting a distinct mechanistic paradigm for understanding protein folding under intracellular mechanical constraints. This insight into the physical basis of chaperone mechanics opens new perspectives on their role in force-driven cellular processes.



IT 91

Deciphering the Modulation of α-Synuclein Amyloid Assembly by β_2-Microglobulin Conformers

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Amyloid formation due to protein misfolding is increasingly recognised for its association with debilitating human ailments such as Parkinson's disease, Dialysis-related amyloidosis and Type II Diabetes mellitus. α-Synuclein (α-syn) and β_2-microglobulin (β_2m) are such proteins that undergo a profound conformational switch to form higher order cross-β-sheet structures, resulting in amyloid formation, which is linked to the pathophysiology of Parkinson's disease (PD) and dialysis-related amyloidosis (DRA), respectively. The present status of research reveals that PD progression may be linked with many other diseases, such as kidney-related disorders. Unraveling the link between PD and non-neurological diseases may help in early detection and a better understanding of PD progression. In most disease conditions, amyloid formation proceeds *via* distinct on-pathway conformers such as oligomers and protofibrils. However, the detailed mechanism by which monomers transform into different species and contribute to disease progression remains an area of intense research. This study identifies and characterizes distinct β_2m conformers, namely, oligomers, protofibrils, and fibrils. Additionally, the interaction between α-syn with different conformers of β_2m is explored to understand how this crosstalk influences PD progression. The insights will lead to early diagnosis and new therapeutic approaches for Parkinson's disease. The finding could also shed light on the unraveling of the yet unknown potential linkage of PD with kidney-related disorders.

References:

1. K. Rani et al. *J. Mol. Biol*. 2023, 22;435(22):168285.

IT 92

Single Molecule Conformational Dynamic of DNA Holliday Junction and T-cell Receptor

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DNA Holliday Junctions (HJ) are crucial intermediates in genetic recombination and genome repair processes, characterized by dynamic nature and transitioning among multiple conformations on the timescale ranging from sub-millisecond to second. While the influence of ions on HJ dynamics has been extensively studied, precise quantification of thermodynamic feasibility of transitions and detailed kinetic cooperativity remains unexplored. Understanding heterogeneity in providing stochastic gene recombination is extremely difficult to characterize using ensemble averaged experimental techniques because of its lack of ability to differentiate dynamics and function in a high spatiotemporal resolution. In this talk, I will discuss how DNA HJ changes conformation in three different patters of resonance, coherence, and decoherence. In the second part of talk, I will discuss how immune cells distinguish between self and foreign antigens at single molecule level in real time. The process of immune cells recognition is fast and finally it is translated to a complex cell signalling. Understanding the conformation dynamics of protein molecules at the surface of immune cells is extremely important. We apply various imaging techniques to capture ligand-receptor interaction dynamics on cell surface.

References:

1.Pratibha Agarwala.et.al. *Nanoscale* **2024,** (Accepted, In press)

2.Pratibha Agarwala, et.al.***2024,*** *Under Review*

3.Dibyendu K Sasmal.et.al. *Cellular and Molecular Immunology,**2020** (Nature Publishing Group)**, 17, 203-217.***

IT 93

Insights into an Extremophile Protein's Structural and Functional Attributes

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Crenarchaea thrive in extreme environments characterized by high temperatures, hypersalinity, and hyperacidity. Proteins from these organisms display exceptional structural stability, allowing them to withstand such harsh conditions. This stability is attributed to their unique amino acid compositions. Cren7, a DNA-bending protein derived from these extremophiles, is noted for its beta-pleated structure with extended loops. Studies employing UV melting techniques on Cren7-DNA complexes have demonstrated that Cren7 enhances the thermal stability of DNA, protecting it from denaturation. The stability of the Cren7-DNA complex is supported by both electrostatic and non-electrostatic components of free energy. Through fluorescence-based reverse titration, it has been shown that non-electrostatic interactions play a significant role in stabilizing this complex under hypersaline conditions, with Trp26, an exposed tryptophan residue, being a major contributor.

Cren7 shows a preference for binding to AT-rich DNA, exhibiting micromolar-range binding affinity. Circular dichroism (CD) studies indicate that Cren7 binding induces cooperative structural changes in the DNA, although Cren7 itself does not undergo structural alterations upon binding. Electrostatic interactions between Cren7 and DNA are primarily facilitated by lysine residues at their interface. The positioning of these lysine residues is critical for both binding affinity and complex stability. Biolayer interferometry and fluorescence anisotropy studies have highlighted the significant roles of lysine residues K31 and K27 in modulating the DNA binding affinity of Cren7.

The hydrophobic core of Cren7 is composed of residues that engage in π-stacking interactions. The C-terminal tyrosine (Y58) acts as a gatekeeper for the core's stability, and its mutation results in complete destabilization of the protein. Alanine mutagenesis experiments, involving substitution of aromatic residues, have confirmed that core residues are essential for correctly orienting the surface-exposed tryptophan (W26). These findings underscore the importance of Cren7's core residues in facilitating DNA binding and bending.

References:

1. Geethika et.al. *Biophys J*. **2023**, *122*, 1701.
2. Geethika et.al. *Phys. Chem. Chem. Phys*., **2022**, *24*, 19401

IT 94

Emergence of Dynamic G-Tetraplex Scaffold: Uncovering Low Salt-Induced Conformational Heterogeneity

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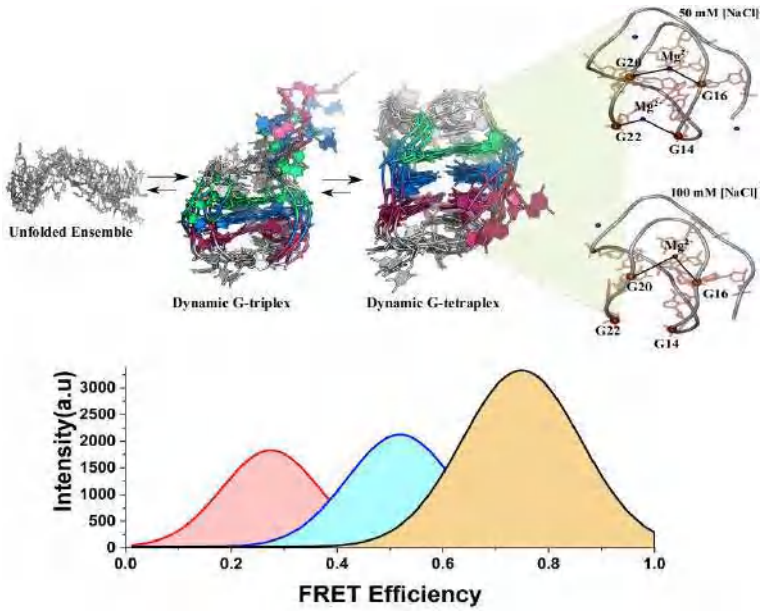
The topological diversity of human telomeric G-quadruplex structures is intrinsically related to their folding mechanisms, and is significantly modulated by ion-atmospheric conditions. Unlike previous studies that focused on higher Na+ or K+ concentrations, this study explores G-quadruplex folding and dynamics under low NaCl conditions (≤100 mM) using single-molecule FRET microscopy and advanced structure-based DNA simulation techniques. The smFRET data reveal three distinct populations; unfolded, intermediate dynamic triplex, and dynamic tetraplex structural ensemble. The broad distribution of the folded population highlights the dynamic nature of the quadruplex structure at low salt conditions. In agreement with smFRET result, free energy simulations show that with increase of NaCl concentration, the population shifts towards the folded state, and differentiates all intermediate structural ensemble. The dynamic equilibrium between the triplex and tetraplex scaffolds explain the microscopic basis of conformational heterogeneity within the folded basin. Simulations also reveal that the flexibility of dynamic tetraplex bases depends on the equilibrium distribution of ions underpinning a few ion-mediated dynamic non-native interactions in G-quadruplex structure. Contrary to the previously held belief that Na^+ induces minimal structural heterogeneity, our combined experimental and simulation approaches demonstrate and rationalize the structural variability in G-quadruplexes under low NaCl concentrations.



IT 95

Mapping Atomic Motions with Ultrabright Electrons: Fundamental Space-Time Limits to Imaging Chemistry and Biology

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One of the long-sought objectives in science has been to watch atomic motions on the primary timescales governing structural transitions. From a chemistry perspective, this capability would give a direct observation of reaction forces and probe the central unifying concept of transition states that links chemistry to biology. With the development of ultrabright electrons capable of literally lighting up atomic motions, this experiment has been realized (Siwick et al Science 2003) and efforts accelerated with the onset of XFELs (Miller, Science 2014). A number of different chemical reactions will be discussed from electrocyclization with conserved stereochemistry, intermolecular electron transfer for organic systems, metal to metal electron transfer, to the direct observation of a bimolecular collision and bond formation in condensed phase for the classic I_3^- system, in a process analogous to a molecular Newton's cradle. These studies have discovered that these high dimensional problems, order 3N (N number of atoms in the reaction volume) representing the number of degrees of freedom in the system, distilled down to atomic projections along a few principle reaction coordinates. The specific details depend on the spatial resolution to these motions, for which <.01 Å changes in atomic position (less than the background thermal motion) has now been achieved on the 100 fs timescale. Without any detailed analysis, the key large-amplitude modes can be identified by eye from the molecular movies. This reduction in dimensionality appears to be general, arising from the very strong anharmonicity of the many body potential in the barrier crossing region. The "magic of chemistry" is this enormous reduction in dimensionality in the barrier crossing region that ultimately makes chemical concepts transferrable. How far can this reductionist view be extended with respect to complexity? The spatial-temporal correlations discovered in this work provide new insight into how chemistry scaled up to biological systems. Similar spatial relationships must exist inside the cell. New concepts in determining molecular distributions inside the cell, to directly observe the free energy gradients leading to living systems, will be discussed.

IT 96

Hydrogen bonding and non-covalent electric field effects in the photoconversion of phytochrome

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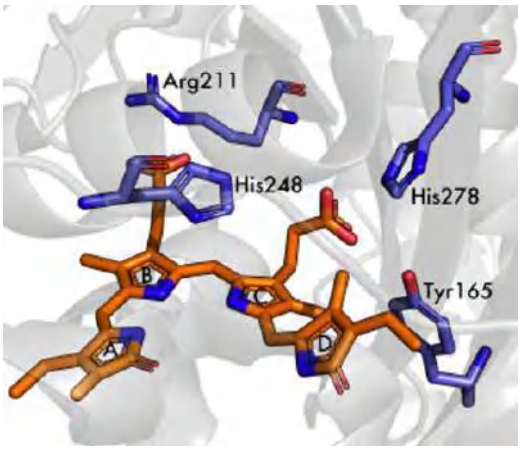
Phytochromes are biological photoreceptor found in all kingdoms of life. Light sensing is based take place at an open-chain tetrapyrrole that is covalently attached to the protein matrix. Upon light irradiation, phytochromes can be transformed between an inactive and an active state. This conversion is initiated by photoisomerization of the tetrapyrrole cofactor, followed by conformational changes of the chromophore and protein matrix that finally leads to the formation of the signaling state [1,2]. A profound understanding of protein structure and mechanism requires dedicated experimental and theoretical tools to elucidate electrostatic and hydrogen bonding interactions in proteins. In this presentation I will introduce you to an approach that exploits the spectroscopic properties of nitrile probes commonly used as reporter groups of the vibrational Stark effect [3,4] to disentangle non-covalent and hydrogen-bonding electric field changes during the reaction cascade of a multi-domain protein, i.e. the phytochrome Agp2.



References:

1. Rockwell, N. C et al. *Annu. Rev. Plant Biol.* **2006**, *57*.
2. López, M. F. et al *Phys. Chem. Chem. Phys.* **2022**, *24* (19), 11967.
3. Kraskov, A et al *Biochemistry* **2021**, *60* (40), 2967.
4. Fried, S. D et al. *Acc. Chem. Res.* **2015**, *48* (4), 998.

IT 97

Designing an Artificial Light Harvesting System and Monitoring Conformational Dynamics of i-motif DNA Using Förster Resonance Energy Transfer

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Dipole-dipole interaction among chromophores facilitates the transfer of excited state energy to the other chromophore: a phenomenon popularly known as Förster Resonance Energy Transfer (FRET).[1] This purely non-radiative energy transfer is governed by the distance (2-10 nm) between the FRET pair, and hence, it is referred to as "spectroscopic nanoruler".[1] Being a sensitive fluorescence technique, it has occupied the central stage in the field of biophysical studies, nanoscience, material science and several interdisciplinary research. FRET is also extensively employed to a wide variety of systems ranging from designing artificial bioinspired light harvesting system (LHS) to investigate the conformational dynamics of intercalative-motif (i-motif) DNA. Nature has beautifully assembled its light harvesting pigments within the protein scaffolds that ensures a very high energy transfer.[2] Motivated by nature's own architecture for light harvesting complexes, a highly efficient artificial LHS is generated using a self-assembled nanostructure of a homopolypetide (poly-D-lysine, PDL) making use of their β-sheet structure in aqueous solution.[3] Such homogeneous β-sheet structure acts as an assembly hub to align two chromophores in close proximity. The chromophores used are compatible for a highly efficient energy transfer process, are non-fluorescent in aqueous medium but exhibit high fluorescence intensity when bound to the nanostructure of PDL and generates white light emission. On the other hand, the effect of confinement on folding pathway of noncanonical (i-motif) DNA under physiological condition is extensively studied by single-molecule FRET (smFRET).[4] Such kind of noncanonical DNA has been considered as a novel drug target in the field of anticancer research.[5] Hence, it is pertinent to have a better understanding as to how various drugs interact with the highly dynamic structures of noncanonical DNA. In view of this, a detailed illustration of the structural dynamics of *c-MYC* promoter-based i-motif conformation has been investigated at physiological condition within microemulsions having various nanodimensions. It is concluded that the folding of such motifs under cellular-like confined environment is not a direct transition between unstructured random coil to a structured i-motif, rather it occurs through a partially folded intermediate depending, on the confined dimension.[4]

References:

1. J. R Lakowicz *Springer*, **2006**.
2. Q Song, et al. *J. Am. Chem. Soc*. **2021**, *143*, 382.
3. A Nandy et al. *J. Phys. Chem. Lett*. **2022**, *13*, 6701.
4. L Khamari et al. *J. Phys. Chem. Lett*. **2022**, *13*, 8169.
5. M Zeraati et al. *Nat. Chem.* **2018**, *10*, 631.

IT 98

(Cryo-)vEM and (cryo-)CLEM at the CLF Octopus facility

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Cryogenic ultrastructural imaging techniques such as cryo-electron tomography have produced a revolution in how the structure of biological systems is investigated by enabling the determination of structures of protein complexes immersed in a complex biological matrix within vitrified cell and model organisms. However, this is mostly true of very abundant proteins which reliably form a single type of complex and does not account for the wider cellular context. To understand biological processes related to health, disease or environmental processes, cryo-ET must be complemented by a suite of complementary techniques and correlative workflows that help paint a more complete picture and to pinpoint rare events for more detailed studies that capture biological heterogeneity.

The CLF Octopus facility has developed two super-resolution cryogenic fluorescence techniques, superSIL-STORM [1] and astigmatism-based 3D STORM, and we are working towards high-resolution correlation with FIB-SEM nanomachining to produce detailed maps of cellular and protein ultrastructure. In parallel, we have also developed a portfolio of techniques that enable us to collect serial EM data on larger volumes of samples to understand the spatial relationships between the various components of biological or biocomposite samples. This presentation showcases their application to a selection of scientific questions and discusses their advantages and limitations and their future applicability to other biological questions and technological advancements.

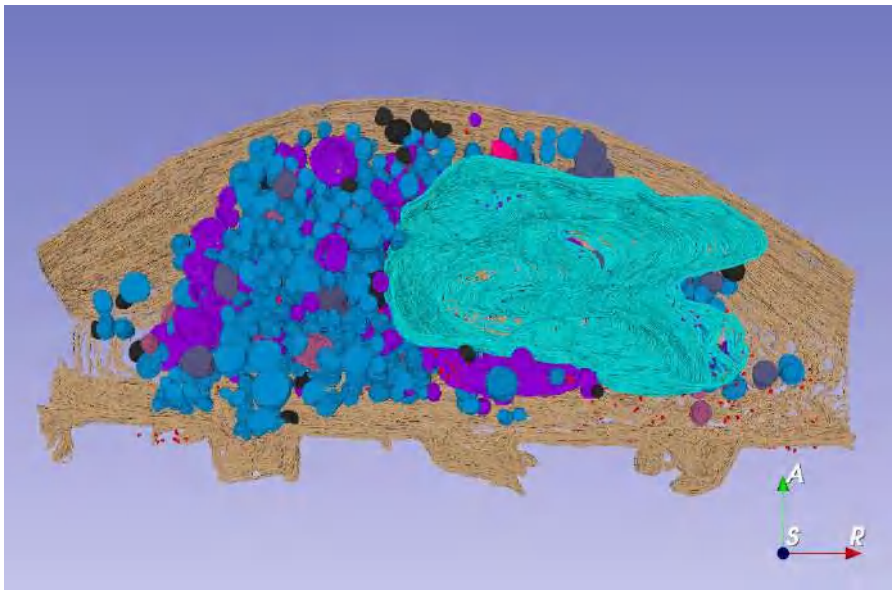

Figure 1. Slicer3D rendered segmentation of a SARS-CoV-2 infected Vero cell, imaged using cryo-FIB-SEM microscopy in slice-and-view mode at 5 nm isotropic voxel size. From Mendonca et al [2]

References:

1. Wang L, et al. *Communications Biology*, **2019** 2(1):74.
2. Mendonça L, et al. *Nat Commun*. **2021**;*12*: 4629.

IT 99

Mapping energy transfer in photosynthetic bacteria in vivo

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The remarkable quantum efficiency of solar energy collection in photosynthetic bacteria is assured by optimized arrangement of chromophores in light-harvesting complexes and by robust connectivity between complexes comprising photosynthetic units. While significant progress has been made through the years in understanding the energy transfer dynamics within isolated complexes, information about their functional connectivity in intact cells is still sparse. A serious challenge for optical studies of energy transfer in intact photosynthetic systems is posed by the great complexity of photosynthetic units and strong light scattering, characteristic of suspensions of photosynthetic cells. To map and characterize light-harvesting processes in intact photosynthetic bacterial cells we use scatter-resistant two-dimensional electronic spectroscopy [1,2]. Here we present physiological temperature measurements of intact cells of the thermophilic green non-sulfur bacterium Chloroflexus aurantiacus and purple bacterium Rhodobacter sphaeroides. Notably, the two studied photosynthetic bacteria feature different architecture of photosynthetic units, but very similar type II reaction centers. We were able to fully map energy transfer processes between the light-harvesting complexes and down to the reaction centers, with ensuing charge separation. As a distinct signature of the last step we observe a clear transient electrochromic shift signal of one of the reaction center absorption bands. Then we apply global fitting procedure to characterize dynamics of all energy transfer channels in the intact photosynthetic machinery of both bacteria.

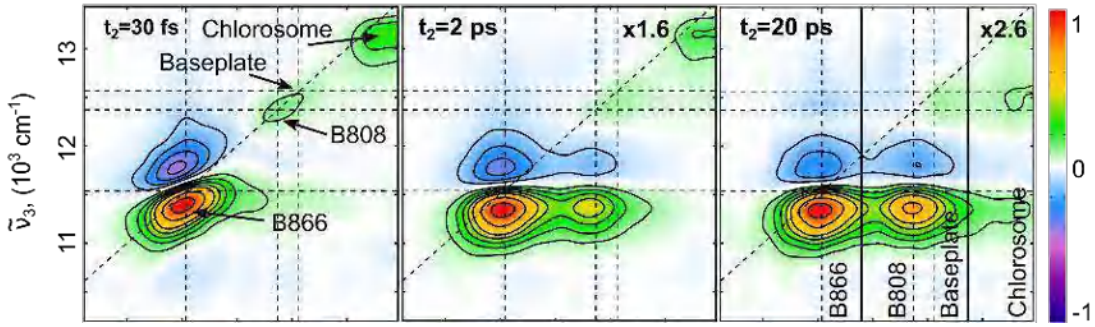

Figure. 2DES spectra of *Cfx. aurantiacus* cells at room temperature.

References:

1. R. Augulis et al. *Opt. Express*, **2011**, *19*, 13126.
2. J. Dostál, et al., *Nature Chem*., **2016**, *8*, 705.

IT 100

Artificially Intelligent Nanopores for High-Throughput DNA Sequencing

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The potential for enhanced rapid genome sequencing methods could revolutionize preventive healthcare. Recently, solid-state nanopores have garnered significant attention for their ability to facilitate rapid DNA sequencing. We have employed various artificial intelligence and machine learning tools to investigate the sequencing of individual nucleotides. In this discussion, we will provide a critical overview of the role of machine learning in advancing third-generation DNA sequencing through solid nanopore-embedded electrodes (see Figure).

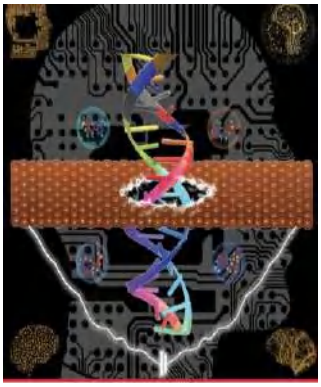

Figure: Solid State Nanopore for Rapid DNA Sequencing

References:

1. M. K. Jena et.al. *Nano Lett.*, **2023**, *23*, 2511.
2. S. Mittal et.al. *ACS Materials Lett.*, **2023**, *5*, 1570.
3. M. K. Jena et.al. ACS Materials Lett., **2023**, *5*, 2488.
4. S. Mittal et.al. Digital Discovery, **2023**, *2*, 1589.
5. M. K. Jena et.al., *J. Phys. Chem. Lett.*, **2022**, *13*, 11818.

IT 101

Biomolecular Condensation of Trypsin Prevents Autolysis and Promotes Ca^{2+}-Mediated Activation of Esterase Activity

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Metal ions play key roles in many cellular processes including metabolic regulation, cell signalling, energy conversion, osmotic regulation, biocatalysis, and cell membrane potential.[1] Therefore, living cells precisely regulate the homeostasis of various metal ions for their optimal cellular functions. Understanding these fundamental regulatory steps for cellular function is important to target various disease associated steps. More fundamentally, metal ions regulate the structural and functional integrity of proteins and enzymes.[2] While the role of metal ions in many metalloenzymes are well documented in the literature, the regulatory role of Ca^{2+} ions on the structural and catalytic activity of serine proteases is poorly understood.

The presence of Ca^{2+} ions is known to facilitate the activity of trypsin-like serine proteases via structural stabilization against thermal denaturation and autolysis. Herein, we report a new and hidden regulatory role of Ca^{2+} in the catalytic pathways of trypsin and α-chymotrypsin under physiological conditions.[3] We discovered that macromolecular crowding promotes spontaneous homotypic condensation of trypsin via liquid–liquid phase separation to yield membraneless condensates over a broad range of concentrations, pH, and temperature, which are stabilized by multivalent hydrophobic interactions. Interestingly, we found that Ca^{2+} binding in the calcium binding loop reversibly regulates the condensation of trypsin and α-chymotrypsin. Spontaneous condensation effectively prevents autolysis of trypsin and preserves its native-like esterase activity for a prolonged period of time. It has also been found that phase-separated trypsin responds to Ca^{2+}-dependent activation of its esterase activity even after 14 days of storage while free trypsin failed to do so. The present study highlights an important physiological aspect by which cells can spatiotemporally regulate the biocatalytic efficacy of trypsin-like serine proteases via Ca^{2+}-signaling.

References:

1. N. Nelson, *EMBO J* **1999**, *18*, 4361.
2. D. Schilter, *Nat. Rev. Chem.* **2017**, *1*, 0081.
3. Patel et al. *Biomacromolecules* **2024**, *25*, 6082.

IT 102

Drug-resistant EGFR mutations promote lung cancer by stabilizing interfaces in ligand-free kinase-active EGFR oligomers

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Activating mutations in the catalytic kinase domain of the Epidermal Growth Factor Receptor (EGFR) are crucial drivers of non-small cell lung cancer (NSCLC). Our understanding of the structural changes induced by such mutations has evolved alongside the rational design of targeted tyrosine kinase inhibitors (TKIs), leading to improved antitumor responses through appropriate patient stratification. However, many challenges remain, including therapy adaptation and acquired resistance, which are driven by the complexity of EGFR's signalling networks and exacerbated by a poor understanding of how and why cancer mutations amplify EGFR auto-phosphorylation signals to enhance cell survival and proliferation.

Using a super-resolution method we have developed, Fluorescence Localization Imaging with Photobleaching (FLImP) (Figure 1), we have found that drug-resistant EGFR mutations manipulate the assembly of kinase-active oligomers to promote and stabilize canonical active dimer sub-units within them, thereby circumventing the need for ligand binding. Combining FLImP [1] with large-scale simulations of various membrane embedded dimer interfaces, we have revealed the structure and assembly mechanisms of these oncogenic oligomers, uncovering oncogenic functions for hitherto orphan transmembrane and kinase interfaces, and for the ectodomain tethered conformation of EGFR.

Importantly, we find that the active dimer sub-units within ligand-free oligomers are the high affinity binding sites competent to bind physiological ligand concentrations and thus drive tumor growth, revealing a link with tumor proliferation. Our findings provide a framework for future drug discovery directed at tackling oncogenic EGFR mutations by disabling oligomer-assembling interactions [2].

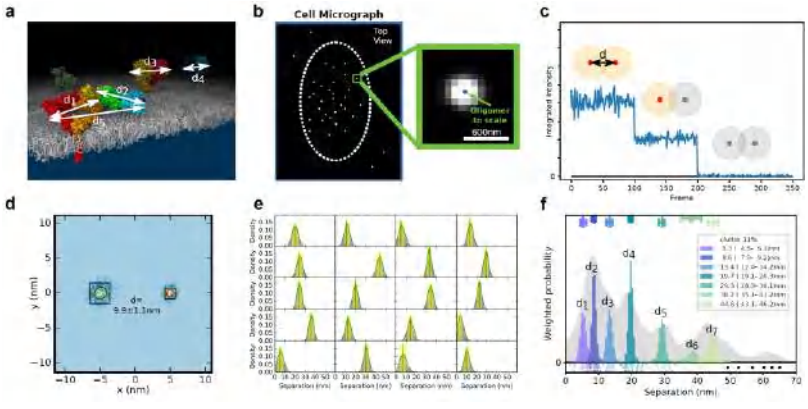

Figure 2: Key stages in the FLImP data acquisition and analysis process.

References:

1. Needham et al. *Nat Comms* **2016**, *1*, 13307.
2. Iyer et al. *Nat Comms* **2024**, 15, 2130

IT 103

Generalized Einstein Relations between Absorption and Emission: a Theory of Fluorescence, Excited State Thermodynamics, and Extreme Stokes' Shifts

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We have derived single-molecule relationships between absorption and thermal equilibrium emission spectra by using detailed balance with Planck blackbody radiation and the quantum electrodynamic connection between stimulated and spontaneous emission.[1] These thermodynamic relationships between spectra resolve the conflict between infinitely narrow lines, a finite spontaneous emission rate, and the time-energy uncertainty principle and contain Einstein's relationships for line spectra as a limiting case. They predict the Stokes' shift between broadened absorption and emission spectra and allow direct measurement of the standard free energy change upon electronic excitation. These single-molecule relationships do not apply directly to inhomogeneously broadened 1D spectra, but their validity can be probed with 2D spectroscopy.[2] For molecules that satisfy three criteria, they supply a theory of ensemble fluorescence that connects it to absorption without adjustable parameters, thus allowing us to build on the NIST and BAM calibration procedures for fluorescence spectrometers.[3] For other molecules, the ensemble Stokes' shift can provide information on molecular heterogeneity and the single-molecule lineshape. We replace Förster's approximate cycle for excited state proton transfer by a true thermodynamic cycle with spectroscopic accuracy. Finally, the relations predict Stokes' shifts so extreme that the forward and reverse transitions are both absorptive; molecular examples of this will be discussed.

Acknowledgment: This material is based upon work supported by the National Science Foundation under award number CHE-2155010.

References:

1. Jisu Ryu et.al., *Proceedings of the National Academy of Sciences of the USA* **2024**, *121*, e2410280121.
2. Jisu Ryu et.al., *Science Advances* **2021**, *7*, eabf4741.
3. Ute Resch-Genger et.al. *Pure and Applied Chemistry* **2012**, *84*, 1815.

IT 104

The Optical Properties of Charged Amino Acids: New Avenues for Label-Free UV-Visible Spectroscopy of Biomolecules

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UV-visible electronic absorption spectra is a useful characterization tool in protein biophysics and biochemistry. However, its application relies on synthetic dyes which absorb in the near UV and visible or a few intrinsic chromophores which may not present in all proteins. I will highlight our discovery of a new optical charge transfer (CT) band spanning 180-800 nm in proteins which significantly expands the set of UV-visible active intrinsic chromophores [1,2]. Our group has shown that this Protein Charge Transfer Spectra (ProCharTS) arises due to the intrinsic electronic properties of charged amino acids and is highly sensitive to their interactions imposed by protein folds [3]. Our conclusions are supported by experimental data from collaborators and other groups. ProCharTS can explain many instances of intrinsic absorption and emission phenomena reported in monomeric protein solutions, biomolecular condensates, and peptide aggregates. I will highlight challenges and future directions for ProCharTS including some exciting possibilities on modelling the spectra of entire proteins.[4]

References:

1. Prasad et al. *Chemical Science* **2017**, *8*, 5416.
2. Mandal et al. *Journal of Physical Chemistry B* **2019**, *123*, 10967.
3. Mandal et al. *Faraday Discussion* **2018**, *207*, 115.
4. Devi et al. *Manuscript-in-preparation*.

IT 105

Using FCS to uncover a two-component molecular motor driven by a GTPase cycle

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ATPases are a group of enzymes that can cyclically convert the free energy of ATP hydrolysis into mechanical work. GTPases are another class of enzymes that are predominantly associated with signal transduction processes but their role in mechano-transduction is less established. It was previously shown that binding of the GTPase Rab5 to the tethering protein EEA1 induces a large conformational change in EEA1 from a rigid and extended to a flexible and collapsed state. This entropic collapse of EEA1 gives rise to an effective force that can pull tethered membranes closer. It currently remains unclear if EEA1 can return from the collapsed to the extended conformation without the aid of chaperone proteins. Here, we show that EEA1 in bulk solution can undergo multiple flexibility transition cycles driven by the energetics of Rab5 binding and unbinding and GTP hydrolysis. Each cycle can perform up to 20 $\rm k_BT$ of mechanical work. Hence, Rab5 and EEA1 constitute a two-component molecular motor driven by the chemical energy derived from the Rab5 GTPase cycle. We conclude that tethering proteins and their small GTPase partners can have active mechanical roles in membrane trafficking.

IT 106

Role of pN level molecular tension in immune cell pathogen interactions

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Immune cells recognize and destroy any pathogenic foreign material in our body by constant surveillance. Macrophages are a type of innate immune cell that interact with pathogenic materials and extracellular matrix (ECM) with tiny submicron size F-actin-rich protrusion structures called podosomes [1]. Macrophages use podosomes as tiny sensors during pathogenic recognition. The molecular level understanding of how podosomes mechanically interact with the external substance was unclear because of their fast dynamics, very small force, and tiny size. Using fluorogenic DNA probe, capable of sensing and reporting diminutive force as low as a few piconewton (pN), we have studied the integrin molecular force in podosome and its effect on the structural maturation of podosome. We found that the presence of membrane-bound DNase X, that can destroy dsDNA, the canonical DNA-based tension probe can not be used in this case [2, 3]. We replace dsDNA with DNA/PNA hybrid that not only robust under DNase environment but also possess all the features of dsDNA sensor [4]. We revealed that the formation or structural maturation of podosome unlike focal adhesion, is independent of external integrin tension.

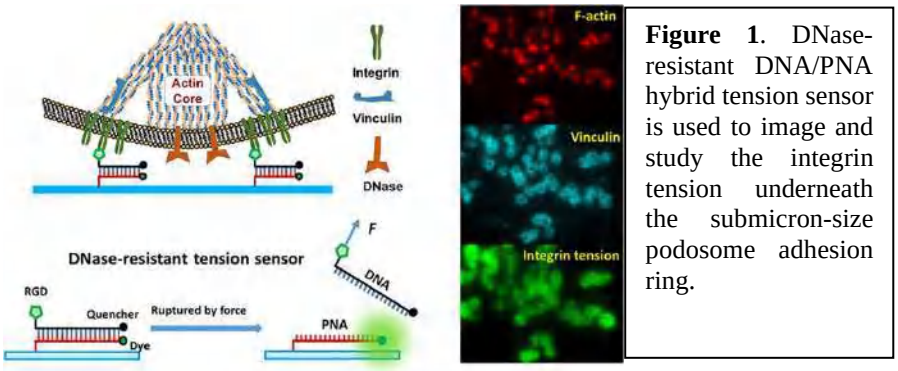

Figure 1. DNase-resistant DNA/PNA hybrid tension sensor is used to image and study the integrin tension underneath the submicron-size podosome adhesion ring.

Later we employed the DNase resistant tension sensor and phagocytosis model to answer the following fundamental question: how do macrophages perform phagocytosis when the pathogen is strongly adhered to the surface or so-called frustrated phagocytosis? It is found that the cumulative integrin tension is being used by the macrophages in phagocytic cup to overcome the physical barrier and engulf the pathogenic materials [5]. pN level integrin tension generation is found to be essential for the successful phagocytosis of surface bound pathogenic materials, and amount of force is dependent upon the strength of adherence. Overall, the discovery of a new role of integrin force generation at phagocytic cup opens a new area of mechanobiology where one can consider integrin as a molecular player beyond cell adhesion and migration.

References:

1. R. Buccione *et al. Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 647.
2. K. Pal et al. *J. Cell Biol.* **2021**, *220*, e202008079.
3. Y. Zhao *et al. J. Am. Chem. Soc.*, **2020,** *142*, 6930.
4. K. Pal *et al. ACS Nano* **2022**, *16*, 2481.
5. K. Pal *et al. bioRxiv* **2023**, https://doi.org/10.1101/2023.08.01.551462.

IT 107

Ultrafast Charge Transfer Chemistry in Metalloproteins and Biomimetic Nanocages

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Charge transfer (CT) states form the basis for multitude of chemical reactions, and has become relevant due to its ubiquity in all light energy conversion paradigms.[1] In order to discover new materials with optimized charge transfer rates at molecular interfaces for energy conversion technologies, it is imperative to diagnose the structure-function correlations "in operando". Tracking the non-equilibrium nuclear dynamics leading up to the charge transfer states and probing the subsequent separation of charges requires time-resolved spectroscopy with structural sensitivity [2, 3]. In this talk, I will discuss the utility of transient Raman spectroscopy as a tool to structurally probe the hidden lengthscale of the photochemistry inside the active site of metalloproteins [1] along with our attempts to carry out biomimetic C-H activation reaction in water [2]. Both frequency domain and time-domain methods will be elaborated with emphasis on challenges of real-time Raman detection during chemical reactions.

References

1. Sajjad Dadashi-Silab et al. *Chem. Rev.* **2016**, 116, 17, 10212–10275;
2. Alexey V. Akimov et al. *Chem. Rev.* 2013,113, 6, 4496–4565.
3. Palas Roy et al. *Nature Comm*. **2017**, 8, 1716.
4. Shreetama Karmakar et al. *J. Phys.Chem. Lett*. **2020**, 11, 12, 4842–4848.
5. Soumyajit Mitra et al. *J. Phys. Chem. B* **2022**, 126, 29, 5390–5399
6. Sundnita Paul et al. *to be submitted.*

IT 108

Energy transfer in molecular wires: new insights

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We investigate energy transfer in a molecular wire mimicking FMO complex in this work. Using a combination of numerically exact simulations (HEOM) and mixed quantum classical approach (surface hopping), we present surprising results of small diabatic coupling significantly affecting rates of energy transfer[1]. We provide evidence that electronic coherence plays a critical role in determining the rates of energy transfer. Further, we show that the coherences are unaffected in presence of strong vibrational unharmonicity[2], further demonstrating that the coherence are not of vibrational origin.

References:

1. A. Sindhu et al. *Chem. Phys. Chem.* ***2022***, *23,* e202200392.
2. A. Sindhu et al. J. *Chem. Phys.* ***2024***, *160, 204117*.

IT 109

UV-Visible Spectra in Proteins arising from Charge Transfer: A useful Intrinsic Probe to investigate Changes in Protein Structure

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Luminescence arising from non-aromatic groups in amino acids, monomeric proteins, protein aggregates, amyloid fibrils and like have attracted a lot of attention from several research groups recently. In a similar context, our group has experimentally investigated the origin of UV-Vis absorption and associated luminescence arising from monomeric proteins rich in charged amino acids like Lys, Glu, Arg, His and Asp. Using time-dependent density functional theory calculations, our collaborator, Venkatramani's group, has shown that photoinduced electron transfer from donors like anionic Asp/Glu; polypeptide backbone (PPB) to acceptors like cationic Lys/Arg/His; PPB can give rise to Protein Charge Transfer Spectra or ProCharTS [1]. Charge recombination among such charge separated excited states, can account for luminescence observed in proteins.

Our group has utilized ProCharTS absorption and luminescence as a *label-free* probe to investigate events like disruption of charged residue contacts during early stages of protein unfolding; tracking initial stages of Ab-peptide fragment aggregation where new contacts established among charged residues in oligomers enhance ProCharTS intensity; monitoring assembly of Hepatitis B viral capsids with icosahedral symmetry; and binding of nucleic acid to protein.

In this talk, I shall present our recent experimental results on application of ProCharTS to detect post-translational modifications which alter the charge on the Lys sidechain of protein.

References:

1. Prasad, S., et al. *Chemical Science* **2017**, *8*, 5416.

IT 110

Molecular probes for protein oligomers and fibrils

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Protein aggregation is primarily responsible for several neurological diseases. Generally, aggregation of protein, which finally leads to the formation of amyloid fibrils, follows sigmoidal growth kinetics. Several types of intermediates are formed during the growth of the amyloid fibrils. Several reports confirm that the soluble protein oligomers, formed at the initial stage of amyloid fibril formation, are principally responsible for the pathogenesis of neurological diseases, and their levels are more important in the progression of such diseases. Hence, finding an effective method for the detection of such aggregated proteins is not only important for the treatment of diseases associated with amyloids but also technologically relevant for the storage of high concentrations of protein. Considering high sensitivity and simplicity, fluorescence based methods for the detection of proteins at different stages of their aggregation are very promising. In the last several years we have identified several molecular fluorescent probes with significantly higher sensitivity and selectivity than the gold standard amyloid probe, Thioflavin-T. Several probes have been investigated for detecting protein oligomers and a methodology has been proposed to distinguish mature fibril from soluble oligomers. In the present talk, we will discuss our recent work on screening some molecular probes for efficient detection of protein aggregates at different stages of their aggregation. Detailed photophysics of these probes will be discussed to understand the underlying mechanism for their sensing of protein aggregates.

IT 111

Unlocking gene expression mechanisms via next-generation single-molecule imaging

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University of Oxford

Single-molecule studies offer unprecedented direct access to biologically important heterogeneity and dynamics; this holds true for reactions involving purified biochemical components, as well as for reactions inside the complex biological milieu of living cells. During the past few years, we have developed and used such methods (single-molecule FRET, super-resolution imaging, and single-particle tracking) to answer long-standing questions in gene expression and repair.

Here I will discuss a new approach to perform highly multiplexed single-molecule fluorescence measurements in order to analyse the sequence-dependence of rate-limiting steps during the initiation of transcription; this approach, which is generally applicable to protein-DNA interactions, also involves a novel way to sequence single DNA molecules based on transient hybridization. I will also briefly discuss examples of our latest in vivo single-molecule work inside living bacteria, including our new ability to track single RNA polymerase molecules for up to 20 min in a single cell. Our methods and biological observations are general and should apply to many systems and organisms.

IT 112

Thermally Activated Delayed Fluorescent Probes for Elucidating Interorganelle Interactions and Time-resolved Imaging of Lysosomal Polarity

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All-organic thermally activated delayed fluorescence (TADF) emitters with long-lived emission properties are emerging materials for biomedical applications.[1,2] However, the hydrophobicity, low solubility, aggregation caused quenching in the aqueous medium, and non-radiative deactivation of the triplet state at ambient conditions hinder their potential for time-resolved bioimaging.[2,3] I shall present the development of a donor-acceptor (D-A) type polarity-sensitive and lysosome-specific TADF molecule, DC-Lyso (Figure 1).[4] The probe showed solvatochromism, triplet harvesting phenomenon, delayed fluorescence emission at the microsecond time domain, rapid internalization in live cells, and highly selective localization into lysosomes. The linear dependence of fluorescence lifetime as a function of solvent polarity was employed to decipher micropolarity changes of lysosomes during autophagy and aging through fluorescence lifetime imaging microscopy. Further, water-dispersible nanoaggregates of DC-Lyso with enhanced delayed fluorescence emission were employed for the time-resolved imaging of live cells in the microsecond time domain.[4] The present study paves the path for TADF probes for specific intracellular sensing and imaging.

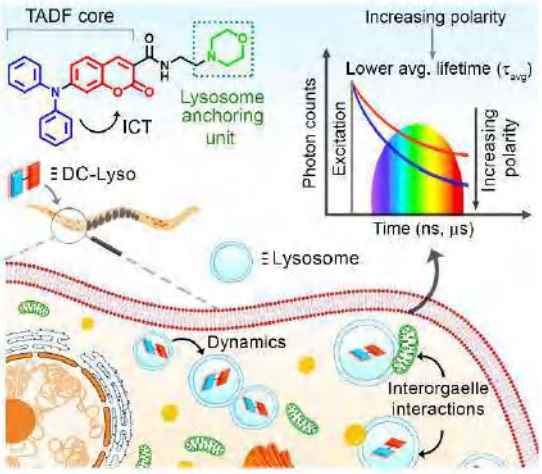

Figure 1 Schematic illustration depicting the design-strategy of coumarin-diphenylamine-based thermally activated delayed fluorescence (TADF) probe, DC-Lyso. The localization of DC-Lyso within the lysosomes and unveiling the micropolarity changes during lysosome-organelle interactions employing time-resolved imaging has been investigated.

References:

1. F. Fang et al. *Adv. Sci.* **2021**, *8*, 2102970.
2. S. Jaiswal et al. *J. Mater. Chem. C* **2022**, *10*, 6141.
3. S. Kundu et al. *Chem. Commun.* **2023**, *59*, 8017.
4. S. Das et al. *Chem. Sci.* **2024**, *15*, 102.

IT 113

Exploring the interface between DNA structures and fluorescent nanomaterials

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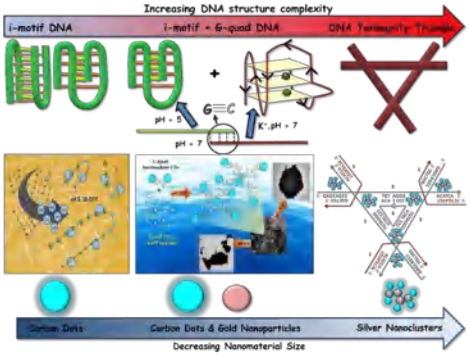
Apart from the well-studied B-DNA structure, single stranded DNAs with repeats of homopurine or homopyrimidine stretches, can fold into various other non-canonical secondary structures like G-quadruplex and i-motif. I-motif or intercalated motif DNA are cytosine-rich four stranded quadruplex DNA structures that may develop in the regulatory regions of human genome and are widely found in telomeres and oncogene facilitators. Therefore, it is of immense biological interest to either stabilise these structures or dissolve it depending on its location and function. We found that folic acid derived carbon dots have a destabilising effect on i-motif structure formation [1]. G-rich G-quadruplexes and C-rich i-motifs are complementary to each other. Along with forming classical Watson-Crick base pairing, these sequences, under different stimuli can fold to their more complex secondary structures mediated by Hoogsten base pairing. We designed a fluorescent stimuli responsive supramolecular network using the C-G base pair complementarity and tagged the G-rich G4 forming DNA with blue fluorescent carbon dots and C-rich i-motif forming DNA with gold nanoparticles. Upon mixing these two DNA coated nanoparticles, we found a μM sized supramolecular assembly. The carbon dots and gold nanoparticles formed a donor-quencher pair which helped us to investigate the assembly formation with FLIM and FRET. Since the C-rich forming sequences are basically proton aptamers, the DNA assembly tears at pH 5 and undergoes compaction at pH 8. These networks also generate reactive oxygen species (ROS) [2]. In 2004 Dickson et al. reported silver nanocluster formation in DNA template [3]. It is difficult to use ds-DNA as a ligand for cluster synthesis as all the favoured binding sites of Ag^+, except the N7 location of the purines, are already involved in hydrogen bonding. This problem can be resolved by designing mismatches in the sequence, using hairpin loop or putting metal mediated DNA base pairing sites, typically C-C sequences in the strands that helps in capturing Ag^+ in an exact location. However, these techniques compromise the integrity, rigidity of the ds-DNA structure. Triplex H-DNA formation alleviates this problem. Mao et al. published a nanotriangle DNA tile with sticky-end cohesion using tensegrity strategy [4] that can form 3D designer crystals. In our work, we have synthesised Ag nanoclusters in the small tricuspid floret pockets of rhombohedral 2T7 tensegrity triangles and have seen the effect of triplex-forming oligonucleotide (TFO) at various pH.



References:

1. Mukherjee et al. *ACS Appl. Nano Mater.* **2023**, *6*, 6380.
2. Mukherjee et al. *J. Phys. Chem. C* **2024**, *128*, 15194.
3. Petty et al. *J. Am. Chem. Soc.* **2004**, *126*, 5207.
4. Liu et al. *J. Am. Chem. Soc.* **2004**, *126*, 2324.

IT 114

Fancying 10,12-Pentacosadiynoic Acids as Multipurpose Dual-output Chemosensors

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10,12-Pentacosadiynoic acid (PCDA) has emerged as a promising platform for designing multipurpose dual-output chemosensors. Known for its unique polymerization and chromatic properties, PCDA can respond to various analytes with both colorimetric and fluorometric outputs, providing a versatile tool for rapid and sensitive detection. This dual-output capability arises from the acid's conjugated polymer backbone, which undergoes structural and electronic changes upon interaction with target analytes, leading to distinct visual and fluorescent signals. The dual-sensing nature of PCDA-based chemosensors makes them ideal for applications in environmental monitoring, biomedical diagnostics, and chemical safety, where rapid and reliable detection of analytes is critical. This talk will covers the use of PCDA in chemosensing applications, highlighting its adaptability, sensitivity, and potential for integration into portable detection systems.

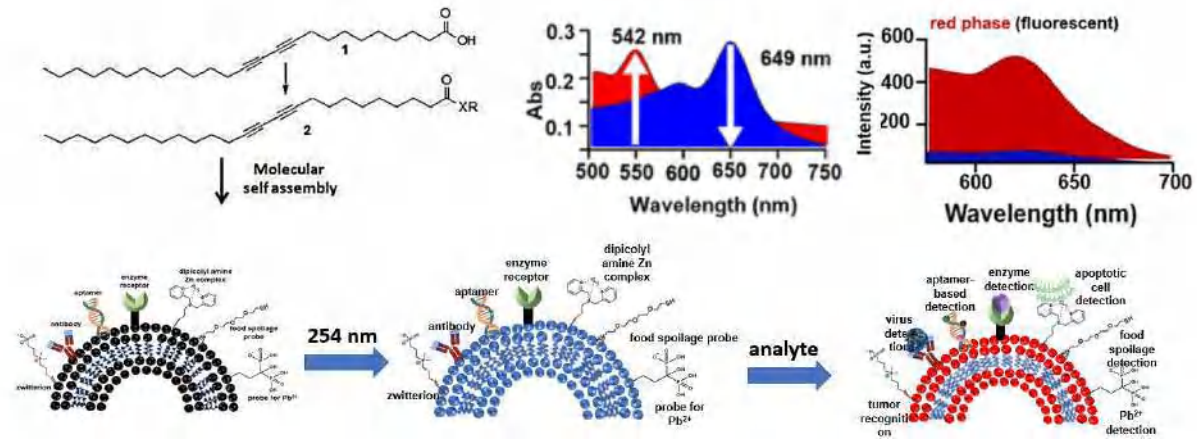

Figure 1. Schematic representation of sensing mechanism of polydiacetylene-based sensors.

References:

1. A. Thakuri et al. *Sensors and Actuators B: Chemical*, **2024**, *409*, 135573.
2. A. Thakuri et al. *ACS Appl. Polym. Mater.* **2024**, *6*, 1023.
3. A. Thakuri et al. *Analyst*, **2022**, *147*, 3604.
4. A. Thakuri et al. *Langmuir* **2024**, *40*, 17613.

IT 115

Bright-field imaging techniques inspired by super-resolution microscopy

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Super-resolution microscopy has gained widespread attention after impressive success of fluorescence-based imaging modalities such as SIM (structured illumination microscopy), STED (stimulated emission depletion), STORM (stochastic reconstruction microscopy) and their variants over the last two decades. While super-resolution techniques have gained prominence in the research literature, many applications in microscopy and consumer photography continue to operate in simple brightfield mode without the use of fluorescence. I will discuss two specific examples from our recent work along these lines:

(a) Extended field of view (FOV) imaging: This work is **inspired by the SIM modality** with a twist that we exchange the role of real and Fourier space. It is shown that Fourier space structuring via a designer phase mask leads to a camera that can "see" beyond the physical sensor boundary. While SIM aims to achieve extension of Fourier space within the same FOV, our system demonstration achieves an effectively extended detector (or FOV) at the same diffraction-limited resolution. The system can be considered to provide single-shot panorama and has multiple applications.

(b) High contrast computational imaging with vortex phase diversity: This work is **inspired by the STED microscopy** principle. We record two brightfield images of the same scene with an open aperture and a vortex phase aperture, without using any fluorescence depletion concepts. The two recorded images are shown to provide a diversity of information that can be combined algorithmically to obtain an effective point-spread function (PSF) with a super-oscillatory character. The central lobe of the PSF is 0.6 times smaller compared to the diffraction-limited PSF. The resultant computational imaging system has an enhanced high spatial frequency response, and the images therefore show a significantly enhanced contrast.

The two illustrations above suggest that the enhanced space-bandwidth product feature offered by super-resolution techniques can be carried over to routine bright-field microscopy as well. The resulting microscopy systems can be built at moderate additional hardware costs and can benefit wide ranging microscopy applications.

References:

1. R. Malik et al. *Journal of the Optical Society of America A* **2023**, *40*, 1066.
2. M. P. Singh, et al. *Journal of Physics B: Atomic, Molecular and Optical Physics* **2024**, 57, 225402.

IT 116

Developing peptide-based broad-spectrum fusion inhibitors as an antiviral strategy

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Enveloped viruses enter the host cells by endocytosis and subsequently fuse with the endosomal membranes, or fuse with the plasma membrane at the cell surface. The crucial stage of viral infection, regardless of the route taken to enter the host cell, is membrane fusion. Our work aims to develop peptide-based fusion inhibitors that prevent membrane fusion by modifying the properties of the participating membranes, without targeting a protein. This would allow us to develop a fusion inhibitor that might work against a larger spectrum of enveloped viruses as it does not target any specific viral fusion protein. With this goal in mind, we have designed multiple derivative peptides of coronin 1, a phagosomal protein, that helps to avoid lysosomal degradation of mycobacterium-loaded phagosomes. We have evaluated the fusion inhibitory ability of these peptides in model membrane fusion assays with varying lipid compositions [1,2]. As proof of our concept, we have further evaluated the efficacy of these peptides against viral infection [3]. The Tryptophan-aspartic acid-containing peptides displayed micromolar EC50 value against influenza and other viral infections.

References:

1. Pandia et al. *J. Phys. Chem. B,* **2024,** *128, 4986.*
2. Pandia et al. *J. Phys. Chem. B,* **2024,** *128, 9163.*
3. Panda et al. *RSC Med. Chem.,* **2024,** *Advance Article (https://doi.org/10.1039/D4MD00523F)*

IT 117

Suitably decorated 2,4,6-tristyrylpyrillium salts in detecting crucial biological amines through diverse optical responses

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Utilization of strong electrophilicity of *aryl*pyrylium salts was limited for detecting ammonia or methylamines and the only biogenic amine (BA) *i.e.*, putrescine. [1] With our recent interest in detecting BAs [2], we present structurally twisted triphenylamine-linked *2,4,6-tristyryl*pyrylium *salt* **PyTPA** as a single dye to recognize various aliphatic/aromatic BAs, nicotine, and guanidine rapidly in nanomolar concentrations (Fig 1). Such a new tri*styrylpyrylium* design suits specific electronic conjugations, steric/geometric constraints, and decent thermal/photostability, facilitating crucial diverse amines detection. The deep-violet solution/solid dye reacted quickly at 298 K with decoloration against vital biologically relevant amines that include BAs like *putrescine, cadaverine, spermidine, spermine and a few other neurotransmitters such as histamines, serotonin, and 2-phenylethylamine.* Further, this dye could detect nicotine at 313 K and guanidine at 298 K distinctively with diminished absorption and unexpected, red-shifted emission enhancement. Mechanistic pathway variations are determined in detecting amines holding mono/di-NH_2 groups and short/ long alkane chains, interpreted by mass, ^{1}H-NMR, FT-IR, SEM, PXRD, and XPS findings. The distinguished detection of key BAs in different phases is employed for on-the-spot applications to perceive the freshness of chicken and fish (Tuna). Nicotine in natural tobacco leaves was detected. Such newly constructed pyrylium salt supports quick signs of progress in this class of molecules in finding and classifying diverse biologically crucial amines.

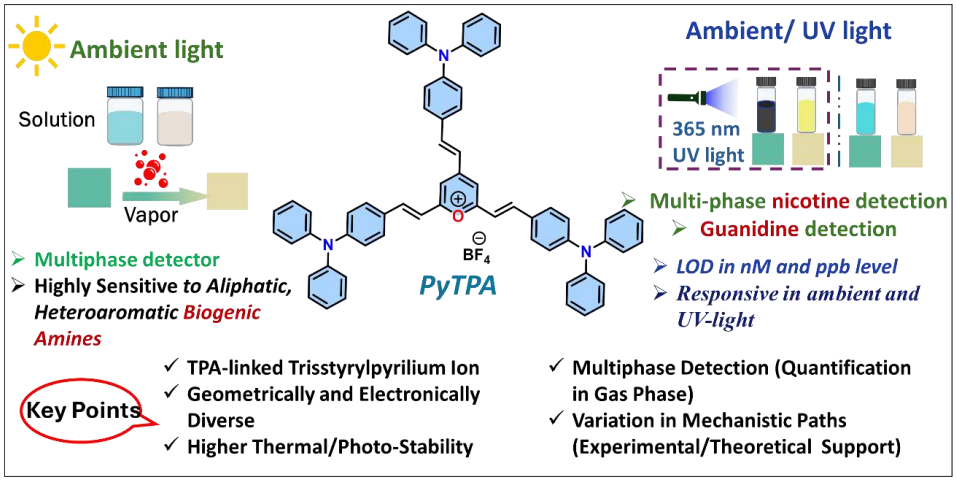

Fig 1: The dye **PyTPA** and its unique features in a nutshell

References:

1. Yin, W et al. *Analyst*, **2022,** *147*, 3451.
2. Basavaraj, D. et al. *ACS Appl. Bio. Mater*, **2020**, *3*, 772.
3. Prusti, B. et al. *ACS Appl. Mater. Interfaces.*, **2023**, *15*, 16492.
4. Chakraborty, M. et al. *J. Mater. Chem. B.*, **2024,** *12*, 2746.
5. Jain, A. et al. *J. Org. Chem.*, **2024,** *89*, 4384.

IT 118

Location-agnostic site-specific protein bioconjugation via BHoPAL

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Proteins labelled site-specifically with small molecules are valuable assets for chemical biology and drug development[1-4]. The unique reactivity profile of the 1,2-aminothiol moiety of *N*-terminal cysteines (*N*-Cys) of proteins renders it highly attractive for regioselective protein labelling. In this talk, I will discuss our work that resulted in the development of a rapid and high-yielding protein bioconjugation technology based on 1,2-aminothiol chemistry that we refer to as Baylis Hillman adduct-orchestrated protein aminothiol labelling (BHoPAL). We built this technological platform by leveraging our discovery that 1,2-aminothiols react extremely rapidly (rate constants > 10^3 $M^{-1}sec^{-1}$ under physiological conditions) with rationally designed isatin-derived Baylis Hillman (IBH) adducts to form novel C=C containing bis-heterocyclic scaffolds[5]. BHoPAL overcomes one of the most enduring open challenges in protein bioconjugation—achieving rapid and quantitative protein labelling with sub/low micromolar concentrations of both the protein and the labelling reagent. Indeed, we achieved the quantitative site-selective labelling of the 1,2 aminothiol moiety of a series of recombinant *N*-terminal cysteine (*N*-Cys)-containing proteins within minutes at room temperature in near-neutral aqueous solutions by treating low-micromolar concentrations of these proteins with merely 1–3 equivalents of our IBH adducts. These results constitute a dramatic improvement over existing bioconjugation technologies that employ large excess of labelling reagents and extended reaction times—conditions that lead to undesirable consequences such as cytotoxicity and protein precipitation. Importantly, the protein conjugates afforded by our chemistry are homogeneous, retain long-term stability, and can be generated under both *in vitro* and live-cell conditions. Additionally, I will also discuss a lipoic acid ligase-based chemoenzymatic approach that we have developed for installing the 1,2-aminothiol moiety at any desired site of a protein of interest. This technology when coupled with BHoPAL enables location-agnostic bioconjugation of proteins not limited to their *N*-Cys residues[5].

References:

1. E. A. Hoyt et al. *Nat Rev Chem* **2019**, *3*, 147.
2. J. A. Prescher et al. *Nat Chem Biol* **2005** *1*, 13.
3. T. Tamura et al. *J Am Chem Soc* **2019**, *141*, 2782.
4. N. Stephanopoulos et al. *Nat Chem Biol* **2011** *7*, 876.
5. H. Mir Mudassir et al. *Nat. Commun.*, **2024**, *15*, 859.

IT 119

Peptide-PAINT: A Transfected Docker Simplifies Live and Fixed Cell Super-resolution Imaging

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Point Accumulation and Imaging in Nanoscale Topography (PAINT) is a single-molecule technique for super-resolution microscopy that uses exchangeable single-stranded DNA oligos or peptide-pair, named as docker and imager strand/peptide, to create fluorescence blinking. This characteristic distinguishes PAINT from other widely employed techniques such as Stochastic Optical Reconstruction Microscopy (STORM) and Photo-Activated Localization Microscopy (PALM). PAINT exhibits various potential advantages when compared to STORM and PALM, such as improved localization precision, increased multiplexing capability, and simplified imaging procedure. Furthermore, Peptide-PAINT has several other advantages over DNA-PAINT. The introduction of the docker peptide into the protein of interest without using any intermediate labelling probe, as opposed to the normal procedure of externally introducing it in DNA-PAINT, enables the attainment of comparable spatial resolution while streamlining experimental protocols and facilitating its utilization in living cellular systems. Deciphering the ultrastructure details of various cellular organelles in fixed mammalian cells and of surface proteins in the live neurons using Peptide-PAINT will be discussed in my talk.

References:

1. Maity et al. Small methods, **2023**, 7, 1.

IT 120

Functional Transbilayer Coupling of Plasma membrane Leaflets in Live Cells Revealed by Imaging Fluorescence Correlation Spectroscopy

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Plasma membrane (PM) leaflets of eukaryotic cells are compositionally asymmetric. Since the leaflets are always vertically stacked to establish any viable cell membrane, it is hypothesized that the biophysical properties of the two leaflets are coupled. However, the complexity of PM arising from collectives of weak interactions of nearly thousands of lipid species in each leaflet poses significant limitations in testing transbilayer coupling in live cells. To address this, we developed a novel extracellular lipid delivery technology for controlled modification of lipid composition of a specific leaflet in live cells and monitor the corresponding changes of membrane order in the opposing leaflet by measuring lateral diffusion of lipid probes by Imaging Fluorescence Correlation Spectroscopy (ImFCS). We show that when the outer leaflet membrane order (more viscous) is increased by adding lipids with saturated acyl chain the inner leaflet diffusion slows down and vice versa. Our results thus prove that two leaflets, while maintaining an asymmetric lipid composition, are biophysically coupled. Furthermore, we showed that increasing the resting state order of the inner leaflet by transbilayer coupling increases the degree of stimulated kinase recruitment during antigen-mediated immunoreceptor signaling via PM-localized FcεRI receptors in mast cells. Overall, our results highlight a novel link of biophysical properties of plasma membrane in cellular signaling.

IT 121

High-Precision Myosin II Step Size Measurement with Single Quantum Dot Tracking in Motility Assays

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Abstract:

Myosin II motors are nano-sized protein machines that convert chemical energy from ATP into mechanical work, resulting in nanometer-scale displacements. This force generation is crucial for processes such as cargo transport, cell division, cell locomotion, and larger-scale movements like running. A key limitation of current In Vitro Motility Assays (IVMAs) for tracking actin filaments is their resolution limit of ±50 nm over 50 ms. To overcome this, we developed a single-molecule IVMA where actin filaments are labeled with quantum dot (Qdot) nanoparticles and visualized with super-resolution microscopy at 100 fps. Qdot nanoparticles were attached via biotin-streptavidin conjugation: biotin-conjugated actin filaments were tagged with streptavidin-coated quantum dots. The biotin labeling and Qdot concentrations were optimized to ensure smooth motility in the IVMA. Quantum dots were precisely localized using 2D Gaussian fitting, achieving tracking accuracy of 1.5 nm over 10 ms intervals. At low ATP concentrations, we observed discrete ~7 nm steps, increasing in multiples of 7 nm with higher ATP concentrations. To our knowledge, this is the first study to directly measure the step size of myosin II motors resolved in an ensemble system.

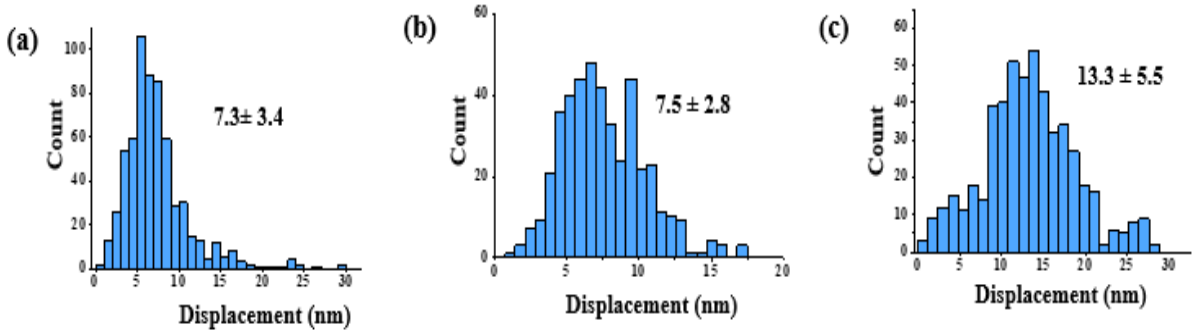

Figure : Histogram of displacement observed per frame at lower ATP concentration. (a) 0.001 mM ATP (b) 0.025 mM ATP (c) 0.05 mM ATP. A minimum of 40 moving quantum were used for histogram analysis.

References and Notes:

Unpublished work

IT 122

Design of Fluorescent Probes for Cellular Imaging and Theranostic Applications

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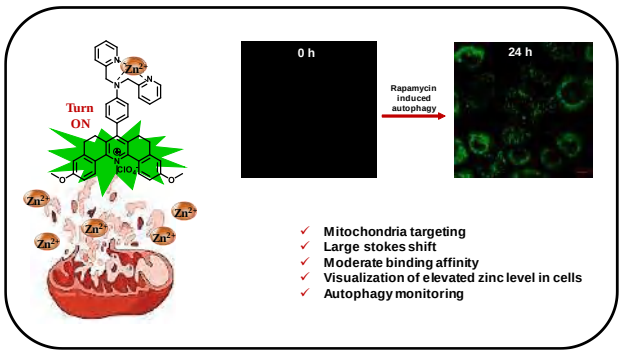
Molecular probes for sensing, imaging, and theranostic applications play a crucial role in clinical diagnostics and disease management. Given the low inherent fluorescence of biological entities, current fluorescence techniques heavily depend on fluorescent dyes. Small molecule-based probes are especially favored due to their compact size, ease of chemical modification, reproducibility, and compatibility with biological systems. Traditional chromophores such as coumarin, pyrene, 1,8-naphthalimide, xanthenes, boron dipyrromethene difluoride (BODIPY), and diketopyrrolopyrrole have been widely employed across various fields, including sensing, enzyme analysis, cellular imaging, and therapy. Each dye presents unique photophysical and physicochemical properties, along with its own set of advantages and limitations. Therefore, the development of new fluorescent probes with superior optical properties, biocompatibility, targeting abilities, and low toxicity is highly valuable for medicinal and biological applications. In our lab, we focus on designing novel fluorescent molecules that can monitor cellular microenvironments by selectively detecting specific analytes, such as Zn^{2+}, OCl^{-}, and SO_3^{2-}. For instance, we developed a pentacyclic pyridinium-based probe, PYD-PA, equipped with a pendant N,N-di(pyridin-2-ylmethyl)amine (DPA), for detecting Zn^{2+} in the cellular environment, which was further used to monitor autophagy. Additionally, we investigated dinuclear Ir(III) complexes as imaging probes and photodynamic therapy (PDT) agents for cancer treatment. PDT is a non-invasive therapy that utilizes light to activate a photosensitizer (PS) in the presence of molecular oxygen, producing cytotoxic reactive oxygen species (ROS) that destroy cancer cells. In this regard, we designed a 2-(2,4-difluorophenyl)pyridine-based dinuclear Ir(III) photosensitizer for imaging and PDT applications. Our research efforts in developing new, brightness-optimized fluorophores for cellular sensing and imaging will be discussed during the talk.



References:

1. Chan, J. et al.., *Nat. Chem.*, **2012**, *4(12)*, 973.
2. Liu, H. W. et al, *Chem. Soc. Rev.*, **2018**, *47(18)*, 7140.
3. Thomas, A. et al., *J Photochem Photobiol B.*, **2024**, *259*, 113006.
4. Neelambaran, N. et al, *ACS Appl. Bio Mater.*, **2023**, *6, 12*, 5776.

IT 123

MitRatiNa: A fluorescent reporter for measuring mitochondrial sodium

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Mitochondrial sodium (Na^+) levels regulate mitochondrial function and help cells to survive salt stress, hypoxia or cytosolic Ca^{2+} stress [1-3]. The discovery of the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) and its key role in several pathologies [4,5], necessitated quantification of mitochondrial Na^+ in situ. In my lecture, I will discuss the development and use of a ratiometric fluorescent reporter for mitochondrial Na^+, denoted *MitRatiNa*, the readout of which is independent of the mitochondrial membrane potential. We discovered that Na^+ levels at single mitochondria resolution depended on cell types and are highly heterogeneous, ranging from 1-40 mM on average. Our experiments showed that mitochondrial Na^+ is elevated on metabolic inhibition as well as when cells experience cytosolic Ca^{2+} elevation. We found a high Na^+ mitochondrial population in skin fibroblasts derived from healthy humans which was absent in patient-derived cells with mitochondrial diseases such as Kearns-Sayre syndrome and Pearson's syndrome. The ability to map absolute Na^+ levels in a single mitochondria enables deeper understanding of the Na^+ regulatory mechanisms in health and disease and potential identification of new therapeutic avenues.

References:

1. P. Hernansanz-Agustín et al. *Antiredox Signal* **2022**, *37*, 290.
2. Y. J. Chung et al. *Nat Commun* **2024**, 15, 4277.
3. P. Hernansanz-Agustín et al., *Nature*, **2020**, 586, 287.
4. R.Palty, *PNAS*, **2009**, 107, 436.
5. T. S. Luongo et al. *Nature* **2017**, 545, 93.

IT 124

Two-dimensional fluorescence lifetime correlation spectroscopy: Recent development and applications

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To understand the working mechanisms of biomolecular systems, it is crucially important to observe their dynamics in aqueous environment. Single-molecule FRET (smFRET) has been widely employed for elucidating conformational distributions and transitions of biomolecules in solution. Based on smFRET, we invented two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS) that enables us to study microsecond conformational dynamics by utilizing fluorescence correlation spectroscopy (FCS) and the information of fluorescence lifetime [1]. To date, 2D FLCS has been applied to a variety of proteins and nucleic acids and revealed the conformational transitions in the microsecond time scale involved in their folding as well as ligand binding processes [2,3]. In this talk, I will present recent development of 2D FLCS for improving applicability as well as a new application of 2D FLCS.

Firstly, I will talk about incorporation of the pulsed interleaved excitation (PIE) scheme to 2D FLCS. Because 2D FLCS uses only a single-color excitation for the FRET measurement, it has been difficult to distinguish a species with a vanishingly low FRET efficiency from only-donor-labeled molecules. To address this problem, we introduced the PIE scheme, in which the donor and acceptor dyes are excited alternately by using temporally interleaved pulses with different colors. Applications of this PIE 2D FLCS to simulated and experimental photon data showed that it readily distinguishes doubly-labeled and singly-labeled (donor-only and acceptor-only) species and quantitatively evaluates the contributions of the donor-acceptor crosstalk [4].

In the second part, I will present the combination of the anti-Brownian electrokinetic (ABEL) trap and 2D FLCS. Dynamics observed in conventional 2D FLCS is an average over a large number of molecules. By utilizing the ABEL trap, we can apply 2D FLCS to single biomolecules through capturing individual single molecules for several seconds in solution. We applied this ABEL-2D FLCS to the structural dynamics of a FRET-labeled hairpin DNA and found that the dynamics of each molecule can be interpreted as a transition between two states, and that there is heterogeneity in the FRET efficiency of the hairpin structure [5].

References:

1. K. Ishii and T. Tahara, *J. Phys. Chem. B* **2013**, *117*, 11414 & 11423.
2. T. Otosu, K. Ishii, T. Tahara, *Nat. Commun.* **2015**, *6*, 7685.
3. B. Sarkar, K. Ishii, and T. Tahara, *J. Am. Chem. Soc.* **2021**, *143*, 7968.
4. B. Sarkar, K. Ishii, and T. Tahara, *J. Phys. Chem. B* **2024**, *128*, 4685.
5. A. K. Foote et al., *ACS Phys. Chem. Au* **2024**, *4*, 408.

IT 125

Fluorescence Sensors for Clinically Relevant Analytes: Harnessing Aggregation-Induced Emission for Enhanced Detection

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Fluorescence-based sensors are playing an increasingly pivotal role in biomedical diagnostics due to their high sensitivity, rapid detection capabilities, and cost-effectiveness—qualities essential for advancing modern healthcare technologies. In this talk, I will explore the design and application of fluorescence-based sensors aimed at detecting a range of clinically significant bio-analytes, including Heparin, Albumin, Trypsin, and Alkaline Phosphatase. These analytes are integral to disease diagnosis and therapeutic monitoring, making their precise detection critical for clinical practice. By leveraging aggregation-induced emission (AIE) fluorophores, these sensors exhibit distinct fluorescence changes upon interaction with target biomolecules, enhancing their specificity and adaptability. In addition, the use of non-covalently assembled systems offers further modularity in sensor design.

In this presentation, I will showcase a series of fluorescence-based platforms and assemblies designed for the efficient, real-time detection of the aforementioned bio-analytes. These systems reduce reliance on complex instrumentation and minimize analysis time, offering streamlined and practical solutions for clinical applications. The talk will emphasize how innovative material design and fluorescence modulation can drive impactful advancements in biomedical diagnostics.

References:

1. J. Kaur et al. *J. Mat. Chem. B*, **2024**, 12, 3786
2. S. Bais et al. *ACS Applied Bio Materials*, **2023,** 6, 64146
3. S. P. Pandey et al., *Analytical Chemistry*, **2023**, 95, 6341
4. S. P. Pandey et al, *Sens. Actuators, B: Chem*., 353 **2022**, 353, 131016

IT 126

Highly sensitive Raman measurements of biomolecules in a liquid droplet formed by liquid–liquid phase separation

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We have quantified intracellular environments and biological molecules using the Raman band of water [1–7]. In this talk, I show our recent Raman results on evaluating the chemical properties of liquid-liquid phase separation (LLPS) and the application of LLPS to highly sensitive Raman measurements.

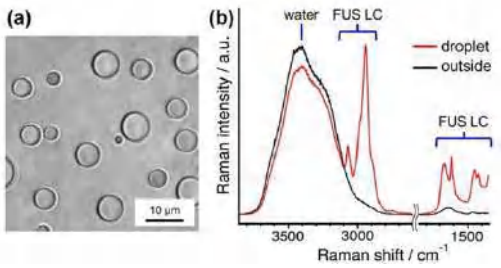

Fig. 1. (a) A bright field image of FUS LC droplets. (b) Raman spectra of inside and outside of the droplet.

[LLPS] LLPS is a phenomenon in which certain biomolecules form a highly concentrated liquid state (droplet); LLPS has been used to explain various biological phenomena, including the pathogenesis of neurodegenerative diseases. We have proposed that Raman microscopy is very useful for studying LLPS [2-7]. In particular, we have demonstrated a label-free method to evaluate the concentration of proteins in a single droplet using the Raman bands of water [3-5,7]. We applied this concentration quantification method to LLPS of FUS LC, one of the ALS-related proteins (Fig. 1) [3]. Our concentration quantification method indicates that FUS LC was highly concentrated (13-15 mM) inside the droplet and that the protein concentration inside decreased as the solution environment such as pH and salt concentration became unfavorable for droplet formation.

[Highly sensitive method] We developed a new method for measuring Raman spectra of biomacromolecules such as protein and nucleic acids at μM level [6]. We succeeded in concentrating almost any biomacromolecules to 1–10 mM in droplets formed by LLPS of water and PEG, allowing us to easily obtain the Raman spectra of biomacromolecules with high S/N ratio by focusing the excitation beam to a droplet. This method has made it possible to analyze low-concentration solutions of proteins and nucleic acids using Raman scattering. (Figs. 2 and 3).

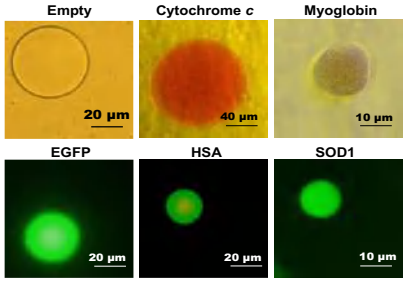

Fig. 2 Protein-encapsulated droplets. Proteins were concentrated in a water droplet formed by a PEG aqueous solution. An empty droplet is also shown at the upper left.

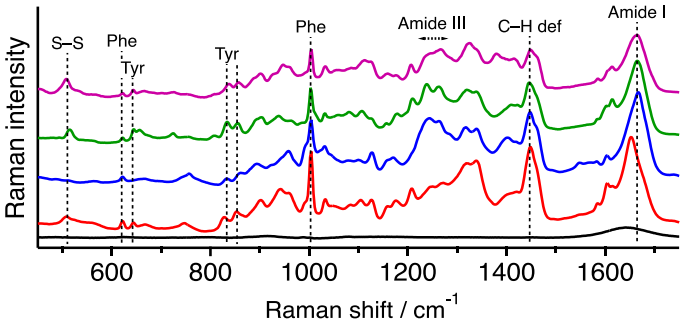

Fig. 3 Raman spectra of a protein droplet of trypsin inhibitor (purple), RNase A (green), SOD1 (blue), HSA (red) in a PEG solution, together with a dispersed solution of HSA at 30 μM (black).

References:

1. T. Sugimura, et. al. *Angew. Chem. Int. Ed.* **2020**, *59*, 7755.
2. K. Murakami, et. al. *Chem. Sci.* **2021**, *12*, 7411.
3. K. Yokosawa, et. al. *J. Phys. Chem. Lett.* **2022**, *13*, 5692, *Chem. Phys. Lett.* **2023**, *826*, 140634.
4. M. Machida, et. al. *J. Phys. Chem. B* **2023**, *127*, 3851.
5. L. Kageyama, et. al. *Chem. Phys. Lett.* **2024** ,*856*, 141671.
6. R. Tobita, et. al. *Chem. Comm*. **2024**, *60*, 8732.
7. M. Shibuya, et. al. *Anal. Chem.* in press.

IT 127

Bend it, Functionalise it and Sense the World Around

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The modulation of light (either absorbance / quenching properties, refractive index or wavelength shift) by materials can be used as highly sensitive sensors in the biological, chemical, environmental and security domains. In that regard optical fibers, aided by nanostructures (or fluorescent molecules) on, or in close proximity to the fiber, can be used as either an active transducer or a passive light guide. It has also been see that these are typically much more sensitive than simple colorimetric sensors. However, few have these have crossed the boundaries of the laboratory and have shown significant promise of being used in points-of-requirement outside of the laboratory. One of the reasons is that creating gratings (i.e. Bragg gratings of long-period gratings) on the fibers require very high-end manufacturing facilities and are expensive in the hands of the user. The other reason might be that the penetration depth of light into the material or environment of interest is quite limited.

Our lab has overcome a significant part of such limitations by developing U-bend, S-bend geometries or even three U bends in a series. The conversion of the lower order modes into higher order modes at the bend(s) give rise to high penetration depth, which can probe the molecules which are in reasonable proximity to the fiber (10-s of nanometers) with high degree of sensitivity, while at the same time preserving specificity using receptors, and the fact that very few “other” molecules would come that close to the fiber surface. We will give examples from detection of bacteria, antibiotics, protein (or aggregates) markers of diseases, etc.

IT 128

Tracking, photobleaching & correlating: How to catch small mobile molecular condensates

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It has become clear over the past decade that liquid-liquid phase separated protein condensates are omnipresent in cells. Particularly in the nucleus, mounting evidence suggests that transcriptional condensates form on gene promoter regions, introducing high concentrations of transcriptional factors. Well studied protein condensates are large enough to be clearly visualized in fluorescence images. Smaller ones, whose size might be below the resolution limit of a conventional light microscope, and which are both dimmer and more mobile than their larger counterparts, are harder to detect and to characterize. I will discuss several techniques such as single particle tracking, fluorescence recovery after photobleaching and fluorescence correlation spectroscopy, and examine how they can be used to characterize small mobile condensates *in vivo* based on their mobility. We have applied these techniques to the study of two transcription factors that play an essential role in pattern formation during early fruit fly embryo development. We find that both the transcriptional activator, Bicoid (Bcd), and the transcriptional repressor, Capicua (Cic), form oligomeric clusters ranging from low stoichiometry and high mobility clusters to larger, DNA-bound hubs [1]. Our observations are consistent with the hypothesis that both Bcd and Cic are included in transcriptional condensates, although their change in abundance and size with change in transcription factor concentration and over developmental time shows that a simple phase transition cannot explain their formation.

References:

1. Zhang et al. *Biophys J*, **2024**, *in press*.

IT 129

Elucidation of the Role of Electronic Effect on Doubly Locked GFP Chromophore Analogues to Help Design Improved Fluorophores

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The search to yield synthesized GFP chromophore (GFPc) with high quantum yield is still a topic of research, even after so many advancement in the field.[1] Besides, use of GFPcs as fluorogenic probes for RNA-aptamer imaging is getting popular. We have synthesized a series of doubly locked GFPc of three different classes, with varying electron donor and acceptor substituents.[2, 3] These compounds showed large Stokes shifted emission, hence useful in bio-imaging (Figure 1a). The electronic effect of the substituents and polarity of the solvent do alter the fluorescence quantum yield as well as the non-radiative pathway appreciably. Quantum Chemical calculations show twisted intramolecular charge transfer (TICT) from 2,3-dihydro indenyl moieties (donor) to imidazolinone rings (acceptor), as well as intramolecular excited state proton transfer (ESIPT) from hydroxyl proton to nitrogen atom, associated with the molecules. It was found that the molecules were effective in non-linear optical application as found from Z-scan (Figure 1b), the poorly fluorescent ones being more effective than the highly fluorescent ones. Besides, the compounds were found to show appreciable cell viability and can be used in cell imaging (Figure 1c).

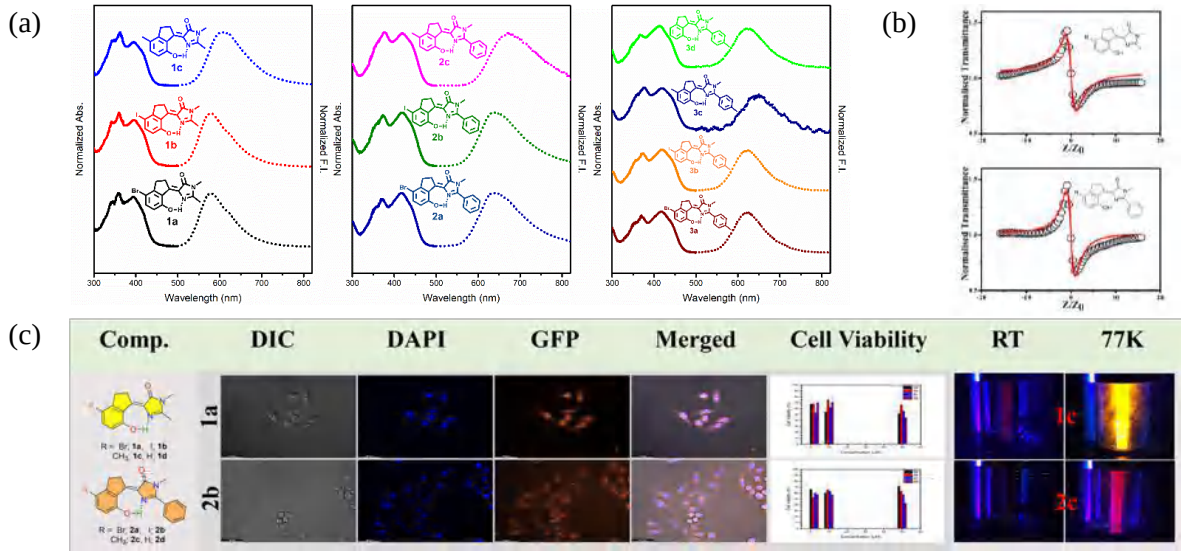

Figure 1. (a) Absorption spectra and emission spectra in toluene (b) Closed-aperture Z-scan measurements of two compounds in DMSO at ~532 nm. (c) Cell-images, cell viability and low temperature fluorescence for some selected compounds

References and Notes

1. Dedecker, P. et al. *J. Am. Chem. Soc.* **2013**, *135*, 2387.
2. Hsu, Y.-H. et al. *J. Am. Chem. Soc.* **2014**, *136*, 11805.
3. Chatterjee, S. et al. *J. Am. Chem. Soc.* **2019**, *142*, 738.

IT 130

DNA supercoiling-mediated G4/R-loop formation tunes transcription by controlling the access of RNA polymerase

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RNA polymerase (RNAP) is a processive motor that modulates DNA supercoiling and reshapes DNA structures. The feedback loop between the DNA topology and transcription remains elusive. Here, we investigate the impact of potential G-quadruplex forming sequences (PQS) on transcription in response to DNA supercoiling. We find that supercoiled DNA increases transcription frequency 10-fold higher than relaxed DNA, which lead to an abrupt formation of G-quadruplex (G4) and R-loop structures. Moreover, the stable R-loop relieves topological strain, facilitated by G4 formation. The cooperative formation of G4/R-loop effectively alters the DNA topology around the promoter and suppresses transcriptional activity by impeding RNAP loading. These findings highlight negative supercoiling as a built-in spring that triggers a transcriptional burst followed by a rapid suppression upon G4/R-loop formation. This study sheds light on the intricate interplay between DNA topology and structural change in transcriptional regulation, with implications for understanding gene expression dynamics.

IT 131

Triplet Energy Migration in the Cytoskeleton

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Dexter energy transfer (DET) of triplet electronic states is used to direct energy in photovoltaics, quench reactive singlet oxygen species in biological systems, and generate them in photodynamic therapy. However, the extent to which repeated DET between aromatic residues can lead to triplet energy migration in proteins has not been clarified. Here, we computationally describe DET rates in microtubules, actin filaments and the intermediate filament, vimentin. We discover instances where inter-aromatic residue Dexter couplings within individual protein subunits of these polymers are similar those of small molecules used for organic electronics. However, inter-aromatic residue coupling is mostly weak ($< 10^{-3}$ eV), limiting triplet energy diffusion lengths to 5.9, 0.5 Å and 1.0 Å in microtubules, actin filaments and respectively. On the other hand, repeated Förster Resonance Energy Transfer (FRET) between aromatic residues leads to singlet energy diffusion lengths of 12.4 Å for actin filaments and about 8.6 Å for both microtubules and vimentin filaments. Our work shows that singlet energy migration dominates over triplet energy migration in cytoskeletal polymers.

IT 132

Probing Molecular Interaction with Single Molecule Sensitivity

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Molecular interactions are fundamental to both chemical and biological processes. Traditionally, these interactions have been explored through ensemble spectroscopic techniques, which provide valuable insights into various systems. However, in recent decades, single-molecule spectroscopic techniques have emerged, offering the ability to investigate molecular interactions with unprecedented sensitivity. These methods allow for deeper exploration of molecular structure, function, and kinetics, paving the way for a more precise understanding and control over desirable outcomes. Our laboratory is focusing on the development of single-molecule spectroscopy and super-resolution functional imaging techniques. These advanced methodologies are used to study the fundamental interactions between molecules and their environments—interactions that are essential for proper molecular function. In particular, we employ a variety of single-molecule sensitive imaging techniques, such as spectral and orientation measurements, to unravel complex behaviors. One of our key focuses is spectrally resolved orientation-localization nanoscopy, where we simultaneously analyze the orientation of molecular dipoles in relation to specific target structures and polarity. This approach enhances our understanding of the dynamic relationship between molecular interactions and their structural context. In this presentation, we will discuss our recent findings involving the use of fluorescent dyes in these experiments, highlighting the potential of these techniques to unravel molecular processes with extraordinary detail.

IT 133

Plug-and-play fluorophores for Squash RNA aptamer allow mRNA imaging in multiple colors

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mRNA imaging tags are important for visualization of mRNA dynamics, transport and processing. Most of the mRNA imaging tags described previously are protein-based. Tagging the mRNA with fluorescent proteins can lead to unwanted consequences such as reduced degradation of mRNA during mRNA processing with exonucleases. Therefore, tags that are made entirely of RNA are preferable to visualize mRNAs in cells. Fluorogenic RNA aptamers are important tool for visualization of RNA in live cells with the help of fluorogenic dye. Typical fluorogenic dyes shows very low fluorescence but can increase its fluorescence significantly when bound to the fluorogenic RNA aptamer. Recently, we have reported a fluorogenic RNA aptamer named Squash which was evolved from a bacterial adenine-binding riboswitch using *in vitro* evolution or SELEX[1]. Squash was developed using two fluorogenic dyes named DFHBI and DFHO which are cell-permeable small molecule mimics of the chromophores of the Green Fluorescent Protein (GFP) and Red Fluorescent Protein respectively. Upon further inspection, we found that Squash can bind to several other fluorogenic dyes which are mimics of different Fluorescent Protein chromophores. This promiscuous nature of the Squash aptamer expanded the fluorescence emission of Squash from 500 nm to 640 nm.

Next, we developed mRNA imaging tag by using concatenated Squash aptamers for imaging mRNA dynamics in multiple colors. Our initial attempt to develop concatenated Squash tag failed due to insufficient mRNA production. Further investigation uncovered that Squash aptamer on its own destabilize mRNA when fused to its 3'-UTR. However, when incorporated into F30 scaffold, Squash aptamer allows mRNA tagging and imaging. We also found that the length of the linker joining each F30-2xSquash unit plays an important role in mRNA stability with longer linker allowing facile mRNA imaging. We further used these Squash-based tags to image β-actin mRNA in stress granules.

References:

1. S K Dey et. al. *Nature Chemical Biology*, **2022**, *18* (2), 180.

IT 134

Imaging and Analysis of Biocolloidal Taxis and Catalysis in Gradient of (Oilgo)nucleotides

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Biology used external stimuli to regulate feedback loops and modulate enzymatic reactions within cells both as a function of time and space.[1] Therefore, incorporating catalysis along with functions like – adaptability, stimuli responsiveness, dynamic specificity towards certain receptors or reactions and precise spatial and temporal control can make a synthetic system much closer to complex natural processes. To develop such systems, a catalyst must autonomously be relocated in different zone and toggled between on/off state. Importantly, gaining control over this process will be crucial for the development of spatially segregated chemistry to synthesize novel products or complex structures that are not accessible using conventional methods. Herein, we addressed the above-mentioned issue by modulating phoretic behaviour of colloids through temporally altering multivalent interactivity with nucleotides. Importantly, the catalytic property of the colloid can be autonomously controlled in a temporal fashion by the convertible multivalent scaffold around it. Therefore, multivalent interactivity can control both the phoretic motion of the colloid to dictate spatial location and the temporal control over catalytic process. In summary, we realize and control life-like behaviours in a synthetic system by tailoring multivalent interactions of adenosine nucleotides and catalytic microbead.[2,3] The research results can find applications - in programming the delivery of colloids for chemical processes (e.g. catalysis or drug release) as a function of space and time by switching on or off phoretic activity. Moreover, these active catalysts can function as sensors in far-off places because they don't need any external power sources.

References:

1. A. S. Lubbe et. al. *Tetrahedron* **2017**, *73*, 4837.
2. E. Shandilya et. al. *Nature Communications* **2024**, *15*, 3603.
3. E. Shandilya et. al. *ACS Nano* **2023**, *17*, 5108.

IT 135

Material properties determine the dynamics of tissue shape transitions

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Developing tissues during embryogenesis attain specific shapes that in turn drive the function of that tissue. For example, the intestine loops dramatically from a simple flat epithelial structure that increases the overall surface area of the tissue aiding in nutrient absorption. Not so surprisingly, understanding how tissues attain specific shapes has therefore been a long-standing problem in developmental biology. Beginning from an initial shape, tissues are generally morphed into specific shapes that depend on multiple processes including oriented cell division, cell death and directed cell rearrangements, which are driven by biochemical signalling processes. In this talk, using developing zebrafish embryos as a model system, I will discuss shape transitions in blastoderm explants and somites as a function of their material properties.

IT 136

Defining a nascent protein conformation on the ribosome

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The regulated expression of functional proteome is crucial for a healthy cell. The functional expression of a diverse family of proteins, like – enzymes, structural proteins, transcription/translation factors, etc. is tightly controlled at various stages, viz. during transcription, post-transcriptional modification, translation, post-translational modifications etc. During translation, the peptidyl transfer reaction allows amino acids polymerization at the peptidyl transferase center (PTC) of ribosome. we used a combination of biochemical, biophysical and cell biology approaches to explain protein folding mechanism of nascent sf-GFP (super folder GFP) and Nano-Luc (Nano luciferase). Our results indicated that a population of full-length nascent sf-GFP and Nano-Luc yields functionally active structures while still attached to the last P-site tRNA at the PTC. The nascent protein sequence's regulated sequestration inside the peptide exit tunnel appeared crucial to form its functionally active conformation.

IT 137

Small molecule Fluorescent Probes for imaging Subcellular Organelles

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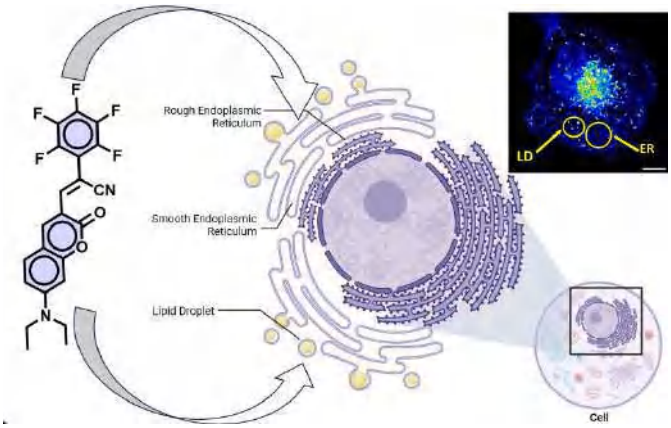
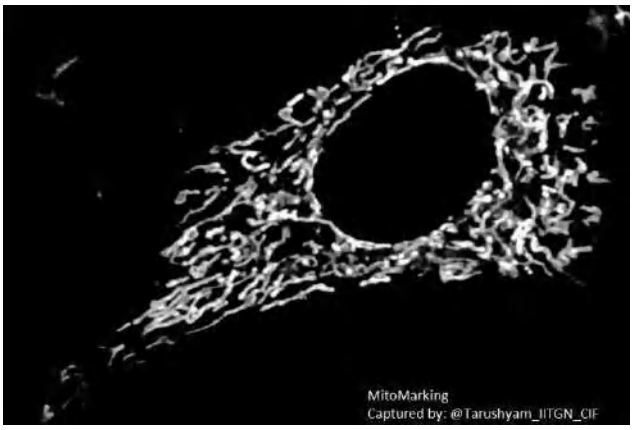
Abstract

Donor-acceptor (D-π–A) conjugated organic molecules possess desirable chemical, optical, electrical, and biological properties, making them an attractive platform for designing innovative functional materials. Conjugated systems that emit in lower energy regimes and exhibit charge transfer characteristics are particularly valuable for diagnostic and analytical applications. Our research focuses on synthesizing these fluorophores and investigating their photoresponsive properties for biological imaging. By precisely tuning various auxochromes, we have developed a range of fluorophores capable of selectively staining sub-cellular organelles, including the plasma membrane, lipid droplets, endoplasmic reticulum, lysosomes, and mitochondria. Additionally, these fluorescent probes allow us to monitor physiological processes, such as viscosity changes and inter-organelle interactions, enhancing their utility in medical diagnostics. This presentation will cover the design principles and findings related to sub-cellular imaging applications





References:

1. Deeksha Rajput et. al. *Org Biomol Chem*, **2023**, *21*, 8554.
2. Shabnam Mansuri et. al. *J. Mat. Chem. B*, **2024**, *12*, 2028.
3. Deeksha Rajput et. al. *J. Mat. Chem. B*, **2024**, *12*, 4698.
4. Yogesh Dubey et. al. *J. Mat. Chem.B*, **2024**, *12*, 4962.
5. Mohini Ghorpade et. al. *ChemBioChem*, **2024**, e202400273.

IT 138

Phase Separation by the HEY1::NCOA2 Fusion Oncoprotein Drives Transcriptional Rewiring in Mesenchymal Chondrosarcoma

Bappaditya Chandra[1*], Kelly Barnett[2*], Simranjot Bawa[1], Robert Mobley[2], Wenqing Qi[4], Kashi Raj Bhattarai[2], Eva Papachristou[3], Chandra Sekhar Reddy Chilamakuri[3], Zhaohong Yin[4], Hazheen Shirnekhi[1], Cheon-Gil Park[1], Clive D'Santos[3], Lu Wang[3], Dan Savic[2**], Richard Kriwacki[1,5**]

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The HEY1::NCOA2 fusion oncoprotein, observed in ~80% of mesenchymal chondrosarcoma cases, forms nuclear condensates associated with aberrant gene activation. We demonstrate that these nuclear condensates are transcriptional hubs, formed through phase separation by a C-terminal intrinsically disordered region (C-IDR) of NCOA2. Both DNA binding and condensate formation are essential for HEY1::NCOA2-dependent gene activation and resulting oncogenic cellular phenotypes. Importantly, HEY1::NCOA2 condensates incorporate CBP and p300, enhancing chromatin acetylation at activated genes. Through multi-site mutagenesis of the C-IDR, we show that graded changes in condensate formation correlates with similarly graded change in chromatin acetylation and gene expression, without affecting interactions with CBP and p300. While global chromatin structure remains unaltered, HEY1::NCOA2 expression caused focal DNA looping at activated genes, dependent on both its DNA binding and condensate formation. Remarkably, substitution mutagenesis showed that the physicochemical properties of the C-IDR, rather than its specific amino acid sequence, govern HEY1::NCOA2-dependent gene expression. These findings reveal how HEY1::NCOA2 exploits phase separation to focally remodel the chromatin landscape and reprogram transcriptional networks.

Student Fellowship Talks
&
Invited Short Talks

Student Fellowship Talks
&
Invited Short Talks

Posters

P 1

Disordered Regions, Ordered Outcomes: IDR Contributions to Bacterial Microcompartments

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Proteins are typically folded to achieve functionality, but recent research has highlighted the critical role that unfolded regions, known as intrinsically disordered regions (IDRs), play in influencing protein function. These IDRs remain largely unexplored in prokaryotes. Bacterial microcompartments (BMCs), protein structures with an outer layer of shell proteins that surround metabolic enzymes, are an ideal model to study prokaryotic IDRs as they consist of completely disordered to completely folded proteins. In this study, we explore how deletion of IDRs from the structural protein will impact the protein-protein interaction and overall function. A combination of advanced imaging and spectroscopic techniques, including fluorescence quenching, circular dichroism (CD) spectroscopy, and bio-layer interferometry (BLI), was employed to elucidate the structural and functional roles of IDRs in the outer shell protein PduA, a key component conserved across various BMCs. Additionally, transmission electron microscopy (TEM) was utilized to assess the morphological changes resulting from IDR deletions. Our findings reveal that IDRs at the N- and C-termini of PduA are essential for proper protein self-assembly and stability. Fluorescence quenching and BLI provided quantitative insights into the disruption of native protein interactions upon IDR deletion, while TEM imaging highlighted significant morphological alterations in the BMC structure. These observations were further validated through in vivo studies in both heterologous and homologous hosts, demonstrating the essential role of IDRs in maintaining wild-type protein properties. This work offers new insights into the functional importance of IDRs in prokaryotic structural proteins, underscoring their role in BMC assembly and stability.

P 2

A Zinc complex as an NIR emissive probe for real-time dynamics and *in vivo* embryogenic evolution of lysosomes using super-resolution microscopy

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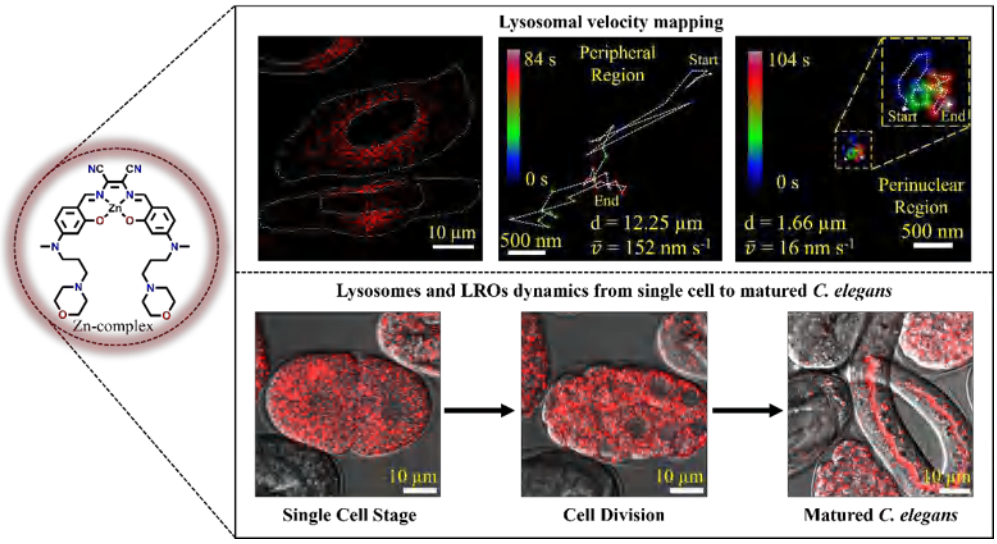
Zinc (Zn) based fluorescent metal complexes have gained increasing attention due to their non-toxicity and high brightness with marked fluorescence quantum yield (QY). However, they have rarely been employed in super-resolution microscopy (SRM) to study live cells and in vivo dynamics of lysosomes. Here, we present an NIR emissive highly photostable Zn-complex as a multifaceted fluorescent probe for the long-term dynamical distribution of lysosomes in various cancerous and non-cancerous cells in live condition and in vivo embryogenic evolution in *Caenorhabditis elegans (C. elegans)*. Apart from the normal fission, fusion, and kiss & run, the motility and the exact location of lysosomes at each point were mapped precisely. A notable difference in the lysosomal motility in the peripheral region between cancerous and non-cancerous cells was distinctly observed. This is attributed to the difference in viscosity of the cytoplasmic environment. On the other hand, along with the super-resolved structure of the smallest size lysosome (~77 nm) in live *C. elegans*, the complete in vivo embryogenic evolution of lysosomes and lysosome-related organelles (LROs) was captured. We were able to capture the images of lysosomes and LROs at different stages of *C. elegans*, starting from a single cell and extending to a fully matured adult animal.



References:

1. A. Salam et al., *Chem. Sci.*, **2024**, 15, 15659
2. N. Gustafsson et al., *Nat. Commun.*, **2016**, 7, 12471
3. D. E. Johnson et al., *J. Cell Biol.*, **2016**, 212, 677
4. Jankele et al., *Elife*, **2021**, 10, e61714

Probing of disease-specific exosomes using Raman spectroscopy and SERS

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Cancer often evolves silently and is realized only in the advanced stages of metastasis. Also, the most practiced diagnostic tools like MRI, CT scan, PET scan, etc, often require costly infrastructure and/or trained personnel. These techniques come with their own detection limits and are also susceptible to produce false positive results. We urgently need more accurate and cost-effective diagnosis techniques. In recent years, markers for various cancers have been identified in the contents of exosomes. Exosomes originating from cancer cells carry genetic materials, metabolites, and proteins that can significantly differ from exosomes originating from healthy cells. We are in the process of establishing a Raman spectroscopy-based comparison of exosomal contents obtained from various cancerous and non-cancerous cell lines, utilizing multivariate statistical methods such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) [1].

In patient samples, disease-specific exosomes are typically present in very low concentrations amidst a variety of other exosome types. Detecting these subtle differences requires a high degree of sensitivity and selectivity, which classical Raman spectroscopy lacks. Instead, Surface-enhanced Raman spectroscopy (SERS) offers a solution by greatly amplifying Raman signal intensities from analytes near plasmonic surfaces, enabling the precise detection of specific biomarkers [1-3]. For example, the human epidermal growth factor receptor 2 (HER2), which is overexpressed in various cancers including breast, cervical, ovarian, and gastro-oesophageal cancers, serves as a potential exosomal biomarker. HER2 can be specifically targeted using a 42-base DNA aptamer known as the Anti-HER2 aptamer (HApt) [4]. In this study, we utilized a modified HApt with a disulfide bond at the 5' end and Raman/fluorescence tags at the 3' end. These modified aptamers were grafted onto silver nanoparticles (AgNPs) to create various nanoconstructs. We hypothesized that binding with HER2 would cause the HApt molecules to reorient on the AgNP surface, leading to detectable changes in the SERS signal from the Raman tags. We observed significant SERS enhancement with our nanoconstructs exclusively in the presence of exosomes isolated from HER2-overexpressing SKOV3 cells. In contrast, no notable changes in SERS characteristics were detected with exosomes from SiHa cells, which do not overexpress HER2. This technique thus holds promise as a highly sensitive and cost-effective platform for the early detection of HER2-overexpressing cancers.

References:

1. Chalapathi, D. et al. *J. Phys. Chem. B* **2020**, *124*, 10952
2. Karthigeyan, D. et al. *Proc. Natl. Acad. Sci. USA*, **2014**, 02695.
3. Bhowmik, D. et al. *ACS Nano* **2015**, *9*, 9070.
4. Bhowmik, D. et al. *ACS Nano* **2019**, 13, 13637.

P 4

How Molecular Crowders Influence Ligand Binding Kinetics with G-Quadruplex DNA? The Role of Bound Water

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The formation of basket-like G-quadruplex DNA (GqDNA) structures resulting from the folding of guanine-rich single-stranded DNA sequence in the presence of monovalent cations (mainly, K^+ or Na^+), typically found in the telomere and promoter regions, play important roles in various biological processes [1,2]. These DNA structures contain specific binding sites for small drug-like molecules (ligands) which make them capable of inducing anti-cancer effects upon interacting with GqDNA within the cell. Thus, understanding the kinetics of ligand interaction with GqDNA in cell-like crowded environment is of paramount importance in biology and pharmacology, as it elucidates how molecular crowders influence the reaction rates governing these interactions, which are mostly unknown. In this study, we have used fluorescence correlation spectroscopy (FCS) and molecular dynamics (MD) simulations, aided by other techniques, to look at the kinetics of a benzophenoxazine-moiety ligand (cresyl violet, CV) binding to human telomeric (3+1) hybrid GqDNA in the absence and presence of commonly used crowders like ethylene glycol (EG), PEG200 and PEG6000 at two different physiological crowder concentrations, i.e., 10% (w/v) and 20% (w/v). Experimental results show a decrease in ligand binding affinity to GqDNA with increasing the crowder size as well as concentration, driven by viscosity-induced reduction of association rate (k_+) and a concomitant increase in dissociation rate (k-) of the ligand. MD simulations reveal the critical role of electrostatic forces and long-lived water-mediated hydrogen-bond-bridges in stabilizing the ligand/GqDNA complex, which are significantly disrupted by crowders, leading to the destabilization of the complex. Although, the binding of the ligand (CV) to these crowders is very weak, their effect on the destabilization CV from its stable complex with GqDNA is quite substantial which gets enhanced with concentration as well as size of the crowders. Unlike polysaccharide crowders, these EG/PEG crowders affect both the association and dissociation rates, which needs special attention while choosing them as crowding agents in cell-like *in vitro* experimental conditions [3,4]. We believe, these results will help researchers in designing GqDNA targeted anti-cancer drugs as well as choosing proper (inert) crowders to mimic cellular environment.

References:

1. Spiegel, J. et al. *Trends Chem.* **2020**, *2*, 123.
2. Rhodes, D. et al. *Nucleic Acids Res.* **2015**, *43*, 8627.
3. Clovis, N.S. et al. *J. Photochem. Photobiol. A Chem.* **2023**, *437*, 114432.
4. Alam, P. et al. *Methods Appl. Fluoresc.* **2024**, *12*, 045002.

P 5

Slowdown of Solvent Structural Dynamics in Aqueous DMF Solutions

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Understanding the hydrogen bonding interactions between amide functional groups and water is essential for studying liquid–liquid phase separation in biological systems. Even simple amides like N,N-Dimethylformamide (DMF) and formamide (FA) exhibit microheterogeneity and distinct hydrogen bonding behaviour in aqueous solutions, forming dynamic equilibria that impact solvation properties.[1, 2] We employed a multidisciplinary approach—integrating FTIR and 2D IR spectroscopy, density functional theory (DFT) calculations, and molecular dynamics (MD) simulations—to investigate the interactions and ultrafast dynamics of these heterogeneous systems. Our findings reveal a notable slowdown in solvent fluctuation dynamics at the maximum viscosity observed in specific amide-water molar ratios, attributed to stronger amide-water hydrogen bonds compared to water-water bonds. Hydrogen-bond autocorrelations from MD simulations corroborate the experimental spectral diffusion timescales, providing a comprehensive molecular view of solvation structure and dynamics.[3] This study offers critical insights into hydrogen bond dynamics and sets the stage for exploring complex solvation phenomena, such as coacervation.

References and Notes

1. Bai, Y. et al. *J. Chem. Phys B* **2022,** *126*, 9663
2. Tomar, D. et al. *J. Chem. Phys.* **2020,** *152*, 114707.
3. Pathania, A. et al. *Chemical Physics Impact* **2024,** *9*, 100711.

P 6

Design and Synthesis of Water Dispersible Red Emissive Carbon Dots and their Applications

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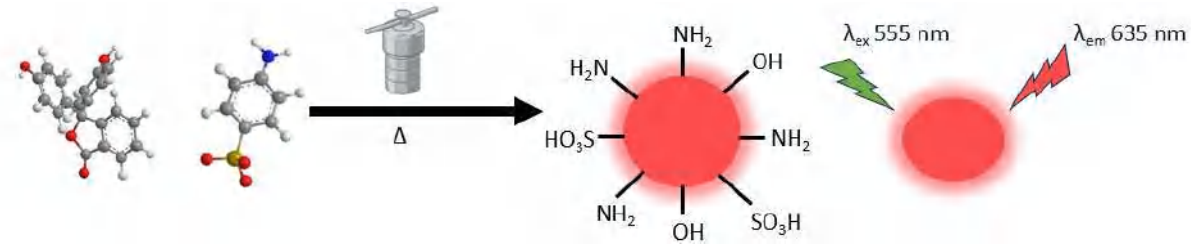
Carbon dots (CDs), fluorescent carbon nanoparticles smaller than 10 nm, have been extensively used in sensors, bioimaging, catalysis, light-emitting (LEDs), and photoelectronic devices. This is due to their unique characteristics, including low toxicity, biocompatibility, high photostability, ease of surface modification, and up-conversion fluorescence. Carbon dots (CDs) have proven effective in revealing the 3D structures of biological samples, distinguishing between normal and cancer cells, and detecting various analytes inside cells. Most of the carbon dots are blue-green emitting CDs. The practical use of blue-green-emitting carbon dots (CDs) is limited by their shallow penetration, photobleaching, and autofluorescence issues. The synthesis of red-emissive carbon dots (CDs) is highly sought after for sensing applications, but challenges remain in precursor preparation and product purification. Red-emissive carbon dots (RCDs) have been developed to address these issues. They offer deep tissue penetration, reduced photodamage, low autofluorescence, and high imaging contrast. Herein, we designed and synthesized RCDs with high water dispersibility, low toxicity, and excellent optical properties using hydrothermal method. These features make them ideal for a range of biological applications.



References:

1. Amanda A Pinheiro et al. *ACS Applied Nano material* **2024**, *7*, 19184.
2. Ankit Thakuri et al. *Journal of Hazardous Chemistry* **2024**, *469*, 133998.
3. Sharanabasava D. Hiremath et al. *ACS Applied Nano material* **2022,** *5*, 17373.
4. Sharanabasava D. Hiremath et al. *Sensors and Actuators: B. Chemical* **2021**, *339*, 119918.
5. Sharanabasava D. Hiremath et al. *Journal of Photochemistry & Photobiology A: Chemistry* **2020**, *389*, 112258.

Charge Polarity effect on the hydration shell water of π-electron containing hydrophobes: Observed by Raman Hydration Shell Spectroscopy

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π-electron containing hydrophobes are vital in many biological, chemical and industrial processes. Interaction of such hydrophobes with water (e.g., π···HOH and CH···OH_2) are generally weak but widespread. Experimental understanding of these weak interactions in aqueous bulk medium is limited due to typically low solubility of such hydrophobes in water and the associated technical challenges in their selective identification. Here, we monitored these interactions in the aqueous solution of structurally similar but oppositely charged molecular ions (tetra-phenyl borate (TPB^-) and tetra-phenyl phosphonium (TPP^+)) and uncharged benzene (Bz), using Raman difference spectroscopy with simultaneous curve fitting analysis (Raman-DS-SCF) [1].

DS-SCF analysis of the Raman spectrum of aqueous solution of a solute with respect to the Raman spectrum of neat water provides the solute-perturbed water spectrum which is considered as the spectrum of the perturbed water in the hydration shell (HS) of the solute. The left panels of Figure 1 show the OH stretch spectra of water pertaining to the hydration shell of Bz, TPB^-, and TPP^+ (obtained after Raman-DS-SCF analysis). The hydration shell spectra differ from spectrum of the neat water (black curve), indicating its interaction with the solutes. The HS water around Bz shows two component bands, centered at 3515 cm^{-1} and 3625 cm^{-1} (green dashed curves, top left panel) which corresponds to water approaching a Bz-ring equatorially and axially, respectively. The equatorial water engages in (Bz)CH···OH_2 interaction and axial water engages in (Bz)π···HOH interaction. For the negatively charged TPB^-, the OH stretch band corresponding to the π···HOH interaction is 45 cm^{-1} red shifted than that of Bz, suggesting stronger π···HOH interaction with TPB^-. For TPP^+, the corresponding π···HOH band is significantly blue-shifted and of lower intensity from that of Bz or TPB^- (left panel Figure 1c), signifying weaker π···HOH interaction with TPP^+. Thus the strength of π···HOH interaction varies as TPB^- > Bz > TPP^+. In addition, the appearance of a major component band around 3210 cm^{-1} for the HS of TPP^+ reveals the collective nature of the OH stretch vibration of water in the HS of TPP^+, which is a unique feature observed only for the cationic hydrophobe [1,2]. The modulation of the (phenyl)CH···OH_2 interaction with the equatorial water is monitored by the CH stretch band of the corresponding hydrophobes (right panels in Figure 1). The blue-shift of aromatic ring-CH band corresponds to the (phenyl)CH···OH_2 interaction, known as blue-shifted H-bond interaction. The strength of the (phenyl)CH···OH_2 interaction varies as TPB^- < Bz < TPP^+, which is opposite to the trend of their corresponding π···HOH interaction. Altogether, the π···HOH and CH···OH_2 interactions are modulated in aqueous medium depending on the sign of charge on the hydrophobe.

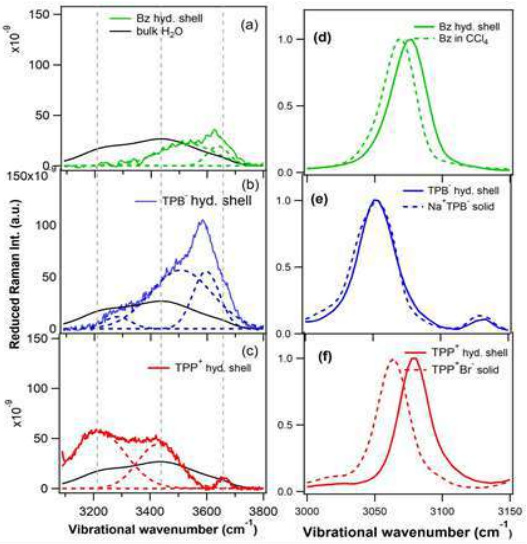

Figure 1: left panel: OH stretch Raman-DS-SCF hydration shell spectra (solid lines) and component bands (dashed lines) for Bz (a), TPB^- (b) & TPP^+ (c). Right panel: CH stretch Raman spectra of Bz (d), TPB^- (e) & TPP^+ (f) in presence (solid) & absence (dashed) of water.

References:

1. Bandyopadhyay,A. et al. *J.Mol.liq* **2023,** *390*,122987.
2. Bandyopadhyay,A. et al. *J.Phys.Chem.B* **2023,** *127*,7174.

P 8

Intramolecular Charge Transfer Dynamics in Persubstituted Perylenediimide

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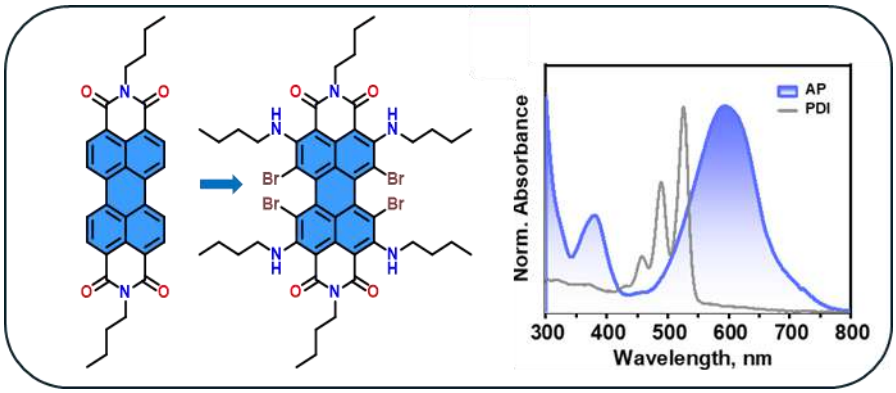
The charge transfer property of an organic chromophore can be tuned by incorporating electron donating/withdrawing functional groups. Amination at the ortho positions of perylenediimide induces intramolecular charge transfer (ICT) character to the chromophore.[1] Herein, we have synthesized and characterized the perylenediimide derivative having ortho substitution with amino groups and bay substitution with bromine atoms. The ICT effect of the amino groups on the ortho positions dominates over the heavy atom effect caused by the bromine atoms on the bay positions of the persubstituted perylenediimide.[2,3] The charge transfer character of the molecule is confirmed via transient absorption measurements. The lifetime of the ICT state reduced as the polarity of the solvent increased. The theoretical and experimental observations suggest that the electronic properties of the perylenediimide core are largely influenced by the position at which the substitution has been made.



References:

1. G. Battagliarin et al. *Org. Lett.* **2011**, *13*, 3012
2. K.Nagarajan et al. *J. Phys. Chem. C* **2016**, *120*, 8443
3. A. Jozeliūnaitė et al. *Synthesis* **2017**, *49*, 5176

P 9

Ultrafast exciton dynamics in $FAPbI_3$ nanorods

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Formamidinium lead triiodide ($FAPbI_3$) nanocrystals are attractive because they emit in red and near-infrared spectral regions.[1]. However, their inherent instability poses a major challenge in fabricating devices based on these materials. The present work reports a serendipitous synthesis of stable rod-shaped oleylamine-capped nanocrystals of $FAPbI_3$. The nanorods first excitonic peak and emission maximum occur in the red region. Their photoluminescence decays are bimodal, with a 14-16 ns component ascribed to band-edge recombination and a 35-40 ns component ascribed to trap-assisted recombination.[2]. Hot exciton relaxation plays a major role in this context. The dynamics of this process in the nanorods have been investigated by ultrafast transient absorption spectroscopy at different pump fluxes.

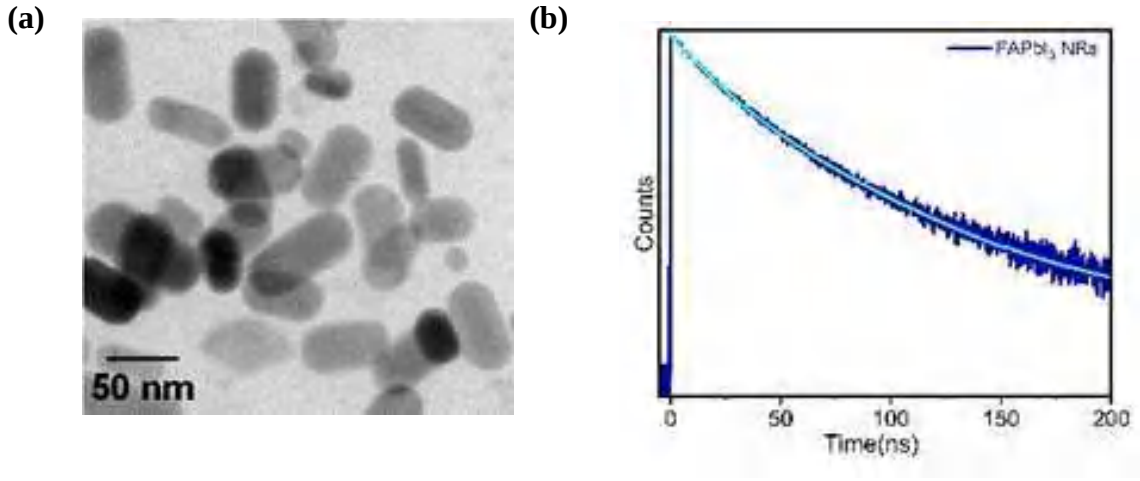

Figure 1 (a) TEM images of $FAPbI_3$ NRs, (b) PL Decay of $FAPbI_3$ NRs

References:

1. L. Protesescu.et al. *ACS Nano*, 2017, **11**, 3119
2. P. Papagiorgis.et al. *ACS Nano*, 2019, **13**, 5799

P 10

Advanced Techniques for Probing the Effect of Microplastics on Lipid Membranes

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Cellular membranes, critical for maintaining homeostasis and regulating molecular transport, are increasingly threatened by environmental pollutants, particularly microplastics. These micron-sized particles can integrate into lipid bilayers, disrupting membrane fluidity and organization. Such disturbances are implicated in diseases like Alzheimer's and Parkinson's. Due to the complexity of membrane systems, conventional fluorescence-based techniques lack the sensitivity to capture the dynamic nature of these interactions. This work focuses on the development of a two-channel confocal-AFM setup for real-time assessment of membrane indentation force and lipid order, integrating mechanical and molecular measurements. The system's design, development, and capabilities are outlined, alongside preliminary data on model membranes. Further optimization is needed to fully understand microplastic-induced membrane disruptions. This work represents a significant step toward real-time, high-resolution analysis of membrane-molecule interactions.

P 11

Amorphous Aggregates in Crystal Nucleation

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Crystallization from solution is at the heart of many natural and industrial processes, from the production of pharmaceuticals and nanomaterials to the formation of minerals, bones and teeth. The earliest phase of the crystallization process begins with nucleation which determines the structure, size and shape distribution of the crystals. For last two decades it has been observed that crystal nucleation from supersaturated solution occurs via formation and reorganization of prenucleation clusters where classical nucleation theory is challenged and modified [1]. These prenucleation clusters are metastable and nanoscale to mesoscale solute-rich structures. Understanding their origin, composition and structure is one of the pertinent and lingering questions in the fundamentals of crystal nucleation. Here, we have shown that these prenucleation clusters or amorphous aggregates are universally present in the supersaturated solutions of a range of amino acids and small peptides [2]. Using light scattering, nano-ESI mass spectrometry and *in-situ* terahertz Raman spectroscopy, we have identified their structures and dynamical behaviour in the aqueous solutions. One of the significant findings disclose that these aggregates encompass a wide variety of length scales, from the dimers to ~30-mers to nanometre and even micrometre scale, implying a continuous distribution throughout this range. Larger amorphous aggregates are seed points of spontaneous or laser-induced crystal nucleation. We have provided a novel perspective of crystal nucleation and non-classical pathways where barrierless nucleation of amorphous aggregates is followed by nucleation of crystals inside the solute-enriched aggregates. We will also discuss the dynamical behaviour of these amorphous clusters with the help of fluorescence dyes. The fluorescence response of the dyes will hint towards the solute-solvent structuring in the clusters and selective segregation of the solutes leading to crystal seed formation.

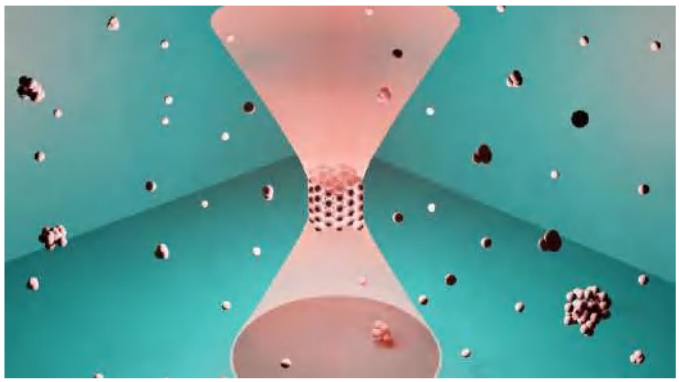

Figure: Amorphous aggregates are present in a wide distribution of sizes in the supersaturated solution of amino acids and peptides, adapted from Ref. 2.

References:

1. D. Gebauer et al., *Chem. Soc. Rev.*, 2014, **43**, 2348.
2. Z. Liao et al., *Chem. Sci.*, 2024, **15**, 12420.

P 12

Cholesterol and small biomolecules affect membrane properties and cellular signaling.

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Abstract

Exocytosis is crucial for neuronal communication, which involves membrane remodeling. Therefore, the biophysical properties of lipid bilayers may influence this process. Cholesterol, a lipid found in both secretory vesicles and cell plasma membranes, changes lipid bilayer properties and therefore has the potential to influence this process. Here, we investigate how changes in membrane cholesterol levels regulate exocytosis in both artificial membranes and in living cells.

We demonstrate that increasing cholesterol fraction from 0 to 50 mole % of the lipid in a mixed lipid membrane modulates membrane properties. Membrane order measurements performed with fluorescence lifetime and spectral shift of indicator dyes (e.g. Flip-tR, Nile Red and Prodan respectively) show an increase of membrane order with increasing cholesterol, as may be expected. However, in an apparent contradiction, these changes do not correlate with the lowering of the force required to indent the membrane with an AFM tip. Significantly, the AFM indentation force measurements correlate with an increase in the fusion kinetics of vesicles to artificial lipid bilayers, measured using a Total Internal Reflection Fluorescence Microscope. This suggests that membrane indentation force can act as a robust assay for vesicle fusion propensity, and membrane order measurement techniques can be misleading in this respect.

We then tested the biological significance of this effect. In a live cell, the fusion machinery may override any impact that the changes in membrane properties may have on vesicle fusion. However, on using mβCD to reduce cholesterol from cellular membranes, we observe that vesicular exocytosis indeed slows down with the reduction of cholesterol. Given the ubiquitous use of cholesterol-lowering drugs by the aging population, this may have clinical implications. We have also examined the effect of some small signaling molecules (related to serotonin) on membrane properties and vesicular fusion since serotonin has already been shown to affect the membrane (1,2). N-acetyl serotonin (NAS) and 5-hydroxy tryptophan (5HTP) are two examples. Our goal is to identify the specific characteristic of these tiny molecules that most strongly influence how they interact with membranes. Using Indentation force measurements and measurements of vesicle fusion and exocytosis using TIRF microscopy, we find that 10 mM of NAS, but not 5HTP, increases the rate of endocytosis in a neuronal cell line by 36%. This is likely a hitherto unknown accessory pathway for neuromodulation. These findings can potentially pave the way for the discovery of small molecules, which can be used to alter membrane properties and therefore influence exocytosis without interacting with any receptors.

References:

1. S. Dey et al. *Chem. Eur. J*., **2021**, *27*, 1
2. D. S. Roy et al. *bioRxiv*, **2024**.01. 20.576155

P 13

Protein function modulation via decoy peptides

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A rationally designed drug molecule has to recognize the protein of interest, bind to it with high affinity, and modulate its function. Developing such a drug is a laborious, time-consuming, and resource-intensive process. We propose a novel strategy using 'decoy-peptides'[1] which provides an efficient shortcut. Decoy-peptides are separate near-identical copies of fragments of the target protein. They can modulate protein function based on their folding topology. We have demonstrated the strategy using a fluorescent protein EGFP. The decoy peptides of EGFP showed no effect on the fluorescence activity of folded EGFP but the presence of decoy-peptides during the folding process of EGFP strongly reduced its fluorescence. FCS and TCSPC measurements indicated the binary nature of fluorescence inhibition upon decoy peptide interaction. The reduction of fluorescence was much less when the sequence of the decoy-peptide was randomized, demonstrating its specificity. The efficacy of this strategy in the biological context was evaluated using an *in vitro* translation assay. We show that the GFP fluorescence can be silenced when the decoy is present during the ribosomal synthesis of the protein. Further, we applied this strategy to inhibit a critical protein of pathogenic bacteria, ß-barrel assembly machinery A (BamA). The decoy is strongly toxic to the bacteria, showing a potential route to antibiotics[2]. Using in vitro assays, we show that BamA decoys can directly inhibit the assembly of BamA client proteins, even in the absence of BamA. In conclusion, decoy-peptides provide a promising new pathway for drug discovery.

References:

1. A. Das et al. *J. Am. Chem. Soc.* **2021**, *143*, 18766.
2. Xu et al. *Molecules.* **2023**, *28*, 3758.

P 14

Unravelling the Phototherapeutic Potential of Pristine 2D Borophene Towards Destruction of Bacteria and Biofilms

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The increasing prevalence of bacterial infections and biofilm formation on clinical wounds pose a significant global health challenge. In this regard, light-mediated phototherapy in the near-infrared (NIR) region has emerged as a promising, non-invasive solution against the surgical modalities. However, conventional dyes used in phototherapy suffer from rapid photobleaching and are primarily activated in the UV-visible range, limiting their clinical utility. Herein, we demonstrate the potential of borophene nanosheets as an exceptional phototherapeutic nanoagent with a photothermal (PTT) conversion efficiencies of 32% at 808 nm (NIR-I) and 26.3% at 1064 nm (NIR-II), along with robust photodynamic (PDT) capabilities across all three NIR windows. Notably, borophene's ability to inhibit biofilm formation and eradicate mature biofilms offers a groundbreaking approach to manage biofilm-associated infections, a critical issue in clinical settings. Our results elucidate the possible mechanisms of borophene-bacterial/biofilm interaction involved in efficient elimination. Furthermore, in vivo studies using a zebrafish model showed that borophene exhibited minimal toxicity, suggesting strong potential for safe clinical translation. This research highlights the potential for eliminating the need for surgical interventions and providing a robust, non-invasive approach to infection control, borophene could pave the way for advanced antimicrobial clinical devices. Our findings provide a strong foundation for future development of borophene composites and hybrids, positioning them as a versatile tool in combating bacterial infections in medical environments.

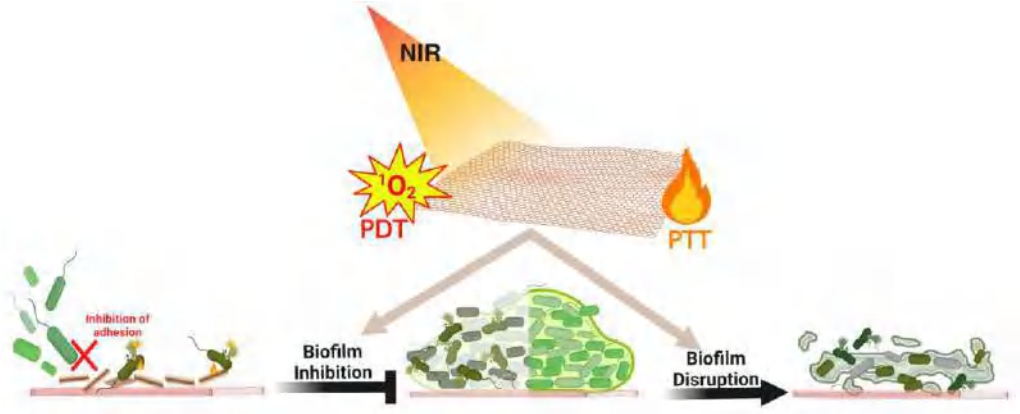

Figure 1: Schematic illustration of the phototherapeutic property of borophene for biofilm inhibition and disruption upon NIR photon irradiation.

References:

1. Ling Xiao et al. *ACS Nano* **2011,** 5,
2. Yu Cheng et al. *ACS Langmuir* **2010**, *26*, 4.
3. Allie Clinton el al. *Laboratory Medicine, 46,* 277.

P 15

Probing the Modulation in Condensate Material Properties using Fluorescence Anisotropy-based HomoFRET Imaging

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Biomolecular condensation into non-canonical, membrane-less organelles has gained prominence as a critical phenomenon in orchestrating complex cellular biochemistry and spatiotemporal organization across organisms. These regulatable supramolecular assemblies are thought to form via a multitude of transient, intermolecular forces that sequester intrinsically disordered proteins and other biomolecules into non-stoichiometric, liquid-like compartments. However, unregulated formation and abnormal maturation of these dynamic, reversible assemblies into irreversible gel-like or solid-like aggregates is implicated in the pathology of a range of neurodegenerative diseases. Thus, the pathophysiological functions of these cellular assemblies are governed by their material properties, necessitating the development of novel tools and methodologies for the detection of altered condensate properties in response to various small molecule regulators and post-translational modifications. Utilizing homo-Förster Resonance Energy Transfer (homoFRET) imaging, we capture the changes in molecular packing within the biomolecular condensates of an archetypical phase separating protein Fused in Sarcoma (FUS). Our single-droplet anisotropy-based homoFRET measurements within the condensates of fluorescently-tagged FUS shed light on the changes in the nanoscale architecture and the resulting modulation in droplet properties upon RNA, ATP, and post-translational methylation. We further extend this in vitro application of homoFRET imaging to probe intracellular phase transitions of nuclear and cytoplasmic FUS within mammalian cell lines.

References:

1. A. Joshi*, A. Walimbe* et. al. *bioRxiv*, **2024** (*Contributed equally) (Accepted in *Nat. Commun.* 2024).

P 16

Aggregation in Deep Eutectic Solvents (DESs): Formation of Polar DES-in-Nonpolar DES Microemulsions

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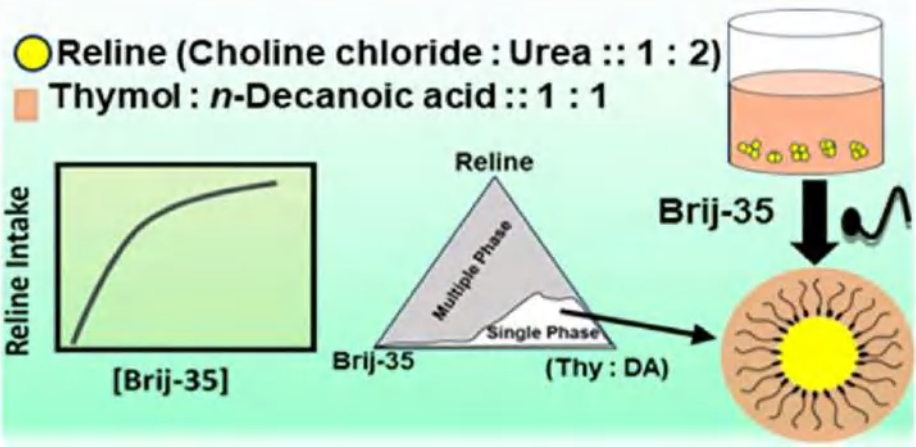
The versatility of environmentally-benign and inexpensive deep eutectic solvents (DESs) lies in their widely varying physicochemical properties. Depending on its constituents, a DES may be highly polar or highly nonpolar in nature. This offers enticing possibility of formation of novel nonaqueous microemulsions (MEs). Evidence of the presence of polar DES-in-nonpolar DES MEs is presented with reline (formed by mixing choline chloride and urea in 1 : 2 T single-phase region where MEs may be forming. Dynamic light scattering (DLS) confirms the presence of MEs of 2 - 10 nm size. Even as up to 2.5 M (ca. 0.35 mole fraction) reline, whose dynamic viscosity (η) and electrical conductivity (κ) are very high, is added to 100 mM Brij-35 solution of Thy : DA, the η and κ of the solution increases insignificantly thus conforming formation of MEs in the solution. FTIR absorbance spectra and fluorescence probe responses further indicate that reline is not dispersed in the medium, it rather forms polar pools of the MEs. These novel nonaqueous polar DES-in-nonpolar DES MEs will not only expand application potential of DESs, they also offer new class of organized media with widespread potential.



References:

1. Van Horn et. al. *J. Am. Chem. Soc.* **2009**, 8030.
2. Baker et. al. *Chem Commun* **2003**, 2932.
3. Correa et. al. *Chem. Rev.* **2012**, 4569.
4. Eastoe et. al. *J. Am. Chem. Soc.* **2005**, 7302.

P 17

Understanding Htt Protein Aggregation in Cell Mimicking Environments

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The intracellular milieu, characterized by molecular intricacy and crowding, heavily relies on proteins as fundamental biological entities. Approximately one-third of the human proteome comprises intrinsically disordered proteins (IDPs). Huntington's disease (HD) arises from the aggregation of mutant Huntington protein (HD39Q), which is one such IDP containing 39 glutamine repeats. This investigation explores the effects of macromolecular crowders—Dextran, Ficoll, and polyethylene glycol (PEG)—on the aggregation process of HD39Q. Using fluorescence-based assays, including concentration-dependent Thioflavin T (ThT) fluorescence, we analyzed the kinetics of protein aggregation, observing distinct influences of each crowder and binary mixtures of crowders on the aggregation mechanism. Nano-particle Tracking Analyzer (NTA) studies further confirmed the modulation of aggregation kinetics by different crowders, showing a reduction in smaller oligomers and a concurrent increase in larger aggregates over time. Fluorescence correlation spectroscopy (FCS) studies, performed subsequently, revealed dynamic changes in oligomer diffusion times and hydrodynamic radii, providing insights into the early stages of aggregation. Confocal microscopy confirmed that different crowders significantly affect the morphology of the resulting aggregates, with fibrillar structures forming more rapidly in crowded environments. The use of BODIPY TMR-labeled HD39Q, along with crowders in various combinations, further unveiled crucial insights into liquid-liquid phase separation (LLPS) and its role in modulating aggregation within distinct phases formed by crowder mixtures. This study highlights the critical role of macromolecular crowding in modulating both the kinetics and morphology of Huntington protein aggregation.

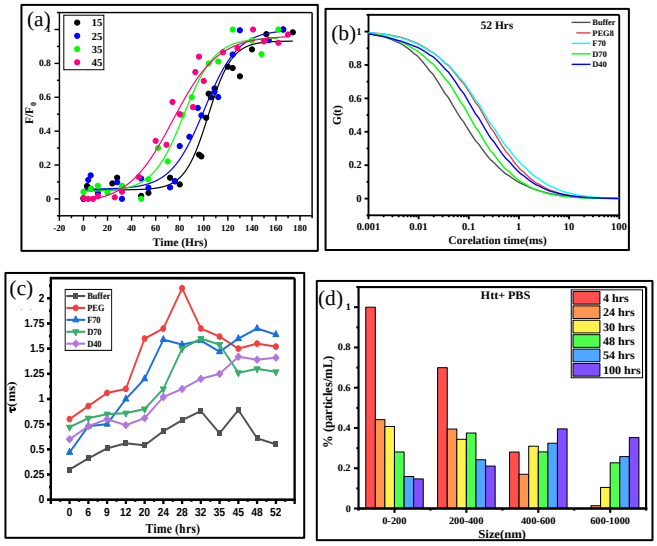

Figure: (a) Th-T intensity overlap (F/Fo) vs time for different concentrations of Htt protein (b) Fluorescence correlation curves shift to longer lag times indicating the increase in the size of the aggregates (c) Change in diffusion time monitored as a function of incubation time (d) Changes in hydrodynamic radii as observed with NTA

References:

1. Larissa A. Munishkina et. al. *Biochem*, **2008**, 47, 8993.

P 18

Quantification of forces involved in cytoplasmic streaming in plants and their temperature dependence

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Cytoplasmic streaming is a phenomenon observed in plants where the circulation of cellular components occur around the central vacuole. It is crucial for the spatio-temporal distribution of organelles in plant cells and thus has an important role in plant growth. Myosin XI, a plant motor protein has been implicated in the transport of organelles during cytoplasmic streaming. Myosins are a family of ATP-dependent motor proteins that move on actin filaments. Although cytoplasmic streaming in plants has been widely studied, the forces involved in this phenomenon remain to be quantified. We have performed in vivo optical trapping in the model organism of our choice-onion cells and measured the forces involved in transporting organelles during cytoplasmic streaming. Cytoplasmic streaming has been previously shown to increase with temperature in plants, but the cause of this dependence is not known. In our study, we have measured the forces involved in this phenomenon at different temperatures in order to study the correlation of myosin force generation and temperature dependent increase of cytoplasmic streaming. It has been found that enhanced cytoplasmic streaming yielded higher growth of plants and better foliage. Understanding how this phenomenon is regulated can help us to develop plants that are able to utilize nutrients more efficiently, thus increasing their yield and survival.

References:

1. M. Tominaga et. al. *Curr Opin Plant Biol*, **2015**, *27*, 104.
2. M. Tominaga et. al. *EMBO J*, **2003,** 22, 1263.

P 19

Elucidation of the excited state dynamics of organoboron fluorophores from ortho-substituted phenolic Schiff bases

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Conformational relaxation of the flexible molecular skeleton and photoisomerization along C=N bond as major non-radiative pathways in solutions of a family of Schiff.[1-3] Chelation of metal ions, especially Al^{3+} and Zn^{2+} by these Schiff bases hinders such relaxation, leading to significant fluorescence enhancement, which is favoured in rigid microenvionments[1,4] The better performance of Al^{3+} than Zn^{2+}in this context prompts the quest for other elements, that may be even more efficient. Boron is a natural first choice, belonging to the same group as Al. In the present work, photophysical properties of three organoboron complexes, two of 2-((2-hydroxybenzylidene)amino)phenol (HBAP) and one of 2,2′-((butane-1,4-diylbis(azaneylylidene))bis(methaneylylidene))diphenol (salbn). are investigated. The organoboron complexes of HBAP, OB1 and OB2 are hardly fluorescent in non-viscous solvents, but exhibit fluorescence enhancement in glycerol implying that the effect of chelation is offset by the introduction of new nonradiative channels by the phenyl group, which is a rotor. However, OB3, the complex of salbn, which is devoid of such a rotor group, is found to be strongly emissive and exhibit room temperature phosphorescence.

References:

1. S. Dasgupta et al. *Phys. Chem. Chem. Phys.* **2021**, *23*, 19494.
2. A. Chowdhury et al. *Chem. Phys. Impact* **2021**, *3*, 100057.
3. J. Dobkowski et al. *Chemistry - A European Journal* **2015**, *21*, 1312.
4. T. Khan et al. *J. Phys. Chem. B* **2016**, *120*, 10319.

P 20

Thermally Activated Delayed Fluorescent Probe for Simultaneous Imaging of Lipid droplets and Endoplasmic reticulum

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Organelles, the essential subunits within eukaryotic cells, are crucial for carrying out various complex biological functions to sustain life.[1,2] Multiple organelle-specific tracker dyes are used to visualize organelles and elucidate their interactions. However, issues such as significant spectral cross-talk, high cytotoxicity, and complicated operational procedures often limit their effectiveness in live-cell imaging.[3] Thus, developing single fluorescent probe capable of simultaneously and selectively visualizing two organelles and their interactions can be a unique approach reducing the overall incubation time which in turn reduces the cytotoxic effects and spectral crosstalk. In this regard, despite this necessity, only a few probes or effective design strategies have been reported so far.[4] Conventional luminescence imaging may suffer from the autofluorescence from the surroundings. In this context, organic thermally activated delayed fluorescent probes could eliminate this shortcoming due to their long-lived emission properties.[5] Herein, we have developed a delayed fluorescent probe DCBPY having diphenylamine as the donor unit and ketocoumarin as the acceptor unit. DCBPY displayed delayed fluorescence properties due to a minimal singlet-triplet energy gap, as observed from the time-resolved spectroscopic studies. The aqueous dispersions of DCBPY nanoparticles displayed enhanced delayed fluorescence properties and a higher quantum yield (15%), and was employed for intracellular imaging. The self-assembled nanoparticles localised within lipid droplets (LDs) and endoplasmic reticulum (ER) simultaneously as confirmed from the colocalization studies with the commercially available tracker dyes. Further, we aim to study the inter-organelle interaction between ER and lipid droplets in luminescence as well as in time-resolved mode due to their close association in diverse physiological pathways.

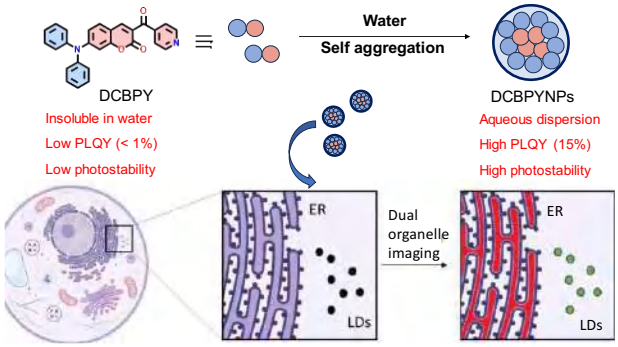

Figure 1: Schematic illustration depicting the fabrication of self-assembled nanoparticles of thermally activated delayed fluorescent probe DCBPY and its application as a dual organelle targeting probe in live cells.

References:

1. Gao et. al. *Chem. Sci*, **2019**,*10*, 6035
2. Hao Zhu et. al. *Acc. Chem. Res*, **2016**, *49*, 2115
3. Jaiswal et. al. *J. Mater. Chem. C*, **2022**, *10*, 6141
4. Guo et. al. *J. Am. Chem. Soc*, **2021**, *143*, 3169
5. Das et. al. *Chem. Sci*, **2024**, *15*, 102

P 21

Photoactivated plasmonic nanohybrid fibers with prolonged trapping of excited charge carriers for SERS analysis of biomolecules

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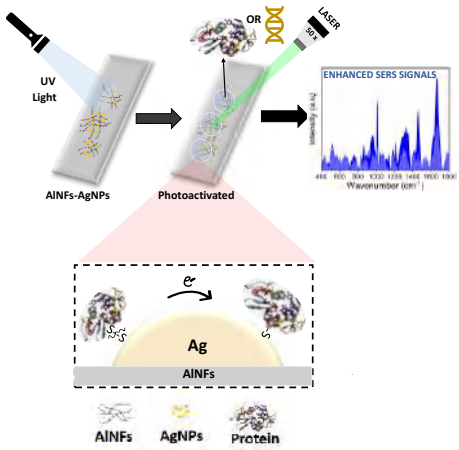
The pursuit of enhancing Raman spectroscopic signals through the strategic design of plasmonic substrates has facilitated the identification and characterization of pharmaceutically significant molecules with low-scattering cross-sections, including amino acids and proteins. This advancement has made significant strides in the realm of biomedical sciences. This study introduces a straightforward approach to fabricating silver nanoparticles-incorporated alumina nanofibers (Ag–AlNFs) using controlled microwave synthesis. The aim is to amplify the surface-enhanced Raman chemical enhancement factor by inducing photo-induced charge accumulation at the plasmonic–dielectric interface. The plasmonic–dielectric fibers effectively trap charge carriers, as evidenced by ultrafast transient absorption spectroscopy studies. Beyond chemical enhancement, the augmented electronic surface charge facilitates the capture of protein disulfide bonds by these electrons, forming a transient disulfide electron adduct radical that transforms into a free thiol radical upon dissociation. This mechanism enables protein molecules to bind to the nanoparticle's surface via a favorable silver thiol bond, resulting in enhanced surface affinity and greater SERS enhancement. The proposed Ag–AlNFs offer a cost-effective material that holds the potential for label-free probing of biological systems by photoactivating the SERS substrate to achieve higher enhancement factors.



Scheme: Schematic for the enhanced chemical enhancement in protein Raman signals after shining UV light on Ag-AlNFs substrate

References:

1. S. Ben-Jaber et. al. *Nat Commun*, **2016**, 7, 12189.
2. Q. D. Mai et. al. *ACS Appl. Nano Mater*, **2022**, 5, 15518.

P 22

Pyrediyne Quantum Dots (PDYQDs) in gel

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Quantum dots (QD) are zero-dimensional semiconductor nanocrystals that has distinct mechanical, catalytical, electrical and optical properties when compared to their corresponding bulk materials. The unique properties of the traditional semi-conductor based quantum dots were analysed in different media and exploited in practical applications such as solar cells, light-emitting devices (LEDs), computer tomography, magnetic resonance imaging, bio-imaging and etc.[1] However, cytotoxicity, low thermal stability and presence of surface defects, limits the applications of traditional semiconductor quantum dots.[2] Even though a core-shell nanostructure can reduce surface traps and consequently the blinking effect, widely used core-shell QDs contains cytotoxic moieties such as cadmium and selenide.[3] To overcome these flaws, PDYQDs, the newest member of the carbon quantum dots family was synthesized. Unlike its bulk counter-part, PDYQDs exhibited good photoluminescence properties such as appreciable quantum yield, high fluorescence lifetime and solvent sensitive emission behaviour. Photophysical properties of QDs can be enhanced further by incorporation of functional groups, structural modification and immobilization of quantum dots in a gel medium. Thus, in the current study PDYQDs (figure 1a) were incorporated into a transparent, thermo-reversible leucine based organogel (OG-leucine) (figure 1b) and the photophysical properties of PDYQDs in gel media were analysed using steady-state and time-resolved fluorescence measurements. OG-Leucine gelator and PDYQDs were synthesized according to a reported literature protocol.[4,5] As shown in the figure 1c, PDYQDs exhibits excitation wavelength dependent emission behaviour in ethanol due to the presence of QDs with varied size and surface defects.[4] The emission peaks at 410 to 430 nm are the characteristics of the monomeric precursor of the PDYQDs, tetraethynyl pyrene (TEP). The longer wavelength emission peaks are observed as a result of QDs with different sizes and formation of static dimers.[4] However, PDYQDs did not exhibit excitation wavelength dependent emission properties and the longer wavelength emission peaks are diminished in gel media (figure 1d). The emission spectra of the PDYQDs resembles the emission spectra of PDYQDs in toluene (figure 1e) which implies that the QDs resides in the toluene pool of the gel, the uniformity of QDs size is enhanced, the formation of static dimers was restricted in gel media. It is evident from the current study that incorporating pyrediyne quantum dots in gel media aided in diminishing the non-uniformity in QDs particle size. The methodology can be extended in future to obtain size-controlled defect free QDs with narrow emission bandwidth which can be used for various applications.

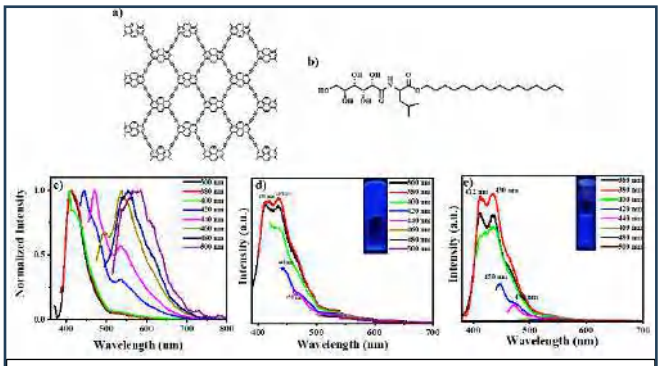

Figure 1. Structure of PDYQDs (a) and OG-leucine (b), emission spectra of PDYQDs in ethanol (c), toluene (d) and (e) at different excitation wavelengths.

References:

1. D. Bera et. al. *Mater,* **2021**, 4, 2260.
2. H. Moon et. al. *Adv. Mater*, **2019**, 34, 1804294.
3. A. M. Derfus et. al. *Nano Lett,* **2004**, 1, 11.
4. J. S. Arya et. al. *Adv. Mater.Interfaces,* **2020**, 15, 1902209.
5. N. Minakuchi et. al. *Langmuir,* **2012**, 25, 9259.

P 23

Carbon Quantum Dots: A Promising Factor for Hydrophobic Drug Delivery and Live Cell Imaging

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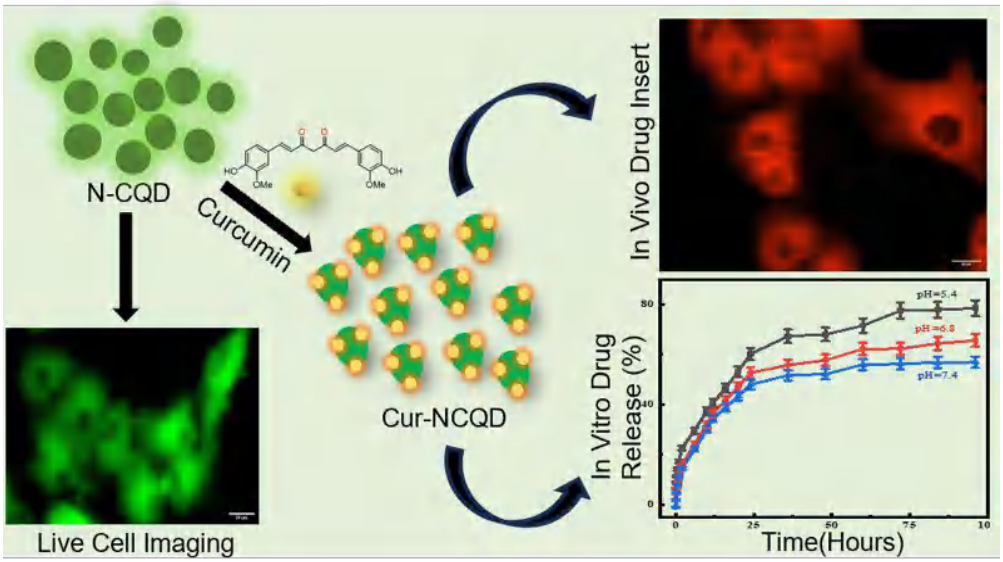
Due to unique physicochemical properties like excellent biocompatibility, photostability, and surface functionality, Carbon Quantum Dots (CQDs) nowadays emerged as a promising nanomaterial for the delivery of hydrophobic drugs and for live cell imaging.[1] My study investigates the potential of N-doped CQDs (N-CQDs) as carriers for hydrophobic drug molecules such as Curcumin, which alone often face challenges like bioavailability and efficacy due to its chemical instability, poor absorption, and rapid systematic elimination.[2] The N-CQDs produced in this study exhibit excellent water solubility, remarkable stability, and high biocompatibility. By functionalizing N-CQDs with hydrophobic drugs, the nanocarriers significantly improve drug solubility, enhance cellular uptake, and allow controlled release within targeted cells.[3] Simultaneously, the intrinsic fluorescence of N-CQDs and cur-NCQs enables high-resolution imaging of live cells. Experimental findings on live cells demonstrate the efficacy of N-CQDs in overcoming the solubility limitations of hydrophobic drugs within the live cancer cell without inducing cytotoxicity. This dual function of N-CQDs in both drug delivery and live cell imaging can make it as a versatile platform for advancing therapeutic delivery systems and diagnostics in nanomedicine.[4]

Graphical



References:

1. Jana et. al. *Mater. Today Commun*, **2022,** *32*, 104068.
2. Sohn et. al. *Pharmaceutics*, **2021,** *13* (12), 2102.
3. Guo et. al. N*anomaterials*, **2024,** *14* (13), 1088.
4. Rong et. al. *Int J Nanomedicine.*, **2021**, 16, 7195.

P 24

Effect of commercial fertilizers in the growth of microgreens: Through the Terahertz lens

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Microgreens have become increasingly popular as a nutrient-rich food source, requiring only 7-14 days to harvest, with the global market expected to grow at a CAGR of 11% to USD 2.6 billion in 2031 from 2022 [1]. With seeds readily available in household kitchens and accessible growth media such as water, tissue-paper, coir, soil, microgreens are being widely cultivated in kitchen gardens due to their short shelf lives and cost effectiveness. Terahertz (THz) radiation, spanning the 0.1 to 10 THz, has shown promise in agriculture due to its low ionization energy, which is harmless to plants, its high sensitivity to substances with unique spectral fingerprints, and its absorption properties in the presence of polar molecules like water.

This work, therefore, investigates the growth dynamics of various microgreens, with and without the application of commercially available fertilizers containing nitrogen, phosphorus, and potassium, using a THz time-domain imaging system in a transmission mode to monitor their growth in terms of girth and length of primary roots. The microgreens examined include chia (*Salvia hispanica*), mustard (brown, *Brassica juncea*; yellow, *Sinapis alba*), fenugreek (*Trigonella foenum-graecum*), and red lentils (*Lens culinaris*). Preliminary results indicate that THz imaging effectively distinguishes the growth patterns of plants in different media, proving to be an effecting tool in the growth and health monitoring of microgreens. Future studies will extend this research to explore the role of THz in evaluating the impact of nutrient variation and fertilizer dosage on the maturity and development of microgreens, aiming for a detailed analysis.

References:

1. Straits Research. *Microgreens market analysis, growth, forecast to 2030* (Report Code: SRFB1654AB). Straits Research. (**2023, December 15**). https://straitsresearch.com/report/microgreens-market (Accessed: September 8, 2024)

P 25

Deciphering effect of having different branch lengths in Gold nanostars in their ability to avoid sequestration by protein-corona

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Gold nanoparticles (AuNPs) have a strong potential to be used as nanocarriers for diagnosis and therapeutics due to their biocompatibility, stability, and robust surface chemistry [1,2]. The aptamer-loaded AuNPs, for example, have emerged as promising nanoconstructs targeting specific receptors on the cancer cell membrane. These nanoconstructs, however, need to overcome the sequestration effect caused by the non-specific adsorption of serum proteins (i.e., formation of protein-corona) on their surface to efficiently target specific receptors involving NP-surface ligands [3]. Therefore, designing AuNPs that can circumvent the effect of protein corona to target cancer cells effectively is crucial. Several recent studies have indicated that anisotropic gold nanostars (AuNS) offer benefits over spherical gold nanoconstructs in projecting out the functional moieties, even in the presence of protein corona [3-5]. Anisotropic AuNS particles have branches of different lengths that taper to form sharp tips. These sharp branches likely play a key role in reducing the impact of the protein corona. To determine the best designs, it is essential to evaluate how different branch lengths influence the receptor-targeting efficiency of AuNS. However, performing this comparative analysis is challenging as the seedless synthesis of anisotropic AuNS results in a diverse mixture of star-shaped nanoparticles with uneven branch lengths and quantities. In this study, we have successfully synthesized AuNS with precise control over their branch lengths and branch numbers. We then functionalized them with DNA aptamer molecules capable of targeting Human Epidermal growth factor Receptor 2 (HER2) on cancer cell membranes to trigger apoptosis. TEM imaging clearly showed that the longer branches in AuNS were efficiently protruding out of the protein corona. The *in vitro* cytotoxic efficacies of these AuNS were then compared with that of anisotropic AuNS and spherical AuNPs, which clearly showed that the nanoconstructs with longer branches were significantly more efficient in causing cytotoxicity in HER2 overexpressing SKOV3 cells. These AuNPs, when functionalized with a control aptamer (with no HER2 specificity), did not cause any cytotoxic effect, as expected. This research illuminates the complex interplay between the branch lengths of AuNS and their effectiveness in targeted drug delivery, providing valuable insights for developing more efficient nanomedicines in the future.

References:

1. A. E. Nel et. al. *Nat Mater*, **2009**, 8, 543.
2. P. Kumthekar et. al. *J Clin Oncol*, **2019**, 37, 3012.
3. D. Bhowmik et. al. *ACS Nano*, **2019**, 13, 13637.
4. P. Choo et. al. *J Am Chem Soc*, **2021**, 143, 4550.
5. B. Diloknawarit et. al. *ACS Nano*, **2024**, 18, 12537.

P 26

Enzyme-free Detection of Uric Acid using Fluorescent Carbon Quantum Dots

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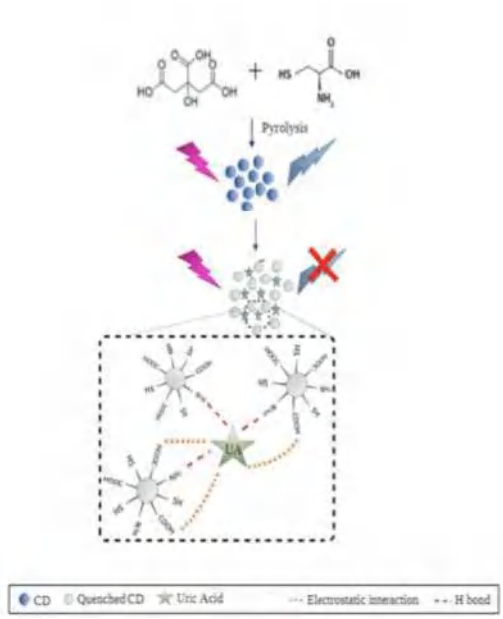
Since uric acid (UA) is involved in several metabolic pathways, abnormal levels of it in the human body is indicative of disease conditions like gout, Alzheimer's, multiple sclerosis, and renal failure, among others [1]. It also plays a role in pro-inflammatory pathways [2]. The conventional and standard methods for UA detection utilise enzymes, which poses difficulties in remote areas and point-of-care applications, due to the specific transport and storage conditions they require. This work focuses on developing carbon quantum dots (CQDs) that can detect the target analyte without enzymatic reaction through a fluorescence-based detection approach. We synthesised blue emitting fluorescent CQDs following one-pot pyrolysis method, further characterization of the CQDs were done with spectroscopy and microscopy. Upon analysis with UA-CQDs, we found an interesting fluorescent behaviour that showed fluorescence quenching at 390nm excitation wavelengths. The limit of detection (LOD) obtained was 96.32µM for UA. The observed finding suggests a potential application of the synthesised CQDs in developing an optical-based UA biosensor, without enzymes.



References:

1. Youssef M. Roman. *J. Pers. Med*, **2023**, *13*, 1409.
2. Mihai-Emil Gherghina et. al. *Int J Mol Sci*, **2022**, *23*, 3188.

P 27

Novel Lipid Corona Formation around Nanoparticles

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While protein corona formation is well-known, the "lipid corona" is relatively new and its dependency on lipid property and stability is yet to be explored. At first, we reported a novel lipid corona formation and its underlying mechanism using aromatic amino acid-functionalized gold nanoparticles (Au-AA NPs). Our study demonstrated that in the presence of high lipid concentration, the Au-AA NPs intrinsically tow the lipid molecules from the lipid vesicles and decorate themselves by lipid leading to unique lipid corona formation. In contrast, at low lipid concentration, the Au-AA NPs underwent lipid-induced aggregation. Significantly, we found that the colloidal property of these lipid-coated nanoparticles (lipid corona) was immune to resist extreme harsh conditions, that is, high acidic pH, several repetitive freeze–thaw cycles, and high salt concentration. Next, we investigated the effect of lipid phase states on lipid corona formation and lipid-induced aggregation of phenylalanine-functionalized gold NPs (Au-Phe NPs). Based on the stability, for the first time, we classify lipid corona as "hard lipid corona" and "soft lipid corona".

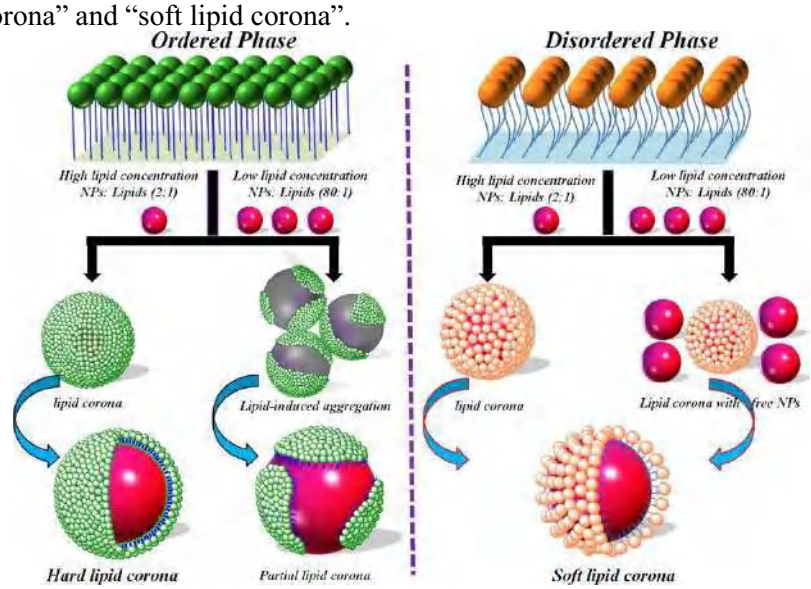

***Figure**: Schematic representation of lipid corona formation around nanoparticles in presence of different concentration and phase state lipid vesicles.*

References:

1. Avijit Maity et al. *J. Phys. Chem. B,* **2021**, *125,* 2113.
2. Avijit Maity et al. *J. Phys. Chem. B,* **2022**, *126,* 2241.

P 28

Development of A Novel Mitochondria Targeted BODIPY-Based Macrocyclic Ratiometric Fluorescence Sensor for Mn^{2+} ions

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Mn^{2+} ions play crucial roles in living systems including regulating immune response, brain function, metabolism, defence against oxidants, and photosynthesis [1]. Thus, tracking this essential metal ion is important to elucidate the *in cellulo* localization and dynamics of Mn^{2+} ions both under physiological conditions and pathological disorders. However, poor binding affinity of Mn^{2+} ions to ligand-scaffolds and fluorescence quenching by the metal ion leads to turn-off sensors that are not applicable for in vivo imaging. Datta and co-workers have reported boron-dipyrromethene (BODIPY)-based penta-aza macrocyclic "turn-on" fluorescent sensor **M1**, and a water soluble, cell permeable sensor **M4** to visualise the manganese dynamics in live mammalian cells [2,3]. The next step in Mn^{2+} sensing is taking these probes to the intra-organelle level to track the dynamics of Mn^{2+} ions within cellular compartments. We are developing a novel, water-soluble, reversible, Mn^{2+} ion selective ratiometric fluorescent sensor, **A2**, which targets the mitochondria of living cells. The design, synthesis, and characterization of the probe will be presented.

References:

1. K. Barnese et al. *Proc. Natl. Acad. Sci. U.S.A*, **2012**, *109*, 6892.
2. S. Bakthavatsalam et al. *Chem. Commun*, **2015**, *51*, 2605.
3. S. Kahali and S. K. Das et al. *Chem. Sci*, **2024**, *15*, 10753.

P 29

Photocatalytic Oxidation of Organic Sulfides inside Water-soluble Nanocages

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Aromatic sulfoxides are very important building blocks for the synthesis of fragrances, and pharmaceutical ingredients[1]. Traditionally the synthesis of sulfoxides generally requires stoichiometric use of oxidizing agents (H_2O_2), metal oxides, and photosensitizers[2]. Although they give moderate to good yield in reaction turnovers but the often associated with the formation of toxic sulfone as side product and use of transition metal-based catalyst and organic solvent generate toxic wastes. To address these challenges and to synthesize aromatic sulfoxides, we have chosen to synthesize a supramolecular cage with a capping ligand. We focused into utilizing the donor-acceptor CT interactions formed between electron-rich aromatic sulfide molecules and electron-deficient cavities. The cavity synthesis was done in three steps as per the literature protocol[3]. We optimized the reaction condition for various substrates with achiral nanocage and after optimization of the photocatalytic reactions, we found the selectivity of sulfoxide products going up to 100 % from GCMS with TON reaching up to 50. We wanted to know about excited state dynamics of this reaction for that we probed the formation of radical cations on the sulfides[4] and anion radicals on the triazine panel[5] from transient absorption measurements that suggest a single electron transfer event.

References:

1. X. Yin. *Organics*, **2023**, 4, 173
2. E. Skolia. *Chempluschem*, **2022**, 87
3. Y. Nishioka. *J. Am. Chem. Soc*, **2008**, 130, 8160
4. S. Tojo. *RSC Adv*, **2016**, 6, 109334
5. Y. Furutani. *J. Am. Chem. Soc*, **2009**, 131, 4764

P 30

Biophysics and Spectroscopy in Tandem: Investigating Structural Stability And New Functionalities Of YedX

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Functional amyloids are a special class of amyloidogenic proteins that serve an important biological function, unlike disease-related amyloids [1]. One such bacterial functional amyloid is curli, produced by *Escherichia coli* during biofilm biogenesis. Biofilm is a complex, well-structured bacterial community that adheres to various surfaces and is encased in a self-produced extracellular matrix (ECM). ECM is majorly composed of polysaccharides, lipids, and proteinaceous heteropolymers of curli-specific proteins [2]. The precursors of curli, CsgA and CsgB are produced in cytoplasm as intrinsically disordered proteins (IDPs). CsgA and CsgB travel to periplasmic space and then secreted out in extracellular space where they polymerise and form amyloid fibrils to strengthen the biofilms [2]. A class of molecular chaperones inside the cytoplasm keeps a check on CsgA and CsgB oligomerisation inside the cell to avoid cytotoxicity [3]. In the periplasmic space, another protein belonging to csg operon, CsgC, keeps CgsA and CsgB in natively unfolded structure. CsgC has also been studied to modulate human amyloidogenic proteins such α-synuclein and reported to prevent its aggregation [3]. Recently, CsgC has been reported to possess structure similarity to human transportational protein, transthyretin (TTR) [4]. TTR also has been studied to possess anti-amyloid activity against bacterial amyloids such as CsgA and CsgB as well as human amyloidogenic proteins such as Aβ [5]. Transthyretin-related proteins (TRPs) are a group of proteins that exhibit sequence and structural similarity to TTR and exist in a wide range of species [6]. TRP from *E. coli*, also known as YedX, is also localized in the cytoplasm and function as hydrolase in purine metabolism [6]. Since TTR and YedX exhibit high structure similarity, we hypothesize that YedX may also have other biological functionalities. In this study, we have utilized various spectroscopy techniques such as UV absorbance and fluorescence spectroscopy and circular dichroism spectroscopy to evaluate the stability of YedX and its anti-amyloid activity. Our biophysical and biochemical studies suggest that YedX modulates the CsgA amyloid assembly and keeps CsgA in its native soluble form. Our findings hypothesize the unrevealed function of YedX, previously known as a hydrolase enzyme, to now we shed light on the possible chaperone-like activity of YedX, which is also present in the cytoplasm and may aid the stable protein homeostasis within the cell.

References:

1. Balistreri et al. *Microorganisms*, **2020**, *8*, 1951.
2. Hung et al. *mBio*, **2013**, *4*(5), e00645-13.
3. Evans et al. Prion, **2011**, 5, 323–334.
4. Jain et al. PNAS, **2017**, 114, 12184.
5. Costa et al. FEBS Letters, **2008**, 582 1873.
6. Eneqvist et al. Eur J Biochem, **2003**, 270, 518.

P 31

Living Biosensors for Selective and Ultrasensitive Detection of Biomarker Nitric Oxide

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Nitric oxide (NO) can be defined as an essential disease marker relevant to numerous chronic inflammatory conditions and malignancies. A fully characterized nitric indicator system is helpful for the prompt advancement of bacterial treatment and synthetic biology. In this work, we designed a group of NO-inducible biosensors with the help of the PnorV promoter and its NorR regulator in the norVW operon and characterized and optimized the circuits in Escherichia coli.

Here we have taken advantage of synthetic biology tools for generating an array of genetically engineered whole cells. Along with this, we describe a set of modular and gain-tunable genetic amplifiers constructed in Escherichia coli that can amplify transcriptional signals with a high control in the cascaded-gene networks. The devices are constructed by using hrpRS, hrpV, and PhrpL which are orthogonal genetic elements from the hrp gene regulatory network in Pseudomonas syringae.

Since whole-cell biosensors (WCBs) have great potential to specifically detect these compounds and provide information about the bioavailability and toxicity of these di-atomic compounds on human health. The combinatorial approach of synthetic and structural biology has enabled us to design “living sensors” with fluorescence outputs. However, initial testing revealed poor detection sensitivity, which was addressed by incorporating transcriptional and translational synthetic control modules. These modules significantly reduced background fluorescence and shifted the sensor response threshold to ~sub nanomolar concentrations, while augmenting the dynamic range.

Quantitative NO estimation directly from whole cells can open new avenues for detecting NO at the cellular level in situ. Through site-directed mutagenesis of the active centre, the activity of the protein can be increased leading to enhanced outputs. It is one of the methods for in-situ detection of such compounds/gases. This will be a low-cost biosensor suitable for on-site monitoring of a range of such compounds.

P 32

The disordered C-terminal region of eukaryotic translation initiation factor 4B dynamically binds RNA

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The eukaryotic translation initiation factor 4B (eIF4B) plays an essential role in translation by enhancing the eIF4A helicase activity in unwinding the structured motifs of mRNA. It is particularly crucial for the translation of mRNAs with long and structured 5'-end untranslated regions, such as those coding for many proto-oncogenes. The eIF4B is predicted to be predominantly disordered, except the folded RRM domain. The disordered C-terminal half of eIF4B (eIF4B-CTR) is essential for RNA binding, as it possesses a non-canonical RNA binding motif, enriched in arginine residues. However, the exact mechanisms of these interactions are currently unknown.

Hence, we characterized the eIF4B-CTR alone and in complex with RNA, employing a combination of single-molecule Förster resonance energy transfer (smFRET), nanosecond fluorescence correlation spectroscopy (nsFCS) and nuclear magnetic resonance (NMR) spectroscopy. Our results show that eIF4B-CTR is disordered and flexible, with significant presence of intrachain interactions [1, 2]. The RNA binding to eIF4B-CTR is highly dynamic, with fast exchange between the molecules forming the complex, and is highly sensitive to ionic strength, implying an electrostatic mechanism of interactions. Upon RNA binding, the N-terminus of eIF4B-CTR compacts, whereas the C terminus expands. After binding to RNA, eIF4B-CTR remains disordered. The interaction of eIF4B-CTR is dependent on the length and sequence of the RNA. The eIF4B-CTR has higher binding affinity for single stranded RNA than double stranded RNA suggesting binding preference to single stranded RNA over structured RNA. Overall, our study provides detailed understanding of the RNA interaction with eIF4B-CTR.

References:

1. S. Mondal et al. Biomol. NMR Assign, **2023.** 17, 199.
2. B. C. Swain et al. bioRxiv, **2024**, 2024.06.21.600094.

P 33

Ion Concentration Effects on Conformational Dynamics of DNA Holliday Junctions: Insights from Fluorescence Correlation Spectroscopy

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DNA Holliday junction (HJ) is a four-way-stranded DNA intermediate that plays a crucial role in genetic recombination. It exists in generally two configurations, i.e., "X-stacked" and "open planar". The conformational changes of HJ depend on the electrostatic interactions of negatively charged chains and salt ions.[1] To enhance the stability of these configurations, Mg^{2+} ions are utilized.[2,3] It has been already reported that at lower ion concentrations HJ exchanges its conformation faster between *iso-I* and *iso-II* whereas at higher concentrations stacked conformation is prominent.[4] In this work, we used fluorescence correlation spectroscopy with different ion concentrations of Mg^{2+} to investigate the diffusion and conformational dynamics on a millisecond time scale.[5] We have found that as the concentration of Mg^{2+} increases the diffusion of DNA HJ decreases. This decrease observed can be due to multiple factors such as increase in the hydrodynamic radius, viscosity of the solution, and ion acting as a crowding agent as it is present in very high concentration. In our study, we have mainly focused on the interactions of DNA HJ phosphate backbone with $Mg^{2+}$and after the binding how hydrodynamic radius increases resulting in slower diffusion. Also, we have observed the conformational dynamic components in µs time scale. There are three components that we assume correspond to three different isomeric conformations.

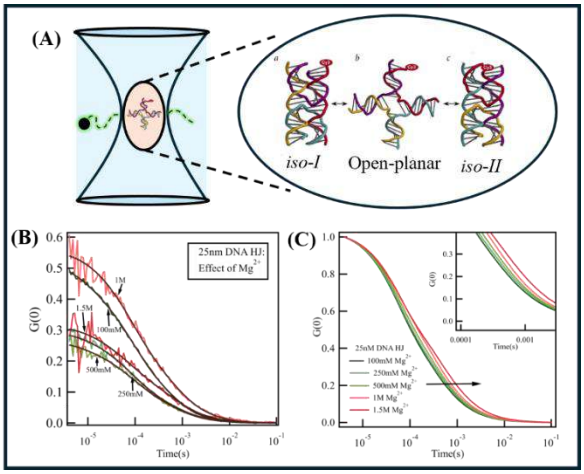

Figure: (A) Diffusion and conformational dynamics of DNA HJ through confocal volume in fluorescence correlation spectroscopy. **(B)** Unnormalized autocorrelation curves of 25 nM DNA HJ in 100 mM, 250 mM, 500 mM, 1 M and 1.5 M of Mg^{2+}. **(C)** Normalised and fitted autocorrelation lines of 25 nM DNA HJ in 100 mM, 250 mM, 500 mM, 1 M and 1.5 M of Mg^{2+}.

References:

1. Qinqin Song et al. *Int. J. Mol Sci*, **2022**, 23, 9730
2. Richard Owczarzy et al. *Biochemistry*, **2008**, 47, 5336
3. Chirlmin Joo et al. *J Mole Biol*, **2004**, 341, 739
4. Sahil Hasan Kabir et al. *bioRxiv*, https://doi.org/10.1101/2024.08.23.609282
5. Dibyendu Kumar Sasmal et al. *J. Phys. Chem. B*, **2011**, 115, 13075

P 34

Designing a bioluminescent fusion protein switch for detection of bacterial virulence factors

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Many pathogenic bacteria responsible for deadly diseases require rapid identification for timely prevention and treatment. Diverse methods have been developed, differing in sensitivity, specificity, cost, and duration, and are based on serological, microbiological, visual or optical, nucleic acid-based, or biosensor-based techniques.[1] In particular, cell-free synthetic biology approaches are highly appealing due to their compactness, low cost, portability, and sensitivity.[2] Herein, we describe the design of a cell-free protein synthesis/IVT-based platform that exploits the transcription regulator RfaH to control the expression of a small subset of genes involved in bacterial virulence across several pathogenic strains.[3] During its functional cycle, RfaH transitions from an auto-inhibited, closed state to an active, open state.[4] This conformational change, along with its DNA-binding specificity during the transcription of target genes, makes RfaH an attractive candidate for detecting genes associated with virulence factors in various pathogenic bacteria. In this study, we present a luminescent reporter construct of RfaH (NanoBiT-RfaH) capable of detecting virulent genes in bacterial species such as *E. coli*, *Salmonella*, *Shigella flexneri*, *Yersinia enterocolitica*, *Vibrio cholerae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* that exploits the open-to-closed conformational conversion reaction.

References:

1. M. Nemergut et. al. *Nat Commun*, **2023**, 14, 7864.
2. A. Shakhmin et. al. *Org. Biomol. Chem*, **2017**, 15, 8559.
3. E. Eremeeva et. al. *Int J Mol Sci*, **2020**, 21, 5446.
4. P. K. Zuber et. al. *Nat Commun*, **2019**, 10, 702.

P 35

Slow relaxation of oil film on water-air interface

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The behavior of droplets deposited on substrates is critical to a wide range of applications, including coating technologies, inkjet printing, microfluidics and phytosanitary treatments. Understanding the dynamics of oil spread on calm water is an active area of research, driven by the need for better control of oil pollution. In this work, we examine the instability that results from the deposition of a two-component oil droplet consisting of vegetable oil and a volatile alkane on a water-air interface. Presence of the volatile alkane forces the vegetable oil drop to spread into a thin film of a much larger radius than its equilibrium radius. The ensuing spatiotemporal evolution of the oil film is recorded and examined, demonstrating the existence of a radial flow that pushes the droplets towards the water bath's edge. Eventually the oil drop breaks into several smaller droplets. We study the relaxation dynamics of the oil film forced to spread on the water surface towards the equilibrium configuration consisting of tiny droplets. The boundary plays a critical role in this process, with the binary mixture exhibiting different wetting behaviors depending on the size of the water bath. After interacting with the boundary, the liquid forms a thin layer over the interface, which eventually undergoes dewetting. The system's propensity to minimize surface energy drives the formation and coalescence of holes as a result. Interestingly, the binary mixture sometimes enters a dynamic loop, oscillating between minimizing surface area and other parameters, which lengthens the time required to reach equilibrium. Analytic scaling laws provide an adequate description of the intricate relationship between fluid dynamics, wetting, and evaporation.

P 36

Looking for Alternative to BODIPY: A Mn Selective Fluorescence Sensor Containing a Naphthalimide Dye

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Manganese, an indispensable trace metal crucial for all living organisms, plays a pivotal role in the catalytic function of multiple metalloenzymes, sustains brain function, acts as an antioxidant, and engages in various metabolic processes.[1] Excessive accumulation of Mn can lead to a neurological disorder resembling Parkinson's disease, known as Manganism.[1] Therefore, elucidating Mn ion homeostasis under physiological and pathophysiological conditions is necessary. Detecting Mn^{2+} ions within living cells requires a non-invasive technique with precise spatial (μm) and temporal (ms) resolution. Fluorescence confocal microscopy fulfils these criteria. However, designing a selective Mn^{2+} binding ligand is challenging due to the low affinities of Mn^{2+} ions for most ligands with N-, O-, and S-donor atoms. Additionally, the paramagnetic nature of Mn^{2+} ions can quench fluorescence. Our group has successfully addressed these challenges by developing Mn^{2+} ion sensors, utilizing BODIPY as a responsive unit and a PeT-based sensing modality.[2,3] Due to challenges associated with BODIPY, such as synthetic complexity and limited photostability, we are exploring the use of naphthalimide dyes as an alternative.[4] Naphthalimide offers advantages like chemical stability, easy synthesis, higher photo-stability, tuneable emission wavelength, and increased fluorescence quantum yields.[5] In this context, we have designed a Mn^{2+} responsive fluorescence sensor using Naphthalimide dye as the reporter unit. In my poster, I will present the design, synthesis, characterization, and in vitro studies of the novel sensor.

References:

1. Das, et.al. A. *Dalton Transactions* **2019**, *48* (21), 7047–7061
2. Bakthavatsalam, S.et.al. *Chemical Communications* **2015**, *51* (13), 2605–2608.
3. Kahali, S.et.al. Chemical Science **2024**, *51*, 1007–1017.
4. Wüstner, D.et.al. D *Molecules* **2014**, *19*, 11096-11130
5. Kagatikar, S.et.al. *J Mater Sci* **2022**, *57, 105–139*

Spectroscopic study of Thiamine and its detection using HPLC LED-IF

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Thiamine, also known as Vitamin B1, is a water-soluble nutrient found in various food items such as fish, meat, nuts, seeds, beans, soy products, etc. It is one of the eight essential B vitamins for the regular functioning of the central and peripheral nervous systems. It helps the body's conversion of food (fat, carbohydrates, and protein) into energy. The structural formula for thiamine is 2-methyl-4-aminopyrimidine linked to a thiazole ring by a methylene group.
The detection of thiamine is mainly done by the fluorescence. Even though thiamine is a weakly fluorescing molecule, it can be oxidized to form thiochrome, which is strongly fluorescing, using suitable derivatizing agent. Potassium ferricyanide is the most commonly using derivatizing agent. Thiochrome is unstable and degraded when exposed to light and heat. So, it has to be kept in dark and temperature-controlled environments. The stability and fluorescence intensity of thiochrome also depend on the pH [1,2].

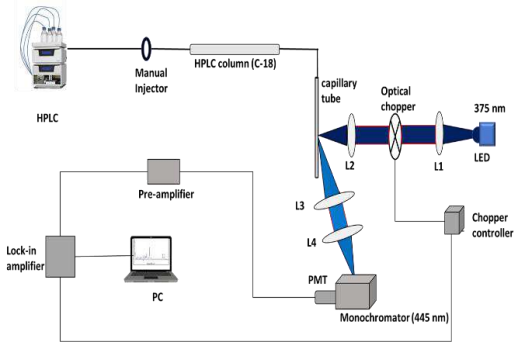

Figure 1: Schematic diagram of HPLC with fluorescence detection.

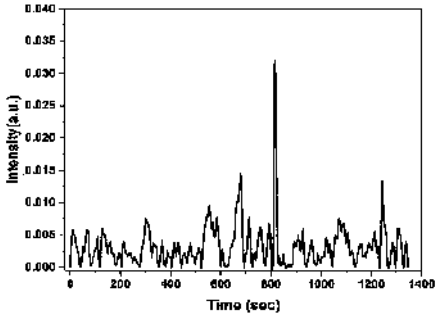

Figure 2: Chromatogram of 10 nM Thiochrome

HPLC (High Performance Liquid Chromatography) methods are generally used for the detection and quantification of metabolites including thiamine. Pre-column derivatization combined with fluorescence detection is the most common method [3]. We propose the HPLC LED-IF (LED Induced Fluorescence) technique for the quantification of thiamine.

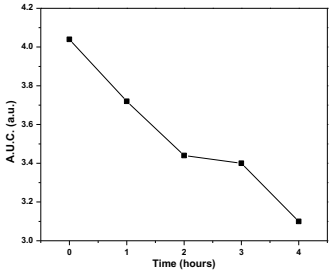

Figure 3: Plot of A.U.C. of chromatogram of 150 nM thiochrome v/s time after derivatization

We were able to detect up to 10 nM concentrations of TDP (Thiamine diphosphate) using the developed system. We have also done the stability study of thiochrome. It was observed that fluorescence intensity is decreasing even after 1 hour of derivatization.

References:

1. Edwards KA et.al. ChemistryOpen. **2017**;6(2):178-91.
2. Kraft CE, et.al. The Quarterly Review of Biology. **2017** Jun;92(2):151-168.
3. Mathew EM et.al. Biomed Chromatogr. **2019**;33(11): e4668.

P 38

Non-Fano-Type Wavelength and Power-Dependent Raman Manifestation of Resonant Electron-Phonon Interaction

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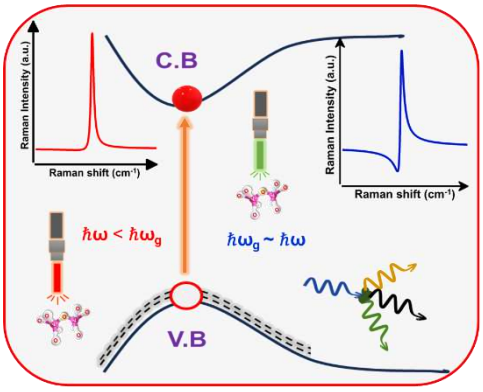
In semiconductors in particular, the existence of Fano resonance in a system frequently turns out to be highly helpful in understanding various electronic and quantum properties of materials. It is challenging to definitively detect its presence and nature because other factors/processes also affect them, even if Fano-type properties, such as asymmetry and antiresonance in spectral line shape, make them identifiable using Raman spectroscopy. When combined with the relevant theoretical analysis, a wavelength- and power-dependent Raman scattering experiment in orthorhombic V2O5 demonstrates the resonant nature of electron–phonon interaction in the Ag Raman mode (994 cm^{-1}). The presence of a Fano interaction is supported by the asymmetric Raman line shape with an antiresonance dip and an electronic Raman background. The electron-phonon coupling strength is quantified by the Fano coupling parameter (q) through theoretical fitting of experimental data. The resonant character of the Fano interaction has been identified by using the excitation wavelength-dependent Raman spectra, which seem to contradict the Fano-type behavior. Because anharmonic effects are involved, the Fano interaction becomes weaker as the excitation power increases.[1]



References:

(1) Rath, D. K. *et.al. J. Phys. Chem. C* **2024**, *128* (36), 15186–15193.

P 39

Unusual Stability of Metastable Vesicles Evolving during the Self-Assembly of Phenylalanine

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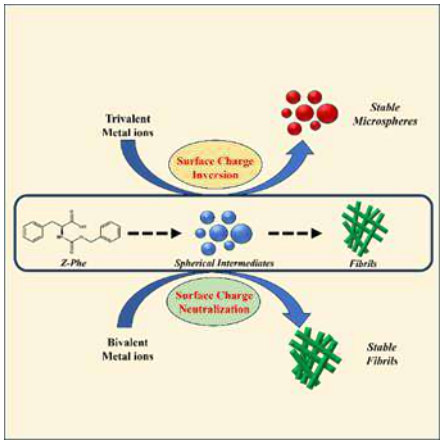
Biomolecular self-assembly is a ubiquitous process in nature, and the self-assembly of proteins and peptides has attracted immense interest due to their ability to form various nanostructures with important cellular organizations and functions. However, the underlying mechanism of the self-assembly process remains elusive despite numerous reports. In this context, a reductionist approach is targeted at understanding the self-assembly of the amino acids, as they serve as the simplest building blocks. Here, we have investigated the self-assembly formation of carboxybenzyl (Z)-protected phenylalanine (ZF). The intermediates formed during the self-assembly process can be stabilized by tuning the metal ion–amino acid interaction. Microscopic and spectroscopic investigations of the self-assembly of ZF reveal that the bivalent metal ions do not affect the self-assembly, as it eventually leads to the formation of fibrillar networks similar to blank ZF. Interestingly, the trivalent ions develop vesicle-like intermediates that do not undergo fibrillation for a prolonged time. The unusual stability of the vesicle-like intermediates in the presence of selective metal ions is well rationalized with the metal ion coordination, metal ion-specific entropy factor, and excess hydrophobicity induced by the trivalent metal ions. Furthermore, the time-lapse measurement of surface charge reveals that the surface charge of blank ZF and in the presence of bivalent metal ions changes from a negative value to zero, implying unstable intermediates leading to the fibril network. Strikingly, a prominent charge inversion from an initial negative value to a positive value in the presence of trivalent metal ions imparts unusual stability to the metastable intermediates.



References:

1. Bagchi et.al. *J. Phys. Chem. Lett.* **2024**, *15*, 4468.

P 40

Oxidative redox intermediates of phenalenone inside water-soluble nanocages

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Redox chemistry is widely used in biological processes e.g. photosynthesis where several enzymes take part in catalytic and electron transfer reactions. Chemists have mimicked the enzymes by creating supramolecular cavities which can serve as active sites for catalysis[1]. In this regard, our group has demonstrated host-guest charge transfer (CT) mediated chemistry to generate cage-confined neutral radicals which can be exploited for catalytic events[2,3]. Hence, we thought to use this host-guest CT paradigm to get access to oxidative intermediates of phenalenone (PLY) chromophores, which have been used previously for carrying out photocatalytic reductive transformations in organic solvents[4]. For this, we have used $Pd_6L_4^{12+}$ supramolecular nanocages to demonstrate a photocatalytic route towards the generation and stabilization of radical cation and neutral radical intermediates on the PLY backbone. Steady-state absorption shows the formation of CT states after PLY encapsulation. Broadband transient absorption (fs-ns) spectroscopy was used to show sequential electron and proton transfer from N-H and O-H bonds to form the corresponding neutral radicals which were further supported by low-temperature EPR studies. We envision that this method of photo-generating oxidative intermediates on PLY derivatives in an aqueous medium will open up new routes for catalytic transformations.

References:

1. R. Breslow. et.al. Acc. Chem. Res. **1995**, 28, 146-153
2. Das, A. et.al, *Sci. Adv.* **2019**, 5, 13.
3. Roy, D. et.al, *Angew. Chem. Int. Ed.* **2023**, 62, e202312500
4. Datta P.et.al. *ACS Catalysis* **2024**, 14, 3420-3433

P 41

Membrane-mediated signalling by monoamine neurotransmitters

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The fundamental event in chemical neurotransmission involves the fusion of synaptic vesicles at the plasma membrane and the subsequent release of its neurotransmitter cargo. The neurotransmitters are known to function only through a receptor-mediated pathway after their release. However, monoamine neurotransmitters have been shown to bind and and increase the fluidity and water penetration of lipid membranes [1]. Here, we show a lipid membrane-mediated action of monoamine neurotransmitters (especially serotonin) to control cellular functions. Using Total Internal Reflection Fluorescence microscopy we find that the interaction between monoamines and lipid bilayers enhance the association of lipid bilayers, and can accelerate vesicular fusion *in vitro* [2]. Further, using a home-built three-photon microscope that enables label-free imaging of serotonin and simultaneous spectral/lifetime imaging of Nile red/FliptR (probes for vesicular membrane fluidity), we show that intra-vesicular serotonin increases the fluidity of vesicular membranes in live neurons. Upon depolarization, this increased fluidity facilitates the exocytosis of mature vesicles which contain higher serotonin concentrations. We conclude that the intra-vesicular monoamine provides 'feedback' through the vesicular membrane which allows it to modulate its own exocytosis. In addition, we also show that the interaction of monoamines with the plasma membrane can control the activity of non-cognate GPCRs [3]. Our results expand our understanding of the effects of monoamines in neurons and suggest that receptor-orthogonal serotonin analogues can be potential drug candidates.

References:

1. S. Dey et.al., Chem. Eur. J., **2021**, 27, 7533–7541.
2. D. S. Roy et.al., **2024**, bioRxiv doi: 10.1101/2024.01.20.576155.
3. D. S. Roy et.al. J. Phys. Chem. Lett., **2024**, 15, 1711–1718.

P 42

Mechanical roles of molecular chaperones observed under Single-molecule force spectroscopic technique

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Protein folding under force invariably generates mechanical output, and while chaperones are well-known for assisting this process, their role in mechanically-tuned cellular energetics has not been thoroughly explored. In this study, we utilized single-molecule covalent magnetic tweezers to investigate the mechanical role of various molecular chaperones [1,2]. Here we mimic the physiological force environment on substrate proteins, keeping the chaperones unperturbed and investigated the role of twelve different chaperones with two structurally different proteins- talin and protein L and proposed a noble mechanism of chaperones. Our findings show that tunnel-associated chaperones (TF, DsbA, DsbC, and PpiD), which act as foldases under mechanical force, assist folding under force and generate energy to facilitate translation or translocation [3]. Conversely, cytoplasmic chaperones (PDI and thioredoxin) and the well-known foldase chaperone DnaKJE do not exhibit this mechanical folding ability. Instead, transferring chaperones (DnaJ, DnaK, SecB, Skp, and Spy) act as unfoldases, preventing misfolding of client proteins and aiding in the translocation of substrate proteins. These results provide new insights into the mechanical roles of chaperones, revealing how they can reshape the energy landscape of client proteins and modulate energy consumption in various biological processes, thus highlighting their significant impact on cellular energetics and protein folding dynamics [4,5,6].

References:

1. I. Popa et.al. *J. Am. Chem. Soc.* **2016**, *138*, 10546–10553
2. Tapia-Rojo, R. et.al. *Nat. Phys.* **2023**, *19*, 52–60
3. Eckels, E. C. et.al. *Chem. Sci.* **2021**, *12*, 11109–11120
4. Chaudhuri, D. et.al. *Biomacromolecules*, **2022**, *23*, 2951–2967
5. S. Chakraborty et.al. *Nanoscale*, **2022**, *14*, *7659–7673*
6. Chaudhuri, D. et.al. *Nanoscale*, **2024**, *16*, 2540-2551

P 43

Anomalous Fluorescence Quenching in Fluorous Solvent-Added Media

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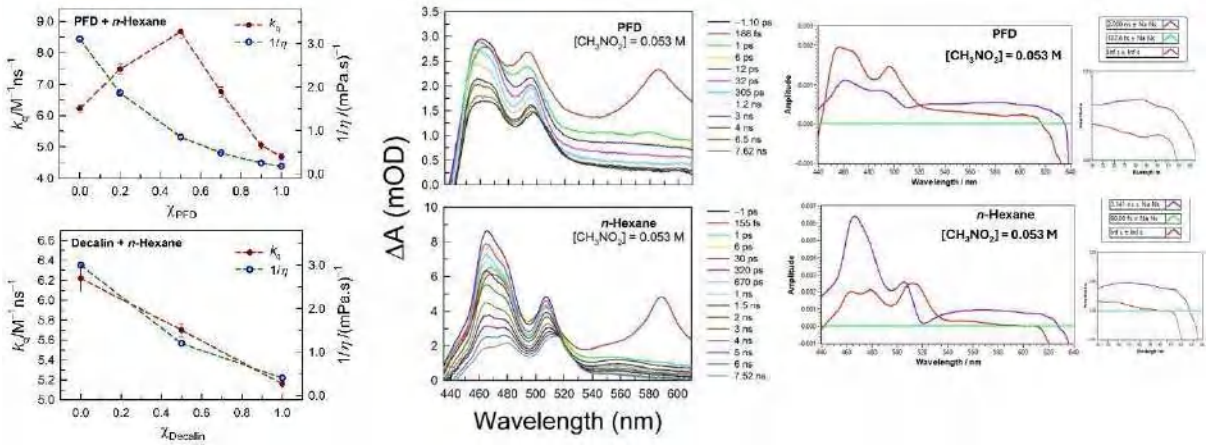
Fluorinated media have potential to exhibit unusual solvation properties in the context of photoinduced electron/charge transfer (PCT). In this study, we have employed binary mixtures of perfluorodecalin (PFD) and *n*-hexane to afford contribution of PFD specifically. Time-resolved fluorescence (TRF) quenching and ultrafast transient absorption (TA) spectroscopy are used for the quantitative interpretation of the diffusion, dynamics, and PCT mechanism. Addition of PFD to *n*-hexane results in unusual charge transfer between polycyclic aromatic hydrocarbons (PAHs) and nitromethane. As more viscous PFD is added to less viscous *n*-hexane, the dynamic viscosity (η) of the media increases. The bimolecular quenching rate constants (k_q) of the PAHs instead of decreasing, increase as PFD is added to *n*-hexane till equimolar mixture composition; k_q exhibits expected decrease only in PFD-rich region of the mixture. It is proposed that highly electronegative fluorines on PFD stabilize partial positive charge (δ^+) that develops on excited PAH during electron/charge transfer to the quencher nitromethane facilitating quenching in the process. The decay constants are similar to that observed for normal kinetic analysis of the transient absorption spectra with no clear evidence of the charge-transfer states. We infer that the initial photoinduced charge transfer is the rate determining step and the charge recombination is faster than the charge separation. Gibbs free energy change [$\Delta G^{(0)}_{PCT}(\varepsilon)$] for the PCT process is calculated using Rehm-Weller equation. k_q follows thermodynamic predictions only in case of neat *n*-hexane using nitrobenzene as quencher. In all other cases the k_q deviates significantly from the thermodynamic predictions. It can be inferred that PCT in the studied systems is kinetically-controlled and the observed charge transfer rate constant should primarily be governed by the reorganization energy of the immediate solvent molecules (outer-sphere) and solute molecules (inner-sphere).



References:

1. Zhang et.al. *Chem. Commun*. **2008**, 5686.
2. Matyus et.al. *J. Photochem. Photobiol. B* **2006**, *83*, 223.
3. Sun et.al. *Sens. Actuators, B* **2018**, *265*, 476.
4. Zhang et.al. *Photochem. Photobiol. Sci*. **2014**, *13*, 917.
5. O'Mari et.al. *Curr. Opin. Electrochem.* **2022**, *31*, 100862.

P 44

Peptide-Based Antiviral Breakthroughs: Mutation-Resistant Drugs

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The rise of antimicrobial resistance (AMR) among viruses has become a notable concern, underscoring the necessity for effective antiviral treatments, sometimes in conjunction with vaccines. The rapid mutation of viruses, poses a significant hurdle in the pursuit of a universally effective vaccine that provides lifelong immunity. Beyond vaccines, antiviral drugs serve as the second line of defence. However, the efficacy of drugs raises concerns, ranging from notable side effects to the emergence of drug-resistant viruses with continued use. To address these issues, the idea of this work is to design antiviral strategies by envisioning potent cocktails of peptide-based virucidal drugs. The aim is to rationally design peptides to be used as targeting moieties onto the scaffold so to make new virucidal antivirals that target various proteins and various parts of proteins on two prominent enveloped viruses, (1) influenza A viruses (IAVs) and (2) severe acute respiratory syndrome coronavirus(SARS-CoV2). Such an approach offers potential therapeutic benefits superior to available approaches especially when it comes to dealing with the mutation-resistance. Through persistent efforts in optimizing and harnessing the unique properties of peptide-based drugs combining both experimental and computational approaches, significant advancements in antiviral therapeutics can be envisioned.

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Probing Brij Surfactant Vesicles Using a New Polarity Probe - AICCN

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The present work involves a comprehensive study on 2-amino-4-(1*H*-indol-3-yl)-4*H*-chromene-3-carbonitrile (AICCN) on vesicular systems. The molecule AICCN (synthesized earlier in our laboratory [1]) has been previously shown to function as an effective polarity probe [2]. Thus, it is expected that AICCN fluorescence can be an effective tool for probing organized assemblies i.e. vesicles. Two types of vesicular systems, niosomes and bilosomes have been prepared. Niosomes have been used as drug delivery vehicles [3] and in cosmetic formulations in the past [4]. The bilosomes are unique in the sense that they can withstand the harsh conditions of the gastrointestinal tract (GIT) [5], unlike the older counterparts i.e. liposomes and niosomes. Three types of non-ionic Brij surfactants have been used to prepare niosomes and bilosomes – Brij S2, Brij S10 and Brij S20. The Brij based surfactants were prudently selected so as to compare the effect of varying head group and HLB on the final vesicular structure and morphology. The fluorescence of AICCN served as an effective tool for studying these systems.

Fluorescence anisotropy, temperature effect on fluorescence, Red Edge Excitation Shift (REES) all indicated that Brij S2 forms robust vesicles with rigid bilayer. Confocal microscopy was used to assess the localization of AICCN within the vesicles. Studies are underway to study entrapment and release of AICCN from the vesicles and use of these vesicles for delivering AICCN to targeted cell lines. There are plans to study the diffusion coefficient of biologically active molecules such as AICCN within these vesicles.

References:

1. Biswas et.al. *Mater. Res. Express* 2015, 2, 065011.

2. Mandal et.al. *Phys. Chem. Chem. Phys.* 2023, 25, 18197.

3. Mondal et.al. *ACS Appl. Bio Mater.* 2022, 5, 8, 3896.

4. Handjani-vila et.al. *Int. J. Cosmet. Sci.* 1979, 1, 303.

5. Conacher et.al. *Vaccine* 2001, 19, 2965.

P 46

Engineering Bacterial Microcompartments: Insights from Shell Protein Interaction Studies

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Bacterial microcompartments (BMCs) are the polyhedral organelles that are composed of shell proteins encapsulating an enzyme core and provides metabolic advantages to bacteria. Outer shell of PduBMC is made up of eight different self- assembling shell proteins, among these PduN is the only pentameric protein that occupy the vertices. PduN is known to interact with the sheet-forming PduA shell protein, influencing the curvature and structural integrity of the microcompartment. Deletion of the pduN gene results in a disrupted, tubular BMC structure. So, here we are understanding the interaction of PduN with other shell proteins in assembling (PduBMC) and engineering them into some useful morphological architectures. We have studied the interaction between PduN with PduA, PduBB' and PduJ (Major shell proteins) using fluorescence based and optical (BLI) techniques. Interestingly, we observed that all these three protein combinations interact and yields diverse morphologies from open to closed compartments and sheets in heterologous host (*E.coli*). The resultant compartments can effectively associate and encapsulate native enzymes while preserving their functional activity. Further, we conducted *in vivo pduN* gene repositioning studies to understand why it is placed after the major shell and enzyme genes in *pdu operon*. We repositioned *pduN* before and after enzyme genes. Our *pduN* gene repositioning studies reveal that changing *pduN* position was unable to rescue the polyhedral structure of BMCs alters BMC structure and function. By integrating genetic engineering with advanced imaging techniques, we have decoded the structure-function relationship within PduBMCs, providing critical insights into microcompartment biogenesis. This approach not only enhances our understanding of BMC assembly but also offers potential for engineering protein nanocontainers and nanoreactors with wide-ranging biotechnological applications.

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Exciton-Polaron in Quasi-1D perovskite-like material-HDABiI5

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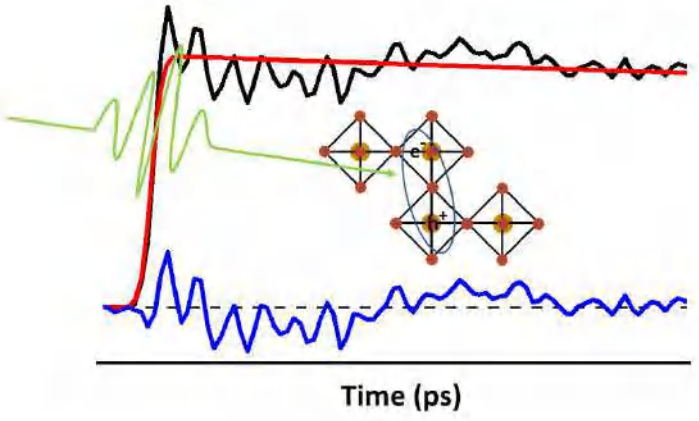
Lower dimensionality invokes properties like quantum confinement in the semiconducting materials[1]. Hybrid Perovskites are rather special in such cases as, along with reduced dimensionality, the organic cations also introduce softness in the lattice. This softness allows for strong coupling between photogenerated charge carriers and the lattice surrounding them[2]. Such interactions in these materials become the basis for next-generation devices for quantum control. A short pulse pump-probe experiment can resolve these coupled lattice modes well. Here, we use time-resolved impulsive Raman spectroscopy[3-6] to investigate the structural deformations in the lattice of organic Bismuth halide compounds on photoexcitation. We find lattice modes at 140 cm-1 & 17 cm-1 corresponding to BiI6 Octahedra stretching and tilting that strongly couples to the generation of the photo-excited states in these materials leading to the formation of Exciton Polaron.



References:

1. O.Yaffe,et.al. Phys. Rev. B, **2015**, 92, 045414.
2. K.Miyata,et.al. Sci. Adv., **2017**, 3, e1701217
3. Y.Yang, Nat. Commun., **2016**, 7, 12613.
4. Thouin, Nat. Mater., **2019**, 18, 349.
5. Ni L, ACS Nano, **2017**, 11, 10834.
6. D.Giovanni,Adv. Sci. **2018**, 5, 1800664.

P 48

TFAM-mediated dual role in DNA dynamics brings about mitochondrial genome compaction and transcriptional regulation via distinct bending states.

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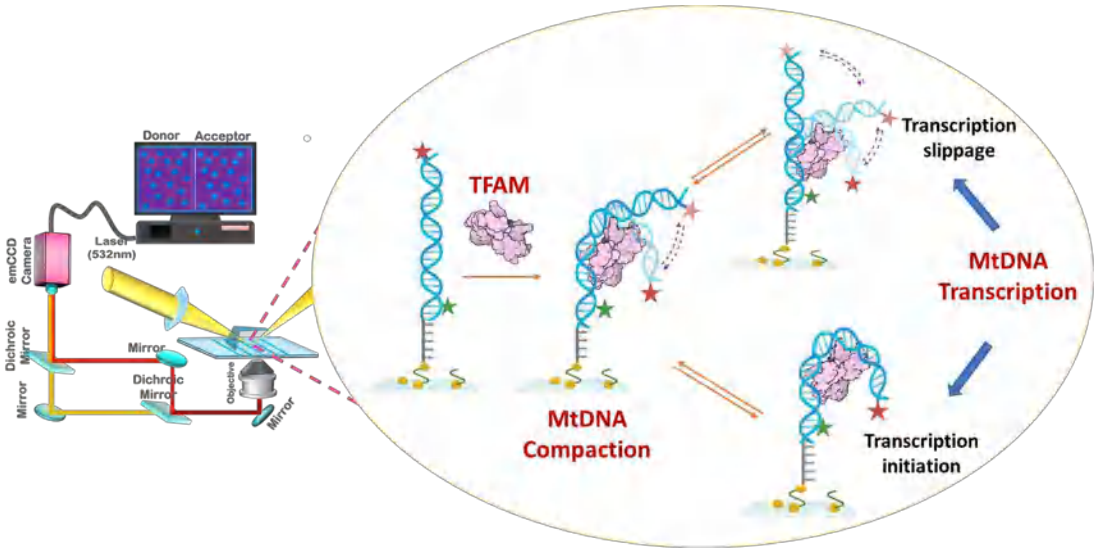
Mitochondrial transcription factor A (TFAM) plays a key role in DNA bending, crucial for both transcription initiation and genome compaction[1]. We studied the binding affinity between TFAM to its specific promoter sequences (LSP & HSP) and also non-specific sequence (NS-DNA). Circular dichroism spectroscopy revealed differential effect of TFAM on these sequences. Using single-molecule FRET, we investigated TFAM's interaction with the light strand promoter (LSP), heavy strand promoter (HSP), and non-specific (NS) DNA. In the absence of TFAM, LSP DNA displayed low FRET efficiency indicating a linear conformation. Upon TFAM binding, mostly a full (F) bent state with occasional transitions to a partial (P) bent state were observed. HSP binding showed three distinct FRET states with majority transitions between linear and partially bent conformations, suggesting TFAM does not stabilize full bending, and thereby somewhat not likely facilitating transcription initiation. In contrast, with NS DNA, TFAM stabilized the fully bent state while also keeping the DNA flexible by allowing transitions into the P-state further supporting its role in DNA compaction. These results highlight TFAM's conformational control over DNA structure, with distinct effects depending on DNA sequence, offering insights into its dual roles in transcription regulation and genome packaging.



Reference:

1. Ngo, H. et al. *Nat Commun*, **2014,** *5*, 3077.

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How robust is studying the structural dynamics of biomolecules by single molecule FRET spectroscopy?

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Biomolecules function by small and large scale structural motions spanning over nanoseconds to seconds. Single-molecule FRET (Foerster resonance energy transfer), is an established technique of single molecule fluorescence spectroscopy when combined with energy transfer between the fluorophores thereby measuring the nanometer distances between them[1]. Previous studies on variety of biomolecules, organic fluorophores, employed set-up configurations, and data analysis makes single-molecule FRET experiments hard to compare.

Recent community wide single-molecule FRET study measuring the nanometer distances between the organic fluorophores attached onto a double stranded oligonucleotides confirmed the robustness of this technique with high accuracy and precision[2]. To extend the study on a more complex biological molecule, we assesses the consistency of the technique to measure the conformational dynamics on millisecond timescale in proteins, where we measured the same protein samples together with the other 19 laboratories. The study reiterated the similar accuracy and precision in the nanometer distance measurements on proteins as on oligonucleotides. Furthermore, by identifying the potential configurations in the measurement set-ups which may introduce the variations amongst different laboratories and avoiding the fluorophore induced effects, dynamic analysis on proteins proved to be reliable in distinguishing the millisecond timescales of sub-nanometer distance changes[3].

References:

1. E. Lerner et al. *Science*, **2018**, *359*, 6373.
2. B. Hellenkamp et al. *Nat. Methods.*, **2018**, *15*, 669.
3. G. Agam et al. *Nat. Methods.*, **2023**, 1.

P 50

Modulation of ESIPT and ICT pathways in symmetrical azines through micro heterogeneous medium

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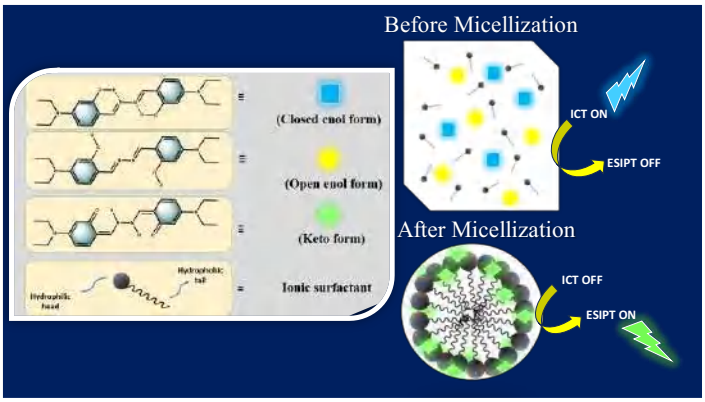
The organic luminescent molecule with tunable emission property has been widely examined due to their well-established role in analysing from single cell to large system. The distinguishable excited state exhibited by luminescent molecules play a dynamic role based on the microenvironment around them. Which are applicable in various fields such as fluorescent sensors, chemical warfare agents, OLED (Organic light emitting diode), and biomarkers (1). The photophysical property of the fluorescent probe is sensitive to the various external factors, particularly, microenvironment around them which influences the various excited state pathways such as PET(Photoinduced Electron Transfer), ICT (Intramolecular Charge Transfer) and ESIPT (Excited State Intramolecular Proton Transfer). Here, we have designed various symmetrical azine based fluorescent probes in the way they possess both ICT and ESIPT mechanisms in the same molecule (2,3,4). Salicylidene based azine molecule (DEASAD) showed dual charge transfer emission due to the presence of open enol (480 nm) and closed enol (510 nm) forms in polar protic solvents (5,6). Upon increasing the concentration of ionic surfactants, there is a significant increase in the emission intensity of both the enol forms of DEASAD until premicellar concentration. After micellization, occurrence of a new anomalous keto form emission through ESIPT was observed around 530 nm in ionic micelles and its intensity changes depend on the micellar surface charge The emission studies revealed the position and interaction of DEASAD with the charge of micellar stern layer as confirmed through interaction of metal ion with the probe and control molecules with and without ESIPT and ICT moieties.



References:

1. Fang M, et al. *Prog. Mater. Sci*., **2022,** *125*, 100914.
2. Thiagarajan V, et al. *Bull Chem Soc Jpn*., **2007**, *80*, 1307.
3. Hao G. et al. *Chem Commun*., **2023**, *59*, 2056.
4. Lu XL et al. *J. Anal Chem*., **2021**, *49*, 184.
5. Damoc M. et al. *J. Phys. Chem. C.*, **2022**, *127*, 99.
6. Suhasini R. et al. *Photochem. Photobiol*., **2024**, *00*, 1.

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SARS-CoV-2 Binding to Terminal Sialic Acid of Gangliosides Embedded in Lipid Membranes

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Multiple recent reports indicate that the S protein of SARS-CoV-2 interacts explicitly with membrane receptors and attachment factors other than ACE2. Which act as an primarily receptors i.e. play an active role in cellular attachment and entry of the virus. In this article, we examined the binding of SARS-CoV-2 virions to three different sialylated gangliosides, i.e., GD1a, GM3, and GM1 incorporated in SLBs. These gangliosides have 2, 1, and 1 SIA, respectively, bonded to a galactose unit of the glycan chain. We traced the binding kinetics of fluorescently labeled SARS-CoV-2 particles to the ganglioside-rich SLBs by recording time-lapse TIRF images of the labeled SARS-CoV-2 at the single-particle level. We observed that the virus specifically binds to sialylated (sialic acid, SIA) gangliosides, i.e., GD1a, GM3, and GM1, as determined from the acquired single-particle fluorescence images using a time-lapse total internal reflection fluorescence microscope. The data of virus binding events, the apparent binding rate constant, and the maximum virus coverage on the ganglioside-rich SLBs show that the virus particles have a higher binding toward the GD1a ganglioside. Enzymatic hydrolysis of the SIA–Gal bond of the gangliosides confirms that the SIA sugar unit of GD1a and GM3 is essential for virus attachment to the SLBs. The structural difference between GM3/GD1a, GM1 is the presence of SIA at the main or branched chain. We conclude that the number of SIA per ganglioside can weakly influence the initial binding rate of SARS-CoV-2, whereas the terminal or more exposed SIA is critical for the virus binding to the gangliosides in SLBs.

Reference:

1. Geetanjali Negi et al., *ACS Infectious Diseases*, ***2023***, *9, 1346.*

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Exploring Chemical Equilibrium Under Vibrational Strong Coupling

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Studies under the collective vibrational strong coupling of the molecules with the electromagnetic cavity modes can provide a unique window into the fundamental processes underlying chemical reactions. [1-2] By coupling the specific vibrational modes of molecules or solvent we can study the role of that specific vibrations in driving chemical transformations. This study has the potential to unlock new knowledge about reaction mechanisms, molecular dynamics and also the possibilities of new reaction pathways. [1-2] We studied the kinetics of the monomerization under the strong coupling of different vibrational modes of the molecules. We observed that this monomer-dimer equilibrium shows shift under vibrational strong coupling.

Reference:

1. Garcia-Vidal FJ et al., *Science*, **2021**, *373*, 6551.
2. Nagarajan K. et al., *JACS*, **2021**, *143*, 16877.

P 53

Deciphering the propionylation on lysine by using a novel label-free tool: Protein Charge Transfer spectra

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Propionylation is a post-translation modification that plays a crucial role in regulating various cellular processes and is implicated in multiple diseases. This modification typically occurs on the cationic groups of proteins and it removes the positive charge of the protein. Traditionally, PTMs have been detected using spectroscopic methods such as protein labelling with external probes, NMR, and mass spectrometry (MS). However, these conventional techniques require expensive instrumentation, increasing the overall cost. Currently, there is no method available that can detect PTMs without labeling, using low-cost instruments, and in quick time. Recently, our group discovered a novel intrinsic chromophore in proteins that exhibits charge transfer transitions, producing a broad UV-Visible absorption band ranging from 250 to 800 nm [1]. This newly identified absorbance band, called **Protein Charge-Transfer Spectra (ProCharTS)** offers a new label-free approach for probing proteins and their interactions. In this study, we explore the chemical propionylation of charge-rich proteins such as α_3C and α_3W using ProCharTS. Initially, we confirmed protein propionylation through MALDI-ToF and analyzed the secondary structure by CD spectroscopy. The ProCharTS spectra of propionylated proteins were recorded, which demonstrated a decrease in intensity for α_3C and α_3W after propionylation. Our studies show that a decrease in the ProCharTS profile after propionylation is indicative removal of charge from the protein.

References:

1. Prasad, Saumya, et al., *Chemical Science*, **2017**, 8, 5416.

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Spectroscopic analysis of Potassium Bromide as host material in Terahertz range

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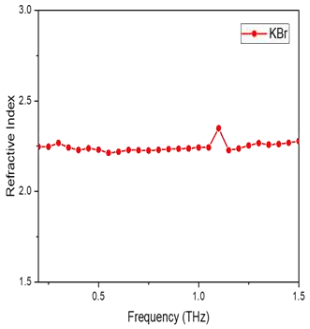
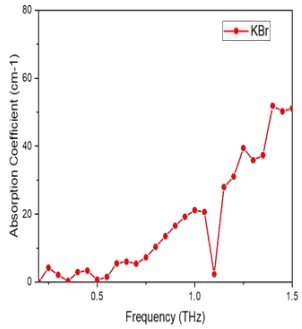
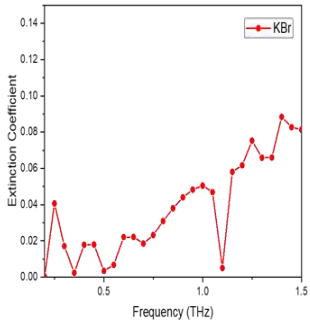
Potassium Bromide (KBr) has been widely used as a host material for various spectroscopic techniques such as Raman spectroscopy, IR spectroscopy etc. In this study, the efficiency of KBr to be used as a host material for THz Time Domain spectroscopy (THz-TDS) has been discussed. Measurements on KBr pellets were performed using THz-TDS spectroscopy. A femtosecond (fs) Ti: Sapphire laser amplifier and two zinc telluride (ZnTe) crystals were used to generate and detect Terahertz (THz) radiation using optical rectification and electro-optic sampling method, respectively. The changes in optical properties of KBr in THz range such as refractive index, absorption coefficient and extinction coefficient have been experimentally shown and discussed in this study.



Refractive index profile of KBr



Absorption coefficient profile of KBr



Extinction coefficient profile of KBr

References:

1. Jens Neu et al., *J. Appl. Phys.* **2018**, *124*, 231101.
2. Zaira Akhvlediani et al., *Wiley Online Library* **2023.**
3. S. Biermann et al., *J. Chem. Phys.* **1996**,*105*, 9754.
4. I Kaplunov et al., *J. Phys.* **2020**: *Conf. Ser. 1697 012253*,
5. Can Koral et al., THz spectroscopy of advanced materials, *Terahertz (THz), Mid Infrared (MIR) and Near Infrared (NIR) Technologies for Protection of Critical Infrastructures Against Explosives and CBRN*, **2021**, *253–273*
6. AK Malik et al., **2010**, *107, 113105*
7. Amit C. Das et al., *Proc. SPIE 9894, Nonlinear Optics and its Applications IV*, **2016**, *98941E*
8. Yeongkon Jeong et al., *Applied Surface Science*, **2022**, *584*, 152611.
9. Emre Aytan et al., *Polymer Bulletin*, **2023.**
10. Paul D. Cunningham et al., *J. Appl. Phys.*, **2011**, *109, 043505.*,

P 55

Designing of gold nanoparticle-cyclodextrin conjugates to assist cholesterol trafficking in Niemann Pick Disease C phenotypic cells

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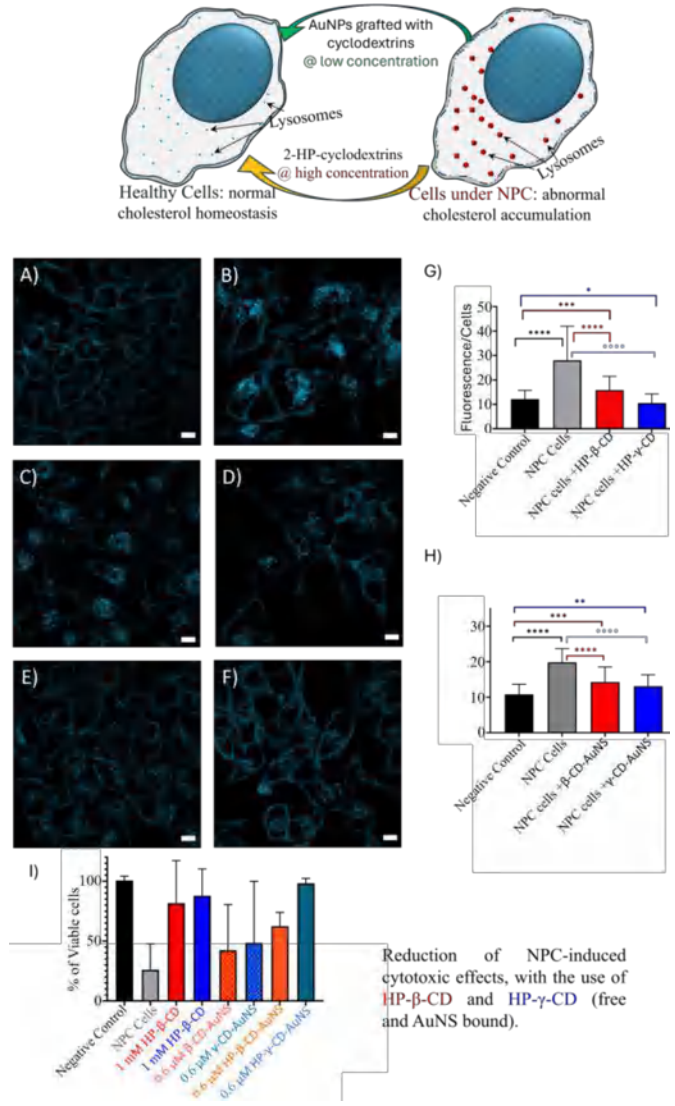
Niemann Pick disease type C (NPC) is a rare genetic disorder caused by mutation in either of the two genes NPC1 and NPC2, which results in abnormal cholesterol accumulation in late endosomes (LE) and lysosomes (LS). To date, no drug has been found with adequate effectiveness to treat NPC and current treatments merely focus on the reduction of some of the complications such as seizures, liver dysfunction, cognitive impairment, etc. Several derivatives of Cyclodextrins (CDs) [e.g., 2-hydroxypropyl β cyclodextrin (HPβCD) and 2-hydroxypropyl γ cyclodextrin (HPγCD)] have been tried against NPC, due to their ability to incorporate cholesterol molecules in their inner cavities. Despite showing early promise in cellular and animal studies, these efforts have failed during clinical trials due to poor pharmacokinetic profiles of the drugs and possible side effects.



Restoration of cholesterol distribution in the NPC-induced cells. Cholesterol distribution in A) normal cells, B) NPC-induced cells, C) and D) nanoconstruct treated NPC-induced cells, E) and F) HP-β-CD and HP-γ-CD treated NPC-induced cells. G) and H) analysis of the imaging experiment. I) Effect on NPC-induced cytotoxicity.

Our work aims to deliver the CDs by loading thiolated derivatives of βCD and γCD on top of biocompatible gold nanostars (AuNS) to improve pharmacokinetics and eliminate the need for high doses. We induced NPC mimicking conditions in SiHa cells by pharmacological inhibition of NPC1 protein. Confocal imaging performed after Filipin staining of cholesterol clearly showed that our conjugates were able to reduce the amount of cholesterol in LE and LS in NPC phenotypic SiHa cells, with the use of ~ 3 orders of magnitude lower concentrations of CDs (loaded on top of AuNS) compared to free HPβCD and HPγCD (Figure A to H). Additionally, the AuNS-conjugates effectively mitigated the cytotoxic effects associated with prolonged NPC1 blockade, requiring approximately 1,000 times lower concentrations of CDs compared to free HPβCD and HPγCD (Figure I).

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Microbial single cell Raman imaging coupled with vibrational tag for monitoring biosynthesis pathways in action

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Microbial single cells are complex, dynamic systems that require advanced multiplexing tools to image biomolecules and track their biosynthesis and turnover. Raman Imaging is a non-invasive vibrational imaging platform, enables the spatial and temporal visualization of various biomolecules—such as amino acids, nucleic acids, proteins, and lipids—simultaneously at the single-cell level. [1,2] In our study, we utilized a combination of reverse stable isotope probing methodology with Raman imaging in which carbon stable isotopes was used as the vibrational tags. The stable isotope labelled biomolecules exhibit distinct shifted peak in Raman spectra when compared to its unlabelled counterparts due to isotopic effect. $^{13}C_6$ labelled microbial cells were grown in a carbon-free culture medium with the unlabelled ($^{12}C_6$) carbon source, and single-cell Raman images were acquired at different time points. In the early incubation time points, the Raman image revealed intense signal distribution from the $^{13}C_6$-labelled biomolecules, while the Raman signal distribution from the unlabelled biomolecules were seen to be weak. As incubation duration progressed, the Raman images of the microbial cell showed diminished intensity signal of the $^{13}C_6$-labelled biomolecules, and the newly synthesized unlabelled biomolecule distribution became increasingly prominent. This approach generated spatio-temporal maps that visualized the turnover and distribution of newly synthesized biomolecules, offering insight into dynamic biosynthetic pathways. Vibrational tag Raman imaging holds significant potential for bioprocess applications in sensing and visualizing the accumulation of commercially relevant metabolites.

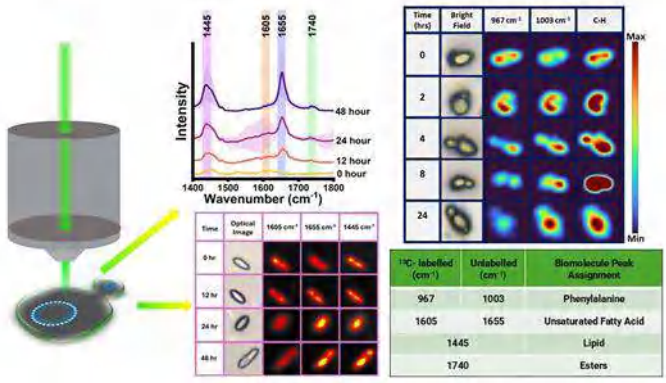

Figure: Microbial single cell Raman images showing time dependent dynamics of different metabolites in situ.

References:

1. Jiro Karlo et al., **2024**, In situ monitoring of the Shikimate pathway: A combinatorial approach of Raman reverse stable isotope probing and hyperspectral imaging. Analyst.
2. Jiro Karlo et al., **2024**, Tracking Trash to Treasure: In situ monitoring of Single Microbial Cell Oil biosynthesis from Waste Cooking Oil using Raman Reverse Isotope Probing and Imaging. ChemRxiv. (Under Review)

P 57

Insights into dynamic properties of beta-lactoglobulin dimer protein disruption by graphene oxide: Spectroscopy and fluorescence lifetime imaging microscopy

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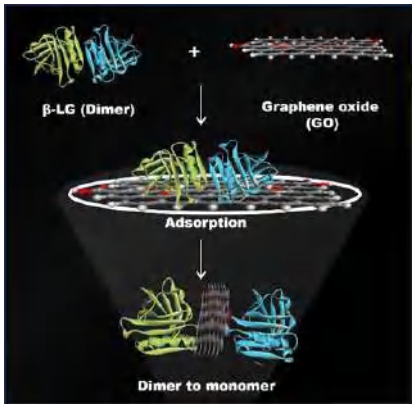
Graphene oxide (GO), a novel applied nanomaterial derived from graphene, has been employed in various biomedical applications due to its unique structure.[1] Nevertheless, their biological applications are severely limited by a lack of comprehensive understanding of GO interactions with biomacromolecules. Herein, we investigated the structure and dynamics of β-lactoglobulin (β-LG), a globular milk whey protein, on the GO surface at two different pHs, 2.5 and 6.2, in connection to the monomer and dimer forms of the protein, respectively. Molecular dynamics simulation (MD), multi-spectroscopic and imaging techniques were employed to monitor the protein interaction with GO. The steady-state results revealed the interaction is significantly stronger in a dimeric form compared to the monomeric form of protein and complexes are driven by hydrophobic forces. Interestingly, the time-resolved fluorescence measurements unveil the significant changes in average lifetime values after adsorbing on GO (from 1.79 ns to 0.20 ns at pH 2.5 and from 1.16 ns to 0.24 ns at pH 6.2). The final lifetime of both forms, with the addition of GO, explains a similar microenvironment around the tryptophan. Furthermore, to verify these changes, the secondary conformation of both forms of protein is elucidated by fourier transform infrared (FT-IR) spectra, which clearly depict the spectral profile of dimeric protein in the presence of GO are closely resembles those of monomeric β-LG at pH 2.5. In addition, comparable morphological changes of GO with protein of both forms were investigated using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Additionally, the exploration of protein interaction with GO was performed using fluorescence lifetime imaging microscopy (FLIM); both forms have a similar distribution of lifetime after adsorbing on GO, which supports the aforementioned results. The plausible reason for this change is that the GO can disrupt the dimer β-LG into a monomer.[2] In order to support the experimental results of dimer protein disruption, we have also performed the molecular dynamic simulation. The simulation results demonstrate that a GO can interfere with hydrophobic protein-protein interaction and can lead to penetrating in between the protein dimer. Hence, our findings can assimilate GO and protein interaction and help to increase the scope for potential biomedical applications.



References:

1. D. Chen et al. *Chemical reviews*, **2012**, 112, 6027.
2. B. Luan et al. *ACS nano*, **2015**, 9, 663.

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Antidepressant Potential Versus Neurotoxicity of Rapidly Acting Antidepressant Drugs (RAADs)

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Rapidly acting antidepressants (RAADs) partially relieve depression in < 1 day and have sustained antidepressant effects for several days. This rapid onset is 10- to 100-fold faster than selective serotonin and norepinephrine reuptake inhibitors (SSRIs and SNRIs). RAADs have varying chemical structures: some RAADs are ketamine enantiomers (R-Ket/S-Ket), metabolites [(2R, 6R)-HNK/(2S, 6S)-HNK], or analogs such as methoxetamine (MXE), and radafaxine (Rad). Other RAADS, such as scopolamine (Scop), are best known as muscarinic antagonists and are structurally unrelated to ketamine. Ketamine (Ket) and scopolamine present something of a paradox for pharmacology, as they exhibit rapid and robust antidepressant activity but have potent neurotoxic potential. However, it is not yet clear precisely which molecular mechanisms mediate these beneficial and deleterious effects; thus, dosing is mostly a trial-and-error affair. Here we carefully dig into the therapeutic and toxic effects of ketamine and scopolamine and their metabolites, shedding light on the mechanisms, cell types, and timescales in cells and tissues.

The first goal of our research program was to develop genetically encoded fluorescent biosensors to follow the import, trafficking, and metabolism of RAADs. We developed a family of "intensity-based RAAD-Sensing Fluorescent Reporter" [iRAADSnFR] indicators. In solution, iS-KetSnFR responds to S-Ket with an EC_{50} ~ 150 nM with maximal fluorescence increase ($\Delta F_{max}/F_0$) ~ 3.2; iS-HNKSnFR responds to (2S, 6S)-HNK with an EC_{50} ~ 110 nM and $\Delta F_{max}/F_0$ ~ 3.6; iR-KetSnFR senses R-Ket with EC_{50} ~ 130 nM and $\Delta F_{max}/F_0$ ~ 4.0; iR-HNKSnFR responds to (2R, 6R)-HNK with EC_{50} ~ 500 nM and $\Delta F_{max}/F_0$ ~ 4.0; and iD-HNKSnFR responds to dehydronorketamine (DHNK) with EC_{50} ~ 160 nM and $\Delta F_{max}/F_0$ ~ 4.0. iScopSnFR senses Scop and displays EC_{50} ~ 510 nM and $\Delta F_{max}/F_0$ ~ 2.8.These sensors will be useful for the study of Ket, its metabolites, and related drugs in a variety of experimental settings. We have previously shown that many drugs unexpectedly act, at least in part, at intracellular targets in various organelles. We wished to assess the potential of RAADs and their metabolites to traffic to, and act on, such intracellular targets. Thus, we incorporated diverse organellar targeting peptides to the iRAADSnFR family and expressed them in cells. Fluorescence microscopy shows that, at the nM concentrations associated with antidepressant activity, RAADs traffic into diverse organelles within seconds, equilibrating with extracellular levels. We next sought to express the iRAADSnFRs *in vivo* in mouse, fish, and worm – the former with adeno-associated virus (AAV) transduction, the latter two with transgenesis. We have expressed the sensors in diverse locations in the central and peripheral nervous systems and subjected the animals to diverse drug regimens. We have also performed complementary experiments using proteomics, immunohistochemistry, biochemistry, and behavior tracking to profile neurotoxicity of ketamine, its metabolites, and related drugs. Together, this research program will help us unravel the complicated mechanisms of action of RAADs in diverse settings, and to design and synthesize next-generation drug analogs with preserved efficacy but minimized side effects.

References:

1. Shivange et al. *2019, 151(6), 738.*
2. Bera et al. *2019, 13, 499*.
3. Beatty et al., *2022, 12(22): e4551*.
4. Blumenfeld & Bera et al., *2024, 49, 246*.

P 59

Parametric optimization for rapid discrimination of *Acinetobacter baumannii* using Surface-Enhanced Raman Spectroscopy (SERS)

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Ubiquitously present Gram-negative bacterium *Acinetobacter baumannii* (*A. baumannii*) has evolved as a highly successful nosocomial pathogen. *A. baumannii* reported to have acquired resistance against almost all clinically important antibiotics. The WHO has listed carbapenem-resistant *A. baumannii* in the top "Critical" tier of Bacterial Priority Pathogens List (BPPL) 2024 – which requires urgent control measures. Rapid detection of clones and antimicrobial resistance (AMR) profile is important for quick decision on treatment options. Raman Spectroscopy is proven to be useful in rapid understanding of the biochemical alterations among the susceptible and resistant microorganism. The spectral fingerprint of Raman spectroscopy is unique for microbial species, however, obtaining high quality Raman spectra is challenging due to the weak signals. These signals can be enhanced to several order of magnitude by placing plasmonic metallic nanoparticles closer to that of the microorganisms and this technique is referred as Surface-Enhanced Raman Spectroscopy (SERS). The present study was initiated to determine the culture conditions, time of culture, and the use of silver nanoparticles (AgNPs) of 60-80 nm (synthesised by Lee-Meisel method) for a reliable SERS measurement that can be useful for rapid differentiation purpose. The bacteria were cultured in LB media at 37 ºC and harvested at different growth phases (6, 8, 12, and 16 hours). Following the cell harvesting, several solutions (distilled water, 1X PBS, 0.9% saline, glucose, and glycerol) were tested for an effective removal of the cellular impurities, which might interfere or shadow with the Raman signals. The samples were casted in triplicates on the aluminium foil covered with a glass slide and each dot was measured thrice in two forms, liquid and dry which resulted in stable spectra with maximum number of peaks that were matched to carbohydrates (589 cm^{-1} and 885 cm^{-1}), phospholipids (1070 cm^{-1}), protein (1202 cm^{-1} and 1654 cm^{-1}). High resolution spectra were best obtained when 1:5 ratio of AgNPs and bacterial cells were used. Both liquid and dry form resulted in comparable peaks, while maximum intensity was observed in the dry form. The results showed that cells harvested at 16 hours and washed with distilled water has given the reproducible spectra with minimum noise.

P 60

Investigating the Mechanical Response of Parallelly Arranged Polyproteins: Mimicking the Mechanical Stress in Biological Systems

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Proteins play numerous roles in cellular processes. The well-defined structure of proteins is attributed to their functional properties executed in various cellular functions.[1] Many proteins are subjected to biologically relevant mechanical forces[2] in several cellular processes, generally called elastomeric proteins. Single-molecule force spectroscopy like atomic force microscopy (AFM) has been employed to investigate the mechanical fingerprint of proteins under external force.[3] Traditionally, the mechanical response recorded from AFM is ascribed to the behaviour of individual protein domains. However, it is important to note that in many biological processes, proteins exist in parallel/antiparallel fashion to execute their biological function.[4] For example, in striated muscle sarcomere, the giant muscle protein titin exists as hexameric bundles to mediate the passive elasticity of muscles.[5,6] To understand how protein domains, arranged parallelly, respond to external forces, a special molecular biology design is necessary which can directly probe the mechanical features of proteins in parallel. In the previous attempt[7] using an alpha-helical coiled-coil domain to create a parallel dimer, it has been shown a doubling of the unfolding force of individual domains. Further study[8] using SpyCatcher-Spytag chemistry and covalent immobilization of proteins on coverslip claimed the ratio of unfolding force in parallel arrangement versus monomer to be always less than 2. Both the previous study has their drawbacks related to the design of the system. In this context, our study provides a suitable molecular biology design to study the mechanical features of proteins arranged in parallel. We used unnatural amino acids to create a junction in the polyprotein construct and kept the alpha-helical coiled-coil domain at the terminus of the construct for the heterodimerization [Fig. 1]. This leads to the formation of a conserved parallel construct to be used to directly probe the mechanical feature through AFM. Moreover, our design can be elongated from parallel dimer to parallel tetramer which can maximize the situation of parallel existence of proteins in biological systems. Our study aimed to have a clear conclusion regarding the change in the mechanical feature of proteins from monomer to parallel dimer and to provide a suitable molecular biology design to probe proteins in parallel arrangement.

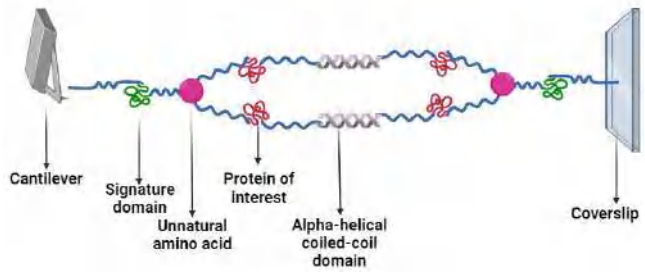

Figure 2: Molecular biology design to probe protein domains in parallel

References:

1. Kessel, A. et.al. *Introduction to Proteins: Structure, Function, and Motion, Second Edition* 2018, 1–932.
2. Halma et. al. *Biophys Rep* 2024, *9* (0), 1.
3. Scholl, Z. N et. al. *Methods in Molecular Biology* 2018, *1814*, 35–47.
4. Ohashi, T et. al. *PNAS* 1999; Vol. 96.
5. Tskhovrebova, L et. al. *Nature Reviews Molecular Cell Biology* 2003, pp 679–689.
6. Rivas-Pardo, J. A. *Emerging Topics in Life Sciences* 2018, pp 681–686.
7. Sarkar, A et. al. *Biophys J* 2007, *92*, 4.
8. Zuo, J et. al. *ACS Nano* 2023, *17* (8), 7872.

P 61

Unveiling Tau K18 and K19 Aggregation through Protein Charge Transfer Spectra (ProCharTS): Addressing Research Gaps and New Insights

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Tau is an intrinsically disordered protein (IDP) which is associated with Alzheimer's disease. Tauopathies are neurodegenerative disorders [1] characterized by its abnormal aggregation, with the K18 and K19 domains consisting of only the four and three repeats of tau protein respectively. They retain the key aggregation-prone regions and it makes them more suitable for studying the early events of aggregation which are critical for understanding the disease onset and progression as compared to the wild-type tau. Traditional spectroscopic techniques like Circular Dichroism (CD), fluorescence spectroscopy, have provided valuable insights into Tau protein aggregation but they often fall short in explaining the nuanced charge transfer dynamics underlying these processes as well as the intricate details involving the early events of aggregation [2]. In this study we introduce protein charge transfer spectra (**ProCharTS**) as a novel and label-free tool [3] to explore the aggregation behavior of Tau K18 and K19 domains thereby providing unique advantages in directly probing the charge transfer interactions within the Tau aggregates which are less accessible through conventional methods. Our investigations have unravelled the previously unrecognized aggregation intermediates and distinct signatures associated with charge transfer for both the K18 and K19 domains. The results not only enhance the resolution of tau aggregation studies but also provide a deeper understanding of the electrostatic contributions to tauopathies. By focussing on protein charge transfer spectra (**ProCharTS**) we unveil critical insights that may guide the development of novel diagnostic and therapeutic strategies. Our results therefore underscore the utility of **ProCharTS** as an intrinsic spectral probe to monitor the various events of aggregation of any protein rich in charged amino acids.

References:

1. Chong, F.P., et al., *Tau proteins and tauopathies in Alzheimer's disease.* 2018. **38**: p. 965-980.
2. Alom, S.E., et al., *Early events during the aggregation of Aβ16-22-derived switch-peptides tracked using Protein Charge Transfer Spectra.* 2024. **1297**: p. 342374.
3. Prasad, S., et al., *Near UV-Visible electronic absorption originating from charged amino acids in a monomeric protein.* 2017. **8**(8): p. 5416-5433.

P 62

The Interplay Between Protein Flexibility, and Associated Water Dynamics in osmolyte solution: Does it Affect macromolecule's thermal stability

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Small organic molecules, or osmolytes, play a crucial role in stabilizing the native conformation of biomolecules within cells, a property that has also been harnessed in biopharmaceutical formulations. Despite their significance, the mechanisms underlying protein-osmolyte interactions remain poorly understood. Water, a key participant in virtually all biomolecular processes, is thought to play a critical role in these interactions as well. In this study, we investigate how osmolytes influence the dynamics of associated water and reveal a striking correlation between modified water behaviour and the stability of proteins across different osmolyte environments. Furthermore, we explore how osmolyte-induced changes in the dynamics of biological water affect the internal flexibility of proteins. Our findings demonstrate that the internal regions of proteins are sensitive to these water dynamics, leading to modulation of their flexibility. In summary, our results suggest that osmolytes interact with proteins by altering the properties of associated water, which in turn affects protein flexibility and is reflected in the thermal stabilization or destabilization of the macromolecules.

P 63

An Activity-Based Fluorogenic Sensor for Cu ions: Deciphering the Sensing Mechanism and Detecting Cu Ions In Vivo

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Activity-based sensors exploit coordination chemistry and metal-assisted reactions for selective and sensitive detection of metal ions in the biological milieu. Activity-based fluorogenic metal ion sensors consist of a fluorophore conjugated to a metal-binding scaffold via a reactive bond.[1] Binding of a specific metal ion to the scaffold results in a metal-catalyzed reaction that leads to the release of the fluorophore thereby increasing fluorescence.[1] The release of fluorophore from the sensor leads to significant fluorescence enhancement and also overcomes fluorophore quenching due to unpaired electrons on paramagnetic metal ions. Further the detection of metal ions that are weak binders based on Irving Williams Series[2] might be feasible via this strategy as an activity-based sensing response does not solely rely on metal-binding to the scaffold. Importantly, activity-based sensors can be synthesized using a modular synthetic approach via separate synthesis of the dye and the scaffold followed by conjugation of two units, facilitating the incorporation of changes in improving the selectivity and sensitivity. Thus, activity-based sensing is an attractive strategy for the detection of weak binding metal ions with unpaired electrons. So far, most activity-based sensors have utilized pyridine donors with di-/tri-/tetra- coordinating N donor sites. These sensors had preferential selectivity towards strong binding metal ions.[1] In this backdrop, to access sensors for the weak binding metal ions, we developed a novel activity-based sensor (**N5-CP)** with 5-N donor sites. **N5-CP** was highly selective toward $Cu^{+/2+}$ ions and gave a 63 times fluorescence enhancement in the presence of Cu^{2+} ions. The sensor could detect Cu ions in both living cells and in a live zebrafish larval model.[3] Interestingly, the sensor worked exclusively in the presence of glutathione and ambient oxygen.[3] Characterisation of intermediates and products hinted towards the involvement of a Cu^{II}-hydroperoxo species in the catalytic mechanism.[3] Since the previously reported activity-based sensors for metal ions also function in the presence glutathione and ambient oxygen, we believe that our proposed catalytic mechanism via a Cu^{II}-hydroperoxo species, supported by experimental evidence, would be applicable to the class of activity-based sensors that function via metal-mediated oxidative cleavage. Finally, the results suggested the need for further fine-tuning of scaffold design to access sensors for metal ions that lie lower in the Irving-Williams series. The sensor design, sensing mechanism, and biological studies will be presented.

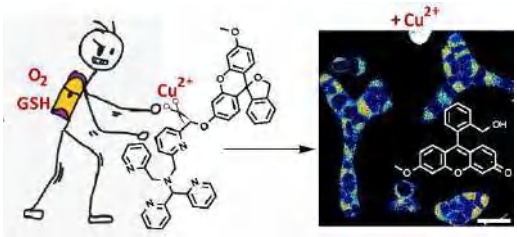

Figure 1: Scheme depicting Cu ion sensing via oxidative cleavage in the presence of glutathione and ambient oxygen

References:

1. D. A. Iovan et al. *Inorg. Chem.*, **2019**, 58, 13546.
2. H. Irving et al. *J. Am. Chem. Soc.*, **1953**, 3192.
3. K. Gupta et al. *Chem. Commun.*, **2023**, 59, 8282.

P 64

$CdTe_{0.25}Se_{0.75}$ Quantum Dots as Efficient Room Temperature Single Photon Source for Quantum Technology

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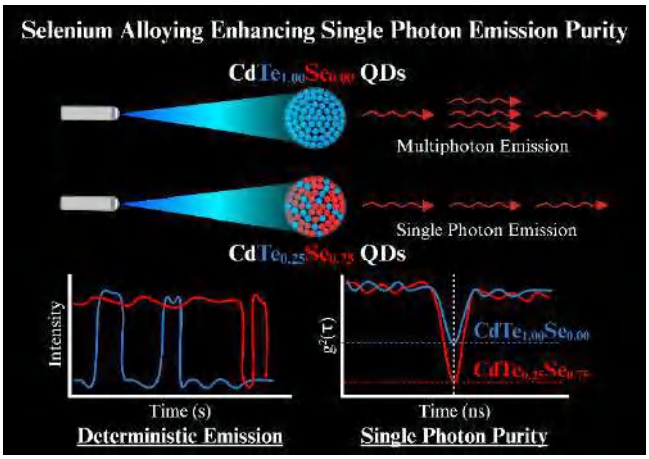
Room temperature single photon sources (SPS) are crucial for developing the next generation quantum technologies. Quantum dots (QDs), recently, have been reported as promising materials as SPS at room temperature. By optimizing the single particle optical properties of a series of water-soluble, $CdTe_xSe_{1-x}$, here we provide an efficient SPS with increased single photon purity. The data revealed that second order photon correlation, $g^2(0)$ value decreases substantially from 0.21 in CdTe to 0.02 in $CdTe_{0.25}Se_{0.75}$ QDs. They also exhibited deterministic emissions with an increase in ON time exceeding 95% of the total time. This was accompanied by an increased photon count rate, substantially reduced blinking events, and extended single particle ON-time. The increased single photon emission in $CdTe_xSe_{1-x}$ is attributed to very fast electron trapping to dense trap states, which suppresses the multiexciton recombination.



References:

1. V. Chandrasekaran et al., *Nano Lett.*, **2017**, *17*, 6104.
2. C. Zhu et al., *Nano Lett.*, **2022**, *22*, 3751.
3. H. J. Chuang et al., *Nano Lett.*, **2024**, *24*, 5529.
4. R. Uppu et al., *Nat. Commun.*, **2020**, *11*, 3782
5. J. Tan et al., *New J. Chem.*, **2015**, *39*, *4488*
6. Y. Arakawa and M. J. Holmes, *Appl. Phys. Rev.* **2020**, *7*, 021309

P 65

Cholesterol-Dependent Membrane Binding of ApoE Signal Peptide: Phosphatidylglycerol as a Switch

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A short segment of signal peptide (SP) located at the N-terminal of the apolipoprotein E (ApoE) protein directs the ApoE protein to the endoplasmic reticulum after its synthesis. Previous research has demonstrated that this peptide exhibits varying binding affinity toward lipid membranes in a manner influenced by cholesterol. However, the specific interaction mechanism is still not fully understood. In our study, we explored how the composition of adjacent lipids affects the binding of the ApoE signal peptide to the membrane. We discovered that a negatively charged lipid, such as phosphatidylglycerol, functions as a switch, reducing the peptide's binding efficiency to cholesterol-rich membranes. It was interesting to note that phosphatidylethanolamine doesn't activate the cholesterol-dependent binding of the ApoE signal peptide, but it does work in conjunction with phosphatidylglycerol to enhance cholesterol sensitivity in membranes containing phosphatidylglycerol. Thus, the binding affinity of a peptide to a membrane is influenced by neighboring lipids rather than the lipid-binding domain of the peptide. Our findings have uncovered a new role of lipid diversity in modulating the membrane binding of the ApoE signal peptide.

P 66

Complex condensation of a prion-like protein and a J-domain protein inhibits aberrant phase transition and amyloid formation

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Eukaryotic cells organize a wide array of biochemical processes by creating membrane-bounded organelles in a spatiotemporal fashion to function properly. In addition to this, cells also contain membrane-less organelles (MLOs) or biomolecular condensates formed via phase separation that are primarily composed of intrinsically disordered proteins/regions and nucleic acids. These biomolecular condensates are involved in a myriad of critical cellular processes and are implicated in various fatal neurodegenerative diseases (1, 2). However, the phase separation of proteins inside the cell is tightly regulated to maintain the metastable state of these condensates and prevent any excessive abnormal aggregation. These MLOs consist of a wide range of J-domain proteins (JDPs), which can play an important role in modulating the sequence of events that govern the intracellular phase transition into liquid-like droplets, further preventing pathological amyloid aggregation (4). This regulation process by JDPs remains largely unexplored. I will discuss our recent results on the heterotypic condensation of a prion-like protein and a JDP into liquid droplets. Utilizing a multidisciplinary approach involving a diverse range of biophysical, molecular biology, and imaging tools, our study delineates the complex network of interactions that govern the heterotypic phase separation of these proteins, which effectively impedes the formation of fibrous amyloid aggregates (5).

References:

1. Boeynaems, S., et al. *Trends Cell Biol.* **2018**, 28.
2. Agarwal, A., et al. *Nat. Commun*. **2022**, 13.
3. Bard, J. A. M., Drummond, D. *Front. Biophys.* **2024**.
4. **Arora, L**., et al. *Biorxiv*. **2024**.

P 67

Partitioning of gentamycin and oxytetracycline in spherical and rod shaped micelles formed by using TTAB, sodium salicylate and pyridoxamine and delivery to bovine serum albumin: Thermodynamic insights

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In this study we have used TTAB micelles representing drug carrier of antibiotic drugs which are gentamicin (GM) and oxyteracycline (OTC). The CMC of TTAB was found to be around 3.5 mM and above 26 mM it was observe to be in rod like shape. We have used two additives namely sodium salicylate (NaSal) which is a hydrotrope and pyridoxamine (PYR), one form of vitamin B6 to check the effect on the premicellar (2.5 mM) and postmicellar (7 mM) concentrations of TTAB surfactant. The intermolecular interactions between cationic surfactant and aromatic salt (NaSal) or essential organic compound pyridoxamine and their subsequent evolution to worm like micelle (WLM) have been investigated. Further, the partitioning of drugs into these systems have been studied by measuring the corresponding energetics involved, using isothermal titration calorimetry (ITC)[1]. GM and OTC both showed two sequential binding profile in the presence of additive. From ITC experiment it was observed that GM is able to partition more in the micellar system having higher concentration of TTAB with addition of hydrotrope NaSal. It has been reported that NaSal promotes the change in the shape of spherical to rod like micelle of TTAB. Imae and Kohnsaka model also suggests that the insertion of salicylate observed to be in between the head groups of the micelle which stabilizes the micellar structure[2,3]. In the presence of NaSal and PYR the first set of OTC and GM molecules showed very low value of partitioning constant (K_1) while K_2 with the high affinity which suggests that the first set of drug molecules might be creating space in palisade layer of TTAB micelle to enter next set of molecules with high affinity. The Stern –Volmer and Benesi-Hildebrand equation from fluorescence and UV-visible spectroscopy method respectively has been used to determine binding constant values which were found to be in accordance with each other. The partitioning of GM into TTAB+ NaSal/PYR systems showed static type while OTC in the same systems showed dynamic type of quenching by using fluorescence and TCSPC technique. From dynamic light scattering experiment it was observed that the addition of NaSal and PYR in TTAB surfactant increases the shape of micelle which we have further confirmed by SEM analysis. It was found that higher molality of TTAB increases the stability maintaining secondary structure of BSA as compared to low molality of TTAB. This change in molality of TTAB and addition of NaSal / PYR further changes the shape of the micelle which results in formation of sphere and / rod shaped micelle. In this sphere and rod shaped micelle GM and OTC are able to partition differently. These micellar systems further can be used as a drug delivery purposes[4].

Reference

1. Judy, E.; Kishore, N. *Langmuir* **2021**.
2. Kabir-ud-Din *J. Dispers. Sci. Technol.* **2009**.
3. Pawar, K.; *J. Dispers. Sci. Technol.* **2012**.
4. Rajput, S. M et al *Colloids Interface Sci. Commun.* **2020**.

P 68

Emergence of Dynamic G-Tetraplex Scaffold: Uncovering Low Salt-Induced Conformational Heterogeneity and Folding Mechanism

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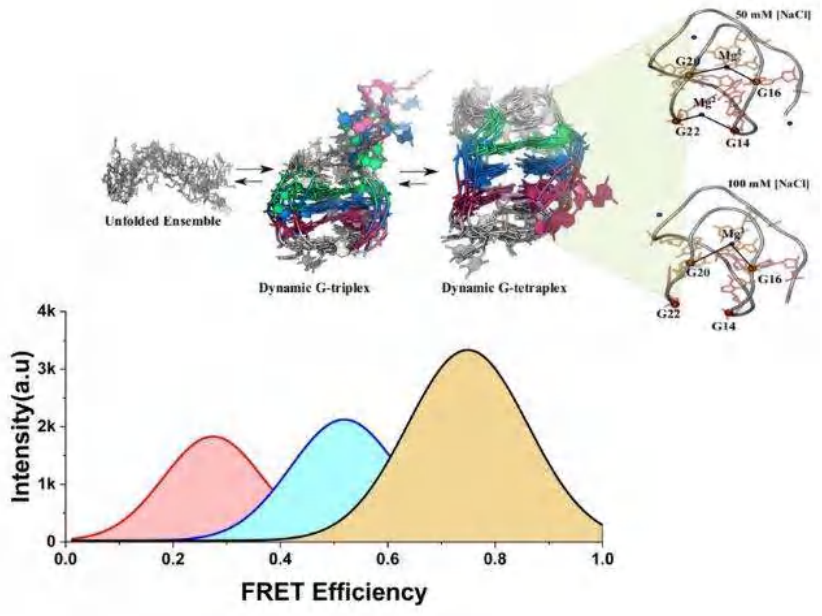
The topological diversity of human telomeric G-quadruplex structures is intrinsically related to their folding mechanisms, and is significantly modulated by ion-atmospheric conditions. Unlike previous studies that focused on higher Na+ or K+ concentrations, this study explores G-quadruplex folding and dynamics under low NaCl conditions (≤100 mM) using single-molecule FRET microscopy and advanced structure-based DNA simulation techniques. The smFRET data reveal three distinct populations; unfolded, intermediate dynamic triplex, and dynamic tetraplex structural ensemble. The broad distribution of the folded population highlights the dynamic nature of the quadruplex structure at low salt conditions. In agreement with smFRET result, free energy simulations show that with increase of NaCl concentration, the population shifts towards the folded state, and differentiates all intermediate structural ensemble. The dynamic equilibrium between the triplex and tetraplex scaffolds explain the microscopic basis of conformational heterogeneity within the folded basin. Simulations also reveal that the flexibility of dynamic tetraplex bases depends on the equilibrium distribution of ions underpinning a few ion-mediated dynamic non-native interactions in G-quadruplex structure. Contrary to the previously held belief that Na^+ induces minimal structural heterogeneity, our combined experimental and simulation approaches demonstrate and rationalize the structural variability in G-quadruplexes under low NaCl concentrations.



References:

1. Aznauryan, M.et al. *Nucleic Acids Research* **2016**, *44* (22), 11024-11032.
2. Noer, S. L et al. *Nucleic Acids Res* **2016**, *44* (1), 464-471.
3. Rajendran, A. et al *Angewandte Chemie International Edition* **2014**, *53* (16), 4107-4112.

P 69

Utilizing coronin 1 as a template to develop peptide-based broad-spectrum entry inhibitors: the importance of tryptophan-aspartic acid repeats

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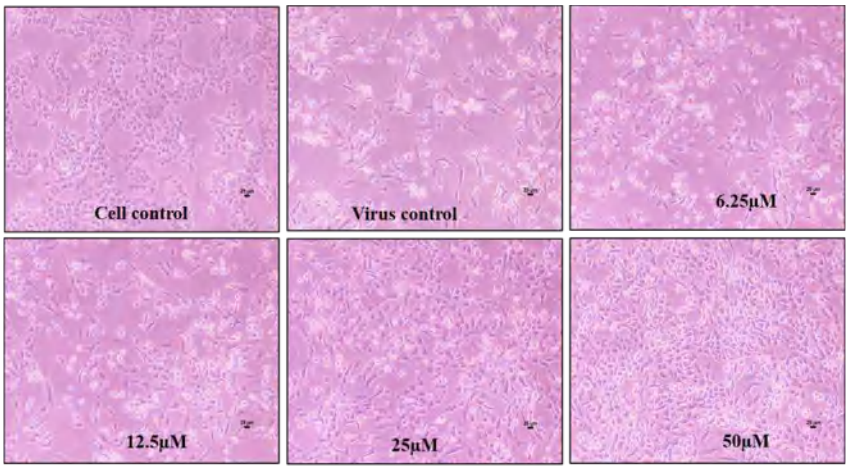
Enveloped viruses can enter the host cells by endocytosis and subsequently fuse with the endosomal membranes, or fuse with the plasma membrane at the cell surface. The crucial stage of viral infection, regardless of the route taken to enter the host cell, is membrane fusion. The present work aims to develop a peptide based fusion inhibitor that prevents membrane fusion by modifying the properties of the participating membranes, without targeting a protein. This would allow us to develop a fusion inhibitor that might work against a larger spectrum of enveloped viruses as it does not target any specific viral fusion protein. With this goal, we have designed a novel peptide by modifying a native sequence derived from coronin-1, a phagosomal protein, that helps to avoid lysosomal degradation of mycobacterium-loaded phagosomes. The designed peptide, mTG-23, inhibits ~30-40% fusion between small unilamellar vesicles containing varying amount of cholesterol by modulating the biophysical properties of the participating bilayers. As a proof of principle, we have further demonstrated that mTG-23 inhibits Influenza A infection in A549 and MDCK cells (with ~EC50 of 20.45 µM and 21.45 µM, respectively), where viral envelop and endosomal membrane fusion is a crucial step. Through a gamut of biophysical and biochemical methods, we surmise that mTG-23 inhibits viral infection by inhibiting viral envelop and endosomal membrane fusion. We envisage that the proposed antiviral strategy can be extended to other viruses that employ a similar modus operandi, providing a novel pan-antiviral approach.



References:

1. G. P. Pattnaik et al. *Chem. Phys. Lipids*, **2018,** 217, 35
2. G. P. Pattnaik et al. *Biochim. Biophys. Acta, Biomembr.*, **2019**, 1861, 183056.
3. S. Pandia et al. *J. Phys. Chem. B*, **2024**, 128, 4986.

P 70

Toward Development of Peptide-based Multi-analyte Fluorescent Sensors and Theranostic Agents

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Biological analytes like phospholipids, reactive oxygen species, H^+ ions, proteins, and metal ions play critical roles in survival and growth of a living system [1-3]. Dynamic changes in the localization and distribution of these bio-analytes transmit and regulate cellular signals, thereby driving ongoing life processes. Furthermore, homeostasis of one analyte is related to that of other analytes present in the biological systems. Disruptions in the correlated distribution of the bio-analytes lead to pathophysiological conditions such as neuro-degenerative diseases [4] and cancers [5]. Hence, it is important to detect multiple analytes simultaneously in living cells to understand their comprehensive role in executing a particular biological process and the effect of dysregulation in their correlated distributions leading to disease conditions. Moreover, the drugs used to treat these diseased conditions can also modulate the levels of one or more bio-analytes in the living system. Owing to regional or genetic variations, concentration and distribution of a particular analyte maybe different in different biological systems as well. This can lead to personalized variations in symptoms and response to treatments. Therefore, it is also important to track the activity of drugs via sensing the levels of the affected bio-analytes in real time. In order to ensure that the drug and the sensing moiety reach the same intracellular localization at the same time, it is necessary to combine the drug and the sensing unit into a theranostic agent [6]. Both the discussed issues involve combining more than one sensing or therapeutic units into a single molecule. To achieve this, we have used peptides as the common platform for attaching different moieties due to their biocompatibility and ease of synthetic modulation [7]. In this backdrop, I will detail the design, synthesis and other studies of a peptide-based multi-analyte sensor and a peptide-based theranostic agent in my poster.

References:

1. G. R. V. Hammond et al. *Curr. Opin. Cell. Biol.* **2020**, *63*, 57.
2. H. J. Forman et al. *Biochemistry* **2010**, *49*, 835.
3. S. Conus et al. *Biochem. Pharmacol.* **2008**, *76*, 1374.
4. K. Jomova et al. *Mol. Cell. Biochem.* **2010**, *345*, 91.
5. V. Ruiz-Rodado et al. *Nat. Methods.* **2022**, *19*, 1048.
6. S. S. Kelkar et al. *Bioconjugate Chem.* **2011**, *22*, 1879.
7. C. Wynne et al. *Sens. Diagn.* **2024**, *3*, 987.

P 71

Investigating Nanoscale Polarity in Thin Polymer Films Using Nile Red Dye and Single Molecule Spectroscopy

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Understanding the polarity of nanoscale environments within thin polymer films is critical for optimizing their performance in various applications, such as in sensors, coatings, and electronic devices.[1] However, the heterogeneity of these environments poses a significant challenge in characterizing local polarity.[2,3] In this study, we use Nile Red,[4] a polarity-sensitive fluorescent dye, as a molecular probe to investigate these nanoscale variations. By employing single-molecule spectroscopy, we gain insights into the distinct nanoenvironments present within the polymer matrix. Our approach enables the detection of variations in polarity at the nanoscale, revealing the complex interplay between polymer morphology and environmental conditions. Preliminary results demonstrate significant heterogeneity in local polarity across different polymer matrices, including PMMA, PVP, PVDF, and Zeonex. These findings highlight the need for single-molecule level analysis to fully understand the heterogeneity within each polymer environment.

References:

1. Palanichamy, K. et al. *Mater Res Express* **2023**, *10* (2)
2. Hess, C. M.et al. *Journal of Physical Chemistry B* **2014**, *118* (29), 8905–8913.
3. Hou, Y. et al. *Journal of Physical Chemistry B* **2000**, *104* (2), 212–219.
4. Freidzon, A. Y et al. *Int J Quantum Chem* **2012**, *112* (18), 3059–3067.

P 72

Elucidating the Aggregation Behaviour of Human β-Synuclein and Its Pathological Mutants :Modulation by Neurotransmitter Interactions

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The misfolding and aggregation of proteins, particularly within the synuclein family, are critical processes underlying the pathology of several neurodegenerative diseases, including Parkinson's disease. Among the synucleins, β-synuclein (β-syn) has been understudied despite its significant role in modulating α-synuclein aggregation and its emerging association with neurotoxicity. This study explores the aggregation properties of wild-type and pathological mutants (P123H and V70M) of recombinant human β-syn under physiologically relevant conditions, focusing on the modulatory effects of various neurotransmitters. It is known that β-syn is resistant to fibrillation under normal cytoplasmic conditions, yet small changes in pH, which frequently occur in cellular environment, can induce fibril formation. Through a series of Thioflavin T (ThT) assays, we observed that catecholamines such as dopamine and epinephrine significantly suppress β-syn fibrillation, a phenomenon further corroborated by ANS binding assays that demonstrated decreased surface hydrophobicity in the presence of these neurotransmitters. Structural and morphological analyses using techniques like Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) provided additional insights into the nature of these fibrils and their modulation by neurotransmitters.

This research highlights the complex role of β-syn in synucleinopathies and underscores the potential of targeting neurotransmitter interactions as a therapeutic strategy. Future work will focus on the differential effects of wild-type and mutant β-syn species on neuronal cell lines, with an emphasis on the chaperonic effects exhibited by these proteins.

Keyword : β-synuclein, Neurotransmitters, Neurodegenerative diseases

P 73

Folate-targeted delivery of doxorubicin in HeLa cells using water soluble ZnS-shelled $CuInS_2$ quantum dots

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Quantum dots have emerged as promising nanocarriers for targeted drug delivery due to their unique optical and electronic properties. The present work explores the prospect of quantum dots for the delivery of a widely used chemotherapeutic agent, doxorubicin (DOX) via a folate receptor mediated pathway. The folate receptor, which is overexpressed in many cancer cells, offers a strategic target for improving the specificity of drug delivery, reducing systemic toxicity, and enhancing therapeutic efficacy. Copper indium sulfide quantum dots with a ZnS shell are functionalized with folic acid to selectively target cancer cells that overexpress folate receptors. DOX added to this system binds to the surface of the quantum dot electrostatically. Fluorescence lifetime imaging microscopy (FLIM) and Fluorescence correlation spectroscopy (FCS) are employed to investigate the intracellular kinetics and interaction of quantum dots and DOX release, providing real-time visualization and quantification of drug accumulation within the cancer cells [1]

References:

1. Suarasan, S. *et al. ACS Appl Mater. Interfac.* **2019**,.11, 7812–7822

P 74

Effect of Vibrational Strong Coupling on Glass Transition Temperature of Polyvinyl acetate

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Vibrational strong coupling (VSC) is mediated by the interaction of the vacuum field in resonance with molecular vibrations. The phenomenon of VSC gives rise to two vibro-polaritonic states that are hybrid of light-matter states.[1,2] This strong light-matter interaction can alter the properties of a molecule such as conductivity[3] and crystallization[4]. It can also modulate the product selectivity and rates of chemical reactions.[5] One such property we would like to study is phase transition such as the glass transition temperature of a polymer (polyvinyl acetate). The experimental results are complemented with transfer matrix method (TMM) simulations. Phase transition is a process that reveals the changes in the physical properties when external conditions such as temperature vary. Studying glass transition with VSC can give insights into inter- and intra-molecular interactions. Here, we are investigating the influence of VSC on the molecular properties like refractive index of the molecules that are undergoing glass transition. Additionally, we are probing if the Fabry-Perot cavity can be used as a tool to study the phase transition of the material. This study would contribute to understanding the mechanism of VSC and how VSC can influence molecular interactions.

References:

1. Garcia-Vidal et. al *Science* **2021**, *373*(6551), eabd0336.
2. Hertzog, M. et al. *Chem. Soc. Rev.* **2019**, *48*(3), 937.
3. Kumar et al. *J. Am. Chem. Soc.* **2024**, *146*(28), 18781.
4. Hirai, K., et. al *Chem. Sci.* **2021,** *12*(36), 11986.
5. Thomas, A. et al. *Science* **2019**, *363*(6427), 615.

P 75

Femtosecond pulse intensity impact on optical nonlinearity of a carbazole-picric acid complex for photonic applications

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The present research article highlights to unveil the nonlinear optical properties of the Carbazole-Picric Acid (Cz-PA) Complexd confirmed through various structural analyses like X-ray crystallography, Infrare (IR) and NMR spectroscopy with UV-Vis absorption spectroscopy. Herein we performed Z-scan experiments (closed as well as open aperture schemes) [1,2] under femtosecond laser pulse excitation (100 fs, 1 kHz) to explore the third-order optical nonlinearity of the complex. The optical nonlinear measurements were performed with the variation of pulse intensities (37-85 GW/cm^2) at 520 nm and the intensity effect on cubic nonlinearity is highlighted. Within the given range of pulse intensity, the material shows the positive absorptive nonlinearity ($\beta > 0$) generated by the two-photon absorption (2PA) process, as well as the electronic Kerr-induced refractive nonlinearity ($n_2 > 0$). We calculated hyperpolarizability (γ) in the order of 10^{-31} esu with the nonlinear susceptibility $\chi^{(3)}$ in the order of 10^{-13} esu. These values increased with intensity. Furthermore, the obtained figures of merit and 2PA cross-section values demonstrate the potential uses of Cz-PA in photonic devices such as ultrafast switching.

References:

1 M. Sheik-Bahae et al. *IEEE J. Quantum Electron.*, 1990, **26**, 760.

2 M. Sheik-bahae et al. *Opt. Lett.*, 1989, **14**, 955.

P 76

Loop Kinematics of Dengue Protease and its role in Allosteric Regulation.

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The Dengue virus (DENV) is the causative agent of dengue fever, and currently, no direct-acting antiviral drugs or approved vaccines exist to combat infections. The NS3 protease, along with its cofactor NS2B, plays a pivotal role in viral replication within human host cells, making them critical drug targets. Previous attempts to design active-site inhibitors have faced challenges due to the relatively shallow active site and surrounding charged residues. To overcome these limitations, the design of allosteric inhibitors is essential for protease inhibition. Our findings emphasize the importance of Finger II motion in opening the claw, which allows the C-terminal region of NS2B to enter, thereby transforming the protease into its active conformation. Further it was established that some known allosteric inhibitors disrupt this motion, effectively inhibiting NS2B/NS3 protease activity and presenting a promising strategy for the development of targeted dengue virus therapeutics.

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Spectroscopic Insights into Beryl Structural Dynamics: A Molecular Approach

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The optical properties of Beryl make it useful in biomedical imaging systems, providing high transparency and durability. Its crystal structure is also explored for potential applications in drug delivery systems and tissue engineering due to its stability and biocompatibility. The optical properties are also utilized in imaging devices, contributing to advancements in medical diagnostics. Hence, molecular orientations and structural distortions are essential for comprehending molecules' responses to variations in temperature (*T*) and pressure (*P*). The features mentioned are also crucial in disciplines such as geology and materials science, where understanding the behavior of materials under different thermodynamic situations is highly significant.

This research comprehensively examines the mineral Beryl utilizing Terahertz (THz), infrared (IR), and Raman spectroscopy techniques at elevated temperatures. These sophisticated spectroscopic techniques enable us to analyze the vibrational modes of Beryl, offering valuable information on its stability and structural features when subjected to temperature stress. Our studies reveal that Beryl retains its structural integrity up to temperatures of 600°C. The vibrational modes of the mineral, which are essential to its behavior in metamorphic rocks, are intimately related to its stability. It is vital to comprehend these vibrational modes to optimize the material's qualities in various geological and industrial applications. Our work adds significant information to geology and materials science disciplines by thoroughly examining its behavior at high temperatures. It clarifies how minerals like Beryl can resist harsh environments without losing their essential characteristics.

P 78

Inhibitory Effect of Diosgenin on α-Synuclein Aggregation: An in-Silico Study

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Parkinson's Disease (PD) is characterized by the accumulation of α-synuclein protein, particularly in its mutated (A30P, A53T, E46K) forms [1, 2]. This aggregation leads to neuronal cell death. To identify the most promising drug candidate, we conducted a high-throughput molecular docking screen of 2000 natural molecules isolated from Indian traditional medicinal plants. Diosgenin emerged as the top candidate, exhibiting the highest binding affinity of -7.5 kcal/mol. Therefore, this study explores the potential of Diosgenin, a phytoconstituent identified through ADMET predictions, to inhibit A30P, A53T, and E46K mutants of α-synuclein aggregation. The temporal and conformational stability of the Diosgenin-protein complex was further validated through extensive molecular dynamics (MD) simulations for 500 ns. MD simulation results at both extracellular (0.145 M) and intracellular (0.015 M) salt concentrations [3] revealed that Diosgenin stabilizes α-synuclein mutants by inducing conformational changes that reduce β-sheet content, a key factor in aggregation. The salt concentration influenced the structural dynamics, with higher salt levels generally promoting more compact and stable conformations. Principal component Analysis (PCA) and free energy calculations further supported the idea that Diosgenin successfully inhibits the α-synuclein mutants investigated here. These results suggest that Diosgenin could be a promising drug for treating PD by preventing α-synuclein aggregation. Further validation can be obtained through wet-lab experiments.

References:

1. Choi, W et al. *FEBS Letters*, 2004. **576**(3): p. 363.
2. Lemkau, L.R., et al. *Journal of Biological Chemistry*, 2012. **287**(14): p. 11526.
3. *Neuroscience*, D.F. Dale Purves , George J. Augustine, Editor. 2018, Sinauer Associates: Oxford University Press, United States.

P 79

Tuning Protein Bioconjugation in Liquid Droplet State

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Understanding diversified role of different form of phosphate-linkage in maintaining biological processes is a topic of interest both in chemistry and biology.[1,2] In this context, we showed here role of nucleotides and phosphates in protein (Bovine Serum Albumin) modification in condensed state.[3] Lysine-specific modification gets inhibited in presence of ATP, but significantly enhanced in presence of monophosphates which allows temporal control over dynamic change in protein functionalization via enzymatic ATP hydrolysis. We believe this temporal modulation of nucleotide-mediated chemoselective behavior in liquid droplet state of protein will find immense application in bioconjugation strategies, protein engineering and also in deciphering altered protein modification behavior in diverse condensed phases.

References:

1. Knouse, K. W. et al. *ACS Cent. Sci.* **2021**, *7 (9)*, 1473.
2. Westheimer, F. H. *Science* **1987**, *235 (4793)*, 1173.
3. Küchler, A. et al. *Nat. Nanotechnol.* **2016**, *11 (5)*, 409–420.

P 80

Enzymatic Dissociation of DNA-Histone Condensates in an Electrophoretic Setting: Modulating DNA Patterning and Hydrogel Viscoelasticity

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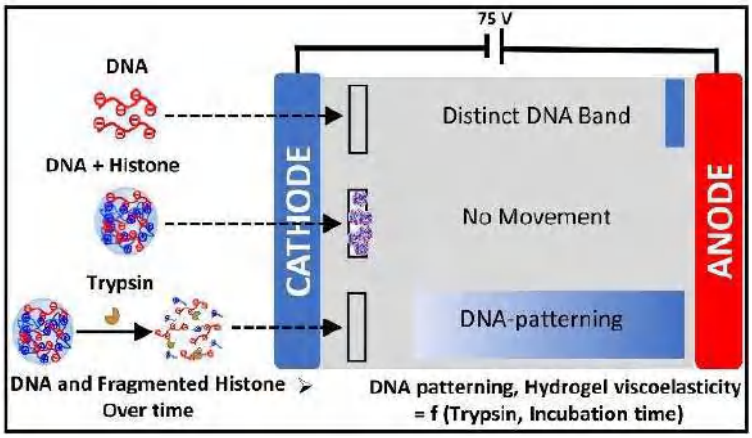
Development of an energy-driven self-assembly process is a matter of interest for understanding and mimicking diverse ranges of biological and environmental patterns in a synthetic system. In this article, first we demonstrate transient and temporally controlled self-assembly of a DNA-histone condensate where trypsin (already present in the system) hydrolyzes histone, resulting in disassembly. Upon performing this dynamic self-assembly process in a gel matrix under an electric field, we observe diverse kinds of DNA patterning across the gel matrix depending on the amount of trypsin, incubation time of the reaction mixture, and gel porosity. Notably, here, the micrometer-sized DNA-histone condensate does not move through the gel and only free DNA can pass; therefore, transport and accumulation of DNA at different zones depend on the release rate of DNA by trypsin. Furthermore, we show that the viscoelasticity of the native gel increases in the presence of DNA and a pattern over gel viscoelasticity at different zones can be achieved by tuning the amount of enzyme, i.e., the dissociation rate of the DNA-histone condensate. We believe enabling spatiotemporally controlled DNA patterning by applying an electric field will be potentially important in designing different kinds of spatiotemporally distinct dynamic materials.



References:

1. Neetu Sivoria et al. *Langmuir* **2024**, *40*, 13505.

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Photo-responsive and shape-switchable MoS_2-Peptide-Hybrid Nano-systems for Enacting Photo-Chemo and siRNA-Mediated Gene Therapy in Glioma

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Photonic nanomedicine includes utilization of a comprehensive range of optical nanomaterials, which upon absorption of photon beam enacts their therapeutic potential. Implementation of light to activate optical materials at the desired site of action has enabled development of successful deep tumor therapeutics. The combination of photochemotherapy with 2D Molybdenum disulfide (MoS_2) nanostructures has attracted attention due to its high surface area and photothermal properties. Here, a novel optically active material-based approach is introduced to induce photothermal anti-glioma effect. Bulk MoS_2 is exfoliated to produce photo-active nanostructures using shape-tunable aqueous peptide scaffolds. Nanofiber and nanosheet like morphology of our self-assembled peptide nanostructures could exfoliate the bulk MoS_2 differentially, altering its ability to generate heat upon NIR photon absorption. Interestingly, exfoliated MoS_2-peptide nanosheets here proves to be an excellent photothermal agent by inducing a temperature elevation up to ~51 ˚C upon 808 nm NIR absorption. Furthermore, ligation of folic acid is carried out to promote tumor-targetability of this NIR-responsive delivery system, that is loaded with anticancer siRNA/drug for treating glioma. Enhanced siRNA/Dox loading onto the 2D flat morphology of MoS_2-peptide nanosheets demonstrates ~90 % cancer cell death in C6 glioma cells under NIR exposure. Expression of an oncogene, galectin-1 also appears to be suppressed following the treatment. Thereafter, *in vivo* analysis in C6 glioma syngeneic rat model demonstrates a remarkable ~11.9 times decrease in the tumor volume with siRNA/Dox loaded nanosystem + NIR as compared to the PBS control group. Taken together, our findings promote the utility of MoS_2-based opto-nanomaterials in conjecture with bio-compatible peptide scaffold as a tri-modal chemo, gene, and phototherapeutic effect in glioma treatment.

References:

1. Wang, Jianling, et al. *Bioactive Materials* 6.11 (2021): 4209.
2. Zhou, Zhan, et al. *Advanced NanoBiomed Research* 2.11 (2022): 2200065.
3. Pang, Bo, et al. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 608 (2021): 125506.
4. Yin, Wenyan, et al. *ACS nano* 8.7 (2014): 6922.
5. Chibh, Sonika, et al. *Nanoscale* 14.3 (2022): 715.

P 82

Tuning the fluorescence emission of DADQ based molecular solids by dielectric environment variation [1]

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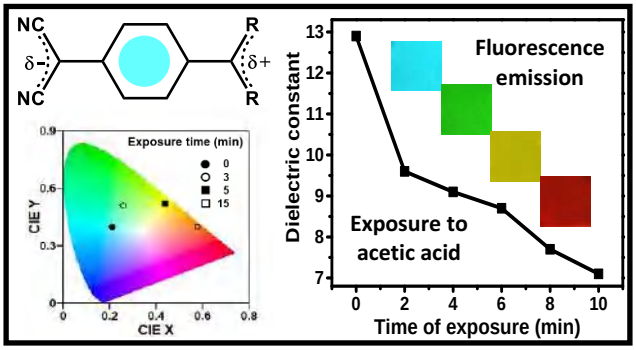
Fluorophores which show enhanced light emission in aggregated or solid-state have been an active area of research due to their applications in fields like optoelectronics [2], information encryption [3], bioimaging [4] and sensing applications [5]. Fluorescence response tuning can be achieved by modification in molecular structure or supramolecular assembly patterns. We show that the fluorescence emission in DADQ based molecular solids can be fine-tuned by short exposure to acetic acid vapors and the effects can be reversed. Contrary to the earlier observation in similar context, there was no structural change observed. Studies based on NMR, mass spectrometry and optical spectroscopy reveal variation results from weak protonation which leads to subtle change in the dielectric environment of molecules. This idea is also supported by computational modelling of electronic ground state and excited state of a DADQ molecule including the standard solvation effect. Dielectric measurement by impedance spectroscopy and surface potential measurement by Kelvin probe force microscopy provide experimental verification of this general concept. The model developed can be useful for broader understanding of fluorescence emission tuning in molecular solids under mild perturbations.



References:

1. Nikita Mahajan et. al. *J. mater. Chem. C* **2024,** 12, 13430.
2. Canbin Oyuyang et. al. *ACS Appl. Mater. Interfaces* **2017**, 9, 10366.
3. Wei Wang.et. al. *ACS Appl. Mater. Interfaces* **2024**, 16, 15096.
4. Senthilnathan N. et. al. *Sci. Rep*. **2017**, *7*(1), 10583.
5. Tianhong Chen. et. al. *J Mater. Chem. A* **2020**, 8, 24695.

P 83

Reduction of membrane cholesterol inhibits neuronal exocytosis

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Exocytosis is essential for neuronal communication and involves significant membrane remodelling. Cholesterol is a strong modulator of membrane properties and can in principle affect exocytosis. However, there are conflicting reports about its effect on exocytosis. This study investigates this puzzle using multiple techniques on both well-characterized artificial bilayers and on living cells.

We measure various physical properties of a lipid membrane (whose composition mimics the synaptic vesicle membrane) as a function of its cholesterol content (from 0% to 50%). As expected, fluorescence lifetime and spectral shift measurements of indicator dyes (e.g., Flip-tR, Nile Red, and Prodan) show that membrane order increases with cholesterol. Surprisingly, the membrane indentation force, as measured by Atomic Force Microscopy, decreases with increasing cholesterol. Importantly, the indentation force correlates with enhanced fusion kinetics involving artificial vesicles and bilayers, as assessed by Total Internal Reflection Fluorescence Microscopy. This indicates that indentation force is a reliable measure of vesicle fusion propensity, while dye-based membrane order measurements may be misleading.

To explore the biological significance, we examined the effect of reducing cholesterol in live cells using mβCD. We found that vesicular exocytosis slows down with decreasing cholesterol, as observed with artificial bilayers. We also investigated whether serotonin-derivatives, such as N-acetyl serotonin (NAS) and 5-hydroxytryptophan (5HTP), can rescue the effect of reducing cholesterol, since serotonin is known to do so[1]. Notably, 10 mM NAS, but not 5HTP, increased the rate of endocytosis in a neuronal cell line by 36%. These insights could aid in identifying small molecules that modulate membrane properties and influence exocytosis without receptor interactions. Though these results are obtained only from *in vitro* cellular systems, they raise potential clinical concerns given that widely used cholesterol lowering drugs cross the blood brain barrier.

Reference and notes:

1. Saha Roy et.al. *J. Phys. Chem. B* 2024, 128, 20, 4975

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Symmetry-Breaking Charge Separation in a Chiral Bis(perylenediimide) Probed at Ensemble and Single-Molecule Levels

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Chiral molecular assemblies exhibiting symmetry-breaking charge separation (SB-CS) are potential candidates for developing chiral organic semiconductors. Herein, we explore the excited state dynamics of a helically chiral perylenediimide bichromophore (Cy-PDI_2) exhibiting SB-CS at the ensemble and single molecule levels (Figure 1). Solvent polarity tunable interchromophoric excitonic coupling in chiral Cy-PDI_2 facilitates the interplay of SB-CS and excimer formation in the ensemble domain.[1] Analogous to the excited state dynamics of Cy-PDI_2 at the ensemble level, single-molecule fluorescence lifetime traces of Cy-PDI_2 depicted long-lived off-states characteristic of the radical ion-pair mediated dark states.[2,3] The discrete electron transfer and charge separation dynamics in Cy-PDI_2 at the single molecule level are governed by the distinct influence of the local environment.[3,4] The present study aims at understanding the fundamental excited state dynamics in chiral organic bichromophores,[5] for designing efficient chiral organic semiconductors and applications towards charge transport materials.

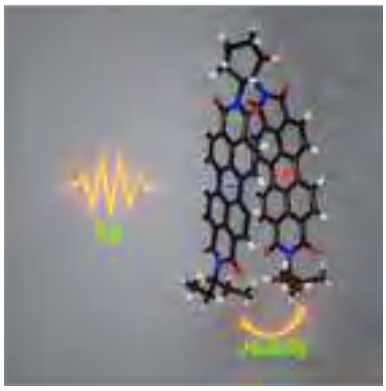

Figure 1: Elucidation of symmetry-breaking charge separation in helically chiral perylenediimide dimer

References:

1. Sebastian et al. ACS *Energy Lett.* **2022**, *7*, 696.
2. Ernst et al. *Chem. Phys. Lett.* **2009**, *482*, 93.
3. Yeow et al. *J. Phys. Chem. A* **2006**, *110*, 1726.
4. Whitcomb et al. *J. Phys. Chem. B* **2015**, *119*, 9020.
5. Haase et al. *Phys. Chem. Chem. Phys.* **2011**, *13*, 1776.
6. Maret et al. *J. Phys. Chem. Lett.* **2023**, *14*, 8667–8675.

P 85

Rapid and sensitive detection of Rhodamine B in cotton candy using surface-enhanced Raman spectroscopy

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Rhodamine B (RhB), a synthetic organic dye, is widely employed in various industries, including textiles, cosmetics, and food coloring. However, its unauthorized use in food products, such as cotton candy, poses significant health risks due to its potential carcinogenic effects. At low concentrations (10^{-7} M), RhB becomes colorless, making its detection challenging. Therefore, developing rapid and sensitive methods for Rhodamine B detection in food products is essential for safeguarding consumer health.

In this study, a cylindrical copper (Cu) substrate combined with silver colloid was utilized for the detection of Rhodamine B in cotton candy using surface-enhanced Raman scattering (SERS) spectroscopy. Various experimental parameters were optimized, including the concentration of the colloid, the colloid-to-analyte ratio, and the volume of the analyte-colloid mixture used for analysis. UV-Visible spectroscopy was employed to characterize the morphology of the SERS substrate. Under optimal conditions, the SERS substrate demonstrated the ability to detect RhB concentrations as low as micromolar levels in an aqueous solution. At an excitation wavelength of 785 nm, the SERS intensity of RhB at 619 cm^{-1} exhibited a linear correlation with the logarithm of RhB concentration, ranging from 10^{-3} to 10^{-9} M, with high reliability ($R^2 = 0.98$). The detection limit (LOD) reached 1×10^{-9} M. Additionally, the RhB spectra at a concentration of 10^{-7} M closely matched that of the real sample.

This study highlights the successful application of SERS for Rhodamine B detection in cotton candy, presenting significant implications for food safety. It contributes to the advancement of rapid, non-destructive analytical techniques for detecting synthetic dyes in food products, ultimately promoting consumer health and safety.

Keywords: Surface Enhanced Raman spectroscopy (SERS); Rhodamine B; Cotton candy;

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Molecular Probe to Image the Intracellular Modulation of NADH Concentration in the Presence of Glycolytic Inhibitor

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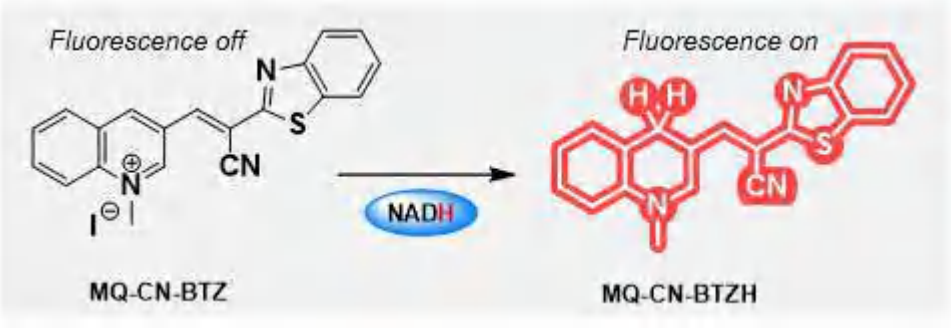
NADH is one of the essential coenzyme participates in various biological processes including maintaining the redox states. To diagnose various pathological conditions at early stages, it is crucial to develop understanding on the intracellular NADH levels. Here, we report the application of a dual channel probe **MQ-CN-BTZ** for fluorescence imaging of intracellular NADH levels. Interestingly, it was observed that by varying the ratio between probe and NADH concentration in solution phase, the probe showed dual emission at ~530 nm and ~660 nm when excited at 475 nm. Also, very large Stokes shift of ~180 nm was observed with respect to the longer emission wavelength. Furthermore, on the basis of good optical response, the probe was explored to image the crucial event of glycolysis pathway by employing glycolytic inhibitor 3-bromopyruvic acid (3-BrPA) that inhibits the activity of glyceraldehyde phosphate dehydrogenase (GAPDH) enzyme. As the depletion of the NADH levels corresponds to the inactivity of GADPH upon treatment with inhibitor, we attempted imaging of the modulation of NADH concentration in cellular system in the presence of 3-BrPA inhibitor indicating the importance of glycolysis step in elevating NADH levels. Overall, the dual channel optical probe was used to image the modulation of NADH concentration in in the presence of glycolytic inhibitor during the glycolysis step.



References:

1. Li, M, et al. *Journal of Materials Chemistry B*, **2021**, *9*, 9547.
2. Pan, X, et al. *Chemical Science*, **2019**, *10*, 8179.
3. Zhao, Y, et al. Analytical chemistry, **2018**, *91*, 1368.
4. Fomin, A, M, et al. *ACS Sensors*, **2016**, *1*, 702.
5. Magesh, K, et al. *ACS Applied Bio Materials*, **2024**, *7*,5679.
6. Wang, X, et al. *Chemical Engineering Journal*, **2024**, *495*, 153652.

P 87

Divalent Cations Modulate Depth-Dependent Polarity and Hydration at Lipid/Water Interface

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Ion binding at the lipid/water interface can significantly impact the structural, functional, and dynamic properties of cell membranes [1-3]. Although ion-lipid interactions have been widely studied, the precise influence of ion binding on the depth-dependent polarity and hydration of the lipid/water interface is still not well understood because of the differential binding of the divalent cations [4,5]. This work investigates the impact of three biologically significant divalent cations Mg^{2+}, Ca^{2+}, and Zn^{2+} on the depth-resolved interfacial polarity and hydration of zwitterionic DPPC lipids in its gel-phase at ambient temperature. To quantify these depth-specific characteristics, a set of solvatochromic fluorescent probes derived from 4-Aminophthalamide, with varying alkyl chain lengths (4AP-Cn; n = 5, 7, 9), was employed. Using steady-state fluorescence measurement and extensive molecular dynamics (MD) simulations, we quantify the minimal or maximal changes in interfacial polarity and hydration caused by divalent cation binding at the lipid/water interface. Our observations show that Zn^{2+} induces a significant blue shift in the steady-state fluorescence spectra of all the 4AP-Cn dyes, indicating a marked reduction in local polarity ($E_T^N \leq 0.05$) at the lipid/water interface compared to Mg^{2+} and Ca^{2+}, which leads to higher polarity ($E_T^N \geq 0.2$) in Richardts' polarity scale. The penetration-dependent steady-state fluorescence spectra of the 4AP-Cn dyes in the presence of Mg^{2+} and Ca^{2+} are similar to those observed for without cations, with only a slight red shift for Mg^{2+}, suggesting a minor hydration at the lipid interface. The MD simulations reveal that cations primarily bind to the phosphate group and glycerol regions of the lipids head groups. Our MD simulations also indicate that Zn^{2+} binds near the phosphate and glycerol group, and causes significant dehydration at the lipid/water interface, as detected by the 4AP-Cn dyes, whereas Mg^{2+} and Ca^{2+} have a much milder impact, with Mg^{2+} inducing slight hydration. Our current study unfolds the unique effects of divalent cations as probed by 4AP-Cn probes at the lipid/water interface, highlighting the potential of these dyes to track penetration-dependent changes in membrane properties induced by external agents or local environmental conditions.

References:

1. Friedman, R *J Membr Biol* **2018**, *251*, 453.
2. Yang, J et al. *J Chem Theory Comput* **2015**, *11*, 4495.
3. Kanti De, S et al. *Physical Chemistry Chemical Physics* **2018**, *20*, 14796.
4. Alsop, R. J et al. *Soft Matter* **2016**, *12*, 6737.
5. Hossain, S. I et al. *Biophys J* **2022**, *121*, 3520.

P 88

NusG displaces σ^{70} from mature transcription elongation complexes

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The *E. Coli* housekeeping sigma factor (σ^{70}) - a crucial component of the RNA polymerase (RNAP) holoenzyme is essential for promoter recognition and transcription initiation. Previous studies revealed that in addition to its classical role in initiation, σ^{70} can continue to influence transcription during later stages by remaining associated with the elongation complex (RDe). Additionally, structural studies revealed that an important transcription factor - NusG – can bind to a site in RDe closely overlapping with the s^{70} binding site in RNAP. Here we used fluorescence correlation spectroscopy (FCS) to develop an assay quantifying the fraction of elongation complexes which retained σ^{70} in presence and absence of varying amounts of NusG and found that NusG enabled release of σ^{70} from stalled RDe in a concentration dependent manner. Further, Single molecule TIRF experiments revealed that the energy barrier for σ^{70} release was greater in case of early elongation complexes (RDe14) in comparison to mature elongation complexes (RDe27). Our results suggest that σ^{70} plays a more prominent role in early transcription elongation, while NusG facilitates its release in later stages.

References:

1. Mazumder et al. *Journal of Molecular Biology*,**2019**, *Vol 431, Issue20.*
2. Kapanidis et al. *Science*, **2006,** *vol 314, Issue 5802.*

P 89

Ultrafast photoinduced electron transfer from Cu-deficient CuInS quantum dots to methyl viologen in water

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Increase in Cu-deficiency leads to the enhancement in photoluminescence (PL) intensity and lifetime in aqueous L-glutathione capped CuInS/ZnS quantum dots (QDs) [1] Such compositional variation is found to affect the rate of ultrafast photoinduced electron transfer (PET) through the ZnS shell from the QDs to methyl viologen (MV^{+2}). The extent of PL quenching by MV^{+2} gradually decreases with increasing Cu deficiency.in QDs. Stern-Volmer plots constructed using PL intensities exhibit upward curvature for all cases and PL lifetime remain unaffected. This observation can be explained by assuming the adsorption of Poisson distributed quencher molecules on the surface of negatively charged QDs. and PET happening in ultrafast regime, post excitation. Femtosecond Transient Absorption Spectroscopy (TAS) confirms the occurrence of ultrafast PET from QDs to quencher as the magnitude of ground state bleach (GSB) of the QDs gradually decrease with increase in concentration of quencher.[2] The dynamics of PET is estimated from the rate of GSB recovery, which becomes faster with decrease in Cu deficiency. As the PET happens through trap states closer to the conduction band edge, the density of these trap states decreases with increasing Cu deficiency due to more incorporation of Zn^{+2} ions in lattice of QDs by ZnS shelling. This observation forms the basis for future experiments on photosensitization of molecular catalysts by these QDs.

References:

1. A. Fuhr et al. *ACS Nano* **2020**, 14, 2212.
2. N. Eliasson et al. *J Phys Chem C* **2021**, 125, 14751.

P 90

Direct visualizing three intermediate conformation in single SARS-CoV-2 spike trimer during entry

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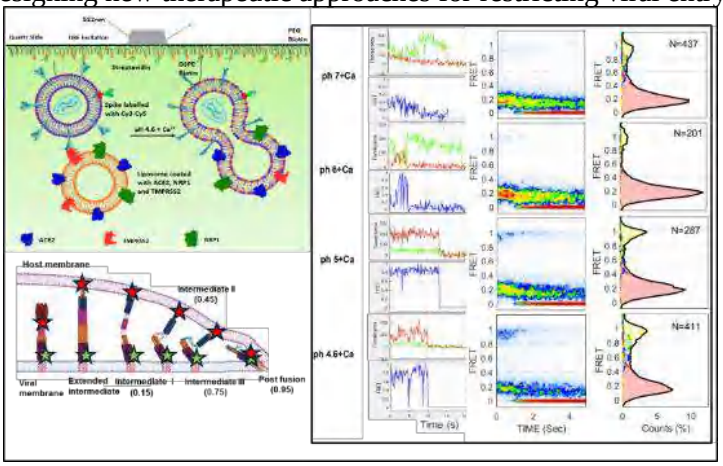
The SARS-CoV-2 is the causative agent for COVID-19 disease. Several variants of SARS-CoV-2 emerged during COVID pandemic. Out of all the variants, the omicron variant (B.1.529.1) was the latest variant of concern and has highest transmissibility owing to its enhanced immune evasion ability [1]. Therefore, understanding the molecular mechanism of cellular entry of SARS-CoV-2 omicron is of huge importance. The entry of omicron virus depends on the trimeric spike glycoprotein, which mediates the membrane fusion between the viral membrane and cellular membrane. The membrane fusion is a multi-step process [2], in which the spike first binds with the receptor protein ACE2 (Angiotensin convertase enzyme 2). Binding of ACE2 with the S1 domain of the spike glycoprotein triggers the spike fusion domain (S2) [3]. Structural study of both the pre and post fusion spike protein suggests a large-scale conformational rearrangement of the S2 domain [4]. Here, using Single molecule foster resonance energy transfer (smFRET) imaging technique, I have directly visualized the conformational dynamics in the S2 domain of individual spike glycoprotein trimers in the surface of the SARS-CoV-2 omicron virion during entry. I discovered that at neutral pH condition, prefusion structure of omicron spike samples three new conformations, which were previously unknown, in reversible manner. Lowering of pH shifts the equilibrium towards a high-FRET intermediate state on pathway to fusion. Finally, addition of Ca^{2+} at low pH shifts the conformational changes of spike to the post-fusion coiled-coil state. I have found that target membrane also allosterically regulates the dynamic intermediates of S2 on the pathway to fusion. My single molecule analysis has determined at least three possible intermediate states of omicron S2 domain on pathway to fusion. My study distinctively depicts the specific interaction between the membrane proximal region (MPR) and fusion peptide proximal region (FPPR) of S2 domain for forming the post-fusion structure. My single molecule assay developed will be utilized for designing new therapeutic approaches for restricting viral entry.



References:

1. Zhang et. al. *Cell Rep*. **2022** ,*39:* 110729.
2. Marcink et. al. *Sci Adv*. **2022**, *8(33)*
3. Singh et.al. *Cell Rep*. **2022**, *39(3):*110694
4. W Shi, et al. *Nature* **2023** *619*,403.

P 91

Probing the oligomerization/de-oligomerization process of *Mtb*'s ATPase, Mpa

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Protein degradation is a naturally occurring cellular process that plays an important role in post-translational protein quality control and maintaining protein homeostasis. Enzyme complexes known as proteases and proteasomes carry out this process[1]. *Mycobacterium tuberculosis* is a type of actinobacteria that causes tuberculosis and has both bacteria-like proteases and eukarya-like proteasomes. Similar to other proteasomal complexes, *Mtb*'s proteasomal complex is made up of two independent compartments: a hexameric ATPase compartment called mycobacterial proteasomal activator (Mpa) and a peptidase compartment called 20SCP. *Mtb*'s ATPase, Mpa, is a homohexameric complex that is a class of AAA+ (ATPase Associated with various cellular Activities) ATPase superfamily. It recognises, mechanically unfolds, and translocates the substrate protein having a degron tag called Pup (prokaryotic ubiquitin-like protein) using ATP as the energy source[2,3]. In contrast to other ATPases of proteases, Mpa can hexamerise spontaneously even in the absence of the nucleotide because of the strong non-covalent interactions (H-bonding, electrostatic interactions, and hydrophobic interactions) between its OB domain[4]. However, the oligomerisation process of Mpa hexamer is not well studied yet. To probe the oligomerisation/de-oligomerisation process of Mpa hexamer, we have used both chemical and thermal denaturation methods. Secondary structure analysis using CD indicates a two-state process from a hexameric state to the unfolded state, while the tertiary structure analysis monitoring the tryptophan fluorescence indicates a transition from the hexameric state to the unfolded state via an intermediate state, plausibly a monomeric state. This transition from hexamer to monomer before unfolding is also supported by the DSC measurements. Interestingly, the chemically refolded state retains its structure and can hexamerize spontaneously but looses its ability to hydrolyse ATP. These studies indicate that Mpa deoligomerizes into only one state, plausibly a monomer, before unfolding but can spontaneously oligomerize upon refolding, although loosing its functional ability.

References

1. De Mot, et al. *Trends Microbiol* **1999**, *7*, 88.
2. M Kavalchuk et al. *Nat Commun* **2022**, *13*, 276.
3. F Striebel et al. *EMBO J* **2010**, *29*, 1262.
4. T Wang et al. *Structure* **2009**, *17*, 1377.

P 92

Evaluating the stability of FtsZ polymers using chemical and mechanical disrupters

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The cell division process is fundamental to all life forms. FtsZ, Filamenting temperature-sensitive Z-mutant, is a conserved protein in all bacterial species, and the importance of its polymers during the earliest stage of cell division has recently been realized [1]. The polymers of FtsZ form when the GTP-binding pocket in the N-terminal of one subunit is in contact with the GTP hydrolyzing domain in the C-terminal of another subunit[2]. These FtsZ polymers further assemble at the mid-cell in a ring called "Z-ring"[3] to initiate the division. One of the proposed mechanical models of cytokinesis indicates the bending of the FtsZ polymers upon GTP-hydrolysis, which induces the inward constriction force in the cell wall[4,5] to form the septum (*figure 1*). Inhibition of Z-ring formation will enlarge the cell into filamentous form, making it more susceptible to lysis/death. In this regard, molecular features that can serve as "chemical disrupters" of FtsZ polymers can be beneficial pharmacophores for antibiotic discovery. In our study, we are testing a hybrid of "Combretastatin" and "Discoipyrrole" that have previously shown good anti-polymerization activity against tubulin (a eukaryotic homolog of FtsZ).
Considering the role of FtsZ as the force generator, It is essential to comprehend the mechanical stability of FtsZ monomers and polymers to construct a qualitative model of the constriction mechanism. We will employ Atomic Force Microscopy (AFM)--based SMFS to understand the mechanical properties of FtsZ by determining the stability of FtsZ monomer and filaments along the direction of polymerization.
I will present our recent results on the chemical and mechanical disruption of Ftsz polymers.

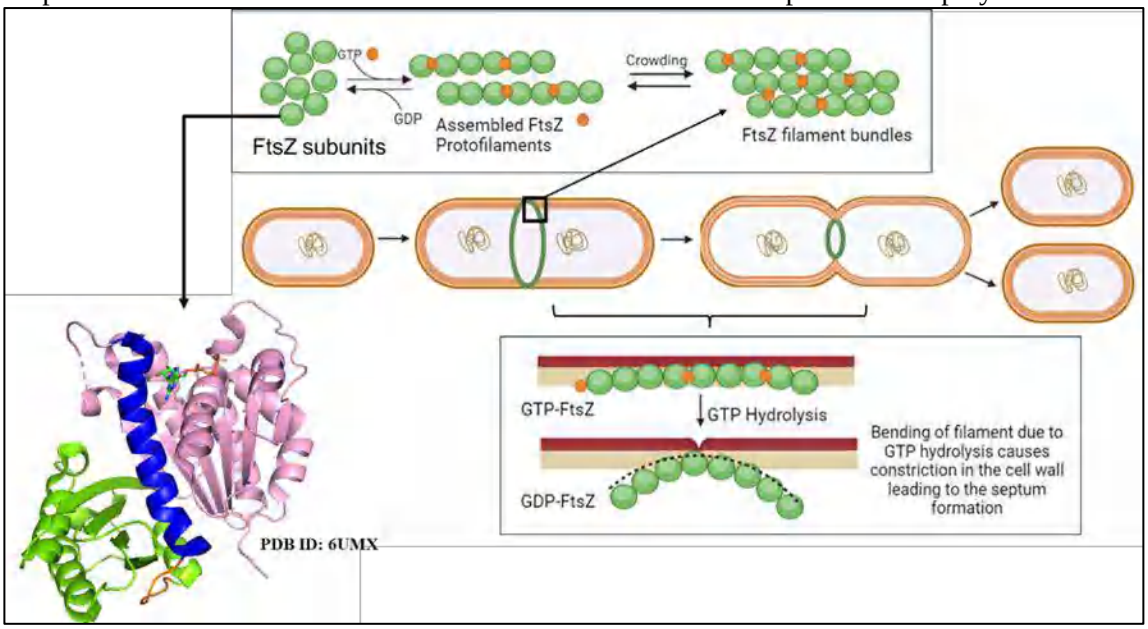

Figure 1. Role of FtsZ in Bacterial Cell wall Constriction

References:

[1] Barrows JM, et al. *Cell Biol* **2021**, *68*, 163.
[2] Löwe J et al. *Nature* **1998**, *391*, 203.
[3] Du S et al. *Trends Microbiol* **2019**, *27*, 781.
[4] Levin PA et al. *EcoSal Plus.* **2021,** *9*, 0022.
[5] Li Z et al. *EMBO Journal* **2007**, *26*, 4694.

P 93

Exploring the Specific Role of Iron Center on the Catalytic Activity of Human Serum Transferrin: CTAB-induced Conformational Changes and Sequestration by Mixed Micelles

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Understanding the fundamentals of conformational changes in a protein and their associated biological activities is an indispensable research topic.[1] This concept becomes more relevant in the context of metalloproteins, owing to the formation of specific conformation(s) induced by internal perturbations (like change in pH, ligand binding, or receptor binding), which may carry out the binding and release of the metal ion/ions from the metal binding center of the protein.[2] Herein, we have investigated the conformational changes of an iron-binding protein, monoferric human serum transferrin (Fe-hTF), using several spectroscopic approaches. We could reversibly tune the cetyltrimethylammonium bromide (CTAB)-induced conformation of the protein, exploiting the concept of mixed micelles, formed by three sequestrating agents: (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) hydrate (CHAPS), and two bile salts namely, sodium cholate (NaC), sodium deoxycholate (NaDC).[3]

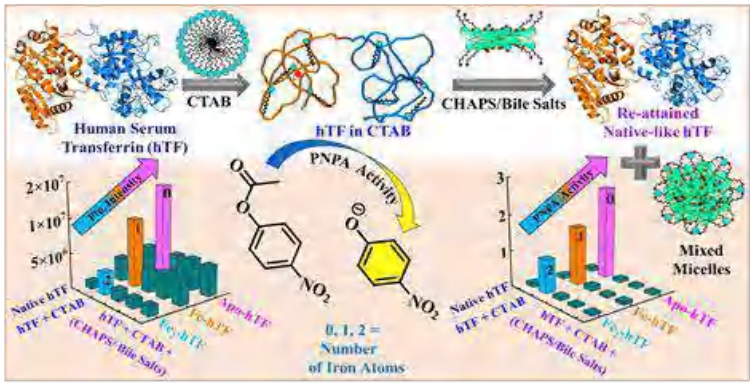

Figure: Pictorial representation of conformational dynamics of human serum transferrin (hTF) induced by CTAB and subsequent sequestration by CHAPS/Bile salts mixed micelles.

The formation of mixed micelles between CTAB and these reagents (CHAPS/NaC/NaDC), results in the sequestration of CTAB molecules from the protein environment, and aids the protein to re-attain its native-like structure. However, the guanidinium hydrochloride-induced denatured Fe-hTF did not acquire its native-like structure using these sequestrating agents, which substantiates the exclusive role of mixed micelles in the present study. Apart from this, we found that the conformation of transferrin (adopted in the presence of CTAB) displays pronounced esterase-like activity toward *para*-nitrophenylacetate (PNPA) substrate as compared to native transferrin. We also outlined the impact of the iron center and amino acids surrounding the iron center for the effective catalytic activity in the CTAB medium. We estimated ~3 times higher specific catalytic efficiency for the iron-depleted Apo-hTF compared to the fully iron-saturated Fe_2-hTF in the presence of CTAB.[3]

References:

1. Harroun et al. *Nat. Methods* **2022**, *19*, 71.
2. Joy et al. *J. Phys. Chem. B* **2023,** *127*, 4374.
3. Yadav et al. *Langmuir* **2024,** *40*, 6172.

P 94

Visualizing Biomolecular Conformational Dynamics using Single-Molecule FRET

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Förster Resonance Energy Transfer (FRET) has been extensively utilized to determine proximity between fluorophore tagged with biomolecules and probe their structural changes or interaction dynamics. However, solution FRET measurements are often inadequate to unveil mechanisms or real-time dynamics owing to ensemble averaging effect. In contrast, single-molecule FRET (smFRET) on a large number of individual donor-acceptor fluorophores attached to surface immobilized biomolecule(s) can reveal intricacies of both intra and inter molecular conformational dynamics, thereby providing new mechanistic insights.[1]

We have assembled a smFRET setup in our lab which involves a home-built total internal reflection fluorescence microscope (TIRFM) equipped with multiple laser lines and a dual-color detection system using an image splitter and an CCD camera. This setup allows us to visualize and track the dynamics a large number dual-dye labelled biomolecules one at a time, when these are either immobilized on a surface or diffuse within the evanescent field. Here, we discuss two projects which utilize inter- and intra-molecular smFRET via labelling distinct subdomains of proteins, with our inferences relying on the analyses of FRET efficiency histograms from several hundred trajectories. First, our studies elucidate interactions between a ribosomal methyl transferase enzyme (Erm) and a precursor (immature) bacterial ribosome during the methylation process of a ribosomal RNA, which forms the basis for antibiotic (erythromyocin) resistance in certain bacteria.[2] Second, our analyses reveals allosteric fluctuations of subdomains within an enzyme involved in the purine biosynthetic pathway (PurL), during the transient formation of a transport tunnel via which the product of one catalytic reaction (ammonia) migrate to the second active site, where ammonia acts as a substrate.[3]

References:

1. Lerner E. et al. *Science*. **2018**, *19*, 359.
2. Schaenzer, A.J. et al.*Trends in molecular medicine*, **2020**, *26(8)*, 768.
3. Sharma, N. et al., *ACS Catalysis*, **2022**, *12(3)*, 1930.

P 95

Engineered MXene Based Photocatalyst for the Selective Photoreduction of CO_2 to Ethanol

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Photocatalytic CO_2 reduction has emerged as a promising strategy, capitalizing on solar energy to drive the conversion of CO_2 into valuable products.[1,2] **CeO_2**, a metal oxide is a promising material for designing highly active catalyst for the photoreduction of carbon dioxide (CO_2RR). It is redox-active in nature and can switch between the oxidation states from Ce^{3+} to Ce^{4+} while exposed to light. Herein, **CeO_2/Ti_3C_2 MXene** hetero-structured photocatalysts have been synthesized hydrothermally that exhibit superior CO_2 reduction to ethanol and methane. The intimate interfacial interactions between **CeO_2** and **Ti_3C_2MXene**, can lead to improved charge separation, faster charge transfer kinetics, and an increased degree of surface sites for CO_2 absorption and activation, in the **Ti_3C_2 MXene**/**CeO_2** heterostructure photocatalysts. The charge separation efficiency and the electron−hole pair recombination process of the photocatalysts can be understood from the photoluminescence (PL) spectra and the charge transfer kinetics can be understood from the time-resolved photoluminescence (TRPL) analysis. In order to optimize the photocatalytic CO_2 reduction efficiency, **Ti_3C_2 MXene** loading amount has been varied and the optimized **5-TC/Ce** shows CO_2 reduction with a drastically enhanced yield of ethanol of the order of 6127 μmolg^{-1} at 5h with 98% selectivity and 7.54% apparent quantum efficiency, which is 6 folds higher than that of ethanol produced by the bare **CeO_2**.[3]

References:

1. K. Niharika et al. *Journal of Materials Chemistry A*. **2023**, *11*, 20839.
2. B. Mishra et al. *Chem Su sChem*, **2019**, *12(14)*, 3383.
3. R. P. Mishra et al. *Langmuir*, **2023**, *39*, 14189.

P 96

Unravelling the LLPS behaviour of SUMO1 protein

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Liquid-liquid phase separation of intrinsically disordered proteins and RNA molecules plays a very important role in many regulatory cellular functions. Our group recently reported small ubiquitin-like modifier (SUMO1) shows LLPS in its native state in the presence of artificial crowders[1]. What are the underlying reasons for its LLPS in its native state are yet to be studied. By confocal and scattering studies, we observed that SUMO1 LLPS is pH-dependent and in a specific pH window, indicating that some residues play a major role in condensate formation. The minimum saturation concentration at which protein starts to show LLPS in the presence of crowders is significantly less for multimeric SUMO1 than SUMO1 monomer. It is biologically relevant because SUMO1 also forms polySUMO1 chains during post-translational modification of various proteins[2,3].

References:

1. S. Arora et al. *Journal of Physical Chemistry Letters* **2023**, *14* (40), 9060.
2. P. Krumova et al. *Journal of Cell Biology* **2011**, *194* (1), 49.
3. A. Princz et al. *Gerontology* **2020**, *66* (2), 122.

P 97

Investigating Kinesin 1 role as a Tether at Lipid droplet and Endoplasmic Reticulum Membrane contact site

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Lipid Droplets (LD) are dynamic organelles which play a crucial role in lipid metabolism and storage. They are known to interact with various organelles, at specific sites known as Membrane contact sites (MCS). LD interacts with endoplasmic reticulum network at specialized domains to carry out various cellular processes such as synthesis, transport, and breakdown of lipids, and facilitates exchange of proteins and lipids across these membranes. Cellular trafficking of LD by motor proteins like Kinesin or Dynein is required for such site-specific interaction. Previously, our lab has elucidated the mechanism by which kinesin motor protein is recruited on LD and drives the LD metabolism towards secretion of VLDL particles at Hepatocytes periphery. In this study, we investigate the role of Kinesin 1 motor protein to convert into a tether via ER-resident protein, Kinectin1 at LD-ER membrane contact site (MCS). Using high spatiotemporal resolution imaging techniques, we assessed the motion of LDs and their distribution in the absence/presence of Kinesin 1 by live cell imaging in COS7 cells and characterize its colocalization with Kinectin 1 at ER-LD MCSs. We hypothesize that the interplay between Kinectin 1, Rab18, and Kinesin 1 is essential for mediating ER-LD tethering and affects the subsequent exchange of lipids and protein between these organelles. To better understand this ternary interaction, we exploit invitro reconstitution assay, biochemical assay, and proximity labelling strategy. Additionally, we will examine the impact of Kinesin1-Kinectin1 complex on lipid droplet biogenesis, growth, and lipolysis. Our findings will contribute to a better understanding of the molecular mechanisms underlying ER-LD interactions and may have potential applications in the treatment of lipid metabolism disorders.

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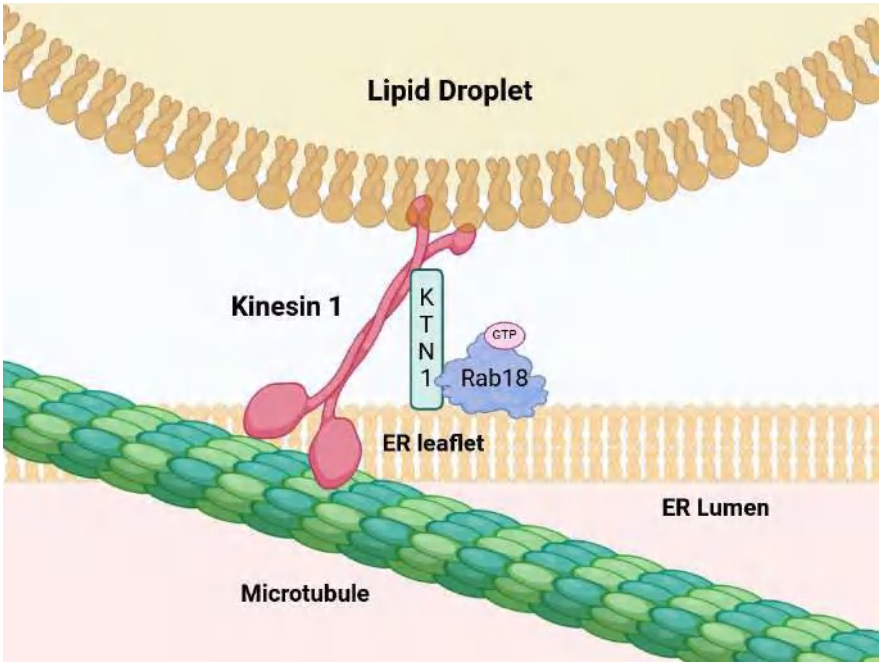

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Designing ECM inspired Bioactive 3D Hydrogel based Biomaterial to fabricate Advanced Tissue Mimics

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In recent times, the main focus of tissue engineering and regenerative medicines has been to obtain advanced biomaterials that can reliably propagate the functionality and properties of the damaged tissues. Precisely, peptide hydrogels owing to their biocompatibility and their ability to mimic the structural and functional complexity of native Extracellular Matrix (ECM) are gaining utmost attention. Mimicking ECM by using biomolecular scaffolds is extremely crucial for facilitating the cellular proliferation, differentiation, and other molecular processes. In this regard, utilization of minimalistic bioactive peptide sequences for the creation of tissue engineering scaffolds offers a significant advantage over traditional design methods, which focused mostly on peptide sequences, far from bearing any suitable biological relevance. In our work, we are mainly focusing on mimicking various proteins present in the native ECM like, laminin and collagen. To this end, we have designed a novel minimalistic peptide hydrogelator to develop scaffolds based on laminin-511 which shows a solvent mediated self-assembly. The secondary structure formed by the self-assembled hydrogels was confirmed using CD, fluorescence and FTIR spectroscopy. These designed hydrogels displayed a nanofibrillar morphology that may create a suitable interface to support cellular growth and proliferation as confirmed using confocal microscopy. Interestingly, these bioactive hydrogels showed optimal mechanical properties which was reflected in significant cellular viability. Furthermore, we adapted a non-equilibrium self-assembly approach to create diverse nanostructures based on a single gelator domain. The rationale behind this strategy stems from the fact that self-assembly of peptides is highly susceptible towards environmental conditions like temperature, pH, ionic concentrations, which leads to diverse nanostructures. Here, we have checked the interaction between collagen mimetic positively charged peptide with biologically relevant anions. The electrostatic interaction between peptide and ions mediate the self-assembly and form distinctive secondary structure which was confirmed by fluorescence, CD, UV-Vis and FTIR spectroscopy. These ion-responsive gels can mediate cellular adhesion and proliferation. We believe the incorporation of short bioactive peptide can effectively mimic ECM proteins to fabricate innovative biomaterials, essential in the field of tissue engineering.

References:

1. R. Jain et al. *Materials Science and Engineering: C* **2020**, *108*, 110483.
2. V.K. Pal et al. *Macromolecular Bioscience* **2022**, *22*, 2100462.
3. V.K. Pal et al. *Langmuir* **2020**, *36*, 1003.
4. R. Jain et al. *ACS Biomaterials Science & Engineering* **2020**, *6*, 2832.

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Effect of solvents on morphological anisotropy of aromatic amino acid-based Aggregation-induced emission (AIE) active system

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The development of fluorogenic materials from amino acids is challenging, but it can be addressed by using derivatives of these amino acids in synthetic protocols. Herein, an aromatic amino acid-derived Schiff base of 1-pyrene carboxaldehyde has been synthesized, exhibiting aggregation-induced emission (AIE). The AIEgen molecule follows a photoinduced electron transfer (PET) pathway. It also demonstrates morphological anisotropy in solvent mixtures, likely due to noncovalent interactions and solute-solvent interactions (e.g., hydrophobicity). This phenomenon has been analyzed through thermodynamic aspects to understand the nucleation-elongation process, as evidenced by variable temperature absorption spectra and the roles of secondary nucleation and cooperative growth, observed through variable temperature circular dichroism. Moreover, the influence of organic solvents such as protic MeOH and the cosolvent H2O on morphological anisotropy has been evaluated using the polar organic solvent DMSO and solvent isotope effects, respectively. Morphological anisotropy can arise from molecular arrangements leading to both amorphous and crystalline phases. These synthesized AIE-active molecules have potential applications in bioimaging, biosensing, OLEDs, and more

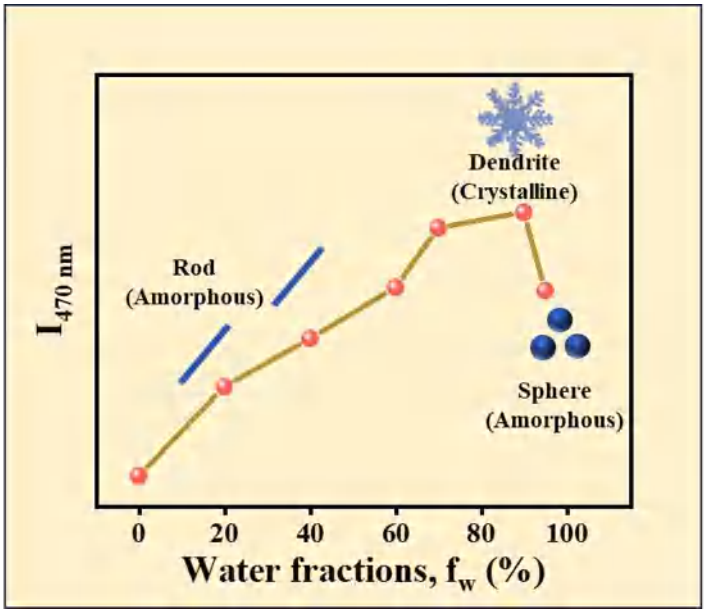

Fig. Aggregation-induced emission and the morphological anisotropy of synthesized Schiff base.

References:

1. Panigrahi et al. *ACS Appl. Mater. Interfaces* **2020**, *12*, 5389−5402
2. Pal et al. *J. Am. Chem. Soc.* **2023**, *145*, 2532−2543

P 100

Lanthanides driven microphase separation of proteins: ions hydration matters.

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Lanthanides, well-known rare earth elements (REE), acquire a major position in the modern world. Their growing application have prompted researchers to study their effects on human life. In presence of Lanthanide (III) cations, proteins exhibit intriguing phase behaviours such as liquid liquid phase separation (LLPS), reentrant condensation, etc which are highly dependent on concentration. In our study, we choose La3+, Nd3+, Gd3+, Ho3+ and Lu3+ ions and BSA protein to observe the phase changes upon the addition of salt to the protein solution. We illustrate the phase diagram which is classified in three regimes; regime I (R1) and regime III (R3) are visually transparent whereas regime II signifies the turbid phase with two critical concentrations C* and C** and the middle point depicts as Cm. The phases are characterized by turbidity, zeta-potential and optical microscopy measurements. Finally, we explore the role of ion hydration in these processes using THz-FTIR (0.2-22.5 THz) measurements. Our experimental results clearly demonstrate that considering only the charge (III) is insufficient to explain the observed phases; ion hydration must also be taken into account.

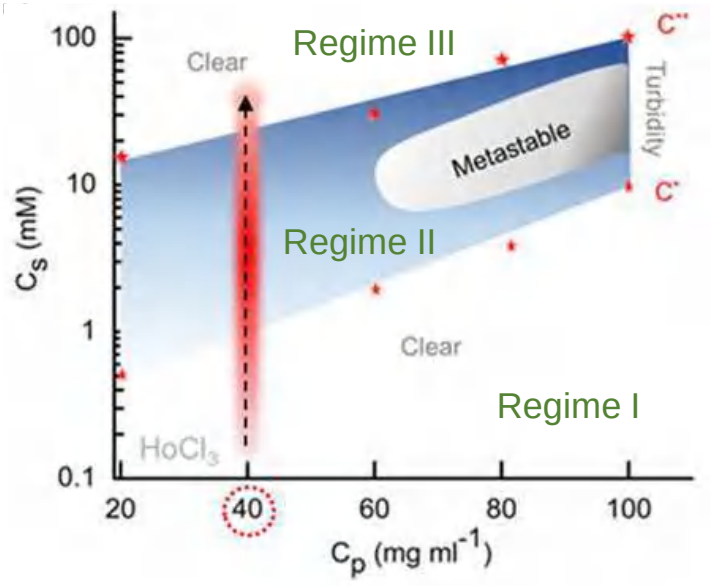

Figure. Representative phase diagram of BSA protein against $HoCl_3$ salt concentration where three regions are mentioned as regime I, II and III depending on appearance and disappearance of turbidity.

References:

1. O. Matsarskaia et al. *Chem Phys Chem* **2020**, *20*, 1742.
2. R. Saha et al. *Phys. Chem. Chem. Phys.* **2022**, *24*, 23661.
3. R. Saha et al. *Langmuir* **2023**, *39*, 4601.

P 101

Encapsulation of Thioflavin T (ThT) by Different Bile Salt Aggregates: Spectroscopic Investigation

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Bile salts are amphiphilic molecules, but their structure is quite different from that of other conventional surfactant molecules. They have a hydrophobic convex side and a hydrophilic concave side. Above Critical micellar concentration, they form primary aggregates via hydrophobic interaction between monomers. At very high concentrations, these primary aggregates form secondary aggregates via hydrogen bonding and hydrophobic interaction. These aggregates are quite unique as they have both hydrophilic and hydrophobic pockets, which can bind different types of guest molecules depending on their nature. In this work, we investigated the binding efficiency of amphiphilic probe Thioflavin-T (ThT) with three different bile salts aggregates, namely Sodium Cholate (NaCh), Sodium Taurocholate (NaTC) and Sodium Deoxycholate (NaDC). The changes in absorption and emission properties of probe molecule (ThT) were found to be sensitive to increasing bile salt concentration. Due to the encapsulation by bile salts aggregates, the emission intensity and emission lifetime of ThT increase significantly. Moreover, we have also investigated the effect of change in the ionic strength of the medium on the spectroscopic properties of ThT inside bile salt aggregates by adding sodium chloride (NaCl). In the presence of NaCl, the fluorescence lifetime of ThT in bile salts increases significantly due to the formation of more rigid aggregates. The encapsulation efficiency of ThT in bile salt aggregates has been assessed by using iodide (I^-) as an external ionic quencher. We found that NaDC aggregates are more efficient in the modulation of the photophysical properties of ThT than the other two bile salt aggregates.

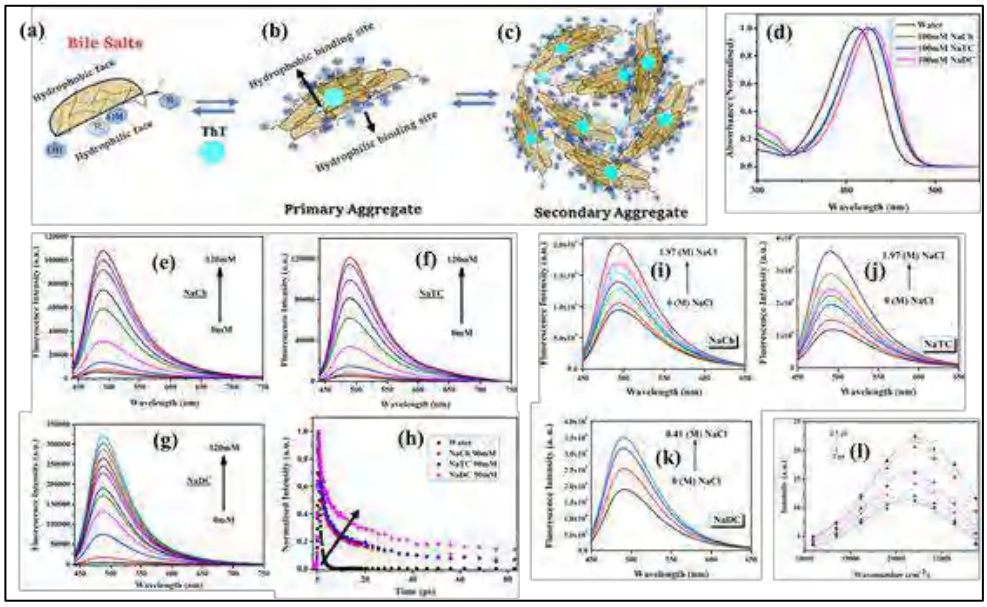

Figure: a,b,c are general structures of monomers and different aggregates of bile salts. d-l represents various spectroscopic properties of ThT inside different bile salt solutions.

References:

1. Ritwik Hazra et al. *Langmuir* **2024**, *40*, 16272.
2. Miguel Gomez-Mendoza et al. *J. Phys. Chem. Lett.* **2011**, *2*, 782.

P 102

Fluorescent Nanothermometer with Exceptional Sensitivity for Intracellular Temperature Sensing

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Abstract

Precise temperature sensing and measurement at the nanoscale level within biological systems is vital for understanding cellular processes such as gene expression, metabolism, and enzymatic activity [1]. In this study, we design and present luminescent niosomes as a novel nanothermometer with exceptional figure of merits. The change in the fluorescence intensity and wavelength shift for common fluorophores are used as indicators for temperature sensing when the fluorophores are entrapped in niosomes, and the niosomes undergo gel-to-liquid phase transition with an increase in temperature. When Coumarin 153 (C153) loaded in Span60-Pluronic L64 (hybrid) niosomes, is excited at 420 nm, the system exhibits a 20 nm blue shift in fluorescence (from 540 nm to 520 nm) with more than double enhancement in its fluorescence intensity at 50 °C, with increasing temperature (20–50 °C), enabling temperature sensing in the very relevant temperature range for biological imaging. The sensor shows excellent reversibility, environmental stability, high relative sensitivity (7.4% $°C^{-1}$), and temperature resolution (0.087 °C) under varying ionic strength and pH conditions [2]. Further, when Nile Red-loaded hybrid niosomes are excited at 520 nm, the system shows an 11-fold increase in fluorescence (highest, 618 nm) with the best temperature sensitivity of 19%$°C^{-1}$ at 42 °C. While Nile Red-loaded pure L64 niosomes exhibit a staggering ~100-fold enhancement (highest, 618 nm) under the same experimental condition with 36.4%$°C^{-1}$ sensitivity at 40 °C. Due to their excellent stability, reversibility, biocompatibility, high-temperature sensitivity, and resolution, the sensors are capable of temperature sensing when loaded inside FaDu cells (Fig. 1). These results highlight the potential of niosome-based fluorescent nanothermometers for biomedical research, allowing precise detection of minute temperature variations associated with cellular processes and diseases such as cancer.

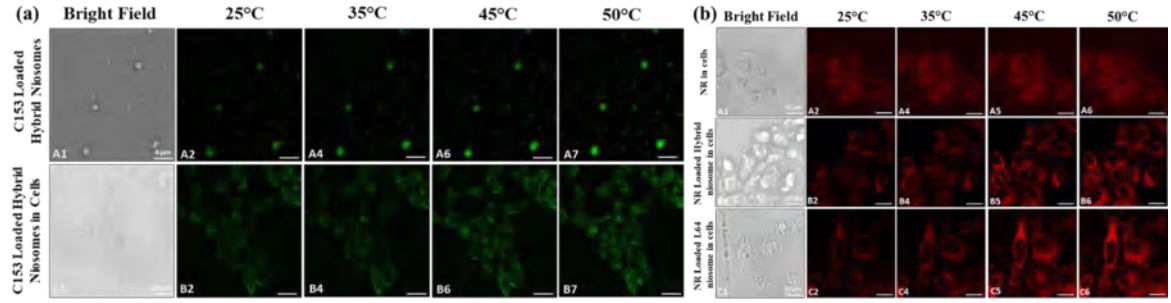

Figure 1. Laser scanning confocal microscopic images at different temperatures: (a) Only C153- loaded niosomes (A1–A7) (scale bar = 4 μm) and (B1–B7) FaDu cells incubated with C153-loaded niosomes (scale bar = 25 μm); λ_{ex} = 405 nm, λ_{em} = 510–530 nm; (b) FaDu cells loaded with only Nile Red (NR) (A1–A6), NR loaded Hybrid Niosomes (B1-B6), NR loaded in L64 niosomes (C1-C6), respectively. (scale bar = 25 μm and 10 μm) (λ_{ex} = 514 nm; λ_{em} = 610–650 nm).

References

1. Albenc Nexha et al. *Nanoscale*, **2021**, 13, 7913.
2. Ronak Lazarus et al. *Analyst*, **2023**, 148, 3169.

P 103

Esterase-responsive Fluorogenic Prodrugs of Aldose Reductase Inhibitor Epalrestat: An Innovative Strategy towards Enhanced Anticancer Activity

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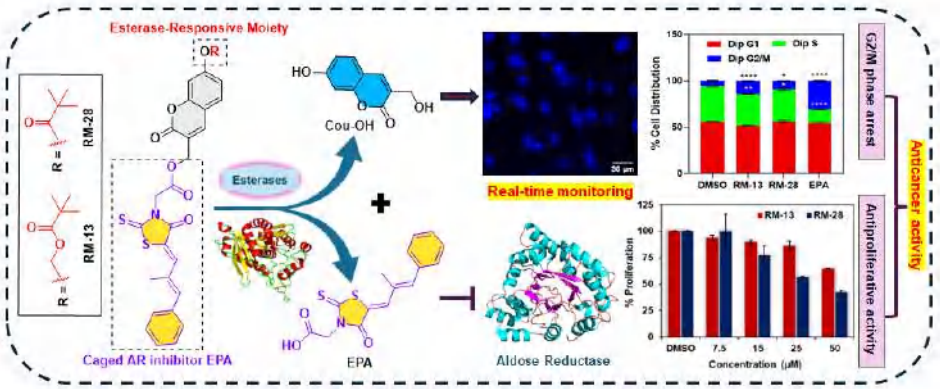
In addition to the conventional chemotherapeutic drugs, potent inhibitors of key enzymes that are differentially overexpressed in cancer cells and associated with its progression are often considered as drugs of choice for treating cancer. Aldose reductase (AR), which is primarily associated with the complications of diabetes, is known to be closely related to the development of cancer and drug resistance.[1] Epalrestat (EPA), an FDA-approved drug, is a potent inhibitor of AR and exhibits anticancer activity.[2] However, its poor pharmacokinetic properties limit its bioavailability and therapeutic benefits.[3] We report herein the first examples of the esterase-responsive turn-on fluorogenic prodrugs **RM-13** and **RM-28** for the sustained release of EPA to the cancer cells with turn-on fluorescence readout.[4] The prodrugs could be activated in the presence of esterases, which are overexpressed in cancer cells. Spectroscopic and HPLC studies revealed a simultaneous release of both the active drug and the fluorophore from the prodrugs over time. While the inhibitory potential of EPA released from the prodrugs towards the enzyme AR was validated in the aqueous medium, the anticancer activity of the prodrugs was studied in a representative cervical cancer cell line (HeLa). Interestingly, our results revealed that the development of the prodrugs can significantly enhance the anticancer potential of EPA. Finally, the drug uncaging process from the prodrugs by the intracellular esterases was studied in the cellular medium by measuring the turn-on fluorescence using fluorescence microscopy. Therefore, the present study highlights the rational development of the fluorogenic prodrugs of EPA, which will help enhance its anticancer potential of EPA with better therapeutic potential.



References:

1. Srivastava et al. *Chemico-Biological Interactions* **2011**, *191*, 330.
2. Bailly et al. *European Journal of Pharmacology* **2022**, *931*, 175191.
3. Banala et al. *Biomaterials Science* **2019**, *7*, 2889
4. Misra et al. *ACS Applied Bio Materials* **2024**, ASAP Article

P 104

Plasmon-driven surface reactions and enzyme catalysis probed using surface-enhanced Raman spectroscopy and 2DCOS

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A circular economy and a more sustainable future depend heavily on catalysis. At the forefront of photocatalysis right now is plasmonic catalysis, which allows one to get beyond the drawbacks of "classical" broad bandgap semiconductors for solar-driven chemistry. Because plasmonic catalysis uses localized surface plasmon resonance (LSPR) stimulation, it can accelerate and control a wide range of chemical reactions [1]. The utility of these plasmonic probes has been restricted at times due to the lack of understanding and control of the reactions which can lead to a diverse set of products. Our work is focused on creating multifunctional plasmonic nanostructures for plasmon-driven catalysis by engineering nanostructured probes and adjusting their optical characteristics. Our proposal in this work is to use in-situ surface-enhanced vibrational 2D correlation spectroscopy (2DCOS) to monitor these reactions in a completely new way [2] [3]. Interestingly, we see a synergistic effect of the reaction time, presence of protic solvents, material of the surface and the laser exposure duration on the nanoparticle surface, and this determines whether the reaction stops at the intermediate or proceed towards the completely reduced product. We have explored the mechanistic underpinnings of intermediate formation on nanoparticle surfaces using a combined spectroscopic and 2DCOS visualization techniques. This spectroscopic visualization technique provided real-time information about the mechanism of the reaction which could otherwise be obtained by a combination of data analytical techniques such as thorough spectral processing and the use of advanced chemometric techniques. We have tried to monitor and control enzyme function through irradiation of plasmonic nanoparticles using UV and visible light as stimuli. We propose an entirely new approach to monitor these reactions which could shed light on the underlying mechanisms and thus help in tuning the conditions to remotely monitor plasmon induced surface reactions and enzyme catalysis at the molecular level.

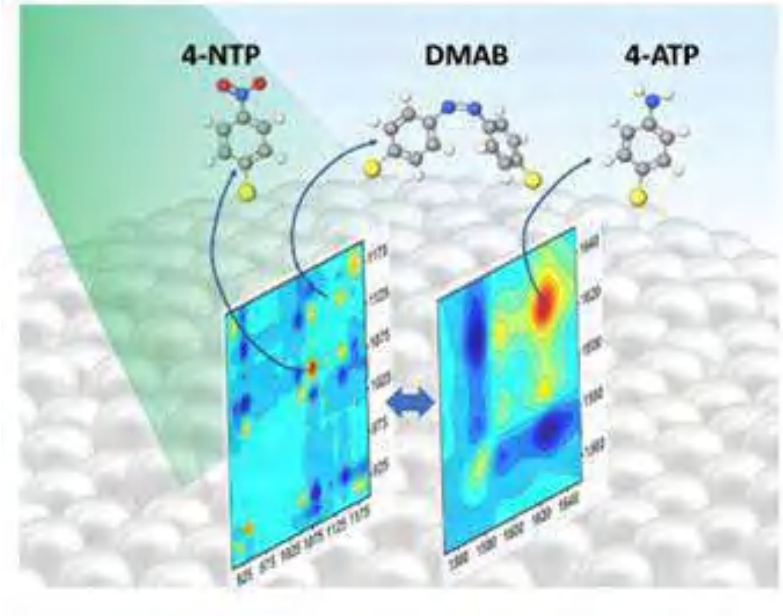

Figure 1: Visualization of plasmon induced surface catalytic reactions using 2DCOS

References:

[1] Z. Yang et al. *Journal of Raman Spectroscopy* **2009**, *40 (10)*, 1343.
[2] I. Noda *Vibrational Spectroscopy* **2012**, *60*, 146.
[3] R. Singh et al.. *Physical Chemistry Chemical Physics*, **2023** *25(8)*, 6032.

P 105

In-situ visualization of growth kinetics of silver nanorods

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Metal nanostructures find extensive applications in diverse fields, such as bioimaging, photothermal therapy, targeted drug delivery, and optoelectronics [1]. As a consequence of their extensive utility, there have been multiple attempts to understand the growth kinetics of these nanostructures. Understanding growth mechanisms allows the control of their size, shape, and morphology, resulting in customising their properties for specific applications [1].

To investigate nanostructure growth mechanisms, we have developed interferometric scattering (iSCAT) [2] based microscopy and correlation spectroscopy, which is a non-invasive, label-free technique often used for applications like single particle tracking and imaging nanoscale entities such as proteins, other biomolecules, metal nanoparticles, semiconductor quantum dots, and single organic molecules [3]. Here, we present a novel approach by employing this technique for real-time, in-situ visualization of growth kinetics of seed-mediated growth of silver nanorods [4]. Using laser light, we promote uniform growth and tailor the aspect ratios of the nanorods, enhancing both their yield and quality. Our observations reveal a more complex growth process than the conventional one-step transformation from spherical seeds to elongated rods. Instead, the growth involves forming larger structures from the seeds, which disintegrate into smaller fragments that reassemble into nanorods. This novel approach offers unprecedented insights into the kinetics of nanostructure formation, which provides significant potential for rational design and tailored synthesis of nanostructures.

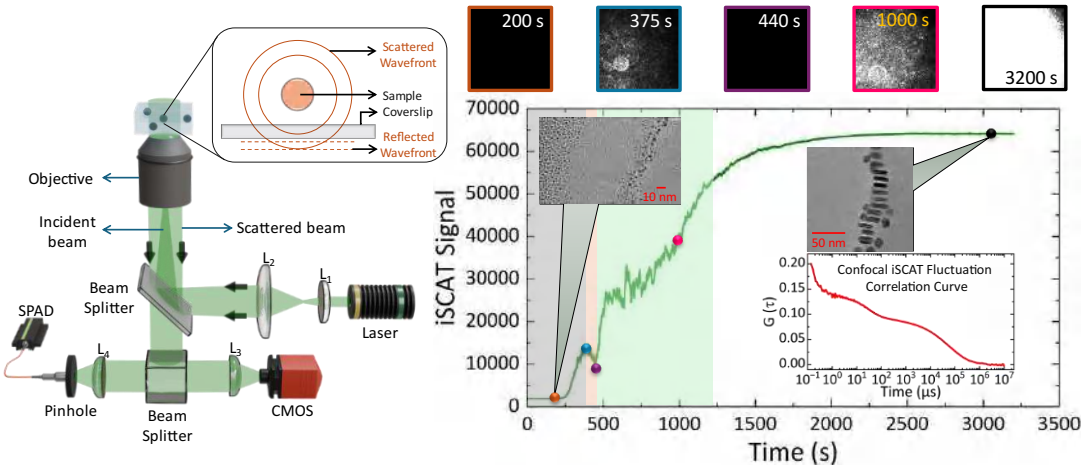

Figure 1: (Left) Schematics of the custom-built Interferometric Scattering (iSCAT) Microscope. (Right top panel) Image frames acquired as a function of time during the nanoparticle growth phase. (Right bottom panel) Frame averaged intensity signals used in visualizing the growth kinetics of silver nanorods. (Right bottom inset) 1D-Fluctuation correlation curve corresponding to the nanostructure growth.

References:

(1) N. Thanh et al. *Chem. Rev.* **2014**, *114* (15), 7610.
(2) J. Ortega Arroyo et al. *Nat. Protoc.* **2016**, *11* (4), 617.
(3) G. Young et al. *Annu. Rev. Phys. Chem.* **2019**, *70*, 301.
(4) N. R. Jana et al. *Chem. Commun.* **2001**, *7*, 617.

P 106

Catalytic Dynamics in Phase-Separated Environments: Contributions of Micro and Macromolecules

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Transient non-covalent interactions between biopolymers (protein-protein or protein-nucleic acid) can induce liquid-liquid phase separation (LLPS) resulting in the formation of distinct dense and dilute phases. The dense phase resulting from the LLPS of catalysts, called the catalytic condensates, can effectively concentrate the catalyst and substrate, thereby significantly accelerating the reaction rate[1]. The functionality of these condensates can be influenced by changes in the local environment which can be modulated by both macro- and micro-molecules[2]. In our current study, we utilize a combination of microscopic and spectroscopic techniques to examine how macro- and micro- molecules can alter the reaction rates by modulating the phase behaviour of these condensates. We have observed that the substrate (a micro-molecule) can induce phase transition within the liquid condensate on increasing its concentration above a critical concentration. Due to this phase transition, the overall reaction rate gets decreased in condensates. In addition to this, macromolecules can induce further phase separation within single phasic condensate leading the formation of multiphasic condensates. We aim to further investigate the effect of such multiphasic condensates, on the reaction kinetics, in the presence of an additional dense phase of macromolecules.

References:

1. Bedi et al. *Chemical Communication.* **2022**, *58*, 8634.
2. Babinchak et al. *Nature communications*. **2020**, *11*, 5574.

P 107

Interaction of human BRAC1 protein with Holliday Junction: Preference for an open X-like conformation

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BRCA1 is a multifaceted tumor suppressor protein involved in key cellular processes such as DNA double-strand break repair and cell cycle checkpoints [1]. Cells deficient in BRCA1 show reduced homologous recombination (HR) activity, leading to an increased reliance on error-prone non-homologous end joining for DNA repair [2–5]. The Holliday junction (HJ) is a crucial intermediate in HR, and while BRCA1 exhibits a strong affinity for HJ and recruits various proteins to DNA damage sites, its binding mode with HJ remains unclear [6,7]. Using single-molecule Fluorescence Correlation Spectroscopy (FCS), we have demonstrated that BRCA1 preferentially binds to an open X-shaped conformation of HJ and has a lower affinity for stacked HJ. Additionally, molecular docking and all-atom molecular dynamics simulations revealed that charged and polar amino acids in BRCA1's DNA-binding region (aa340-554) form a complex with HJ, many of which are reported as crucial sites of missense mutations [8].

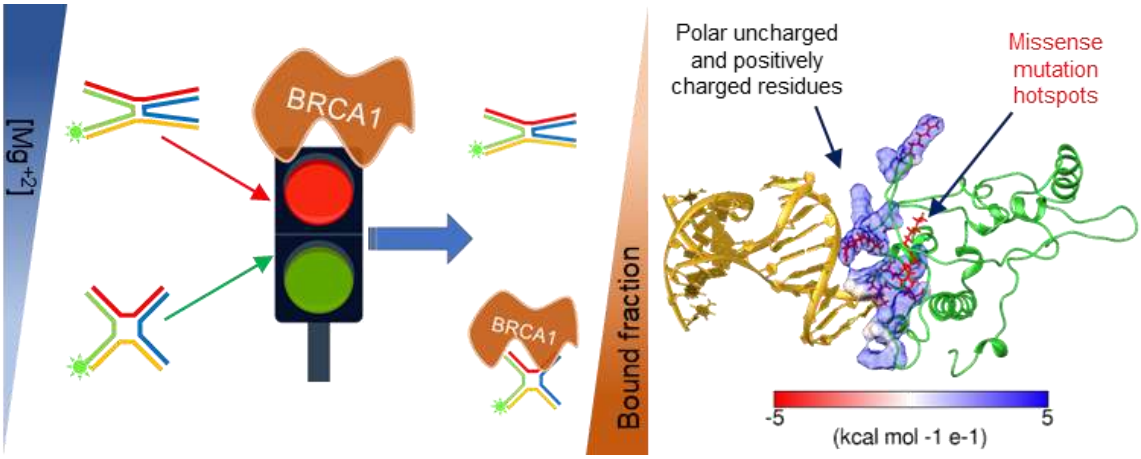

Fig 1: (a) Schematic representation of BRCA1 protein's preference for an open X-like conformation of HJ, (b) Crucial polar uncharged and positively charged amino acids from DNA binding region of BRCA1 protein interacting with a HJ.

References:

1. Li X et al. *Cell Res* **2008**; *18*, 99.
2. Tarsounas M et al. *Nat Rev Mol Cell Biol* **2020**; *21*, 284.
3. Zhao W et al. *Annu Rev Biochem* **2024**, *28*, 221.
4. Venkitaraman AR et al. *Cell* **2002**, *108*:171.
5. Mylavarapu S et al. *Front Oncol* **2018**, *8*, 16.
6. Brázda V et al. *BMC Mol Biol* **2016** *17*, 1.
7. Paull TT et al. *Direct DNA Binding by Brca1* **2001**, *98.11*, 6086.
8. Ramirez CJ *et al. Oncogene* **2004,** *23:1780*,8.

P 108

Effect of Plasma Membrane Cortical Architecture and Protein Crowding on the Transport Properties of Membrane Proteins

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The plasma membrane is a complex mixture of proteins and lipids that serves as an organizing centre for a variety of sorting and signalling processes. It is also heterogeneous and has a composite nature with a variety of barriers to protein and lipid mobility. Understanding sorting and signalling processes at the membrane require an understanding of these barriers. These barriers emerge at the least as a result of protein crowding in the plane of the plasma membrane and the effect of the underlying cortical actin meshwork. They influence the transport properties and as a consequence, the interaction kinetics of all the molecules on the membrane. To understand the effect of protein crowding in the plane of the plasma membrane and the effect of the actin meshwork-based corralling and picketing on the transport properties of membrane bound protein molecules, we have developed an in-vitro, minimal model of the plasma membrane to systematically tune protein crowding and actin meshwork-based picketing. We probe the effect of these mobile and immobile barriers to study the diffusion of membrane molecules by FRAP and Fluorescence Correlation Spectroscopy (FCS) based methods. Further, we have developed a method to correlate these changes in transport properties of a tracer with the spatiotemporal maps of pickets and crowders in live cell membranes.

P 109

Evaluating the inclusion complex of triazolium-based ionic liquid in β-cyclodextrin media by Nile blue fluorescence

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Ionic liquids (ILs) are remarkable chemical compounds with applications in many fields of modern science. The majority of attention has been focused on imidazolium-based ILs and their biological applications [1]. While only a few 1,2,4-triazolium-based ILs have been synthesized and systematically studied. β-cyclodextrin (β-CD) is a type of oligosaccharide composed of glycopyranose units. Encapsulation within β-C alters the chemical reactivity of guest molecules, stabilizes oxygen and light-sensitive substances, and improves solubility [2]. To evaluate the inclusion complexation of triazolium-based IL in β-CD media, the current work will provide an account of the photophysical response of Nile blue chloride (NBC) in presence of both the media. NBC is a versatile, nontoxic visible Oxazine dye that has demonstrated potential efficacy as a photosensitizer for photodynamic therapy of malignant tumors. NBC is also a well-known DNA probe. Understanding how NBC interacts with biological and biomimicking systems is crucial.

Here, we have synthesized 1,2,4-triazolium-based IL, 1-propyl-1,2,4-triazolium trifluoroacetate (1-prop3HTTFA), by simple neutralization method, and structurally characterized by NMR spectroscopy. A comparative analysis of the effect of different concentrations of IL and β-CD on the photophysical properties of NBC was investigated. For NBC in an aqueous solution, emission wavelength is noted at 670 nm. When IL is added to NBC, fluorescence intensity of NBC is increased with a slight red shift from 670 nm to 674 nm. Both IL cationic and anionic parts may interact electrostatically and hydrophobically with NBC. IL and NBC molecules are encapsulated in the β-CD cavity, where they experience a hydrophobic environment. The increased fluorescence intensity of NBC in the IL-β-CD system is due to its solubility of NBC molecules in the hydrophobic cavity, which is protected from quenching by bulk water through proton transfer. Observing changes in NBC absorbances and fluorescence in aqueous IL media with increasing concentrations of β-CD revealed that β-CD and IL form inclusion complexes with 1:1 stoichiometry. The binding constant for inclusion complex formation has been derived using the Benesi-Hildebrand method.

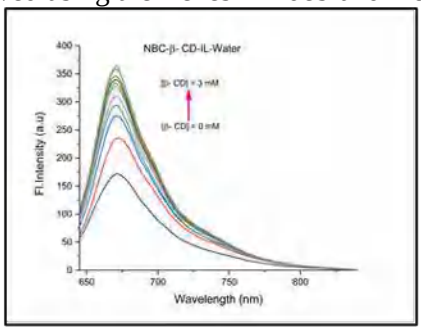

Fig.1 Emission spectra (λex = 635 nm) of NBC in β-CD-IL system

References:

1. Buettner, C.S. et al., *J. Mol. Liq.*, **2022**, *347*, 118160.
2. Sahu, K.M. et al., *Int. J. Biol. Macromol.*, **2023**, *240*, 124338.

P 110

Exploring the Impact of Chromophore Environment on Excited-State Dynamics Leading to Large Stokes Shift in Red Fluorescent Proteins

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Red fluorescent proteins (RFPs) are gaining popularity as genetically encodable bio-probes and biomarkers, with the potential to drive significant advancements in imaging and life sciences. Designing RFPs with enhanced functions or versatility necessitates a deep understanding of their mechanisms. Since fluorescence happens on an ultrafast timescale, the use of a toolkit that includes steady-state and time-resolved spectroscopic methods, as well as computational studies is essential for uncovering the crucial species and dynamic processes that govern large Stokes shift (LSS) RFPs [1]. Among the diverse LSS-RFPs created, a monomeric RFP mKeima is notable for exhibiting one of the largest Stokes shifts (~180 nm) and a unique "*reverse protonation*" effect. The substantial Stokes shift observed in mKeima arises from an ultrafast sequential process involving excited state isomerization followed by proton transfer (ESPT) between the *trans*-protonated to *cis*-deprotonated forms of the chromophore through *cis*-protonated leaving *trans*-deprotonated as a bystander [2,3]. In the recent work we investigate the intriguing photophysics of a site-directed mutant of monomeric RFP mKate (extracted from *Entacmaea quadricolor*) known as mBeRFP (monomeric blue light-excited red fluorescent protein) is noteworthy among other LSS-RFPs for its improved photostability, brightness, and responsiveness to chlorides [4,5,6]. Unlike mKeima, mBeRFP exhibits "*reverse protonation*" at higher pH and remarkable triple fluorescence at ~ 77 K which is the first report on triple fluorescence from a chromophore. In summary, we proposed a schematic of potential energy surface based on our studies by employing steady-state and time-resolved spectroscopic tools and computational calculation and discuss how local environment play a crucial role for distinct photophysical phenomenon with change of temperature and pH [7].

References:

1. Taylor D. Krueger et al. *Chemistry- An Asian Journal*. **2023**, *18*, e202300668
2. Garima Bhutani et al. *The Journal of Physical Chemistry B* **2023,** *3197-3207*, 127.
3. Garima Bhutani et al. *Photochemistry and Photobiology* **2024,** 1.
4. Jie Yang et al. *PLoS One* **2013**, *8*, e64849.
5. Rafael Salto et al. *ACS Sensor*. **2021,** *6*, 2563.
6. Rafael Salto et al. *Sensors and Actuators B: Chemical* **2024**, *410*, 135673.
7. Garima Bhutani et al. (under review)

P 111

Molecular effects behind Drug-induced liver steatosis and mitochondrial dysfunction by lipophilic anti-cancer drugs.

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Drug-induced liver steatosis and hepato-toxicity are a major cause of retraction of anti-cancer drugs from the market. Steatosis is caused by an increase in Lipid Droplets (LDs), LDs being neutral-lipid-containing organelles that can partition away lipophilic anti-cancer drugs. Among many other orally administered lipophilic multi-tyrosine kinase inhibitors, Ponatinib was reportedly partitioned inside LDs due to its hydrophobic nature. In tandem with this, Ponatinib also increases the amount of lipids inside cells and gets sequestered even more creating a vicious cycle for the fate of the drug. Though there are significant studies on the lipid biogenesis pathway being upregulated, little has been reported on lipolysis being affected in the presence of Ponatinib. Lipolysis is largely regulated by the transfer of lipids into the mitochondria from cytosol or through a direct linkage with LDs. LD-mitochondria contacts are modulated by the phosphorylation of Perilipin 5 by Protein Kinase A (PKA) during lipolysis. We observed that PKA activity is inhibited in the presence of Ponatinib and LD-mitochondria contacts are significantly reduced. This may be a previously unknown molecular mechanism at play whereby multi-tyrosine kinase inhibitors like Ponatinib inhibit PKA and block the lipolytic flux of the liver causing drug-induced liver steatosis and hepatic failure. It would thus be interesting to explore the possible interventions to mitigate the effect of Ponatinib whereby co-administration of drugs inducing lipolysis in the context of PKA might alleviate the adverse effects of hepato-toxicity.

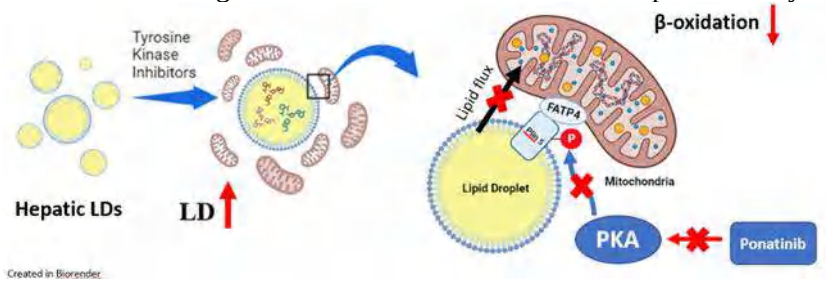

Figure 3: Proposed pathway for disruption of LD-Mitochondria linkage in the presence of lipophilic tyrosine kinase inhibitors like Ponatinib.

References:

1. Englinger, Bernhard et al. *International journal of cancer* **2020**, *147*, 1680.
2. Petan, Toni. et.al. *Reviews of physiology, biochemistry and pharmacology* **2023**, *185*, 53.
3. Yan, Gege et al. *Circulation research* **2024**, *134*, 482.
4. Talari, Noble K., et al. *Nature Communications* **2023**, *14*, 1.
5. Benador, Ilan Y et al. *Cell metabolism* **2018**, *27*, 869.
6. Keenan, Stacey N et al. *Journal of Lipid Research* **2021**, *62*.
7. Najt, Charles P et al. *Molecular Cell* **2020**, *77*, 810.
8. Miner, Gregory E et al. *Developmental cell* **2023**, *58*, 1250.

P 112

Host-Guest Charge Transfer Mediated Disequilibration of Stilbenes inside Water Soluble Nanocage

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Cis-trans photoisomerization is a basic photochemical reaction that is vital to the functioning of many biological processes, including light sensing, ion pumping and vision.[1] Thus far, photoisomerization has been exploited in backbones of molecular switches to activate many artificial light-energy conversion systems and regulate in-vivo biochemical pathways. The secret is in the reaction timescales which are usually ultrafast affecting substantial global conformational change. Although isomerization has been used in diverse applicatons, one fundamental bottleneck is producing 100% yield of one of the isomer after light-activation for any photoswitch. The problem lies in overlap of the absorption spectra of cis-trans forms of the photoswitch.[2] To solve this major challenge, we have developed a new method to control the outcome of cis to trans isomerisation in stilbene derivatives using visible light and water with high efficiency inside confinement. Furthermore, with the help of time resolved spectroscopy, we have tracked the reaction pathway and assigned the intermediates formed to be radical cations of cis and trans stilbene molecules.[3]

References:

1. Christophe D. et al. *Chem. Rev.* **2003**, *103*, 2475.
2. Jolanta K. et al. *Dyes and Pigments* **2023**, *219*, 111558.
3. Yasunao K. et al. *Bulletin of the Chemical Society of Japan* **1993**, *66(6)*, 1852.

P 113

Smartphone based portable devices for cervical and oral precancer diagnosis in clinical conditions

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In recent years, a rapid increase in cervical and oral cancer cases worldwide has been observed. In India, the International Agency for Research on Cancer (IARC) 2022 report estimated 127,000 new cases (9%) and 80,000 deaths (8.7%) due to cervical cancer. For oral cancer, 143,000 new cases (10.2%) and 79,000 deaths (8.7%) were reported [1]. Conventional techniques often fail to predict the severity of the disease at an early stage due to low sensitivity, low specificity, and the lack of real-time prediction. Early diagnosis and effective treatment can enhance patient survival rates. Optical techniques, especially fluorescence spectroscopy and imaging, have the potential to monitor subtle morphological and biochemical changes occurring in tissues with disease progression [2]. These techniques capture the spectral response of various fluorophores (NADH, FAD, collagen, and porphyrin) present in the layered tissue structure [3,4].

We have designed and developed smartphone-based, 3D-printed portable devices for the early diagnosis of cervical and oral cancers using fluorescence spectroscopy and imaging techniques. The device for cervical precancer detection, shown in Figure 1, is based on extracting intrinsic fluorescence from polarized fluorescence and elastic scattering spectra collected from the cervix [5,6]. It utilizes a mutual information and long short-term memory (MI-LSTM) based algorithm to classify different grades of cervical cancer [7-9]. The oral precancer detection device, shown in Figure 2, is a bimodal tool that captures both fluorescence spectra and images on a single platform using a smartphone as the detector [10-11]. Preliminary results from clinical testing suggest that a ratio ($\frac{I_{Porphyrin}}{I_{FAD}}$) based analysis, combined with AI/ML techniques, can effectively discriminate between normal and cancerous oral patients [12].

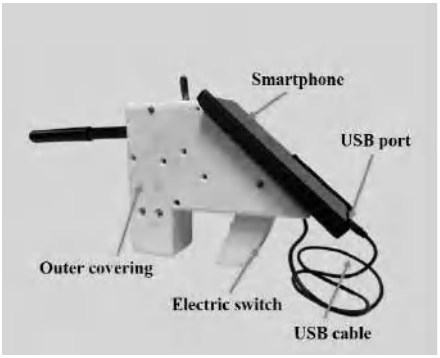

Figure 1: Photograph of the smartphone based cervical precancer detection device

Figure 2: Photograph of the smartphone based bi-modal device for oral precancer detection

References:

1. Ferlay, J. et al. **(2024).** Global Cancer Observatory: Cancer Today (version 1.1). *Lyon, France: International Agency for Research on Cancer. Available from: https://gco. iarc. who. int/today*.
2. Alfano, R. R. et al. **(1989).** Optical spectroscopic diagnosis of cancer and normal breast tissues. *JOSA B*, *6*(5), 1015-1023.
3. Ramanujam, N. et al. **(1996).** Development of a multivariate statistical algorithm to analyze human cervical tissue fluorescence spectra acquired in vivo. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, *19*(1), 46-62.

P 114

Triphenylamine/Phenothiazine-linked 2,4,6-Trisstyrylpyrylium based Probes for Detecting and Differentiating Biologically Relevant Amines

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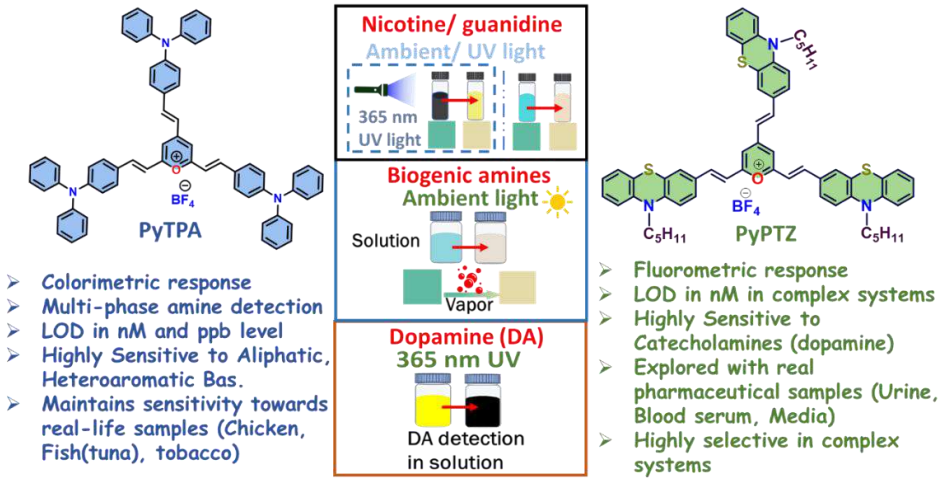
A class of amines, classified as small biomolecules, is generated through the microbial decarboxylation of amino acids in fermented foods and beverages. These amines often play crucial roles in neurological functions. They are categorized as biogenic amines (BAs) and catecholamines, depending on their origin. Given their potential health risks at high concentrations, BAs are considered food hazards, highlighting the importance of monitoring their levels in food samples [1-3]. Herein, we develop two conformationally twisted colorimetric/fluorometric probes, **PyTPA** and **PyPTZ**, where triphenylamine or phenothiazine is linked to 2,4,6-tristyrylpyrylium motif. These probes are designed to detect biogenic amines and catecholamines (dopamine, serotonin) at nanomolar concentrations. This novel and previously unexplored styrylpyrylium probe design provide unique electronic conjugations, steric and geometric constraints, along with good thermal and photostability. These features enable the detection of various amines in distinct and specific ways. The deep-violet **PyTPA** or deep-green **PyPTZ** in solution and solid exhibited a rapid and significant discoloration in response to various aliphatic biogenic amines i.e. putrescine, cadaverine, spermidine, spermine, and also aromatic BAs i.e. histamine, serotonin, 2-phenylethylamine, and dopamine. Notably serotonin and dopamine belong to catecholamine family. The detection of these important amines is remarkable across different phases and has been applied for on-site testing of fresh chicken and fish (tuna). Additionally, **PyPTZ** was utilized for detecting dopamine in human urine and plasma samples. Mass spectrometry played a key role to analyze the products formed from the reactions between amine and pyrylium salts. Further, ^{1}H-NMR, FT-IR, SEM, PXRD, and XPS studies provided a detail insights into the mechanism of this colorimetric/fluorometric detection. We notice the formation of mono- or bis-pyridinium salts after the amine treatment. This newly designed pyrylium salts represents a significant advancement in this class of molecules, offering great potential for detecting and differentiating various biologically important amines.



References:

1. Oguri, S. et.al. *Anal. Chim. Acta.* **2006**, *558*, 326.
2. Patange. S.B. et al. *Food Control*. **2005**, *16*, 465.
3. Munzi, G. et al. *Analyst* **2021**, *146*, 2144.

P 115

Investigating Urea's Role in Protein Denaturation: Insights from Water Dynamics via Two-Dimensional Infrared Spectroscopy

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Osmolytes play a crucial role in cellular function by stabilizing proteins, adjusting osmotic pressure, and maintaining cell volume. However, urea, a unique osmolyte, denatures proteins and counteracts the effects of stabilizing osmolytes like trimethylamine N-oxide. The molecular mechanism behind urea's denaturing ability remains debated, centered around two opposing mechanisms: direct and indirect. The direct mechanism proposes that urea disrupts hydrogen bonds and hydrophobic interactions within proteins, while the indirect mechanism suggests that urea modifies water properties, thereby disrupting protein-protein interactions.[1-3] A key question is whether urea acts as a "structure-maker" or "structure-breaker," akin to how salts are classified by the Hofmeister series. To explore these mechanisms, we investigated water dynamics through the spectral diffusion of the OD stretch in isotopically diluted water in the presence of urea and other osmolytes using two-dimensional infrared (2D IR) spectroscopy. By comparing water dynamics in the presence of urea and other osmolytes to those of neat water, our spectroscopic analysis provides new insights into the water-urea system, shedding light on the intricate mechanisms of protein denaturation by urea.

References and Notes

1. Ding, B. *et al. The Journal of Physical Chemistry Letters* **2018,** *9* (11), 2933.
2. Carr, J. K. *et al. The Journal of Physical Chemistry B* **2013,** *117* (42), 13291.
3. Borkowski, A. K. *et al. The Journal of Chemical Physics* **2023,** *158* (6).

P 116

Development of a Fluorescent Biosensor for Sensitive Trypsin Detection Using a Tetraphenyl Ethylene-Histone Complex

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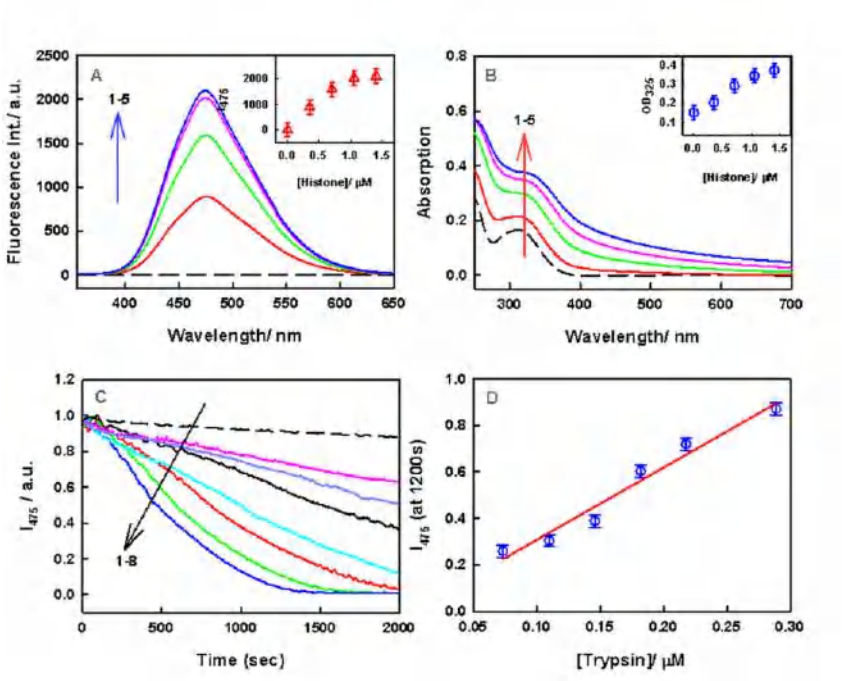
Trypsin is a digestive serine protease that hydrolyzes dietary proteins in many eukaryotic and prokaryotic organisms. It has numerous applications in various fields and is a potential biomarker for diseases, such as pancreatitis, cystic fibrosis, etc [1,2]. The present contribution describes a fluorescence turn- off sensing scheme for Trypsin, which utilizes the phenomenon of aggregation-induced emission (AIE). The sensing scheme of the probing system involves the interaction of a water-soluble Tetraphenylethylene (TPE) fluorophore, sodium 1,2-bis[4-(3-sulfonatopropoxyl)phenyl]-1,2-diphenylethene (BSPOTPE) with Histone. The sensing scheme for Trypsin detection is based on the principle of trypsin-dependent Histone hydrolysis. The interaction between the components of BSPOTPE-Histone-Trypsin system has been thoroughly investigated using various photophysical techniques such as ground-state absorption, steady-state, and time-resolved emission. Besides being simple and selective, the present sensor system is highly sensitive to Trypsin. Moreover, BSPOTPE is a commercially available probe molecule that abstains from time-consuming protocols and provides potential utility for real-life applications. The application for the present sensor system has been demonstrated in real urine matrix also.



References:

1. Kaur Jet al. *J Mol Liq.* **2021**, *333*, 115980.
2. Wang et al. Talanta. **2023**, *259*, 124562.

P 117

Unraveling Lipid Droplet Dynamics during Ferroptosis using a Novel D-A-D Fluorescent Probe

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Lipid droplets (LDs) are crucial cellular organelles involved in lipid metabolism, energy homeostasis, and signal transduction. [1] This work investigates the dynamics and polarity changes of LDs during ferroptosis, a regulated form of cell death marked by iron-dependent lipid peroxidation. [2] Utilizing the terephthalate group based Donor-Acceptor-Donor (D-A-D) fluorescent probe R1(figure 1) , we have conducted real-time visualization of LD behavior in HeLa and A549 cells under various conditions using confocal laser scanning microscopy (CLSM) and Fluorescence Lifetime Imaging Microscopy (FLIM). The results demonstrate that R1 selectively localizes to LDs, providing a robust and sensitive means to monitor LD dynamics. Upon inducing ferroptosis with erastin, a significant decrease in R1 fluorescence intensity as well as lifetime is observed within LDs, indicating an increase in polarity, which is a hallmark of ferroptosis. The probe also effectively tracks the fusion and migration of LDs over time, revealing their dynamic nature. These findings highlight the potential of R1 as a powerful tool for studying LD-related processes, particularly in understanding ferroptosis mechanisms and lipid metabolism disorders. This work establishes the use of R1 for advancing lipidomics research and offers potential applications in disease diagnostics and the development of targeted therapeutic strategies.

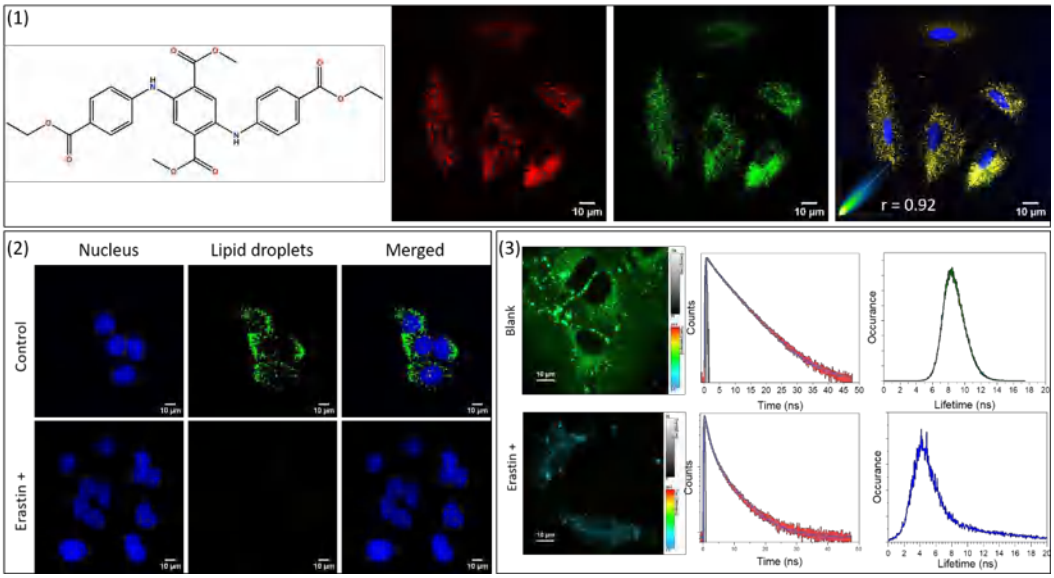

Figure: (1) Structure of fluorophore R1 followed by CLSM images of A549 cells treated with Nile Red, and R1. Inset in the merged image of DAPI, Nile red and R1 shows Pearson's correlation plot (r) of Nile Red and R1 fluorescence intensities, indicating co-localization of lipid droplets. (2) CLSM images of A549 cells. Top: Control cells with normal distribution of nuclei (DAPI) and lipid droplets (R1). Bottom: Cells treated with erastin (10 µM, 6 hours) showing altered lipid droplet distribution and nuclei morphology. (3) FLIM images of A549 cells. Top: Control cells showing normal lipid droplet distribution with corresponding fluorescence decay trace and lifetime distribution. Bottom: Erastin-treated cells (10 µM, 6 hours) displaying altered lipid droplet distribution with corresponding fluorescence decay trace and a shifted lifetime distribution, indicating changes in the cellular environment.

References

1. Beckman, M. *Science* **2006**, *311*, 1232–1234.
2. Yang, W. S. et al. *Trends Cell Biol.* **2016**, *26*, 165–176.

P 118

Elucidation of Origin of Electroluminescence Intermittency in Hybrid Lead Halide Perovskite Crystal

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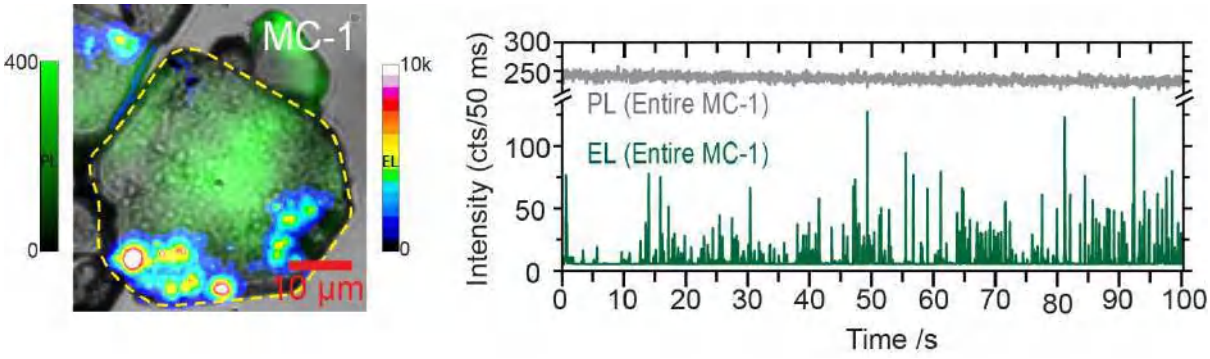
Over the past decades, metal halide perovskites (MHPs) have come up as the most promising materials for both photovoltaic and optoelectronic devices. There are numerous reports on the photoluminescence (PL) intermittency (blinking) of MHP nano/micro-crystals. Reports of MHPs displaying electroluminescence (EL) intermittency, however, are quite rare [1-3]. Herein we report bulk crystals of $MAPbBr_3$ exhibiting intermittent EL, while having temporally stable emission under photoexcitation. Single-particle wide-field imaging allows to probe the temporally unstable EL emission emanating from stochastically changing local emission centers within an individual crystal. The stochastic EL helps to understand the carrier recombination dynamics in an MHP single-crystal on carrier injection and provide a plausible EL blinking mechanism for the observed peculiar behaviour by invoking the concept of ion migration in MHPs under externally applied bias. EL intermittency is detrimental for the working of perovskite light emitting devices as it reduces the EQE. Hence, our study may provide ways to mitigate the consequences leading to EL blinking.



References:

1. D. K. Sharma et. al. *Nat. Commun.* **2019**, *10* (1), 1.
2. S. B. Bhagyalakshmi et. al. *J. Phys. Chem. C* **2022**, *126* (42), 17826.
3. P. Andričević et. al. *ACS Photonics* **2019**, *6*, 967.

P 119

Multifunctional Pyridine-functionalized Coumarin-fused Imidazole Derivative: Ratiometric Sensing and pH Profiling

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Coumarin is a notable fluorophore with many advantages, such as high fluorescence quantum yield, large Stokes shift, good photostability, and low toxicity [1]. The development of chemosensor which is capable of recognizing specific ion in selective manner will be the challenging task for many Researchers. In this study, the pyridine-functionalized coumarin-fused imidazole derivative was successfully synthesized, characterized, and evaluated for its sensing of metal ion capabilities. The probe selectively detects Zn^{2+}, Cu^{2+} and Ni^{2+} with metal ion specific optical output. The binding mechanism of the probe with metal ion was investigated through Job's plot, Benesi-Hildebrand plot, DFT studies and mass spectrometry [2]. Additionally, the probe-Zn^{2+} ensemble exhibited intriguing ratiometric spectral reversibility in the presence of Adenosine Triphosphate (ATP), functioning as a fluorescence "on-off" sensor [3]. The quenching mechanism for Cu^{2+} and Ni^{2+} was confirmed using the time resolved measurement. Additionally, the probe exhibited specific pH sensing in the acidic region, displaying a color change from pale yellow to intense yellow under UV light, attributed to a charge transfer process. This study highlights the potential of combining coumarin with heterocyclic derivatives to create a multifunctional chemosensors having high sensitivity, selectivity, and practical applicability.

References:

1. Asim Mansha et al. *springer* **2022**, *76*, 3303.
2. Sinan Bayindir et al. *Spectrochemical Acta part A* **2019**, *213*, 6.
3. X. Zhang et al. *J. Photochem. Photobiol. B, Biol* **2021**, *223*, 112279.

P 120

Light-Induced Electron Transfer in BMC Shell Proteins: Towards Self-Powered UV Devices

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Specialized protein structures, known as bacterial microcompartments (BMCs), are primarily composed of shell proteins that self-assemble into planar sheets, forming the microcompartment's outer shell. These protein sheets display distinct patterns of electron-dense and electron-sparse regions, which have potential applications in electron conduction. In our study, we investigated PduA and PduBB', key shell proteins from the 1,2-propanediol utilization microcompartment in *Salmonella enterica*. We found that these proteins exhibit low work function and non-linear I-V characteristics, suggesting semiconducting properties. Notably, UV light exposure can induce rapid electron transfer within the shell protein sheets even without an external voltage. Our findings position PduBB' as a promising candidate for bio-photodetectors, with faster rise (0.35s) and decay (0.74s) times. The strategic arrangement of amino acids in shell proteins facilitates the Proton Coupled Electron Transfer reaction, the underlying mechanism behind the photocurrent generation. Thus, these proteins' sensitivity to UV light, coupled with high figures of merit such as responsivity, detectivity, and enhanced quantum efficiency indicates their potential as effective self-powered UV photodetectors.

P 121

Detecting early stages of glycation in Human Serum Albumin using Protein Charge Transfer Spectra.

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Protein Glycation is a spontaneous Post-Translational Modification involving the non-enzymatic covalent attachment of a reducing sugar or sugar derivative to a protein and is prevalent in biological system. Glucose initially reacts with amino groups to form glycosylamine, which dehydrates to a Schiff's base that undergoes Amadori rearrangement to yield fructosamine, classified as early glycation adducts. In later stages fructosamine changes into a stable advance glycation end products (AGEs).[1] There are various method for the detection of AGEs, Such as Immunohistochemistry, ELISA, Western blotting, MALDI-ToF, LC-MS/MS.[2] However only a few detection techniques are available for early stages of glycation, like MALDI-ToF and NBT assay. These conventional techniques either requires expensive instrumentation or external labelling which may changes the characteristic features in the protein. However, detecting glycation using a label-free intrinsic probe is limited. Recently our group discovered a new intrinsic non-aromatic chromophore in a monomeric charged rich protein. The charged residues (Lysine, Arginine, Glutamate, Histidine and Aspartate) participate in photoinduced electron transfer with the peptide backbone or among themselves. This gives rise to broad UV- Vis electronic absorption ranging from 250 to 800 nm called as Protein Charge Transfer Spectra (ProCharTS).[3] Herein we use Human Serum Albumin (HSA) to track the initial glycation stages by using ProCharTS. HSA is known to undergo glycation modification upon prolong presence of Glucose in the blood, a characteristic feature among diabetic patients. Firstly, we confirmed glycation of HSA using MALDI-ToF with the change in the mass of glycated form. We observed an increase in ProCharTS intensity after glycation in HSA signifying similar trends observed in MALDI-ToF. We further analysed the structural changes by using CD spectroscopy and local changes using Tryptophan fluorescence. The other physiochemical parameter are compared by using anisotropy measurement, DLS, Zeta potential.

References:

1. Rabbani N et al. *Amino Acids.* **2012**, *42(4)*, 1087.
2. Rabbani N et al. *Glycoconj J.* **2016**, *33(4)*, 553.
3. Singh S, et al. Chem Sci. **2017**, *8(8)*, 5416.

P 122

Role of SUMO1 in Phase Separation: More Than a Recognition Tag

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Liquid–liquid phase separation (LLPS) plays a crucial role in cellular organization, primarily driven by intrinsically disordered proteins (IDPs) leading to the formation of biomolecular condensates. [1–4] A post-translational modifier protein, SUMO, has recently emerged as a regulator of LLPS.[5–7] Given its compact structure and limited flexibility, the precise role of SUMO in condensate formation remains to be investigated. Here, we ask how SUMO protein having globular folded structure gets recruited into the liquid-like assemblies? Is this recruitment driven by their IDP substrates? Or do they possess an independent tendency to undergo weak interactions required for LLPS? Towards this, we study the phase-separation of SUMO1 protein in controlled crowded environment. We find that SUMO1 can rapidly and independently form micrometer-sized liquid-like condensates in the absence of its IDP substrates or any SUMO-interacting motifs. The liquid condensates undergo subsequent time-dependent conformational changes and aggregation which are probed by label-free methods (tryptophan fluorescence and Raman spectroscopy). Remarkably, experiments on a SUMO1 variant lacking the N-terminal disordered region further corroborate the role of its structured part in phase transitions. Our findings highlight the potential of folded proteins to engage in LLPS and emphasize further investigation into the influence of the SUMO tag on IDPs associated with membrane-less assemblies in cells.

References:

1. Feric M et al. *Cell* **2016**, 65, 1686.
2. Brangwynne CP et al. *Science* **2009**, *324*, 1729.
3. Hyman AA et al. *Cell* **2011**, 21, 14.
4. Hyman AA et al. *Annu Rev Cell Dev Biol* **2014**, *30*, 39.
5. Pountney DL et al. *Exp Neurol* **2003**, *184*, 436.
6. Kirk KE et al. Modification of Huntingtin and Huntington's Disease Pathology **1997**.
7. Dorval V et al. *Journal of Biological Chemistry* **2006**, *281*, 9919–9924.

P 123

Mechanism of Conformational Selection of tRNAArg2 by Bacterial Deaminase TadA

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Base editing is a common mechanism by which organisms expand their genetic repertoire to access new functions. Here, we explore the mechanism of tRNA recognition in the bacterial deaminase TadA which exclusively recognizes $tRNA^{Arg2}$ and converts the wobble base adenosine (A34) to inosine. We quantitatively evaluate the dynamics of tRNA binding by incorporating the fluorescent adenine analogue, 2-aminopurine (2-AP) at position 34 in the wobble base of the anticodon loop. Time-resolved fluorescence lifetime and anisotropy studies revealed that the recognition process is finely tuned. While mutations in residues that partake in assisting deamination (E55A, N42A) does not exhibit a major effect on binding dynamics, the residues that stabilize the region above the active site 'capping residues', and are exclusive to prokaryotic TadAs, such as R149, have a marked effect on binding dynamics and catalytic activity. Moreover, for effective catalysis, peripheral positively charged residues (R70, R94) that are part of the adjacent subunit in the dimeric assembly, are important to splay out the tRNA, assisting in A34 attaining a flipped-out conformation. Perturbations in these extended regions, although 15-20 Å away from the active site, disrupt the binding dynamics and consequently the function, emphasizing the fine regulation of the tRNA recognition process. Analysis reveals that tip of the extended helix where R149 is positioned, acts as a selectivity filter imparting exclusivity towards the deamination of $tRNA^{Arg2}$ by TadA.

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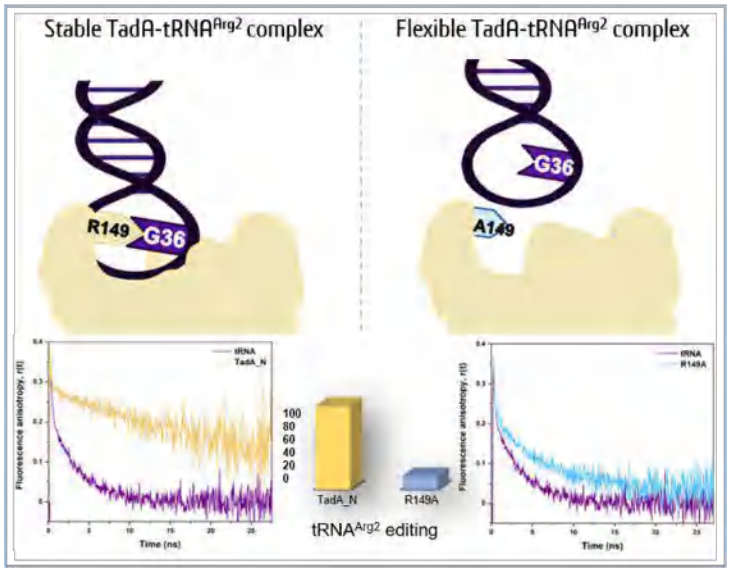

References:

1. Wolf, J., et.al. *EMBO J*, **2002**, *21*, 3841.
2. Losey, H. C., et.al. *Nat. Struct. Mol. Biol*, **2006,** *13*, 153.
3. Elias, Y., and Huang, R. H. *Biochemistry*, **2005**, *44*, 12057.
4. Luciano G. Dolce, et.al. *Nat Commun*, **2022**, *13*,6737.

P 124

Tracking Mn^{2+} Dynamics and Quantifying Labile Mn^{2+} in Living Mammalian Cells Using a Water-soluble, Cell-permeable Fluorescent Sensor

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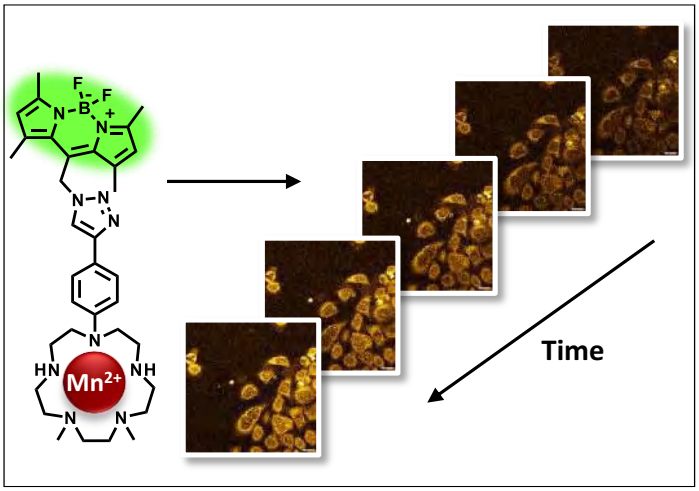
Manganese (Mn^{2+}) ions are indispensable for proper functioning of life, given their crucial roles in brain function, immunity against pathogens as well as photosynthesis.[1] Disruptions in Mn^{2+} ion homeostasis have been linked to neurodegenerations, microbial infection and have also been implicated in cancers.[2-4] This entails the need to not only detect the localization of this metal ion at a specific time, but also track Mn^{2+} dynamics in real time in living cells. For that, fluorescent sensors selective to Mn^{2+}, sensitive to physiologically relevant concentrations of Mn^{2+} ions, and responsive in aqueous media are required. So far, no Mn^{2+} sensor had a combination of all the aforementioned properties. This is because designing fluorescent sensors for Mn^{2+} is challenging. Mn^{2+} is weak-binder to ligand-scaffolds and quenches the fluorescence of fluorophores resulting in turn-off sensors, not conducive for live-cell imaging. To address these challenges, we have developed a novel water-soluble, cell-permeable fluorescent turn-on Mn^{2+}-selective sensor, sensitive to the physiological levels of Mn^{2+}.[6] We have employed penta-aza macrocycle as the Mn^{2+} -binding scaffold, aligning with the native or reported Mn^{2+} binding preferences.[7-8] Photo-induced electron transfer (PeT) was chosen as the mechanism of sensing and a density functional theory (DFT) and time-dependent DFT (TD-DFT) based computational workflow guided the design of the molecule. The computationally designed, PeT-based water-soluble, cell-permeable Mn^{2+} sensor could detect endogenous levels of Mn^{2+} in living cells in both fluorescence intensity and lifetime-based setups. The sensor enabled the visualization of disease-relevant differences in Mn^{2+} uptake dynamics and provided the first ever estimate of labile Mn^{2+} ion concentration in living mammalian cells. I will detail the design, computational studies, in vitro and in cell results achieved with this novel sensor in my poster.



References:

1. S. Das et al. *Dalton Trans.* **2019**, *48*, 7047.
2. S. M. Damo et al. *Proc. Natl. Acad.* **2013**, *110*, 3841.
3. K. Tuschl et al. *Nat. Commun.* **2016**, 7, 11601.
4. P. A. Doble et al. *Metallomics.* **2018**, *10*, 1191.
5. H. Irving et al. *J. Am. Chem. Soc.* **1953**, 3192.
6. S. Kahali et al. *Chem Sci* **2024**, *15*, 10753.
7. A.M. McGuire et al. *Biochemistry* **2013**, *52 (4)*, 701.
8. D. Salvemini et al. *Nat. Rev. Drug Discov.* **2002**, *1 (5)*, 367.

P 125

Bio-waste derived Mg-doped carbon dots: A Fluorescent Probe of Y^{3+} Metal ion and Bio imaging Application

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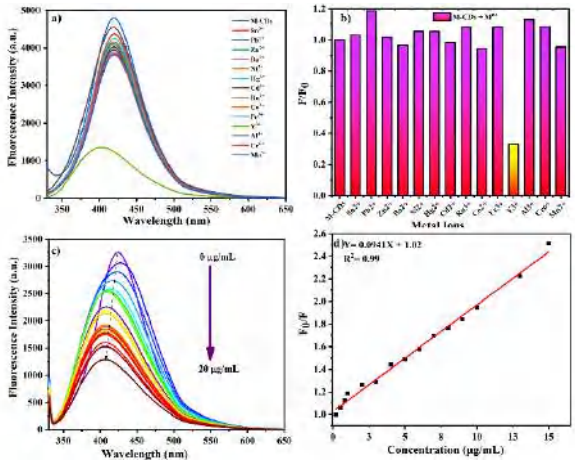
In present study, waste valorisation approach was utilizing for synthesis of Mg-doped carbon dots form cow dung using hydrothermal method. The Mg doped fluorescent carbon dots (M-CDs) having maximum emission at 420 nm upon 310 nm excitation with 20 % quantum yield. The M-CDs synthesis was confirmed using different analytical a characterization technique as well as stable in different pH as well as ionic strength solutions. The M-CDs was highly selective towards Y^{3+} ions with significant blue shift. The LOD of developed probe toward Y^{3+} was 0.019 µg/mL. The study indicates quenching of Y^{3+} was result of dynamic and IFE quenching effect [1] which was analysed by TCSPC (Time-Correlated Single Photon Count) and UV-Visible spectroscopy measurement. Further the interaction of CDs with Y^{3+} ion was investigated. The oxygen containing groups of CDs was responsible for Y^{3+}-CDs bonds [2-3]. The healthy growth of blood vessels in angiogenesis study demonstrated the cytotoxicity of CDs. further the CDs employed for MCF-7 breast cancer cell imaging [4]. The CDs were enable to interact with MCF-7 cell and brighten the fluorescence signal.



References:

1. Panpan Zhu et al. ACS Appl. Nano Mater **2023**, 6, 21194.
2. Xuelian Hu et al. Sen. Actuators B. Chem. **2021**, 338, 129809.
3. Mingxi Yang et al. Part. Part. Syst. Charact. **2019**, 36, 1800315
4. Cintya D'Angelis do E. S. Barbosa et al. Chem. Eur. J. **2015**, 21, 5055 – 5060

P 126

Untangling the degradation mechanism of knotted proteins by ATP-dependent proteases and proteasome

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Knotted protein is a special class of protein where self-entanglement of the poly peptide chain results in a folded protein with a knot. Depending upon the knot topology knotted proteins are classified as *trefoil knotted (3_1), figure of eight knotted (4_1), gordian knotted (5_2), and stevedore knotted (6_1) proteins*[1].

These proteins currently populate almost 1% of known protein structures in the PDB[2]. However, how a protein folds with a knot and what is the functional significance of a knot in a protein remains elusive. Following initial reports of knots providing proteins mechanical stability against cellular degradation by ATP-dependent proteases and proteasomes[3], we have tried to understand the degradation mechanism of model knotted protein substrates (miRFP709 and human UCHL1) by proteases ClpXP and ClpAP and proteasomal system Mpa-20S CP, using steady-state fluorescence loss assay and SDS-PAGE densitometry assays.

ClpAP and ClpXP are known to be a comparable in terms of their protein degradation activity[4]. But, to our surprise, when degraded from C to N terminus, ClpAP fails to degrade knotted protein miRFP, while ClpXP degrades it very easily. To reconcile such unanticipated behavior, we further investigated the role of knots in degradation by using fusion GFP-knotted proteins as substrate. On the other hand, the knotted protein substrate UCHL1 is seen to be resistant to degradation by both ClpXP and ClpAP when degraded from C to N terminal, whereas the Mpa-20S CP proteasome system quite easily degrades UCHL1 from N to terminal.

Thus, our study suggests that knots may contribute to protein stability against certain degradation systems, such as ClpAP. However, knotted proteins are still susceptible to degradation by other systems, including ClpXP and Mpa-20S CP. This duality could reflect evolutionary selection, where knots provide protection from some quality control mechanisms while retaining alternative pathways for maintaining protein homeostasis within the cell.

References:

1. Faisca et al. *Computational and Structural Biotechnology Journal. Elsevier B.V.* **2015**, *459*.
2. Dabrowski-Tumanski et al,. *Proc Natl Acad Sci U S A* **2017**, *114* (13), 3415
3. Xu et al. *Biophys J* **2018**, *115* (9), 1681.
4. Olivares et al., *Nat Struct Mol Biol*. **2014**, *21(10)*, 871
5. Lim et al. *J Mol Biol* **2015**, *427 (2)*, 248

P 127

Structural and functional basis of Erythromycin Resistance Methyltransferase mediated antibiotic resistance

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Antimicrobial resistance is a silent pandemic rapidly engulfing the world, driven by the misuse and overuse of antibiotics[1]. Among the many methods employed by pathogens to evade the effects of antibiotics, target modification is both efficient and straightforward. Erythromycin-resistance methyltransferases (Erms) site-specifically mono-/di-methylate A2058 (*E. coli* numbering), a base located deep within the nascent peptide exit tunnel (NPET) of the ribosome[2]. This modification confers resistance to the macrolide, lincosamide, and streptogramin B classes of antibiotics, which also bind in the NPET to disrupt protein translation. While it is established that Erms act on ribosomal precursors, the true in vivo substrate of these enzymes has remained elusive[3]. Using cryogenic electron microscopy (Cryo-EM) and single-molecule Förster resonance energy transfer (sm-FRET), we have not only captured this elusive substrate but also revealed the complex dynamic nature of this enzyme when interacting with its substrate. Our results highlight key structural determinants, located away from the enzyme's catalytic domain, that govern substrate specificity. Additionally, we observe a unique molecular motion displayed by these enzymes, which facilitates the methylation of the target base. Overall, our study provides a comprehensive understanding, from substrate identification to the elucidation of the enzyme's dynamic nature, which paves the way for drug development against these pathogenic proteins.

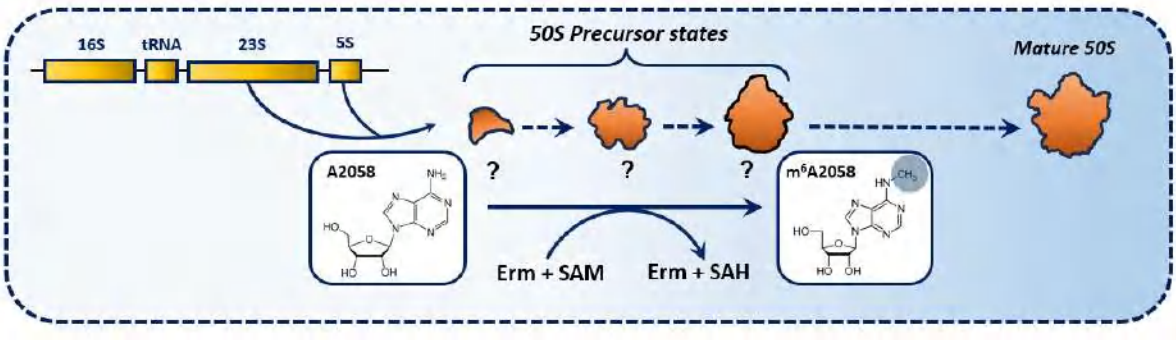

Figure 1: Schematic representing the action of Erms *in vivo*. The true substrate of Erms have remained elusive. Erms methylate an early ribosomal particle and induces drug resistance.

References:

1. Okeke, I. N. et al. The Lancet **2024,** *403*, 2426.
2. Bhujbalrao et al. *J Am Chem Soc* **2019**, *141*, 1425.
3. Pokkunuri, I. & Champney, W. S. *RNA Biol* **2007,** *4*, 147.

P 128

Drug-assisted white light generation *via* self-assembly of a styryl dye

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Fluorescence spectroscopy and microscopy stand as pivotal methodologies in the realm of scientific inquiry, offering profound insights into the intricate molecular properties of both organic and inorganic entities.[1-2] With unmatched sensitivity, fluorescence spectroscopy enables the clarification of molecular structures, interactions, and dynamics through the absorption and subsequent emission of photons by fluorophores. In this investigation, we focused on white light generation *via* the combination of a polarity-sensitive red emitting Styryl chromone 1 (**SC1**)[3] **(Fig.)** and a blue-emitting anticancer and psycotherapeutic drug Norharmane (**NHM**) [4] in a self-assembled neutral adjuvant Triton X-100 (TX-100). Incorporation of both emitters inside the micellar system results an improved fluorescent behaviour and resulting in white light emission due to complementary wavelength overlap. Further spectroscopic investigation allows us to understand the premicellar aggregation process [5] of three different type of surfactants with varying charges using the **SC1** dye. This investigation highlights the significance of co-assembly of **SC1** dye and NHM drug for the generation of a highly stable white light.

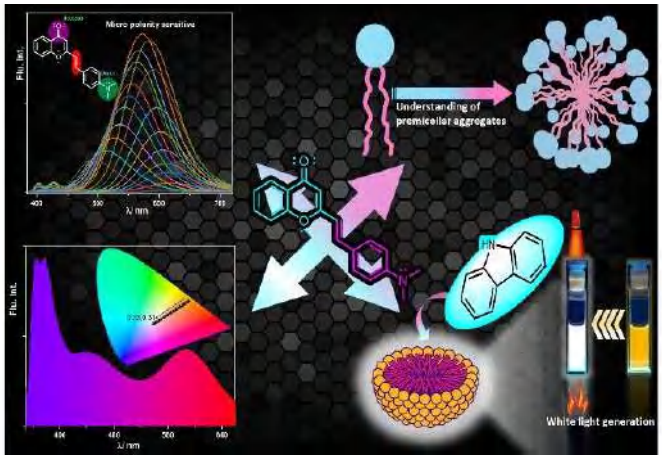

Fig. White light generation using a co-assembly of a micro-polarity sensitivity dye and anticancer drug

References:

(1) Engelborghs, Y. *et al.*, Book on: Fluorescence spectroscopy and microscopy. In *Methods in molecular biology*. Springer, **2014**.
(2) Chatterjee, O., *et al.*, *Adv. Opt. Mater.*, **2022**, *10*, 2201187.
(3) Kavyashree P. *et al.*, *J. Mater. Chem, B* **2022**, *10*, 5071.
(4) Chakrabarty, A. *et al.*, *Langmuir*, **2007**, *23*, 4842.
(5) Niu, X. *et al.*, *Analyst*, **2019**, *144*, 6866.

P 129

Scaling Principles in Chemistry: Elucidating the Reaction Kernel

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Abstract :

The visualization of atomic motion at its fundamental length scale and time scale has long been a 'Gedanken'experiment to develop the conceptual understanding of chemical reactions in condensed matter systems[1,2]. In this regard, an electrocyclic ring-closing reaction or a spin crossover reaction are prototypically classic examples of a bond-making process or an electron transfer reaction with the change of electron spin and density. Hence following the electron with its spin while bonds are being formed, is the ultimate magnum opus of chemistry. In this regard, the conquest of comprehending the reaction mechanism through the barrier-crossing process becomes imperative and even so because of the enormous reduction of dimensionality during a chemical reaction, which still alludes to chemists and physicists all around the world. The idea of only a few key reactive modes out of 3N-6 degrees of freedom (N: number of atoms) driving a chemical reaction through the transition state is still an open quest yet fully to conquer. Observation of these key reactive modes defines the reaction kernel and gives a detailed understanding of the reaction coordinate and the competition among myriads of quantum vibrations or orthogonal coordinates. In this context, we have determined the reaction kernel for the ring-closing reaction in a prototypical molecule called fulgide with three key reactive modes and, also elucidated the reaction kernel for the spin crossover process in a Fe (III) based inorganic molecule consisting of two key reactive modes. For the ring-closing reaction in fulgide, we have been able to disentangle the excited state dynamics of 70 fs followed by a ~140 fs timescale for the ring-closing reaction highlighting the ultrafast nature of the reaction. In the spin crossover process, we have discovered the participation of an intermediate quartet spin state which is populated in ~50 fs limited by the instrument response. In 96 fs, the system depopulates the intermediate state to form the high spin sextate state starting from the initially photoexcited low spin state (Ligand-to-metal charge transfer doublet state). In both systems, we observe a structural reorganization occurring in the sub-ps timescale because of the constrained crystalline environment since molecular motion is restricted. This results in the activation of molecular vibrations dephasing in a sub-ps timescale optimizing the molecule in the product potential energy surface after the ultrafast photoinduced chemical reaction. This present work has also unearthed a new method of reaction mechanism, which involves the non-linear mixing of molecular vibrations giving rise to transient vibrations on ultrafast timescales that light up as coherent photoinduced motions directing the chemical transformation. The molecular vibrations dephasing in the sub-ps timescale undergo non-linear mixing in the molecular framework to generate transient vibrations with growing amplitude. Mechanism of this kind is well known in non-linear optics but is a surprising observation in the molecular framework highlighting the strong anharmonicity within the product potential energy surface. This discovery could open new ways of perceiving chemical reactions, with yet another mechanism collapsing the system onto a few highly nonlinearly coupled coordinates. This new insight is more clearly observable in solid crystalline environments where the initial conditions are well-defined. The elucidation of the structural dynamics and the key reactive modes defining the reaction kernel in the single crystal environment will open new avenues in carrying out reactions in the crystalline environment and inspire new design strategies for better and more improved chemical reactions in comparison to the solution phase. This is possible because of conserved spatial correlations in crystals, inspiring a new field of research in open quantum systems. Current research is underway in analyzing ultrafast electron diffraction (UED) data to unearth the atomic motion leading up to the reactive kernel and visualize the serendipitous discovery of the mixing of molecular vibrations in the crystalline domain. These discoveries can be further expanded to extended systems in biology where proteins (N>1000) effectively carry out chemical transformations to drive function in living systems and our research promises to uncover the secrets of anharmonic couplings and localized motions defining the reaction kernel driving biological functions[1,2].

References

1. Ischenko, *et. al*., *Chem. Rev*. **2017**, *117*, 11066.

2. Miller, R., J., D, *Annu. Rev. Phys. Chem.*, **2014**, *65*, 583.

Complex Interplay of domains in the co-condensation of α-Synuclein and SARS-CoV-2 Nucleocapsid Protein

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Recent studies suggest that SARS-CoV-2's impact extends beyond respiratory tract infections to neurological symptoms including Parkinsonism [1, 2]. Recently, it was reported that the SARS-CoV-2 nucleocapsid protein (N-protein) accelerates the aggregation of α-Synuclein (α-Syn), a protein associated with Parkinson's disease [3]. Liquid-Liquid Phase Separation (LLPS) is key for organizing biomolecules in cells but can also lead to toxic aggregate formation in condensates [4]. α-Syn undergoes nucleation and aggregation via condensation through LLPS [5]. In this study, combining single molecule spectroscopy, microscopy and simulation we tried to understand the underlying molecular mechanism of N-protein-facilitated aggregation of α-Syn. We observed that N-protein promotes LLPS of α-Syn via co-condensate formation. Fluorescence Correlation Spectroscopy (FCS) inside droplets and Fluorescence Recovery After Photobleaching showed hindered dynamics of α-Syn in the co-condensate. Time-domain fluorescence Lifetime Imaging further confirmed the enhanced ageing of the co-condensates. The increased partitioning of amyloid reporter- Thioflavin T inside co-condensates suggested the temporal maturation of liquid droplets into β-sheet rich structures. Confocal microscopy, co-existence coarse-grained simulations and binding affinity measurements elucidated that electrostatic heterotypic interactions through oppositely charged blocks in these proteins can dictate the architecture of the co-condensates. Overall, this study reveals how α-syn and N-protein domains interact to drive co-condensation, shedding light on α-syn amyloid formation and possible reason for COVID-induced Parkinsonism.

References:

1. Cohen, Mikhal E et al. *The Lancet Neurology* **2020**, *19*, 804.
2. Lee, Bina et al. *Cell Reports Medicine* **2024**, *5*, 101570.
3. Semerdzhiev, Slav A et al. *ACS Chemical Neuroscience* **2021**, *13*, 143.
4. Boeynaems, Steven et al. *Trends in Cell Biology* **2018**, *28*, 420.
5. Ray, Soumik et al., *Nature Chemistry* **2020**, *12*, 705.

P 131

Intracellular active forces and out of plane rotation due to motor activity revealed in passive microrheology

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Optical tweezers are a common tool to investigate biological systems such as single cells, applying very low, non-invasive forces. It uses a tightly focusses laser beam to confine a micro particle and simultaneously track its position and orientation. A phagocytosed bead inside a cell is affected by its local environment, cytoskeletal dynamics as well as molecular motor activity which is responsible for transport of cargo inside cells [1-3]. Out of plane rotational motion of a trapped phagosome happens due to molecular motor activity. However, calibrating the data inside cells is a challenge [4]. We show a method to track and calibrate the out of plane rotation of a particle in intracellular environment using passive microrheology. We record Power Spectral Density in the frequency range from 0.5Hz to 1kHz. The lower frequency domain is dominated by active processes whereas thermal fluctuations prevail at higher frequency domain [5,6]. The crossover frequency from active to thermal domain has been a matter of conflict since long [5,6]. Complicacies are increased by the fact that the active processes are random and stochastic, and the cellular environment is highly complex and heterogeneous [7]. We combine the generalized Maxwell model for viscoelasticity with Active Ornstein Uhlenbeck process to derive a model to describe the complete behavior of an optically trapped bead inside a cell at all frequency regimes [8]. This model proves useful to simultaneously extract local viscoelastic parameters along with effective active force and torque exerted on the cargo by teams of molecular motors and activity timescale. The out of plane rotational motion yields interesting information about multiple motor activity elusive to conventional translational tracking.

References:

1. F. C. MacKintosh et. al. *Curr. Opin. Cell Biol*. **2010,** *22(1),* 29.
2. W. W. Ahmed et al. *Biophys. J* **2018**, *114(7),* 1667.
3. C. Leidel, et al. *Biophys. J*. **2012**, *103(3),* 492.
4. Y. Jun et al. *Biophys. J*. **2014**, *107(6),* 1474.
5. H. Turlier, et al. *Nature physics*, **2016**, *12(5),* 513.
6. D. Mizuno et al. *Macromolecules* **2008**, *41(19),* 7194.
7. K. Luby-Phelps et al. *Int. Rev. Cytol*. **1999**, *192,* 189.
8. U. Nakul, et al. *Biomedical optics express* **2023**, *14(10),*5440.

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Probing SARS-CoV-2 spike mediated nascent fusion pore open-close dynamics during single membrane fusion events

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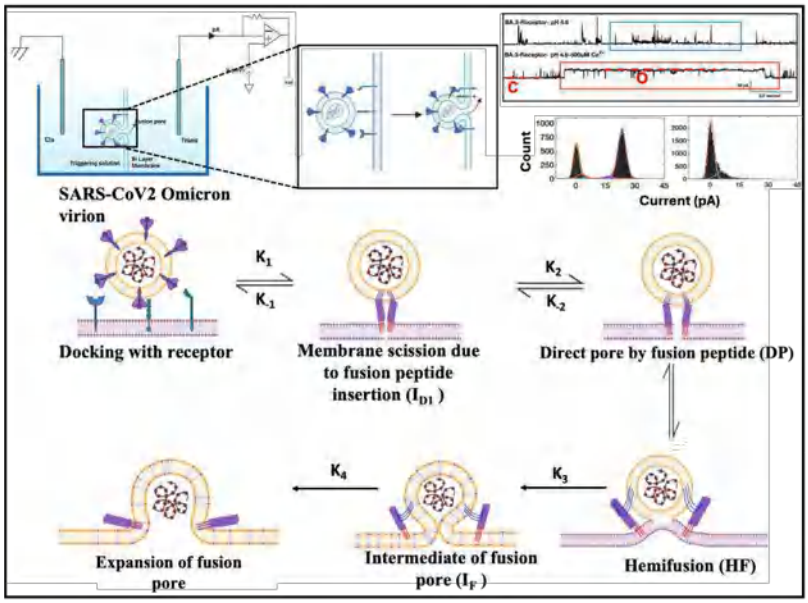
SARS-CoV-2 is the causative agent of the COVID-19 infection that has resulted public health concern worldwide. Cellular entry of SARS-CoV-2 is mediated by the spike glycoprotein fusion machinery[1,2]. During entry, trimeric spike protein facilitates fusion pore formation between viral and cellular membrane to release the viral genetic materials into the host cell[3]. Still, the mechanism of pore formation during membrane fusion by the spike protein is not properly understood. Here, using planar lipid bilayer electrophysiology and fluorescence-based lipid mixing, we directly detected the Omicron spike mediated fusion pore formation in real time at single fusion events. We found that fusion pore has a complex dynamics and it follows distinct open-close states depending on the cellular environment and spike protein localization. We also observed that the pore formation reaction proceeds through sequential intermediates on its pathway to complete fusion. Moreover, our findings also highlight the synergistic effect of lysosomal pH and presence of Ca^{2+} ion in the regulation of pore stability, dwell time and overall pore opening kinetics.



References:

1. Fan et al., *Nat Commun* **2020**, *11*, 3618
2. Yongfei Cai *et al.*, *Science* **2020**, *369*, 1586-1592
3. Singh, P et al., *Cell Rep* **2022**, *39*, 110694

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Interplay of Excited State Lifetime and Redox Potentials for Intracellular Superoxide Anion Radical Generation

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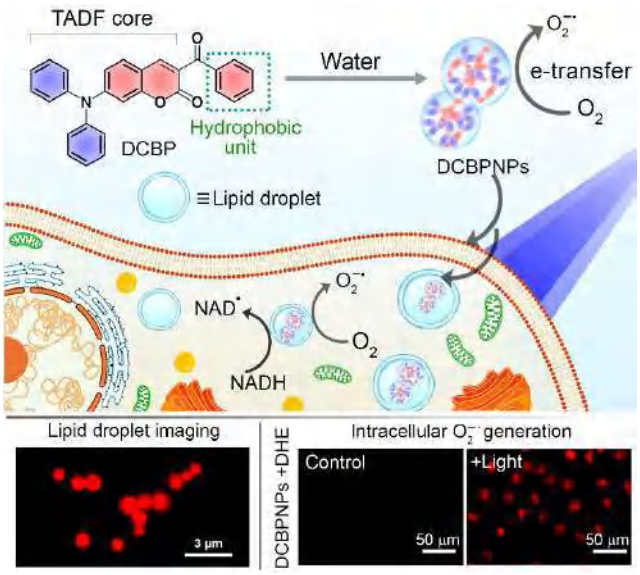
All-organic thermally activated delayed fluorescence (TADF) emitters with long-lived emission properties are emerging materials for time-resolved bioimaging and photodynamic therapy.[1-4] The triplet harvesting phenomenon of TADF materials is promising to generate intracellular reactive oxygen species (ROS) via either electron transfer (ET, Type-I) or energy transfer (EnT, Type-II) pathways for cancer cell ablation.[2,3] However, elucidating the design strategy to develop Type-I photosensitizer with tunable optical and biological properties remains a formidable challenge. Herein, employing keto-coumarin derivatives, ECBP and DCBP, we demonstrate that the presence of a long-lived excited state along with suitable excited state redox potential of the photosensitizer are the two key parameters resulting in the Type-I mode in organic delayed fluorescent materials.[5] Among the two derivatives, DCBP and its self-assembled nanoparticles (DCBPNPs) exhibit triplet harvesting properties and long-lived emission via thermally activated delayed fluorescence due to the presence of a small singlet-triplet energy gap and show promising scope for superoxide anion radical ($O_2^{-\cdot}$) generation (Scheme 1). Electrochemical and time-resolved spectroscopic studies reveal the facile electron transfer from the excited state as a driving force for the photoinduced electron transfer. DCBPNPs also showed electron transfer ability in the presence of biologically relevant analytes like NADH, which is favourable for generating oxidative stress in cells. Thereafter, the high quantum yield, photostability coupled with the inherent hydrophobicity of DCBPNPs, low dark toxicity and high phototoxicity enabled the successful imaging of lipid droplets and targeted generation of intracellular $O_2^{-\cdot}$ for cancer cell ablation. Thus, the present work presents a broad scope for the development of Type-I thermally activated delayed fluorescent photosensitizers in photodynamic therapy.



Scheme 4: Schematic illustration depicting the specific localization in lipid droplets and intracellular superoxide radical anion generation by self-assembled nanoparticles of DCBP up on light irradiation.

References:

1. F. Ni *et. al*. *Adv. Opt. Mater*. **2020**, *8*, 1902187.
2. T. C. Pham *et. al*. *Chem. Rev*. **2021**, *121*, 13454.
3. F. Fang *et. al*. *Adv. Sci*. **2021**, *8*, 2102970.
4. S. Das *et. al*. *Chem. Sci*. **2024**, *15*, 102.
5. S. Das *et. al*. (*manuscript under preparation*).

P 134

Breaking the Refractive Index Barrier: Imaging High Refractive Index Media with a Polarized TIRF Microscopic Approach

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Preferential alignment of nanostructures leads to various optoelectronic properties, which may differ significantly while probing near the interface in comparison to their bulk [1,2]. Total Internal Reflection Fluorescence Microscopy (TIRF) is a technique used to probe areas at interfaces using evanescent wave induced fluorescence, achieving surface selective excitation. [3]. However, the major difficulty arises when attempting to image objects with a very high refractive index. When the refractive index of the material is much higher than that of the substrate, total internal reflection cannot occur, and hence evanescent wave excitation is not possible. In our previous work, we used simulations to show that by introducing an intermediate thin layer with a low refractive index, we can achieve total internal reflection and generate an evanescent wave across the interface [4]. In this work, we prepared a multilayered surface consisting of an optically transparent, low refractive index thin film polymer sandwiched between the glass and the high refractive index $CsPbBr_3$ nanocrystals. These nanocrystals were excited with a 405 nm CW laser beam, and a structural similarity analysis of the widefield and TIRF images was also carried out to identify areas exhibiting significant changes in widefield mode and evanescent field excitation. In addition, excitation polarization was varied, and fluorescence anisotropic imaging was performed in widefield and TIRF modes to measure anisotropy at the interface.

References:

1. Fei Zheng et. al. *ACS Applied Materials & Interfaces* **2022**, *14*(46), 51910.
2. Heyou Zhang, et. al. *ACS nano* **2018**, 12(8) 7529.
3. Daniel Axelrod et. al. *Methods in cell biology* **1989**, *12*(8), 245.
4. Subhajit Chakraborty et. al. *Physica Scripta* **2022**, 98(1), 015014.

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Development of an ^{19}F MRI Probe for Redox Sensing

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Reactive oxygen species (ROS) are free radical entities produced majorly by mitochondria during cellular metabolism and include hydrogen peroxide (H_2O_2), hydroxyl ($HO^{\bullet}$) and hydroperoxy radicals ($HO_2^{\bullet}$), and singlet oxygen ($^1O_2$). ROS play several important roles in biological systems such as providing defence against pathogens, modulating cell signalling, and preservation of cellular homeostasis[1,2]. Overproduction of ROS contributes to oxidative stress which is associated with several pathophysiological conditions such as cancers, cardiovascular diseases, and neurodegenerative disorders[3]. Hence, tracking ROS in living systems can provide both mechanistic insights into pathophysiological conditions and afford diagnostic routes. Fluorescence-based ROS responsive probes have been reported previously but have limited applicability because of the low tissue penetration of fluorescence microscopy[4]. Magnetic Resonance Imaging (MRI) is a popular technique for clinical imaging. MRI is suitable for soft tissue imaging in living systems owing to its non-invasive nature and deep tissue penetration[4,5]. Favourable magnetic resonance properties of the ^{19}F nuclei (I=1/2 Same as ^1H) and low bioavailability of fluorine (< 10^{-6} M) lead to low background signals making it an apt choice for MRI [6]. Transition metal ions like Fe^{2+}, Co^{2+} are oxidized by cellular ROS. This property has been leveraged to develop ROS responsive ^{19}F-MRI probes where oxidation of a metal ion complex containing a fluorine atom leads to modulation of relaxation properties of the fluorine nuclei [3,4]. We have attempted the development of a novel macrocyclic mixed N, O-donor based fluorine substituted metal-binding ligand toward an ^{19}F-MRI based ROS probe. The details of the synthesis, characterization, and initial relaxation measurements will be presented.

References:

1. A Abdal Dayem et.al. *Int. J. Mol. Sci*. **2017**, 18, 120.
2. C Lennicke et.al. *Molecular Cell.* **2021**, 81, 3691.
3. M Yu et.al. *Chem. Commun*. **2016**, 52, 13885
4. R Kadakia et.al. *Chem. Sci.* **2023**, 14, 5099
5. M. L James et.al. *Physiol. Rev*. **2012**, 92, 897.
6. Da Xie et.al. *Acc. Chem. Res.* **2020**, 53, 2.
7. M Yu et.al. *Inorg. Chem*. **2012**, 51, 9153.

P 136

Ultrafast dynamics of nonrigid Ni porphyrin dimer

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Photosynthesis is one of the fundamental processes in nature that efficiently converts light energy to chemical energy. Chlorophylls are arranged in a dimeric structure, called special pair which initiates a multistep electron transfer reaction. Porphyrins have almost similar macrocyclic framework as chlorophylls. Therefore, much effort has been put into mimicking the dimer structure of the special pair.[1]

Here, the photophysical properties of ethane-bridged Nickel octa ethyl porphyrin dimer has been explored and compared with its monomer analogue. Initial photoexcitation is delocalized over porphyrin π ring. However, intramolecular vibrational relaxation is hampered by the presence of intermediate states involving metal d orbitals.[2] Excitonically coupled porphyrin dimer causes an ultrafast deactivation which limits the excitonic lifetime of the dimer.[3] Axial ligand binding and excitation wavelength play a role in vibrational relaxation pathways that give us a better understanding of excess energy dissipation of highly excited Nickel (II) porphyrins into surrounding solvent molecules.[4]

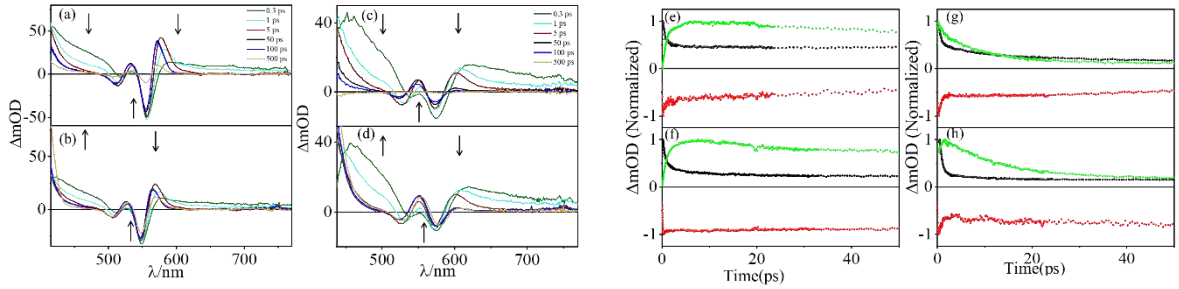

Fig 1. (a) and (b) TA spectra of Ni porphyrin monomer in chloroform and pyridine respectively. (c) and (d) TA spectra of Ni porphyrin dimer in chloroform and pyridine respectively with λ_{pump} = 400 nm. (e) and (f) Kinetics of GSB (red) at 550 nm, ESA (green, black) signal at 450 nm, 565 nm of monomer in chloroform and pyridine respectively. (g) and (h) Kinetics of GSB (red) at 575 nm, ESA (green, black) signal at 470 nm, 600 nm of dimer in chloroform and pyridine respectively.

References:

1. J. Baek et. al. *J. Phys. Chem. C* **2018**, *122*, *13285*
2. M. A. Naumova et. al. *J. Phys. Chem. Lett.* **2024**, *15*, 3627
3. L. Moretti et. al. *J. Phys. Chem. Lett.* **2020**, *11*, 3443
4. H. S. Eom et. al. *J. Phys. Chem A.* **1997**, *101*, 3661

P 137

Surface-enhanced Raman spectroscopy of atomically precise nanocluster

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Noble metal nanoclusters (NCs) are a class of nanomaterials that exhibit unique properties due to their ultra-small size (diameter <2.2 nm) and high surface-to-volume ratio[1]. In recent years, NCs have been shown to be promising candidates for various applications, including catalysis, sensing, and imaging. However, their molecular-like properties have not been fully explored. The molecular nature and atomic preciseness of nanoclusters have been unequivocally affirmed through a comprehensive suite of characterisation techniques, including UV-visible spectroscopy, photoluminescence spectroscopy, and mass spectrometry[2]. To delve further into their properties and unlock their full potential for real-life applications, additional sophisticated methods are imperative. Surface-enhanced Raman spectroscopy (SERS) emerges as a versatile and powerful tool for probing molecular vibrational levels within nanoclusters when perched atop nanoparticles. This symbiotic relationship between nanoclusters and nanoparticles not only enhances the SERS effect but also paves the way for a myriad of applications. Notably, SERS facilitates the observation of minute changes in nanoclusters even within harsh environments, offering unprecedented insights. The Raman features exhibited by clusters such as Cu_4(m-CBT)$_4$ and $[Ag_{17}(o\text{-CBT})_{12}]^{3-}$ are informative, rendering them ideal candidates as Raman reporters in SERS. The synergistic assemblies of anisotropic nanoparticles and nanoclusters, showcasing enhanced Raman capabilities, hold immense promise for sensor applications. The resulting assemblies exhibit remarkable Raman features, opening new avenues for sensor applications, particularly in the realm of biosensors. This research marks a significant stride towards harnessing the unique molecular precision of nanoclusters for practical, real-world implementations.

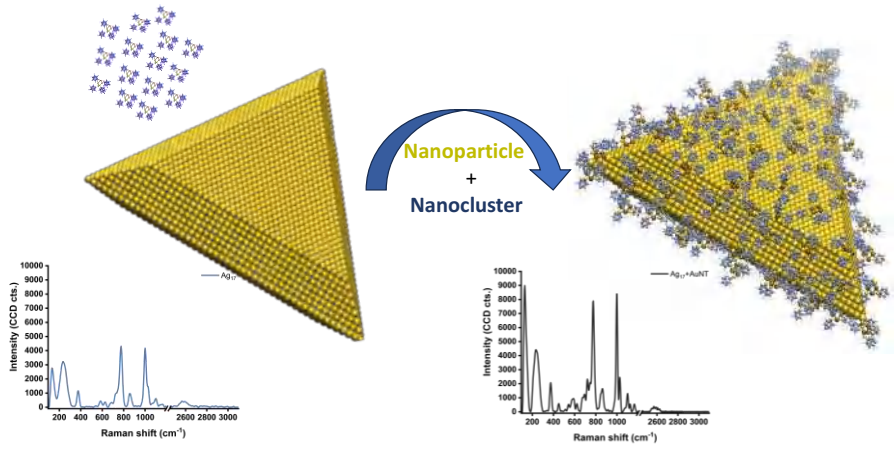

Figure. A representative diagram showing the process and generation of the SERS.

References:

1. Chakraborty, I. et al. *Chem. Rev.*, **2017**, *117*, 12, 8208.
2. Negishi, Y. Et al. J. Am. Chem. Soc., **2005**, *127*, 14, 5261.

P 138

Activity Based Alkyne Tagged Raman (ABATaR) Probes for Detection of Bio-analytes

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Metal ions are essential for biological function.[1] However, mis-regulation of metal ions can lead to severe pathophysiological consequences. The regulation of metal ions within living cells is closely linked to the redox state of a cell. Hence, elucidating the correlated regulation of metal ions and reactive oxygen species[2] can afford key insights into the redox balance within cells under both physiological and pathophysiological conditions. This necessitates multi-analyte imaging. Fluorescent molecular probes in conjunction with fluorescence microscopy have been widely been used to image bio-analytes. Despite providing very high spatio-temporal resolution, the fluorescence imaging platform suffers from signal overlap due to the inherently broad nature of fluorescence spectra of molecular probes which significantly restricts multiplexed imaging of bio-analytes. Raman microscopy on the other hand depends on molecular vibration and hence provides ~50-100[3] times narrower spectral width compared fluorescence which can allow facile multiplexing. In recent years, bio-orthogonal Raman tags like alkyne, nitrile, C/^{13}C-H/D have been developed to visualize bio-molecule localization and distribution inside living cells.[4] However, these efforts are mostly focused on Raman tags which are non-responsive. The development of responsive Raman probes will provide the key handle to multi-plexed imaging and tracking of bio-analytes. Toward this goal we have designed a generalized platform to develop "Activity based" alkyne tagged Raman (**ABATaR**) probes for detection of bio-molecules in a multiplexed approach. Here we have leveraged pH dependent changes in the protonation state of an alkyne tagged phenol to develop **ABATaR** probes for detection of pH, ROS and Cu^{2+} ions in a multimodal fluorescence-Raman imaging platform. In my poster, I will be presenting the design principle, synthesis, in-vitro, and in-cell studies of our novel **ABATaR** probes.

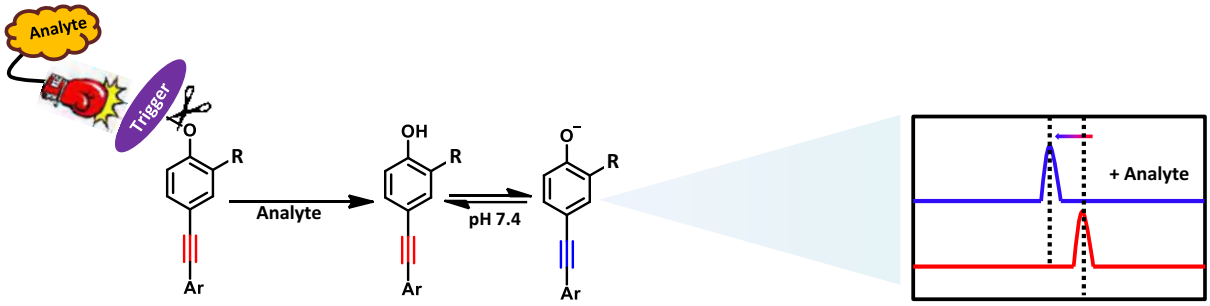

Figure 5: Scheme depicting the working principle of ABATaR .

References:

1. Emily L. Que et. al. *Chem. Rev.* **2008**, *108*, 1517.
2. Helmut Sies et. al. *Redox Biol.* **2017**, *11*, 613.
3. Hanqing Xiong et. al. *Light Sci Appl.* **2021**, 10.
4. Hiroyuki Yamakoshi et. al. *J. Am. Chem. Soc.* **2012**, *134*, 20681.

P 139

Position-Dependent Modulation of Intersystem Crossing in Iodo-Functionalized Salicylideneimine-Boron Compounds

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Intersystem crossing (ISC) is the key factor to generate the molecular triplets, which can be significantly enhanced by the introduction of heavy atoms due to their ability to strengthen spin-orbit coupling (SOC) [1]. However, the influence of the heavy atom's position within a molecule on ISC efficiency remains largely unexplored [2,3]. In this study, we address this gap by investigating the ISC process in three positional isomers of an iodine-substituted Salicylideneimine-Boron compounds: 3ISB, 4ISB, and 5ISB. Using a combination of steady-state and time-resolved absorption and fluorescence spectroscopy, we observed that ISC efficiency varies notably between the isomers. The 3ISB isomer exhibited the most efficient ISC and triplet formation, followed by moderate efficiency in 5ISB and the least efficiency in 4ISB. Quantum chemical calculations suggest that the iodine atom plays a critical role in the electronic transition process. In 3ISB and 5ISB, the HOMO to LUMO excitation effectively redistributes electron density from the iodine atom to other parts of the molecule, enhancing spin-orbit coupling. However, in 4ISB, the electron density remains largely localized on the iodine atom, which significantly reduces the overall spin-orbit coupling within the chromophore, leading to the least efficient ISC in this isomer. Additionally, our theoretical analysis suggests that SOC is not the only parameter governing the ISC process; C–X bond vibrations may also play a role in influencing ISC in such heavy-atom systems. These findings provide a deeper understanding of the positional dependency of ISC in heavy atom-modified molecules and offer valuable insights for the design of highly efficient triplet-state materials, which have broad applications in various fields [4] such as organic electronics and photodynamic therapy.

References:

1. M. A. El-Sayed *J. Chem. Phys.* **1965**, *43*, 2864.
2. Jack T. Ly et. al., *Phys. Chem. Chem. Phys.* **2021**, *23*, 12033.
3. A Das et. al. *J. Phys. Chem. A* **2022**, 126, *9*, 1475.
4. Xiao et. al. *Chem. Soc. Rev.*, **202**1, *50*, 9686.

P 140

Exploring Iridium (III) Complex Nanoparticles Photocytotoxicity Against Hypoxic Cancer

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Near-infrared (NIR) emitters Iridium (III) complexes are an important genre of photosensitizers (PSs) for photodynamic therapy (PDT). [1][2] The current research explores nanoparticles (NPs) encapsulating the NIR-emitting Iridium (III) complex. Initially, RM2 was found to show high singlet oxygen ($^1O_2$) generation efficiency (73% in the solution phase), making it promising for PDT. [3] During the in-vitro study, it was found to have low dark toxicity but significant cell toxicity once exposed to light. Mechanistically, light-irradiated RM2-treated cancer cells exhibited a substantial increase in the intracellular reactive oxygen species (ROS) level. Neutralizing ROS by Vitamin C (Vc) pretreatment inhibited the cytotoxic ability, demonstrating that ROS is an indispensable effector of cell cytotoxicity. After nanoformulations via soluplus micelles [4], RM2 showed good biocompatibility and a significant decrease in cell viability under light irradiation, even under low oxygen conditions with a half-maximal inhibitory concentration (IC_{50}) of 100 nM. This work emphasizes the importance of the Iridium (III) complex as an important class of PS for PDT against hypoxic cancer.

References:

1. Zhijun Zhang et. al. *Chemical Society Reviews* **2022**, *51*, 1983.
2. Shengnan Liu et. al. *Chemical Communications* **2024**, *60*, 9938.
3. Thanh Chung Pham et. al. *Chemical Reviews* **2021**, *121*,13454.
4. Sumit et. al. *ACS Applied Bio Materials* **2023**, *6*, 1445.

P 141

Competition between the hydrogen bond and the halogen bond in a [ROH-CCl_4] (R = CH_3 and C_2H_5) complexes

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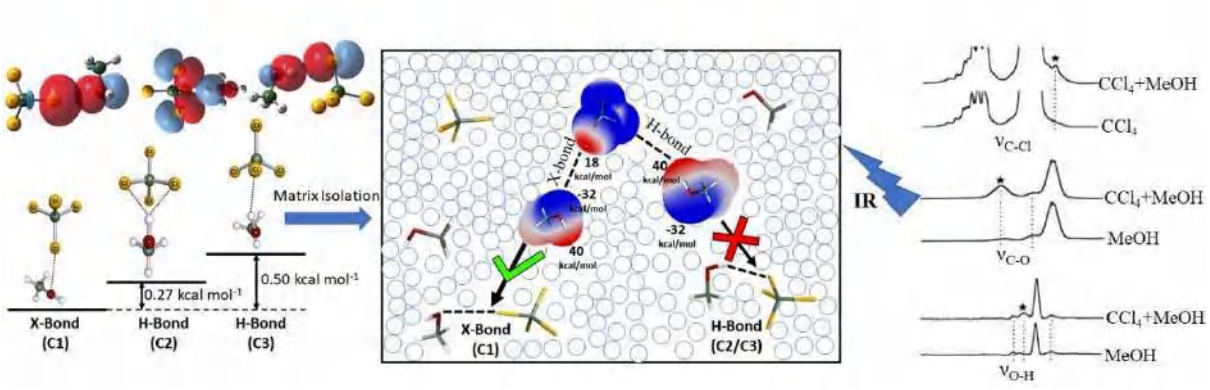
Methanol (CH_3OH) is the simplest alcohol, while carbon tetrachloride (CCl_4) is a widely used solvent in the chemical industry. Both CH_3OH and CCl_4 are significant volatile substances in the atmosphere, with CCl_4 playing a critical role as a precursor in atmospheric ozone depletion. Additionally, mixtures of CH_3OH and CCl_4 are notable as non-aqueous systems due to their significant deviations from Raoult's law [1]. At the molecular level, CH_3OH and CCl_4 can interact in two primary ways: through hydrogen bonding (O-H···Cl) and halogen bonding (C-Cl···O) [2]. The general impression of weakly bound clusters is revolved around H-Bond interaction [3].

We characterized the 1:1 [CH_3OH-CCl_4] complex using matrix-isolation infrared spectroscopy combined with electronic structure calculations. Vibrational spectra were recorded in the C-Cl, C-O, and O-H stretching regions. Our findings confirm the exclusive formation of halogen-bonded 1:1 complex in both argon and nitrogen matrices.

This study is extended to investigate the conformer-specific complexation between ethanol (C_2H_5OH) and CCl_4. Characterizing the [CCl_4-C_2H_5OH] complex is more challenging due to the presence of two conformers of C_2H_5OH, namely anti-C_2H_5OH (A-C_2H_5OH) and gauche-C_2H_5OH (G-C_2H_5OH), each capable of binding to CCl_4.



References:

1. Musso, Maurizio, et. al., *The Journal of Physical Chemistry A*, **2002**, *106(43)*, 10152.
2. Torii, Hajime, *Chemical physics letters*, **2004**, *393(1-3)*, 153.
3. Arunan, Elangannan, et. al., *Pure and applied chemistry*, **2011**, *83(8)*, 1637.

P 142

Achieving High Temporal Resolution in Single-Molecule Fluorescence Techniques Using Plasmonic Nanoantennas

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Single-molecule fluorescence techniques are vital for studying molecular mechanisms in biological processes, but achieving sub-millisecond temporal resolution for fast molecular dynamics remains challenging. Fluorescence brightness, which determines temporal resolution, is limited in conventional microscopes and standard fluorescent emitters. Plasmonic nanoantennas have been proposed to address this, but even with significant fluorescence enhancement, brightness typically stays below 1 million photons/s/molecule, limiting temporal resolution improvements. Here we present a method to enhance temporal resolution using plasmonic nanoantennas, specifically optical horn antennas[1-3]. The study achieves around 90% light collection efficiency and a fluorescence brightness of 2 million photons/s/molecule in the saturation regime, enabling single-molecule observations with microsecond binning times and rapid fluorescence correlation spectroscopy. This work extends the application of plasmonic antennas and zero-mode waveguides in the fluorescence saturation regime, leading to brighter signals, faster temporal resolutions, and improved detection rates, advancing fluorescence sensing, DNA sequencing, and dynamic molecular studies.

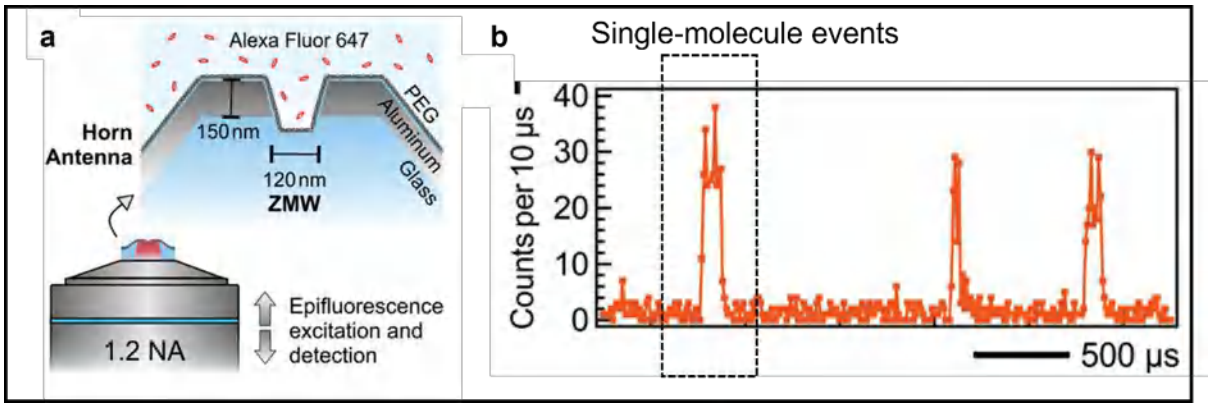

Figure 1: Optical horn antennas to achieve 90% fluorescence collection efficiency from single emitters. a) Scheme of the experimental configuration. b) Single molecules events at a fast-binning rate.

References:

1. Tiwari S, et. al., *Advanced Optical Materials*, **2023**, *11*(13), 2300168.
2. Barulin A, et. al., *Nature Communications*, **2022**, *13*(1), 1842.
3. Roy P et. al., *Nano Letters*, **2023**, *23*(2), 497.

P 143

Mechanistic Insights into the c-MYC G-Quadruplex and Berberine Binding inside an Aqueous Two-Phase System Mimicking Biomolecular Condensates

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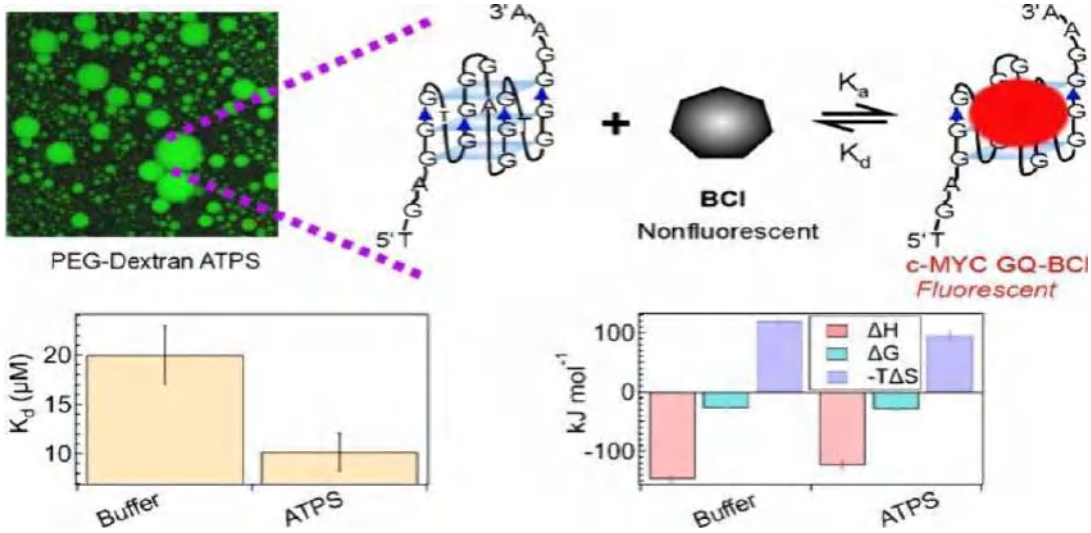
We investigated the binding between the c-MYC G-quadruplex (GQ) and berberine chloride (BCl) in an aqueous two-phase system (ATPS) with 12.3 wt % polyethylene glycol and 5.6wt % dextran, mimicking the highly crowded intracellular biomolecular condensates formed via liquid−liquid phase separation. We found that in the ATPS, complex formation is significantly altered, leading to an increase in affinity and a change in the stoichiometry of the complex with respect to neat buffer conditions. Thermodynamic studies reveal that binding becomes more thermodynamically favorable in the ATPS due to entropic effects, as the strong excluded volume effect inside ATPS droplets reduces the entropic penalty associated with binding. Finally, the binding affinity of BCl for the c-MYC GQ is higher than those for other DNA structures, indicating potential specific interactions. Overall, these findings will be helpful in the design of potential drugs targeting the c-MYC GQ structures in cancer-related biocondensates. [1]



References:

(1) S Pradhan et. al., *Journal of Physical Chemistry Letters*, **2024**, *15*, 8706.

P 144

Single-Molecule Detection of a Terrylenediimide-Based Near-Infrared Emitter

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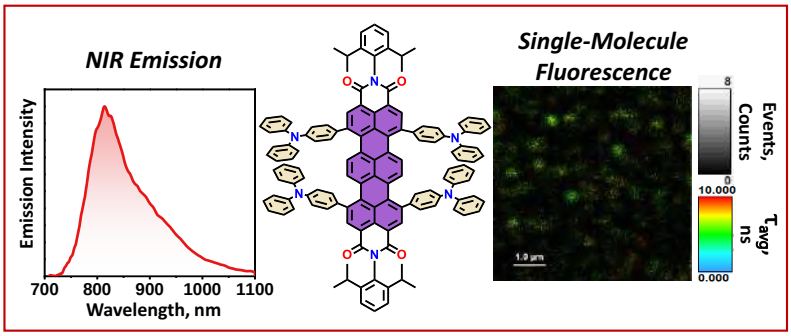
Near-infrared single-photon emitters are an emerging class of compounds for quantum information science, including computing, sensing, and communication.[1, 2] Single-molecule level scrutiny of organic near-infrared (NIR) emitting molecules has recently gained much attention.[3] The efficiency of the NIR organic emitters is limited by the energy gap law; the intrinsic forbidden nature of emission from states with low energy gap results in negligible fluorescence quantum yield.[4] The donor-acceptor systems with charge transfer (CT) character are better alternatives to circumvent the limitations possessed by conventional π-conjugated NIR molecules by narrowing the energy gap for NIR emission.[5] We report a novel NIR absorbing (λ_{max}^{Abs} = 735 nm) and emitting (λ_{max}^{Fl}= 814 nm) terrylenediimide (TDI) based donor-acceptor chromophore (TDI-TPA$_4$), exhibiting polarity-sensitive single-photon emission. By virtue of the charge transfer (CT) character, ensemble-level measurements revealed solvatochromism and NIR emission (ϕ_{Fl}= 26.2%), overcoming the energy gap law. To mimic the polarity conditions at the single-molecule level, TDI-TPA$_4$ was immobilized in polystyrene (PS; low polar) and poly(vinyl alcohol) (PVA; high polar) matrices, which enables tuning of the energy levels of the locally excited state and charge-separated (CS) state. Minimal blinking and prolonged survival time of the TDI-TPA$_4$ molecule in the PS matrix, in contrast to the PVA matrix, possibly confirms the implication of the energy gap law and polarity sensitivity of TDI-TPA$_4$. The existence of the CT state in nonpolar and CS state in polar solvents was confirmed by transient absorption measurements in the femtosecond regime. The current work sheds light on the design principle for NIR single-photon emitting organic chromophores for deep tissue imaging and probing the nanoscale heterogeneity.



References:

1. M. R. Wasielewski et. al. *Nat. Rev. Chem.*, **2020**, 4, 490.
2. F. Fuchs et. al. *Nat. Commun.*, **2015**, 6, 7578.
3. M. Musavinezhad et. al. *J. Phys. Chem. B*, **2023**, 127, 5353.
4. S. J. Jang et. al. *J. Chem. Phys.*, **2021**, 155, 164106.
5. Y.C. Wei et. al. *Nat. Photonics*, **2020**, 14, 570.

P 145

Strategic design of tryptophan-aspartic acid containing peptide inhibitors using coronin 1 as a template: Inhibition of fusion by modulating membrane organisation and dynamics

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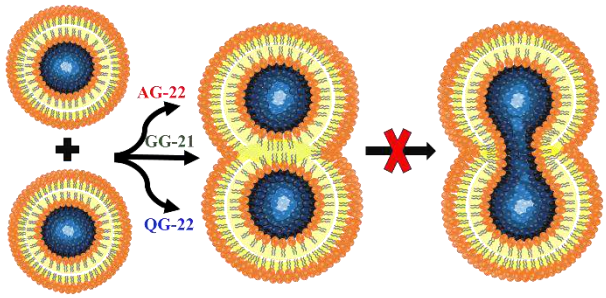
Enveloped viruses enter the host cell by fusing at the cell membrane or by entering the cell by endocytosis and fusing at the endosome. Conventional inhibitors target the viral fusion protein to inactivate it for inducing fusion. These target-specific vis-à-vis virus-specific inhibitors fail to display their inhibitory efficacy against emerging and remerging viral infections. This necessitates the need to develop broad-spectrum entry inhibitors that will be effective against a broad spectrum of viruses. The proposed fusion inhibitors modify the physical characteristics of the viral membrane in such a way that the membrane would be less susceptible to fusion. Earlier it was shown that TG-23, a tryptophan-aspartate-containing peptide from coronin 1, a phagosomal protein, demonstrated inhibition of fusion between small unilamellar vesicles (SUVs) by modulating the membrane physical properties. However, its inhibitory efficacy reduces with increasing concentration of membrane cholesterol. The present work aims to develop fusion inhibitors whose efficacy would be unaltered in the presence of membrane cholesterol. We have designed a tryptophan-aspartic acid (WD)-containing peptide, GG-21, having similar hydrophobic profile to that of TG-23. Interestingly, GG-21 displays inhibitory efficacy by modulating membrane organization and dynamics in a wide variety of lipid compositions despite having a similar secondary structure and physical properties to TG-23. These results advocate that the secondary structure and physical properties of the peptide may not be sufficient to predict its inhibitory efficacy. We have further designed two WD-containing hydrophilic peptides, QG-22 and AG-22, from coronin 1, and evaluated their fusion inhibitory efficacies in the absence and presence of membrane cholesterol. Our results demonstrate that QG-22 and AG-22 inhibit membrane fusion irrespective of the concentration of membrane cholesterol. Our measurements of depth-dependent membrane organization and dynamics reveal that they impede fusion by enhancing acyl chain order. Overall, our results validate the hypothesis of designing fusion inhibitors by modulating the membrane's physical properties.



References:

1. Pandia S., et. al. *J. Phys. Chem. B.* **2024**, *128*, 4986.
2. Pandia S., et. al. *Eur Phys J Spec Top*, 2024.
3. Chakraborty, H., et. al. *Biophys. J.* **2012**, *102*, 2751.
4. Pattnaik, G. P., et. al. *Biochim. Biophys. Acta Biomembr*. **2019**, *1861*, 183056.

P 146

Supramolecular Peptide Hydrogels with Diverse Functionalities for Directing Cellular Behavior

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Supramolecular hydrogels are emerging as advanced functional materials fabricated via classical molecular self-assembly strategy. Particularly, peptide-based hydrogels received exclusive attention due to their inherent biocompatibility and biodegradability. The well-defined fibrillar structures of these hydrogels provide an excellent three-dimensional environment for cellular growth and proliferation. In our work, we attempted to design suitable peptide scaffolds to effectively mimic the structural and functional aspects of the native extracellular matrix (ECM). Herein, we have employed a co-assembly approach to fabricate composite nanostructures mimicking the ECM by using pathway-dependent self-assembly. Our study includes co-assembly of different ratios of a gelator and a surfactant to fabricate diverse hydrogels. The gelation was triggered by utilizing distinct self-assembling pathways that resulted in differential fiber morphology as well as variable stiffness of the fabricated hydrogels which further proved the diversity of the hydrogels. Additionally, the secondary structures of the hydrogels were characterized by using CD, fluorescence, and FTIR spectroscopy. The designed hydrogels demonstrated enhanced cellular proliferation as assessed by confocal microscopy. Thus, these diverse supramolecular structures play a major role in governing cellular behavior in the co-assembled system. Such observation led us to further envisage that the differential self-assembly pathways would be a superior strategy and could eliminate the synthetic challenges associated with developing new materials. In this direction, we further explored metal-ligand coordination by employing cations of variable valency to trigger gelation within a pentapeptide derived from one of the major ECM proteins, i.e., N-cadherin, which mediates cell-cell adhesion, migration, and differentiation. Interestingly, the induction of hydrogels via the incorporation of cations at different concentrations followed the Hofmeister series. This was further confirmed by CD and fluorescence spectroscopy. The hydrogels showed tuneable mechanical stiffness which governed their cellular behavior. These highly tunable multi-component hydrogels attained from diverse designing approaches can be crucial in expanding their potential in various biomedical applications.

References:

1. S. Mohanty et. al. *Biomacromolecules* **2024**, *25*, 3271.
2. H. Kaur et. al. *Appl. Mater. Interfaces* **2020**, *12*, 52445.
3. H. Kaur et. al. *J. Mater. Chem. B* **2021**, *9*, 5898.
4. V.K. Pal et. al. *Macromol. Biosci.* **2022**, *22*, 2100462.

P 147

Role of Associated Water Dynamics on Protein Stability and Activity in Crowded Milieu

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Macromolecular crowding bridges *in vivo* and *in vitro* studies by simulating cellular complexities such as high viscosity and limited space while maintaining the experimental feasibility. Over the last two decades, the impact of macromolecular crowding on protein stability and activity has been a significant topic of study and discussion, though still lacking a thorough mechanistic understanding. This article investigates the role of associated water dynamics on protein stability and activity within crowded environments, using bromelain and Ficoll-70 as the model systems. Traditional crowding theory primarily attributes protein stability to entropic effects (excluded volume) and enthalpic interactions. However, our recent findings suggest that associated water structure modulation plays a crucial role in a crowded environment. In this report, we strengthen the conclusion of our previous study, i.e., rigid-associated water stabilizes proteins via entropy and destabilizes them via enthalpy, while flexible water has the opposite effect.[1] In the process, we addressed previous shortcomings with a systematic concentration-dependent study using a single-domain protein and component analysis of solvation dynamics. More importantly, we analyze bromelain's hydrolytic activity using the Michaelis–Menten model to understand kinetic parameters like maximum velocity (V_{max}) achieved by the system and the Michaelis–Menten coefficient (K_M).[2] Results indicate that microviscosity (not the bulk viscosity) controls the enzyme–substrate (*ES*) complex formation, where an increase in the microviscosity makes the ES complex formation less favorable. On the other hand, flexible associated water dynamics were found to favor the rate of product formation significantly from the ES complex, while rigid associated water hinders it.[2] This study improves our understanding of protein stability and activity in crowded environments, highlighting the critical role of associated water dynamics.

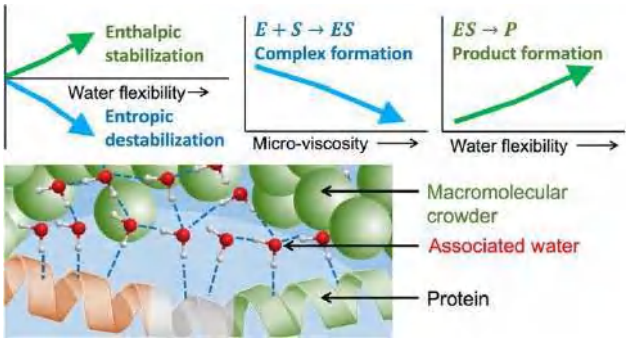

Figure 1: A flexible associated water stabilizes protein by enthalpy and destabilizes through entropy, whereas rigid water has the opposite effect. Micro-viscosity of the medium controls the formation of the enzyme-substrate complex; however, the modulation of associated water controls the product formation from the enzyme-substrate complex, where the flexibility of associated water favors the process.

References:

1. Das et al. *J. Phys. Chem. B* **2023,** *127***,** 3151.
2. Khan et al. *J. Phys. Chem. B* **2024**, *Just published*.

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Mg doping induced modulation of ultrafast exciton dynamics in $FAPbBr_3$ nanocrystals

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Introducing dopants into perovskite nanocrystals has proven to be an effective method for modifying their optical and electronic properties for advanced optoelectronic applications. [1] This study explores the synthesis, characterization, and photophysical properties of magnesium (Mg)-doped $FAPbBr_3$ perovskite nanocrystals. By embedding Mg ions into the $FAPbBr_3$ lattice, we aim to boost photoluminescence efficiency through radiative decay engineering. Morphological analysis via PXRD, XPS, and FETEM reveals that doping causes lattice contraction (from 12 nm to 10 nm), resulting in increased confinement. Mg doping (4.8%) enhances photoluminescence intensity and quantum yield (increasing from 60±3% to 75±3%), along with a higher radiative recombination rate (2.35 times that of undoped nanocrystals). In femtosecond transient absorption experiments, excitation at a wavelength (λ_{ex} = 400 nm) much higher than the nanocrystals' band gap (520 nm) generates hot excitons. [2] The hot-carrier cooling time in undoped nanocrystals is 1094 ± 45 fs, while in the doped system, it is significantly faster (710 ± 26 fs) due to the enhanced coupling of conduction band states with the dopant states (Figure 1). The reduction of trap states and increased trapping-detrapping rate with doping contribute to a faster ground-state bleaching (GSB) recovery. [3] These results offer new insights into doping strategies for perovskite nanocrystals and pave the way for developing high-performance optoelectronic materials.

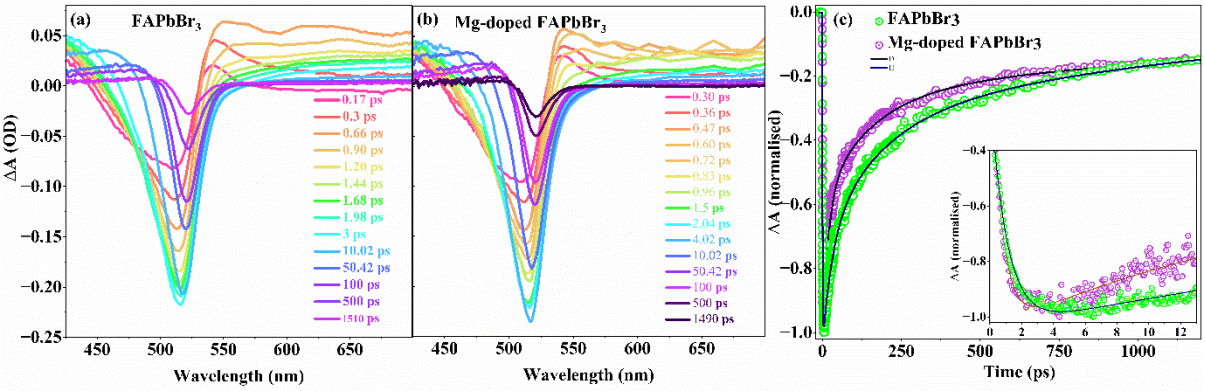

Figure 1. TAS of (a) $FAPbBr_3$ and (b) Mg-doped $FAPbBr_3$. (c) Comparison of GSB recovery kinetics of doped and undoped $FAPbBr_3$, inset depicts the zoomed shorter timescale.

References

1. C. H. A. Li et al. *Chem. Mater.*, **2019**, *31*, 6003.
2. P. K. Singha et al. *J. Chem. Phys.*, **2024**, *161*.
3. S. Das et al. *J. Phys. Chem. Lett.*, **2020**, *11*, 1178.

P 149

An overview of the measurement of elastic moduli of RBCs using microfluidics

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Measurements of the mechanical properties of red blood cells (RBC) are very crucial because changes in these properties can be closely associated with diseases. RBCs are very deformable compared to most other cells in the human body, with their Young's modulus ranging between 2 and 8 kPa [1] [2]. When RBCs become rigid, they can block blood flow in capillaries, which may result in excessive destruction of these cells and this may result in the failure of organs. RBCs lose their flexibility after extended storage [3]. Changes in RBC deformability can be indicative of various diseases, such as sickle cell disease [4], peripheral vascular disease [5], diabetes [6], and malaria [7]. Consequently, measuring the elastic properties of RBCs could be a useful diagnostic tool for detecting and understanding these conditions.

There are several conventional methods by which the deformability of RBC populations can be measured at the level of single cells, such as micropipette aspirations [8], atomic force microscopy (AFM) [9], membrane fluctuations [10], optical tweezers [11], optical stretcher [12], and magnetic bead-based rheology [13], etc. These traditional methods require expensive, specialized equipment and are often limited by low throughput and time-consuming data analysis. To overcome these challenges, researchers have developed microfluidic techniques that improve throughput by utilizing either physical constriction [14] [15] or the hydrodynamic shear stress from a channel [16], cross-sections [17], or T-junctions [18]. For evaluating RBC deformability, typically one monitors real-time shape changes as RBCs navigate constrictions, rather than directly measuring elastic constants. These methods often require high-speed cameras (2,000–4,000 fps) to capture rapid deformations or measure the pressure required for passing through narrow constrictions [19]. Zhu and others [20] developed a numerical approach for microfluidic devices inspired by Rutherford scattering, utilizing a semi-cylindrical obstacle in a funnel to sort spherical capsules based on deformability. Later another group [21] validated this approach, showing its effectiveness in sorting artificial vesicles by size and deformability and introducing a critical capillary number for sorting regimes.

A recent study from our research group [2] modified the design reported by Zhu and others to obtain Young's modulus of healthy and artificially stiffened RBCs. This device has a single channel that opens into a funnel. There is a semi-circular obstacle where the funnel begins. As RBCs move past this obstacle, stiffer RBCs deviate more from their original path compared to softer RBCs. As a result, one needs to monitor only the path taken by RBCs, and not their shape change, which is done using a regular microscope camera at 25–30 fps. The authors also generate a one-time calibration curve by correlating the deflection trajectories of RBCs with Young's modulus values obtained from independent AFM measurements. This calibration curve allows obtaining Young's modulus of unknown RBC populations from only microfluidics measurements.

References:

1.Fay, M. E. et.al. 2016, Proc Natl Acad Sci USA 113, 1987–1992
2.Kumari, S. et.al. 2024, Cell Reports Physical Science 5, 102052
3.Park, H. et.al. 2016, Sci. Rep. 6, 34257
4.Barabino, G. A. et.al., 2010, Annu. Rev. Biomed. Eng. 12, 345–367
5.Reid, H. L. et.al., 1976, Lancet 1, 666–668
6.Tsukada, K. et.al., 2001, Microvasc. Res. 61, 231–239
7.Park, Y. et.al., 2008, Proc Natl Acad Sci USA 105, 13730–13735

P 150

Impact of post-translational modifications on LLPS in intrinsically disordered proteins

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Membraneless organelles like stress granules, p bodies, have drawn the interest of scientists in recent times. These membraneless organelles are referred to as biomolecular condensates which are mostly formed via liquid-liquid phase separation of proteins and RNAs. Liquid-liquid phase separation (Llps) plays a vital role cellular functioning like sequestration, signalling, cellular interactions. In addition to their contribution in the normal functioning of cells, they are also associated with various pathological conditions like Alzheimer's, Parkinson's Disease.

In previous studies, it has been seen that neurodegenerative diseases are the outcome of protein aggregation. But in recent studies it has been determined that they pass through Liquid-liquid phase separated state prior to aggregation. There are several factors that aggravates protein aggregation like post translational modifications. Even though they are considered to be important, their impact on phase separation remains elusive. Alpha-synuclein plays an important role in Parkinson's disease and it has been observed that they do undergo PTMs like sumoylation, phosphorylation which can either enhance [1] or inhibit their aggregation. In addition to this, alpha-synuclein has sumoylation sites and it is found in modified form in aggregates. Moreover, sumoylation targets proteins towards nucleus thereby enhancing its nuclear accumulation [2]. Although alpha-synuclein is present at the nerve terminals, biochemical analysis has identified it in the nucleus of the neurons. Intriguingly, both alpha synuclein [3] and SUMO1[4] exhibit Llps under different conditions but the nature of their interaction is unknown. Thus, the main aim of the study is to determine the effect of PTMs on alpha-synuclein, which is the major aggregating protein in Parkinson's Disease.

References:

1. Abeywardana et.al *Biochemistry* **2015**, 54, 959.
2. Pinho et. al *Hum. Mol. Genet***. 2019**, 28, 31.
3. Ray, S. et al. *Nat. Chem***. 2020,** 12, 705.
4. Arora et. al *J. Phys. Chem. Lett*. **2023**, 14, 9060.

P 151

Understanding mechano-regulation by myomerger in cell -cell fusion

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Myomerger is a small membrane protein – a fusogen known to impart positive curvature to the outer leaflet of lipid bilayers of liposomes[1]. It is crucial for cell-cell fusion during myogenesis[2] but there is little direct evidence of mechano-regulatory mechanisms processes[3] operational in cells. Local curvature induction or other membrane mechano-regulation by myomerger remain poorly explored for differentiating myoblasts. Although myomerger is believed to stress the plasma membrane post hemi-fusion, for fusion pore formation, recent work from the lab has shown that its surface levels is correlated to the surface tension even at early timepoints such that when the surface tension decreases as myomerger surface levels rise[4]. Here, we first present data to demonstrate how myomerger not only correlated but directly affected the cell surface tension. Further, imaging relative height of the basal membrane and myomerger clustering in same cells revealed that the shallow membrane remodelling by myomerger clusters are mostly exvaginating bulges. Other agents that similarly affect the membrane curvature were tested to understand if curvature remodelling affects surface tension. Connecting the mechanical changes imparted by myomerger to cell-cell fusion, we report that as the probability of hemi-fusion increases, a clear tension difference develops between the hemi-fused pair of cells underscoring how myomerger's direct impact of surface tension can tune fusion probability.

Keywords: Myomerger, Myogenesis, hemi-fusion,mechano-regulation, surface tension.

References:

1. Golani G, Leikina E, Melikov K, et. al. *Nat Commun.* **2021**, *12(1*
2. Quinn ME, Goh Q, Kurosaka M, et. al. *Nat. Commun*. **2017**, 8, 15665
3. Mukhopadhyay U, Mandal T, Chakraborty M, Sinha B. *ACS Omega*. **2024**, *9(20),* 21780.
4. Chakraborty M, Sivan A, Biswas A, Sinha B. *Front Physiol*. **2022**, *13*

P 152

Generation of autonomous spinning and gyrating spherical micro-vesicles

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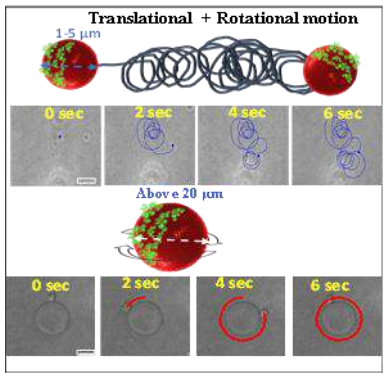
Looking into chiral active particles and rotors is an intriguing and quickly expanding field. Active particles have the potential to be useful tools in the treatment of complex in vivo issues like tissue absorption, wound healing, drug delivery, and cell sensing. In addition to eliminating drawbacks like systemic toxicity and reliance on passive diffusion for transport, active motion delivers cargo more quickly than passive diffusion. Due to the field's rapid development, an increasing number of review articles focusing on the design and construction of different active micro/nano rotors are being published. "Micro/nanomotors" (Gao and Wang, 2014), "micro/nano swimmers" (T. Li et al., 2016), "micro/nano engines" (Sanchez et al., 2011), "micro/nanomachines" (Wang and Putera, 2018), "micro/nano propellers" (Ghost and Fischer, 2009), "micro/nano pumps" (Wong et al., 2016), "micro/nano rockets" (J. Li et al., 2016a), etc. are some of the terms used in the literature to describe these small-scale rotors. In order to power these, scientists either use Janus particles as engines catalyzed by fuels like H_2O_2 or they use symmetry breaking of the chassis material, such as by creating star- or dumble-shaped rotors. However, finding practical propulsion methods for these micro/nanorobotic devices that can be used for biomedical applications is still very difficult. Their primary constraints are their size, poor biocompatibility of the fuels for active motion and the materials used for fabrication, and their mobility in biological fluids. Enzymes are excellent candidates for use as catalysts in biomedical applications because of their high turnover numbers, excellent selectivity in physiological settings, and biocompatibility. Additionally, research revealed that every functioning enzyme can produce enough mechanical force to move itself during catalysis. Here, we create chiral active particles with isotropic symmetry that are coated with enzymes. For the chassis of these active particles, we employed lipid vesicles. Internal energy is created through the transformation of chemical energy. Translating, rotating, or both, these active particles can propel themselves by using their internal energy. Vesicles ranging in size from 1 to 5 µm exhibit both translational and rotational motion, while those larger than 20 µm primarily manifest spinning behaviour.



Keywords: 1; Chiral active particles 2; autonomous rotors 3; Lipid vesicles

Fig. 1 Active enzyme coated GUVs. Upper panel shows the translation and rotational motion of 2 µm sized vesicles. Lower panel showing spinning of 20 µm sized vesicles after catalyzing enzymatic reaction (scale bar: 10µm)

P 153

Understanding stem cell differentiation into neurons using fluorescence microscopy

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Neural differentiation from mouse embryonic stem cells is an excellent model to elucidate key mechanisms in neurogenesis, study disease mechanisms, and screen new drug compounds. For the differentiation of mouse embryonic stem cells, first, the hanging drop method is used for the formation of embryoid bodies; followed by differentiation into neurons using the neuronal differentiation media. The metabolic changes during the differentiation of stem cells into neurons are investigated by measuring the autofluorescence of coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). The ratio of FAD/ NADH provides insight into the metabolic state of a cell and is referred to as the redox ratio. The fluorescence images of NADH and FAD are acquired before induction of differentiation and on the subsequent days using confocal microscopy and two-photon microscopy.

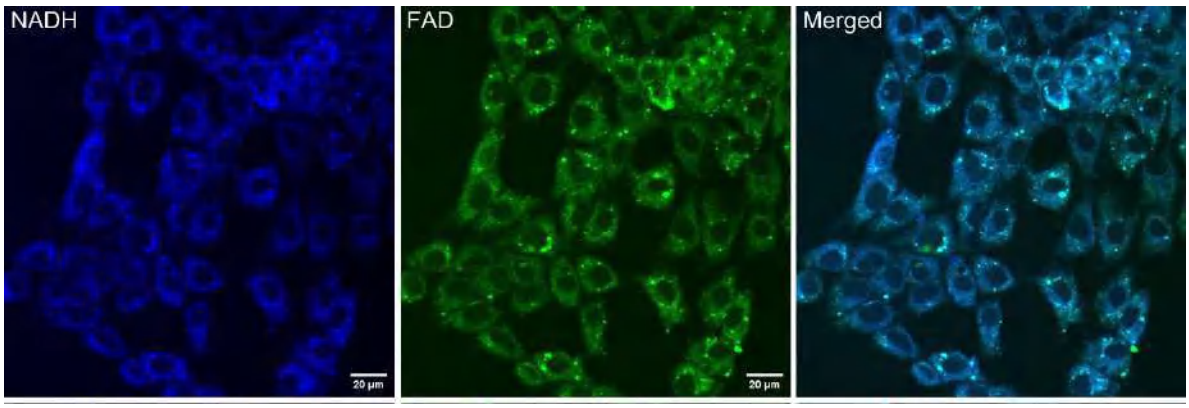

Fig 1: NADH, FAD fluorescence images captured using two photon microscopy in mouse embryonic stem cells.

References:

1. O. I. Kolenc et al. Antioxidants & Redox Signaling, 2019, 30(6):875-889.
2. R. J. Burgess et al., J Intern Med, 2014, 276(1),12–24.

P 154

Detecting labile heme and ferroptosis through 'turn-on' fluorescence and lipid droplet localization post Fe(II) sensing

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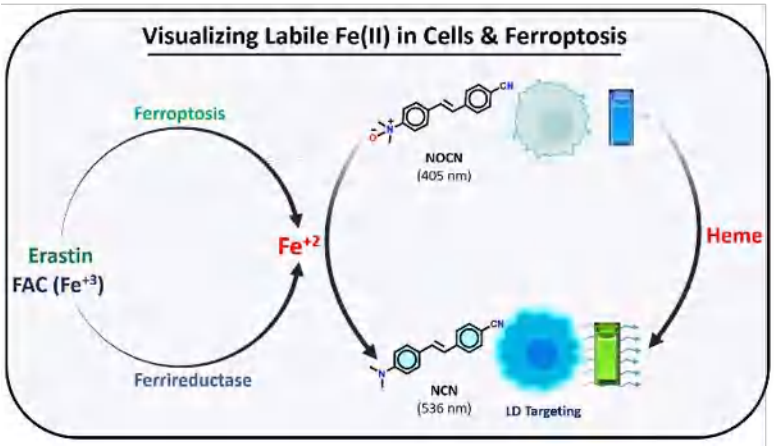
Iron, a crucial biologically active ion, is essential for metabolic processes in living organisms and plays a vital role in biological functions. The World Health Organization (WHO) has set the limit for iron in drinking water at 5.3 µM. However, many groundwater sources are contaminated with Fe(II), and imbalances in iron levels can lead to various diseases. In this study, We have designed two easily accessible, water soluble *N*-oxide based probes (NOPy and NOCN) for the selective detection of Fe(II) in presence of other interfering ion, with clearcolour demarcation. Both the probes exhibit large stoke shifts with an enhancement in fluorescence intensity post Fe(II) detection, with NOCN localizing inside lipid droplets in live*COS-7* cells. NOPy and NOCN are biocompatible and can detect Fe(II) at concentration as lowas 35nM and 42nM respectively. Additionally, NOCN is capable of detecting labile heme inside live cells, generated by aminolevulinic acid and ferric ammonium citrate. NOCN can also be used to visualize ferroptosis process, which is an iron-based apoptosis utilized in cancertherapy.



Keywords: Photophysical Chemistry, Turn-on emission, Bioimaging in live cells, Analyte Detection, Labile Heme, Ferroptosis

Reference:

1. Yogesh Dubey et. al., *Journal of Materials Chemistry B*, **2024**, *12*(20), 4962-4974.
2. T. Hirayama et. al., *Journal of clinical biochemistry and nutrition*, **2017**, *60*(1), 39-48
3. Yogesh Dubey et.al., *Organic and Biomolecular Chemistry*, **2024.**

P 155

Red-edge effects in proteins rich in charged amino acids: A Computational Study

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Protein Charge Transfer Spectra (ProCharTS) is a new and unexplored label-free UV-Vis spectral window suitable for probing proteins rich in charged amino acid content. A convolution of charge transfer transitions within charged amino acid clusters contributes to the ProCharTS spectral window. Previously, our group has proposed a computational framework to investigate the dynamics and optical properties of charged amino acid chromophores within the alpha-3c protein. The studies employed classical atomistic molecular dynamics (MD) simulations to extract the active chromophores possibly contributing to the optical charge transfer transitions. Time-dependent density functional theory was employed to analyse the spectra of a statistical ensemble of charged amino acid chromophores to construct the ProCharTS profile of the alpha-3c protein. [1]

The heterogeneities in charged amino acid clusters lead to red-edge effects. Here, I present a mathematical framework to model REES in multichromophoric charged amino acid clusters considering a two-state model.

References

1. Mandal, I., Manna, S., & Venkatramani, R. *Journal of Physical Chemistry B* **2019** 123(51), 10967–10979.
2. J. Doe, F-Q. Chen and B. White, *J. Chem. Phys.* **2026**, *193*, 34924.

P 156

Elucidating the Role of Chlorophyll-*a* in Cytochrome b_6f Complex

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Cytochrome b_6f (cyt b_6f) complex plays a key role in the electron transfer process from photosystem II (PSII) to photosystem I (PSI) by catalyzing the oxidation of plastoquinol and reduction of plastocyanin or cytochrome c_6 in oxygenic photosynthetic systems.[1,2] Electron transfer through cyt b_6f is coupled to proton transfer, this contributes to the transmembrane proton gradient (ΔpH) utilised for ATP synthesis and photosynthetic regulation. A curious feature of the cyt b_6f complex is the presence of a chlorophyll (chl) *a* molecule which does not directly participate in electron or proton transfer and is of unknown function. Chl molecules are potentially dangerous since they can transfer excitation energy from their excited triplet state to the ground state oxygen, forming singlet oxygen a potent reactive oxygen species which can damage the complex. It has been proposed that the local protein structure may act to quench the singlet excited state lifetime of the chl molecule,[3] although the exact mechanism is not clear. Herein using femtosecond transient absorption spectroscopy, we probed a mutant of cyt b_6f complex (L108W) in the cyanobacterium synechocystis and compared to its wild type. Our primary observations suggest that replacing a leucine, at 4.2 Å distance to the chl, with an aromatic amino acid (tryptophan), quenches the singlet excited state lifetime of the chl molecule from 160 ps to 90 ps. We will discuss our results in the context of the effect of local environment on the chl lifetime in cyt b_6f complex.

References:

1. W. A. Cramer et. al. Annu. rev. plant physiol. plant mol. Boil, **1996**, 47, 477.
2. L. A. Malone et. al. *BBA-Bioenergetics,* **2021**, 1862, 148380.
3. Y. Jiusheng et. al. *Biochem,* **2008**, 47, 3654.

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P 157

Effect of system-bath interaction to control spin-vibronic coupling in intersystem Crossing reaction of a di-platinum complex

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Insights into the fundamental mechanism of intersystem crossing lie at the heart of strategizing design principles to control structure-function paradigm in vitro. In this work, using transient absorption spectroscopic measurements, we have experimentally shown how crystal packing and ligand sphere affect the rate of an intersystem crossing reaction by changing the strength of spin-vibronic coupling in a di-platinum complex. We have proposed a model to describe our observation where an intermediate triplet plays a crucial role to modulate the strength of intersystem coupling via modulation the strength of spin-vibronic coupling in this complex. We found that depending on whether the energy of the intermediate state is tuned in and out of resonance with the singlet state, spin-vibronic coupling and thus the rate of intersystem crossing is strongly affected.

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Conformational Heterogeneity in Intramolecular Singlet Fission

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Singlet fission (SF) is a process of multiexciton generation in which a molecule in singlet excited state shares its excitation energy with a neighbouring electronically coupled chromophore to generate two excited molecules in their triplet state, via a correlated triplet pair intermediate state.[1] This whole event is guided by optimal electronic coupling between two chromophores as well as a thermodynamic requirement of E (S_1) ≥ 2E (T_1). However, triplets can also be generated via ISC, although in much slower timescale. So, to harvest the triplets, it's very important to know the exact origin of the triplets. Herein, we probe into two covalently linked TIPS-pentacene dimers, one of which has been substituted with chlorine atoms to enhance the rate of ISC, and we wanted to see whether the enhanced rate of ISC can compete with the SF process. For that we took the help of both steady state and time resolved spectroscopy to probe the dynamics of these dimers. From the transient absorption spectroscopy, we could see a slower SF dynamic in case of the chlorinated dimer. But, apart from this we got a very interesting result of excimer formation in the chlorinated dimer due to conformational heterogeneity. For the SF vs ISC dynamics, we are taking the help of trEPR which actually shows a different dynamic for both the dimers.

References

1. Miyata, K.; Conrad-Burton, F.S.; Geyer, F.L.; Zhu, X.-Y.; *Chem. Rev.*, **2019**, 119, 4261
2. Smith, M.B.; Michl, J.; *Chem. Rev.*, **2010**, 110, 6891.
3. Feng, X., Casanova, D., Krylov, A.I., *J. Phys. Chem. C*, **2016**, 120, 19070−19077
4. Busby, E., Xia, J., Wu, Q., Low, J.Z., Song, R., Miller, J.R., Zhu, X-Y., Campos, L.M.; Sfeir, M.Y., *Nature Materials*, **2015, 14**, 426–433

P 159

Copper Chelators as Novel Theranostic Agent for Generating ROS in Prostate Cancer Inducing Fenton Reaction

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Theranostic agents, which combine therapeutic and diagnostic functionalities, represent a significant advancement in cancer treatment, offering precise targeting and real-time monitoring. This dual capability enhances treatment efficacy and provides valuable insights into disease progression and response to therapy, addressing the limitations of traditional approaches that often suffer from non-specificity and significant off-target toxicities.

Prostate cancer (PC), a major cause of cancer-related deaths, frequently exhibits resistance to conventional therapies, underscoring the need for innovative treatment strategies. PC cells are characterized by an inherent misregulation of metal ions, particularly copper (Cu), and elevated levels of hydrogen peroxide (H_2O_2)[1]. This dysregulation facilitates the Fenton reaction, which generates reactive oxygen species (ROS) and induces oxidative stress. We propose a novel theranostic agent which integrates copper chelation for inducing ROS generation and a probe that facilitates intracellular tracking, enabling real-time visualization and monitoring of the drug's distribution and efficacy within living systems as diagnostic agent. Additionally, it incorporates a prostate-specific membrane antigen (PSMA)[2] targeting unit ensures selective delivery to prostate cancer cells.

By combining therapeutic and diagnostic capabilities, this theranostic approach will enhance both treatment efficacy and diagnostic accuracy, may provide a optimistic solution for drug delivery system that improves patient outcomes and facilitates more effective management of prostate cancer.

References:

1. Lovejoy, D. B. et al. *Inorg. Chem.* 2012, *51*, 5966.
2. Kularatne, S. A. et al. *Mol. Pharmaceutics* 2009, *6*(3), 780

P 160

A Fluorescent Peptide-Based Sensor for Imaging Signal-Mediating Lipids

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Phosphatidylinositol 3,4,5-trisphosphate (PIP3) is a phospholipid found in the inner leaflet of the cell membrane. It plays a key role in cell signalling by recruiting and activating proteins involved in cell growth, survival, and metabolism. Dysregulation of PIP3 levels is associated with diseases such as cancer, neurodegenerative diseases, and diabetes due to its direct role in the PI3K/AKT pathway, which controls cell proliferation and survival.[1,2] Detecting PIP3 is essential for elucidating its dynamics and regulation within living cells which can aid in the study of disease mechanisms and the development of therapeutic interventions. Our group has recently developed peptide-based sensors that can sense and track PIP3 within living system.[3] The sensors afford ~10-fold higher selectivity toward PIP3 over other structurally similar lipids which is sufficient to detect pathophysiological levels of PIP3. However, under physiological conditions the levels of PIP3 are around 100 times lower than that of other signal-mediating lipids necessitating probes with higher selectivity. Toward this goal, we are developing PIP3 sensors with improved selectivity. We have developed a novel, reversible, ratiometric fluorescent sensor for PIP3 which affords ~40-fold higher selectivity toward PIP3 over other biologically essential phospholipids. I will present the design, synthesis, characterization, and evaluation of the probe.

References:

1. Lemmon, M. A. *Nat. Rev. Mol. Cell Biol*. **2008**, *9(2)*,99.
2. Posor et al. *Nat. Rev. Mol. Cell Biol*. **2022**, *23(12)*, 797.
3. Kundu, R. et al. *JACS Au*. **2024**. *13;4(3)*,1004.

P 161

Efficient, cleaner and automated approach towards single-molecule TIRF assays for investigating DNA replication on flow-stretched DNA substrates.

Daniel Ramirez Montero, Vincent Kruit, Francisco Palmero Moya, **Saurabh Talele**, Nynke Dekker

Single-molecule TIRF microscopy on flow stretched DNA is a powerful technique to probe the dynamics of the DNA replication machinery in real time at high-throughput. One can visualize and track the motion of fluorescently labelled proteins onto the DNA substrate which is tethered at both ends on the surface of the flow cell. The primary limitation for using this technique is protein aggregation either onto the surface of the flow cell or onto the DNA and the inability to wash away the unbound proteins in the solution. Additionally, in context of data analysis, manual labelling of ~ 1000 DNAs may be required for one experiment which is a laborious process. Here we highlight two key advancements in this methodology enabling a clean and efficient measurement along with automated and user-friendly data analysis platform. We demonstrate a hybrid ensemble and single-molecule assay where first, we perform bulk biochemical reactions such as loading, or activation of various replication associated components using desthiobiotin functionalized DNAs and streptavidin coated magnetic beads. This allows us to perform sequence of reactions followed by washing steps to remove non-specifically interacting proteins and finally elute the DNA at high concentration using biotin. In our python-based data analysis pipeline, first, we a use segmentation-based approach that allows us to detect the DNA molecules and determine their length in an automated fashion. For every DNA segment, we quantify the tension and the lateral fluctuations in the DNA molecule to provide mechanical information. To localize fluorescently labelled proteins on the DNA, we have implemented a gradient based spot detection algorithm. Additionally, we use the LAP tracker to track the motion of proteins along the DNA. The analysis pipeline is packaged with a user-friendly GUI using Napari viewer for easy access and implementation

P 162

Bio-waste derived Mg-doped carbon dots: A Fluorescent Probe of Y^{3+} Metal ion and Bio Imaging Application

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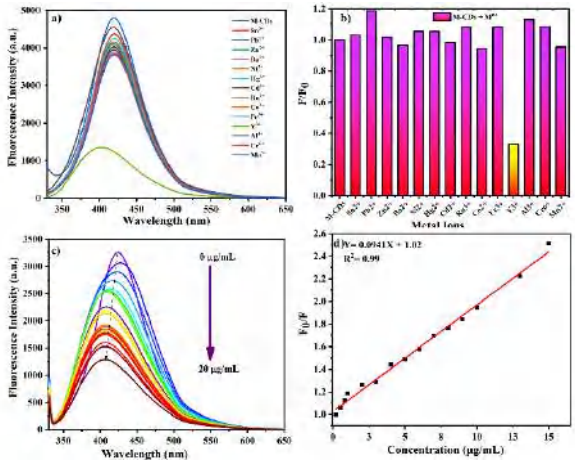
In present study, waste valorisation approach was utilizing for synthesis of Mg-doped carbon dots form cow dung using hydrothermal method. The Mg doped fluorescent carbon dots (M-CDs) having maximum emission at 420 nm upon 310 nm excitation with 20 % quantum yield. The M-CDs synthesis was confirmed using different analytical a characterization technique as well as stable in different pH as well as ionic strength solutions. The M-CDs was highly selective towards Y^{3+} ions with significant blue shift. The LOD of developed probe toward Y^{3+} was 0.019 µg/mL. The study indicates quenching of Y^{3+} was result of dynamic and IFE quenching effect [1] which was analysed by TCSPC (Time-Correlated Single Photon Count) and UV-Visible spectroscopy measurement. Further the interaction of CDs with Y^{3+} ion was investigated. The oxygen containing groups of CDs was responsible for Y^{3+}-CDs bonds [2-3]. The healthy growth of blood vessels in angiogenesis study demonstrated the cytotoxicity of CDs. further the CDs employed for MCF-7 breast cancer cell imaging [4]. The CDs were enable to interact with MCF-7 cell and brighten the fluorescence signal.



References:

1. Panpan Zhu et al. ACS Appl. Nano Mater **2023**, 6, 21194.
2. Xuelian Hu et al. Sen. Actuators B. Chem. **2021**, 338, 129809.
3. Mingxi Yang et al. Part. Part. Syst. Charact. **2019**, 36, 1800315
4. Cintya D'Angelis do E. S. Barbosa et al. Chem. Eur. J. **2015**, 21, 5055 – 5060

P 163

Post Synthetic Solvent Studies on Organic Inorganic Hybrid Perovskites

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Hybrid organic-inorganic perovskites (HOIPs) have been emerged as a superior class of materials for the optoelectronic applications due to their extremely high absorption coefficients, tunable bandgap and several other properties etc.[1,2] In this work, a bigger cation phenylethyl ammonium (PEA) has been used as A site cation to get a layered perovskite structure. The structural configuration is $(PEA)_2PbBr_4$ which is formed due to the interaction between the layered inorganic octahedra and the organic layers.[3,4] We have studied the behaviour of the $(PEA)_2PbBr_4$ in presence of different solvent environments (DMF, CLB, Hex, CAN, IPA etc). After addition of 5% of DMF with high Guttman Donor Number (DN) 26.6, the crystal gets completely disintegrated whereas after adding 22.5% Cyclohexane with 0 DN no. solvent, the crystal structure remains intact and stabilize the structure as well, shown in figure below. This will give a new insight in revealing the interaction and the long-term stability of these materials in their colloidal forms and will also help us to ponder over the interactions between the Pb complexes and the A site cations. [5,6]

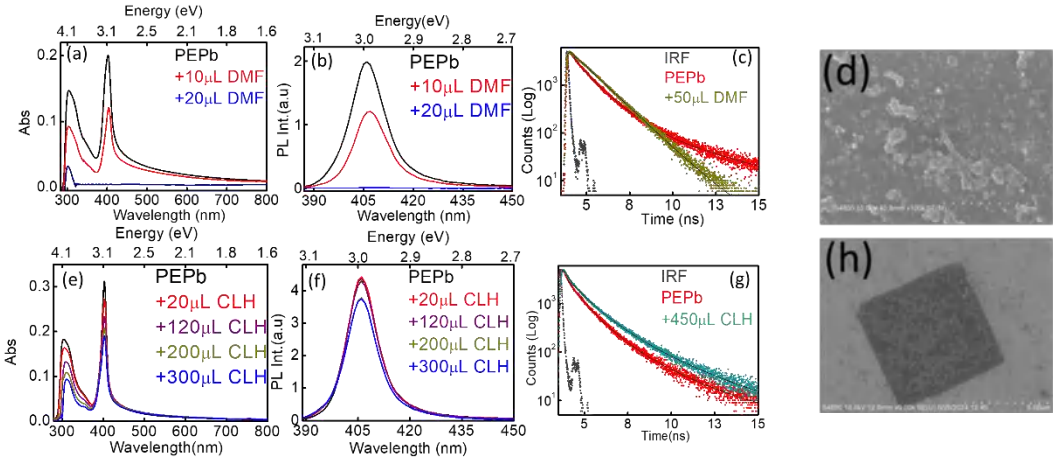

Figure 1: Uv-vis,PL and Lifetime after addition of DMF in a) ,b) and c) respectively, and in Cyclohexane in e),f), g) respectively. d) and h) is the corresponding FESEM images in these solvents

References:

1. Ke-zhao Du et al Inorg. Chem. 2017, 56, 9291.
2. Yu-Tao Li et al ACS Appl. Electron. Mater. 2022, 4, 547.
3. Dong Liang et al ACS Nano 2016, 10, 6897.
4. David B. Mitzi et al J. Chem. Soc., Dalton Trans., 2001, 1.
5. J. Clay Hamill, Jr. et al ACS Energy Lett. 2018, 3, 92.
6. Andrei S. Tutantsev et al J. Phys. Chem. C 2020, 124, 11117

P 164

The Role of Charge Transfer in CsPbBr3 Perovskite Nanocrystals for Cutting-Edge Optoelectronic Technologies

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The success of lead halide perovskite nanocrystals (P-NCs) based optoelectronic devices hinges on the swift and seamless interfacial charge transfer (CT), which dramatically boosts energy conversion efficiency. In this study, we thoroughly explore the CT process in $CsPbBr_3$ P-NCs when paired with ZnO and TiO_2 nanoparticles (NPs), which serve as excellent electron acceptors due to their optimal energy level alignment with the $CsPbBr_3$ P-NCs. By utilizing spectroscopic (steady state and time resolved) and electrical (current sensing atomic force microscopy) measurements, we observe a facile electron transfer (ET) from P-NCs to both these acceptors. Further, to highlight the crucial role of ET in the P-NC based photovoltaic devices, we have conducted simulations comparing the performance of three devices, FTO/$CsPbBr_3$/PEDOT:PSS/Au (control), FTO/ZnO/$CsPbBr_3$/PEDOT:PSS/Au (device with ZnO), FTO/TiO_2/$CsPbBr_3$/PEDOT:PSS/Au (device with TiO_2). We have seen a striking improvement in device performance (efficiency, current density, open circuit voltage and fill factor) with the incorporation of ZnO and TiO_2, surpassing the results from the control device. However, the ET rate, boost in electrical conductivity, and device performance all show marked improvements with TiO_2 over ZnO, thanks to TiO_2's energy levels being more favourably aligned with P-NCs. This study underscores the critical role of tuning interlayer charge transfer in optimizing the performance of photovoltaic devices.

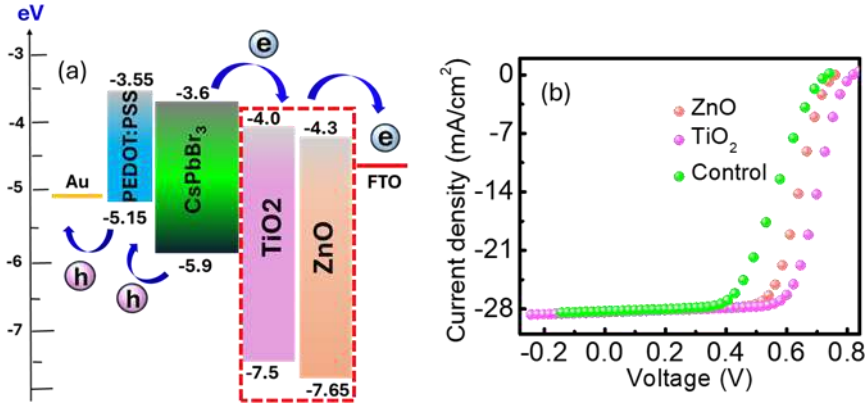

Figure: Energy band alignment of the photovoltaic device (a) and the corresponding current-voltage curve for control device, device with ZnO and TiO_2 (b).

References:

1. QA Akkerman et al. *Nature materials* **2018**, *17*, 394.
2. H Zhang et al. *Nature reviews chemistry* **2023**, *7*, 632.
3. NG park et al. *Nature energy* **2016**, *1*, 16152

P 165

Computational Simulations of Protein Charge Transfer Spectra

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Recent experimental studies have revealed that monomeric proteins rich in charged amino acids exhibit broad absorption profiles spanning the entire UV-Visible spectral band (200-800 nm). Computational investigations attribute this phenomenon to photoinduced charge transfer (CT) transitions involving charged amino acids, such as lysine (Lys), glutamate (Glu), Arginine (Arg), Aspartate (Asp), and protonated Histidines (His) [**1,2**]. This unusual absorption arising from charged amino acid rich proteins is termed Protein Charge Transfer Spectra (ProCharTS). Here, we describe a computational strategy to simulate ProCharTS profiles of entire proteins, combining classical molecular dynamics sampling and time-dependent density functional theory-based spectra calculations. We demonstrate an application of the strategy by investigation of the ProCharTS sensitivity to post-translational modifications (PTMs), specifically acetylation, which quenches the charge of Lys residues in two helical model proteins α3C (no aromatic amino acids) and α3W (wherein the CYS34 residue in alpha3C is mutated to tryptophan). We show that the statistical distribution of charged amino acid clusters changes with the degree of acetylation, resulting in a corresponding decrease in simulated ProCharTS profiles. We will discuss the potential of combining experimental measurements and computational analysis to quantify PTMs in a label-free scheme and establish the resolution and sensitivity limits of this novel approach.

References:

1. Prasad, S., Mandal, I., Singh, S., Paul, A., Mandal, B., Venkatramani, R., & Swaminathan, R. (2017). Near UV-Visible electronic absorption originating from charged amino acids in a monomeric protein. *Chemical science*, *8*(8), 5416-5433.
2. Mandal, I., Paul, S., & Venkatramani, R. (2018). Optical backbone-sidechain charge transfer transitions in proteins sensitive to secondary structure and modifications. Faraday discussions, 207, 115-135.

P 166

Novel Role of Sphingolipids in the Organization of the Actin Cytoskeleton

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Sphingolipids are essential components of eukaryotic cell membranes and constitute 10-20% of total membrane lipids. They are recognized as dynamic regulators of a multitude of cellular processes [1]. Various pathological conditions such as Niemann-Pick disease, Gaucher's disease, Tay-Sachs disease are associated with abnormalities in sphingolipid metabolism [2]. Sphingolipids are distributed heterogeneously in the plasma membrane, majorly contributing to the formation of laterally segregated membrane microdomains (sometimes referred as "rafts") along with cholesterol [3]. These microdomains are believed to serve crucial functions in regulating lateral diffusion of membrane lipids and proteins, which dictates diverse cellular signaling events [4]. Analyzing the spatiotemporal resolution of these domains remains very challenging. An interesting source of heterogeneity in cell membranes exists due to the dynamic confinement created by the underlying actin cytoskeleton [5]. The mobility of membrane proteins such as GPCRs and many microdomains-associated proteins is often regulated by the dynamic actin cytoskeletal meshwork [6,7]. This has given rise to an emerging model regarding the synergistic existence of membrane microdomains and confinement due to the actin cytoskeleton. In this context, we previously explored the role of chronic cholesterol depletion in regulating the organization of actin cytoskeleton [8]. Due to the close interaction of cholesterol and sphingolipids in membrane microdomains, we examined whether sphingolipids play any role in regulating actin organization. In this work, we explored the effect of sphingolipid depletion on cellular actin cytoskeleton organization by using a quantitative confocal microscopy based approach previously developed by us [9,10]. For this, we inhibited cellular biosynthesis of sphingolipids using metabolic inhibitors such as fumonisin B_1 (FB_1) and myriocin in CHO-K1 cells and explored the actin cytoskeleton organization using the assay. Our results show that there was a significant increase in actin polymerization upon inhibition of sphingolipid biosynthesis using FB_1 and myriocin. Notably, the process was not reversed upon sphingolipid replenishment, which could be due to the non-specific effects of the inhibitors. To test this, and to overcome any effect of accumulation of metabolic intermediates, we monitored the organization of the actin cytoskeleton in LY-B cells [11], which are CHO-K1 cells with sphingolipid-auxotrophic mutants defective in the first committed step in sphingolipid biosynthesis. Interestingly, we observed that the increase in F-actin content was reversible upon sphingolipid replenishment in LY-B cells. To the best of our knowledge, our results constitute the first report on the role of sphingolipids in actin polymerization. These results assume relevance in the context of remodeling of the actin cytoskeleton in pathological conditions with altered sphingolipid content.

References:

1. Holthuis JC et al. *Physiol. Rev*. **2001**, *81*, 1689.
2. Rabbo MA et al. *Lipids Health Dis*. **2021**, 20, 44.
3. Brown RE, *J. Cell Sci.* **1998**, *111*, 1.
4. Goñi FM, *Chem. Phys. Lipids* **2019**, *218*, 34.
5. Fujiwara TK et al. *Mol. Biol. Cell.* **2016**, *27*, 1101.
6. Shrivastava S. et al. *Biophys. J*. 2020, *118*, 944.
7. Ganguly, S. et al. *Biophys. J*. 2010, *99*, 1397.
8. Sarkar P et al. *J. Lipid Res*. **2022**, *63*, 100206.
9. Ganguly S et al. *Biochim. Biophys. Acta* **2011**, *1808*, 1921.
10. Sahu SS et al. *Methods* **2024**, *230*, 44.
11. Jafurulla M et al. *Biochim. Biophys. Acta* **2017**, *1859*, 598.

P 167

Structure-function mapping of Salmonella and host cytoskeleton interactions.

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The cytoskeletal network of a cell is key to maintenance of cell shape, uptake and transport of material, cell migration and cell division. The central nature of the cytoskeletal network makes it a key target for pathogens that hijack it for cellular entry, pathogenic replication and spread. Salmonella is a model example of an enteropathogenic bacterium that hijacks cytoskeletal remodeling and transport processes for cellular invasion. Salmonella achieves this feat by secreting pathogenic factors that interact with and modulate the function of cytoskeleton and its associated proteins. These pathogenic factors exhibit "molecular mimicry" wherein they harbor motifs like native cytoskeletal regulatory proteins that allows them to switch cytoskeletal processes towards pathogen invasion. Although several key Salmonella pathogenic factors such as SopB, SopE and SifA have been identified as cytoskeletal regulators, the structural and functional mechanisms of molecular mimicry for these factors is poorly understood. The research being done in our group aims to bridge this gap by an interdisciplinary approach combining reconstitution biology, DNA nanotechnology and FRET-based conformational biosensors to probe the structure-function mechanisms of Salmonella pathogenic factors and identify key protein-protein interactions utilised by these factors for hijacking the cytoskeletal network.

P 168

Inverse adaptation to force modalities in multidomain proteins: The role of interdomain linkers

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The mechano-biome of biological systems exhibits remarkable diversity and dynamism, as complex cellular and tissue processes are orchestrated through intricate mechanical interactions. Central to this mechano-biome are mechanical proteins, which function as both sensors and responders to a broad range of mechanical cues, thereby actively modulating cellular architecture, signalling pathways, and tissue-level mechanics. Architecturally, these proteins are often elongated, modular structures with multiple domains arranged in series, where each domain acts as a mechanically responsive element or "spring."

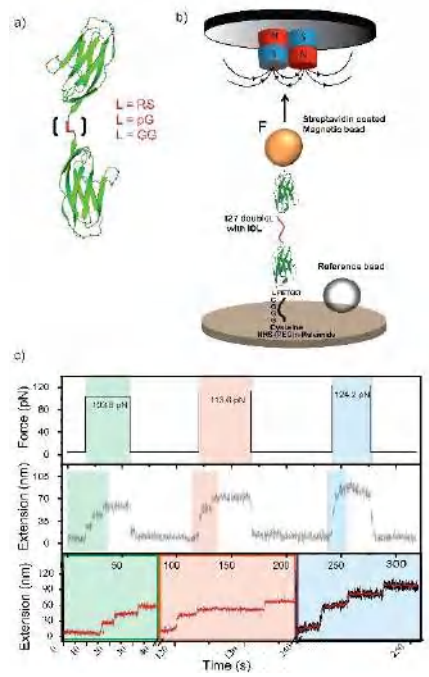

Figure: a) A cartoon representation of three protein models. b) Schematic representation of attachment setup of protein dimer variants in magnetic tweezers. c) A representative extension versus time trace of three protein models at three clamping forces.

Despite significant advancement in our understanding, the adaptive mechanisms by which these domains respond to diverse mechanical stimuli remain poorly understood. In this study, we focus on interdomain linkers (IDLs) and elucidate how chemically distinct linkers modulate the mechanical behaviour of domains within a representative muscle protein under both constant and oscillatory force conditions. Specifically, we investigate the impact of these linkers on the stability, unfolding kinetics, and interdomain interactions of repeat domains, uncovering unique mechano-adaptive roles.

Our findings reveal that IDLs differentially tune domain interactions, thereby regulating the stability and folding kinetics under sustained tension. Notably, IDLs that enhance mechano-stability under constant forces, paradoxically diminish the ability of domains to retain power under oscillatory forces, making them more susceptible to mechanical fatigue. This inverse response to force modalities suggests that linker properties are finely tuned to either sustain constant loading or to adapt to cyclic forces, but not both simultaneously.

Through these insights, we propose that strategic modifications of linker composition offer a means to engineer mechanical protein variants with tailored force responses, providing a potential framework for designing proteins optimized for specific mechanical environments. These findings underscore the critical role of interdomain linkers in the mechano-adaptation of modular proteins and present foundational principles for advancing the design of biomimetic materials like hydrogels and therapeutics.

P 169

Unravelling the Role of Membrane Lipid Constituents in Dengue Virus Membrane Fusion

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Dengue fever, a prevalent vector-borne viral illness, is caused by the Dengue virus. Characterized by its compact, icosahedrally symmetric structure, this flavivirus is enveloped in ninety dimers of the envelope protein (E), crucial for viral attachment and membrane fusion. In this study, we systematically investigate the membrane fusion step, essential for virus entry into host cells, focusing on several key aspects including the influence of the target membrane's lipid composition, the effect of anionic lipids or other oxysterols on fusion, and the potential for direct inhibitors binding to the virus. Furthermore, our findings exhibit that 25-Hydroxycholesterol (25-HC) can reduce viral load in cell culture in a dose-established way. We also look at the mechanism of Dengue virus inhibition with the aid of 25-HC through Time of Addition assay (TOA), function of certain ISGs such as cholesterol 25-hydroxylase (CH25H) in different cell line, and also calculation of synergistic combination with some recognized anti-virals, (Chloroquine, Remdesivir, Picolinic acid, K-22), in the context to decide if the combination of compounds can enhance the effectiveness compared to the usage of them in alone. Utilizing actual-time fluorescence membrane assays, cell based dye de quenching assay, our studies uncovers new insights into how lipid compositions have an impact on dengue virus binding and membrane fusion. This study offers a nuanced understanding of the virus's entry mechanisms and lays the groundwork for potential therapeutic interventions targeting the viral fusion process.

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Heterogeneous Micropatterns of Biological Macromolecules Using Microbubble Lithography

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The recent pandemic highlights the importance of adopting high-throughput and rapid diagnostic techniques for accurate disease identification and optimal healthcare management. Developing diagnostic tools necessitates patterning biomolecules while preserving their biological activity. In contrast, the concept of bio-patterning largely relies on two methods: top-down and bottom-up. Although the top-down approach is advantageous in terms of scalability, it often exposes the biomaterial to high laser energy (in the case of photolithography), high voltage (electron beam lithography), or requires additional chemicals, such as developer-stripper solutions which are very likely to compromise the bioactivity and functionality of the patterned biospecimen seriously.

Microbubble lithography (MBL) is a comparatively recent (about a decade-old) bottom-up method that uses laser-generated and translated microbubbles to self-assemble and pattern materials of choice in real–time. Microbubbles are formed inside optical tweezers due to the high absorption of tightly focused lasers by dispersed particles immersed in or in contact with a fluid [1]. As the microbubble is manipulated by translating the laser, continuous self-assembly of the materials occurs along the direction of translation, forming continuous linear patterns. Changing experimental parameters, including laser intensity and power, translation speed, and sample concentration in liquid dispersion, allows easy control over pattern width. However, despite of its success in patterning mesoscopic entities - to the best of our knowledge – MBL has not yet been employed to develop continuous patterns of living organisms [2, 3]. This is principally due to temperature, adhesion of biospecimens (patterning efficiency) and low signal to noise ratio. Here, we outline a method for printing living matter or biospecimens using self-assembly mediated by microbubbles and report the successful creation of a strategy for immobilizing diverse biospecimens on transparent substrates. Our deployment of fast laser translation speeds ensure no temperature-induced damage to the biospecimens since their contact time with the microbubbles is less than a hundred milliseconds (ms). Overall, this study sets the groundwork for manufacturing multilayer heterostructures of living matter using MBL, and may open a new paradigm in designing bioelectronic chips.

References

1. S. Ghosh et al. *Nano Letters* **2021**, *21*, 10–25.
2. A. D. Ranjan et al, *JMCC*, **2023**, *11*, 17061.
3. A. D. Ranjan et al. *Small*, **2024**, 2401127.

P 1

Disordered Regions, Ordered Outcomes: IDR Contributions to Bacterial Microcompartments

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Proteins are typically folded to achieve functionality, but recent research has highlighted the critical role that unfolded regions, known as intrinsically disordered regions (IDRs), play in influencing protein function. These IDRs remain largely unexplored in prokaryotes. Bacterial microcompartments (BMCs), protein structures with an outer layer of shell proteins that surround metabolic enzymes, are an ideal model to study prokaryotic IDRs as they consist of completely disordered to completely folded proteins. In this study, we explore how deletion of IDRs from the structural protein will impact the protein-protein interaction and overall function. A combination of advanced imaging and spectroscopic techniques, including fluorescence quenching, circular dichroism (CD) spectroscopy, and bio-layer interferometry (BLI), was employed to elucidate the structural and functional roles of IDRs in the outer shell protein PduA, a key component conserved across various BMCs. Additionally, transmission electron microscopy (TEM) was utilized to assess the morphological changes resulting from IDR deletions. Our findings reveal that IDRs at the N- and C-termini of PduA are essential for proper protein self-assembly and stability. Fluorescence quenching and BLI provided quantitative insights into the disruption of native protein interactions upon IDR deletion, while TEM imaging highlighted significant morphological alterations in the BMC structure. These observations were further validated through in vivo studies in both heterologous and homologous hosts, demonstrating the essential role of IDRs in maintaining wild-type protein properties. This work offers new insights into the functional importance of IDRs in prokaryotic structural proteins, underscoring their role in BMC assembly and stability.

P 2

A Zinc complex as an NIR emissive probe for real-time dynamics and *in vivo* embryogenic evolution of lysosomes using super-resolution microscopy

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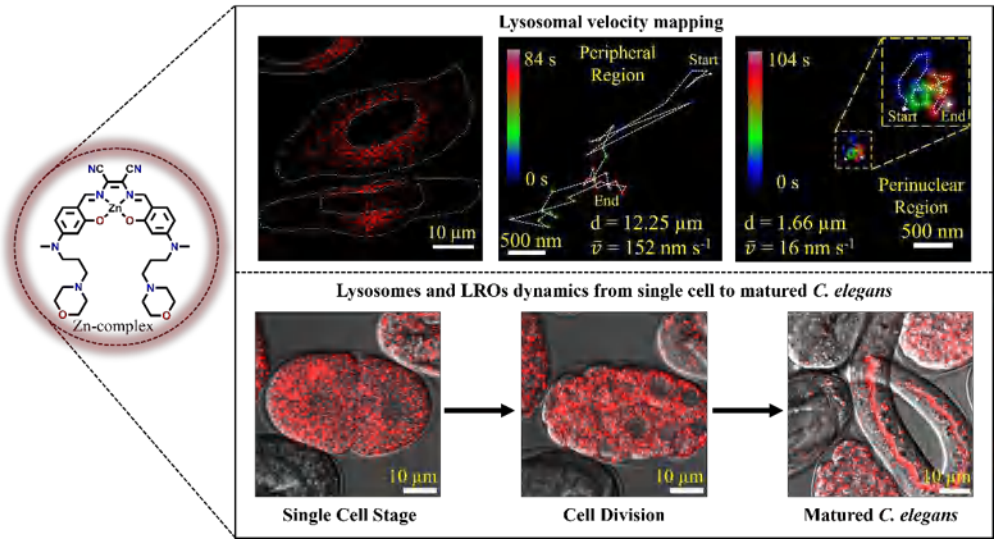
Zinc (Zn) based fluorescent metal complexes have gained increasing attention due to their non-toxicity and high brightness with marked fluorescence quantum yield (QY). However, they have rarely been employed in super-resolution microscopy (SRM) to study live cells and in vivo dynamics of lysosomes. Here, we present an NIR emissive highly photostable Zn-complex as a multifaceted fluorescent probe for the long-term dynamical distribution of lysosomes in various cancerous and non-cancerous cells in live condition and in vivo embryogenic evolution in *Caenorhabditis elegans (C. elegans)*. Apart from the normal fission, fusion, and kiss & run, the motility and the exact location of lysosomes at each point were mapped precisely. A notable difference in the lysosomal motility in the peripheral region between cancerous and non-cancerous cells was distinctly observed. This is attributed to the difference in viscosity of the cytoplasmic environment. On the other hand, along with the super-resolved structure of the smallest size lysosome (~77 nm) in live *C. elegans*, the complete in vivo embryogenic evolution of lysosomes and lysosome-related organelles (LROs) was captured. We were able to capture the images of lysosomes and LROs at different stages of *C. elegans*, starting from a single cell and extending to a fully matured adult animal.



References:

1. A. Salam et al., *Chem. Sci.*, **2024**, 15, 15659
2. N. Gustafsson et al., *Nat. Commun.*, **2016**, 7, 12471
3. D. E. Johnson et al., *J. Cell Biol.*, **2016**, 212, 677
4. Jankele et al., *Elife*, **2021**, 10, e61714

Probing of disease-specific exosomes using Raman spectroscopy and SERS

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Cancer often evolves silently and is realized only in the advanced stages of metastasis. Also, the most practiced diagnostic tools like MRI, CT scan, PET scan, etc, often require costly infrastructure and/or trained personnel. These techniques come with their own detection limits and are also susceptible to produce false positive results. We urgently need more accurate and cost-effective diagnosis techniques. In recent years, markers for various cancers have been identified in the contents of exosomes. Exosomes originating from cancer cells carry genetic materials, metabolites, and proteins that can significantly differ from exosomes originating from healthy cells. We are in the process of establishing a Raman spectroscopy-based comparison of exosomal contents obtained from various cancerous and non-cancerous cell lines, utilizing multivariate statistical methods such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) [1].

In patient samples, disease-specific exosomes are typically present in very low concentrations amidst a variety of other exosome types. Detecting these subtle differences requires a high degree of sensitivity and selectivity, which classical Raman spectroscopy lacks. Instead, Surface-enhanced Raman spectroscopy (SERS) offers a solution by greatly amplifying Raman signal intensities from analytes near plasmonic surfaces, enabling the precise detection of specific biomarkers [1-3]. For example, the human epidermal growth factor receptor 2 (HER2), which is overexpressed in various cancers including breast, cervical, ovarian, and gastro-oesophageal cancers, serves as a potential exosomal biomarker. HER2 can be specifically targeted using a 42-base DNA aptamer known as the Anti-HER2 aptamer (HApt) [4]. In this study, we utilized a modified HApt with a disulfide bond at the 5' end and Raman/fluorescence tags at the 3' end. These modified aptamers were grafted onto silver nanoparticles (AgNPs) to create various nanoconstructs. We hypothesized that binding with HER2 would cause the HApt molecules to reorient on the AgNP surface, leading to detectable changes in the SERS signal from the Raman tags. We observed significant SERS enhancement with our nanoconstructs exclusively in the presence of exosomes isolated from HER2-overexpressing SKOV3 cells. In contrast, no notable changes in SERS characteristics were detected with exosomes from SiHa cells, which do not overexpress HER2. This technique thus holds promise as a highly sensitive and cost-effective platform for the early detection of HER2-overexpressing cancers.

References:

1. Chalapathi, D. et al. *J. Phys. Chem. B* **2020**, *124*, 10952
2. Karthigeyan, D. et al. *Proc. Natl. Acad. Sci. USA*, **2014**, 02695.
3. Bhowmik, D. et al. *ACS Nano* **2015**, *9*, 9070.
4. Bhowmik, D. et al. *ACS Nano* **2019**, 13, 13637.

P 4

How Molecular Crowders Influence Ligand Binding Kinetics with G-Quadruplex DNA? The Role of Bound Water

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The formation of basket-like G-quadruplex DNA (GqDNA) structures resulting from the folding of guanine-rich single-stranded DNA sequence in the presence of monovalent cations (mainly, K^+ or Na^+), typically found in the telomere and promoter regions, play important roles in various biological processes [1,2]. These DNA structures contain specific binding sites for small drug-like molecules (ligands) which make them capable of inducing anti-cancer effects upon interacting with GqDNA within the cell. Thus, understanding the kinetics of ligand interaction with GqDNA in cell-like crowded environment is of paramount importance in biology and pharmacology, as it elucidates how molecular crowders influence the reaction rates governing these interactions, which are mostly unknown. In this study, we have used fluorescence correlation spectroscopy (FCS) and molecular dynamics (MD) simulations, aided by other techniques, to look at the kinetics of a benzophenoxazine-moiety ligand (cresyl violet, CV) binding to human telomeric (3+1) hybrid GqDNA in the absence and presence of commonly used crowders like ethylene glycol (EG), PEG200 and PEG6000 at two different physiological crowder concentrations, i.e., 10% (w/v) and 20% (w/v). Experimental results show a decrease in ligand binding affinity to GqDNA with increasing the crowder size as well as concentration, driven by viscosity-induced reduction of association rate (k_+) and a concomitant increase in dissociation rate (k-) of the ligand. MD simulations reveal the critical role of electrostatic forces and long-lived water-mediated hydrogen-bond-bridges in stabilizing the ligand/GqDNA complex, which are significantly disrupted by crowders, leading to the destabilization of the complex. Although, the binding of the ligand (CV) to these crowders is very weak, their effect on the destabilization CV from its stable complex with GqDNA is quite substantial which gets enhanced with concentration as well as size of the crowders. Unlike polysaccharide crowders, these EG/PEG crowders affect both the association and dissociation rates, which needs special attention while choosing them as crowding agents in cell-like *in vitro* experimental conditions [3,4]. We believe, these results will help researchers in designing GqDNA targeted anti-cancer drugs as well as choosing proper (inert) crowders to mimic cellular environment.

References:

1. Spiegel, J. et al. *Trends Chem.* **2020**, *2*, 123.
2. Rhodes, D. et al. *Nucleic Acids Res.* **2015**, *43*, 8627.
3. Clovis, N.S. et al. *J. Photochem. Photobiol. A Chem.* **2023**, *437*, 114432.
4. Alam, P. et al. *Methods Appl. Fluoresc.* **2024**, *12*, 045002.

P 5

Slowdown of Solvent Structural Dynamics in Aqueous DMF Solutions

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Understanding the hydrogen bonding interactions between amide functional groups and water is essential for studying liquid–liquid phase separation in biological systems. Even simple amides like N,N-Dimethylformamide (DMF) and formamide (FA) exhibit microheterogeneity and distinct hydrogen bonding behaviour in aqueous solutions, forming dynamic equilibria that impact solvation properties.[1, 2] We employed a multidisciplinary approach—integrating FTIR and 2D IR spectroscopy, density functional theory (DFT) calculations, and molecular dynamics (MD) simulations—to investigate the interactions and ultrafast dynamics of these heterogeneous systems. Our findings reveal a notable slowdown in solvent fluctuation dynamics at the maximum viscosity observed in specific amide-water molar ratios, attributed to stronger amide-water hydrogen bonds compared to water-water bonds. Hydrogen-bond autocorrelations from MD simulations corroborate the experimental spectral diffusion timescales, providing a comprehensive molecular view of solvation structure and dynamics.[3] This study offers critical insights into hydrogen bond dynamics and sets the stage for exploring complex solvation phenomena, such as coacervation.

References and Notes

1. Bai, Y. et al. *J. Chem. Phys B* **2022,** *126*, 9663
2. Tomar, D. et al. *J. Chem. Phys.* **2020,** *152*, 114707.
3. Pathania, A. et al. *Chemical Physics Impact* **2024,** *9*, 100711.

P 6

Design and Synthesis of Water Dispersible Red Emissive Carbon Dots and their Applications

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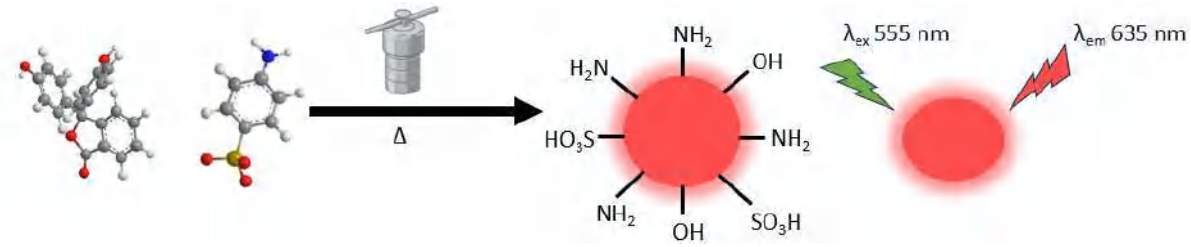
Carbon dots (CDs), fluorescent carbon nanoparticles smaller than 10 nm, have been extensively used in sensors, bioimaging, catalysis, light-emitting (LEDs), and photoelectronic devices. This is due to their unique characteristics, including low toxicity, biocompatibility, high photostability, ease of surface modification, and up-conversion fluorescence. Carbon dots (CDs) have proven effective in revealing the 3D structures of biological samples, distinguishing between normal and cancer cells, and detecting various analytes inside cells. Most of the carbon dots are blue-green emitting CDs. The practical use of blue-green-emitting carbon dots (CDs) is limited by their shallow penetration, photobleaching, and autofluorescence issues. The synthesis of red-emissive carbon dots (CDs) is highly sought after for sensing applications, but challenges remain in precursor preparation and product purification. Red-emissive carbon dots (RCDs) have been developed to address these issues. They offer deep tissue penetration, reduced photodamage, low autofluorescence, and high imaging contrast. Herein, we designed and synthesized RCDs with high water dispersibility, low toxicity, and excellent optical properties using hydrothermal method. These features make them ideal for a range of biological applications.



References:

1. Amanda A Pinheiro et al. *ACS Applied Nano material* **2024**, *7*, 19184.
2. Ankit Thakuri et al. *Journal of Hazardous Chemistry* **2024**, *469*, 133998.
3. Sharanabasava D. Hiremath et al. *ACS Applied Nano material* **2022,** *5*, 17373.
4. Sharanabasava D. Hiremath et al. *Sensors and Actuators: B. Chemical* **2021**, *339*, 119918.
5. Sharanabasava D. Hiremath et al. *Journal of Photochemistry & Photobiology A: Chemistry* **2020**, *389*, 112258.

Charge Polarity effect on the hydration shell water of π-electron containing hydrophobes: Observed by Raman Hydration Shell Spectroscopy

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π-electron containing hydrophobes are vital in many biological, chemical and industrial processes. Interaction of such hydrophobes with water (e.g., π···HOH and CH···OH_2) are generally weak but widespread. Experimental understanding of these weak interactions in aqueous bulk medium is limited due to typically low solubility of such hydrophobes in water and the associated technical challenges in their selective identification. Here, we monitored these interactions in the aqueous solution of structurally similar but oppositely charged molecular ions (tetra-phenyl borate (TPB^-) and tetra-phenyl phosphonium (TPP^+)) and uncharged benzene (Bz), using Raman difference spectroscopy with simultaneous curve fitting analysis (Raman-DS-SCF) [1].

DS-SCF analysis of the Raman spectrum of aqueous solution of a solute with respect to the Raman spectrum of neat water provides the solute-perturbed water spectrum which is considered as the spectrum of the perturbed water in the hydration shell (HS) of the solute. The left panels of Figure 1 show the OH stretch spectra of water pertaining to the hydration shell of Bz, TPB^-, and TPP^+ (obtained after Raman-DS-SCF analysis). The hydration shell spectra differ from spectrum of the neat water (black curve), indicating its interaction with the solutes. The HS water around Bz shows two component bands, centered at 3515 cm^{-1} and 3625 cm^{-1} (green dashed curves, top left panel) which corresponds to water approaching a Bz-ring equatorially and axially, respectively. The equatorial water engages in (Bz)CH···OH_2 interaction and axial water engages in (Bz)π···HOH interaction. For the negatively charged TPB^-, the OH stretch band corresponding to the π···HOH interaction is 45 cm^{-1} red shifted than that of Bz, suggesting stronger π···HOH interaction with TPB^-. For TPP^+, the corresponding π···HOH band is significantly blue-shifted and of lower intensity from that of Bz or TPB^- (left panel Figure 1c), signifying weaker π···HOH interaction with TPP^+. Thus the strength of π···HOH interaction varies as TPB^- > Bz > TPP^+. In addition, the appearance of a major component band around 3210 cm^{-1} for the HS of TPP^+ reveals the collective nature of the OH stretch vibration of water in the HS of TPP^+, which is a unique feature observed only for the cationic hydrophobe [1,2]. The modulation of the (phenyl)CH···OH_2 interaction with the equatorial water is monitored by the CH stretch band of the corresponding hydrophobes (right panels in Figure 1). The blue-shift of aromatic ring-CH band corresponds to the (phenyl)CH···OH_2 interaction, known as blue-shifted H-bond interaction. The strength of the (phenyl)CH···OH_2 interaction varies as TPB^- < Bz < TPP^+, which is opposite to the trend of their corresponding π···HOH interaction. Altogether, the π···HOH and CH···OH_2 interactions are modulated in aqueous medium depending on the sign of charge on the hydrophobe.

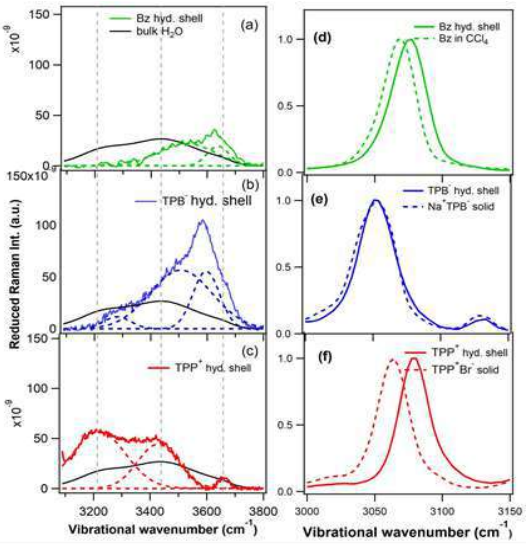

Figure 1: left panel: OH stretch Raman-DS-SCF hydration shell spectra (solid lines) and component bands (dashed lines) for Bz (a), TPB^- (b) & TPP^+ (c). Right panel: CH stretch Raman spectra of Bz (d), TPB^- (e) & TPP^+ (f) in presence (solid) & absence (dashed) of water.

References:

1. Bandyopadhyay,A. et al. *J.Mol.liq* **2023,** *390*,122987.
2. Bandyopadhyay,A. et al. *J.Phys.Chem.B* **2023,** *127*,7174.

P 8

Intramolecular Charge Transfer Dynamics in Persubstituted Perylenediimide

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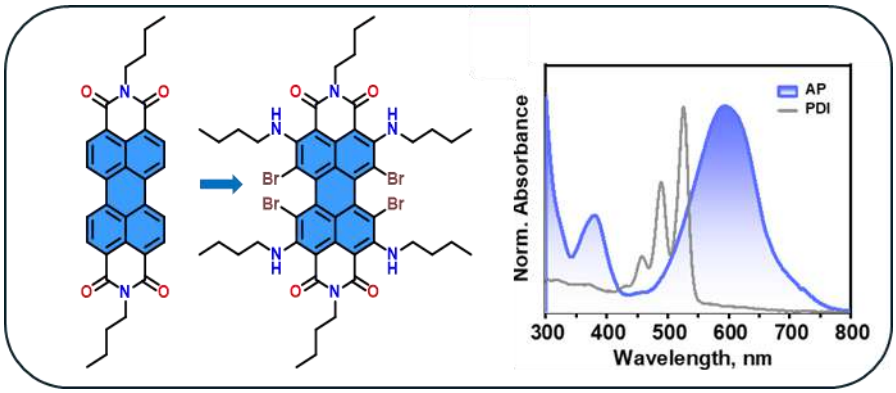
The charge transfer property of an organic chromophore can be tuned by incorporating electron donating/withdrawing functional groups. Amination at the ortho positions of perylenediimide induces intramolecular charge transfer (ICT) character to the chromophore.[1] Herein, we have synthesized and characterized the perylenediimide derivative having ortho substitution with amino groups and bay substitution with bromine atoms. The ICT effect of the amino groups on the ortho positions dominates over the heavy atom effect caused by the bromine atoms on the bay positions of the persubstituted perylenediimide.[2,3] The charge transfer character of the molecule is confirmed via transient absorption measurements. The lifetime of the ICT state reduced as the polarity of the solvent increased. The theoretical and experimental observations suggest that the electronic properties of the perylenediimide core are largely influenced by the position at which the substitution has been made.



References:

1. G. Battagliarin et al. *Org. Lett.* **2011**, *13*, 3012
2. K.Nagarajan et al. *J. Phys. Chem. C* **2016**, *120*, 8443
3. A. Jozeliūnaitė et al. *Synthesis* **2017**, *49*, 5176

P 9

Ultrafast exciton dynamics in $FAPbI_3$ nanorods

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Formamidinium lead triiodide ($FAPbI_3$) nanocrystals are attractive because they emit in red and near-infrared spectral regions.[1]. However, their inherent instability poses a major challenge in fabricating devices based on these materials. The present work reports a serendipitous synthesis of stable rod-shaped oleylamine-capped nanocrystals of $FAPbI_3$. The nanorods first excitonic peak and emission maximum occur in the red region. Their photoluminescence decays are bimodal, with a 14-16 ns component ascribed to band-edge recombination and a 35-40 ns component ascribed to trap-assisted recombination.[2]. Hot exciton relaxation plays a major role in this context. The dynamics of this process in the nanorods have been investigated by ultrafast transient absorption spectroscopy at different pump fluxes.

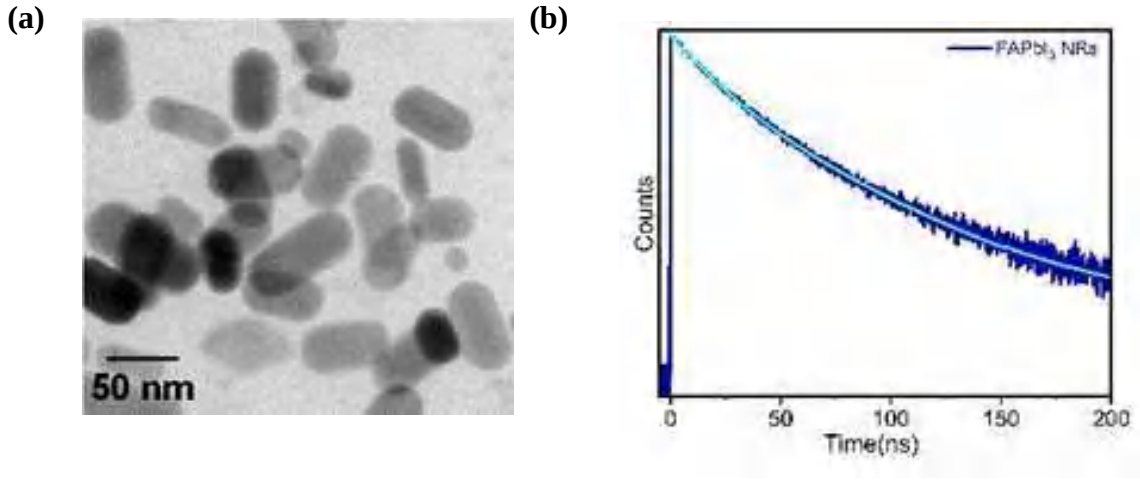

Figure 1 (a) TEM images of $FAPbI_3$ NRs, (b) PL Decay of $FAPbI_3$ NRs

References:

1. L. Protesescu.et al. *ACS Nano*, 2017, **11**, 3119
2. P. Papagiorgis.et al. *ACS Nano*, 2019, **13**, 5799

P 10

Advanced Techniques for Probing the Effect of Microplastics on Lipid Membranes

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Cellular membranes, critical for maintaining homeostasis and regulating molecular transport, are increasingly threatened by environmental pollutants, particularly microplastics. These micron-sized particles can integrate into lipid bilayers, disrupting membrane fluidity and organization. Such disturbances are implicated in diseases like Alzheimer's and Parkinson's. Due to the complexity of membrane systems, conventional fluorescence-based techniques lack the sensitivity to capture the dynamic nature of these interactions. This work focuses on the development of a two-channel confocal-AFM setup for real-time assessment of membrane indentation force and lipid order, integrating mechanical and molecular measurements. The system's design, development, and capabilities are outlined, alongside preliminary data on model membranes. Further optimization is needed to fully understand microplastic-induced membrane disruptions. This work represents a significant step toward real-time, high-resolution analysis of membrane-molecule interactions.

P 11

Amorphous Aggregates in Crystal Nucleation

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Crystallization from solution is at the heart of many natural and industrial processes, from the production of pharmaceuticals and nanomaterials to the formation of minerals, bones and teeth. The earliest phase of the crystallization process begins with nucleation which determines the structure, size and shape distribution of the crystals. For last two decades it has been observed that crystal nucleation from supersaturated solution occurs via formation and reorganization of prenucleation clusters where classical nucleation theory is challenged and modified [1]. These prenucleation clusters are metastable and nanoscale to mesoscale solute-rich structures. Understanding their origin, composition and structure is one of the pertinent and lingering questions in the fundamentals of crystal nucleation. Here, we have shown that these prenucleation clusters or amorphous aggregates are universally present in the supersaturated solutions of a range of amino acids and small peptides [2]. Using light scattering, nano-ESI mass spectrometry and *in-situ* terahertz Raman spectroscopy, we have identified their structures and dynamical behaviour in the aqueous solutions. One of the significant findings disclose that these aggregates encompass a wide variety of length scales, from the dimers to ~30-mers to nanometre and even micrometre scale, implying a continuous distribution throughout this range. Larger amorphous aggregates are seed points of spontaneous or laser-induced crystal nucleation. We have provided a novel perspective of crystal nucleation and non-classical pathways where barrierless nucleation of amorphous aggregates is followed by nucleation of crystals inside the solute-enriched aggregates. We will also discuss the dynamical behaviour of these amorphous clusters with the help of fluorescence dyes. The fluorescence response of the dyes will hint towards the solute-solvent structuring in the clusters and selective segregation of the solutes leading to crystal seed formation.

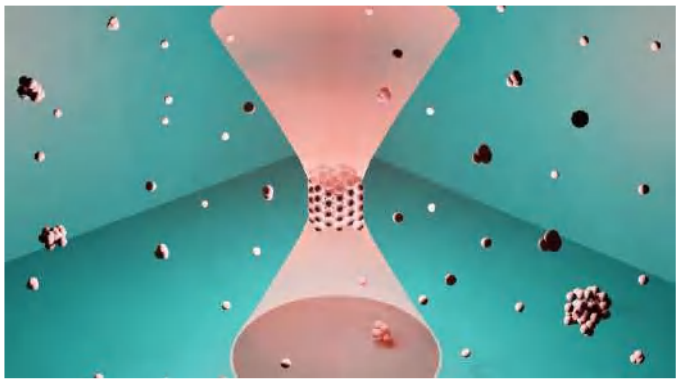

Figure: Amorphous aggregates are present in a wide distribution of sizes in the supersaturated solution of amino acids and peptides, adapted from Ref. 2.

References:

1. D. Gebauer et al., *Chem. Soc. Rev.*, 2014, **43**, 2348.
2. Z. Liao et al., *Chem. Sci.*, 2024, **15**, 12420.

P 12

Cholesterol and small biomolecules affect membrane properties and cellular signaling.

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Abstract

Exocytosis is crucial for neuronal communication, which involves membrane remodeling. Therefore, the biophysical properties of lipid bilayers may influence this process. Cholesterol, a lipid found in both secretory vesicles and cell plasma membranes, changes lipid bilayer properties and therefore has the potential to influence this process. Here, we investigate how changes in membrane cholesterol levels regulate exocytosis in both artificial membranes and in living cells.

We demonstrate that increasing cholesterol fraction from 0 to 50 mole % of the lipid in a mixed lipid membrane modulates membrane properties. Membrane order measurements performed with fluorescence lifetime and spectral shift of indicator dyes (e.g. Flip-tR, Nile Red and Prodan respectively) show an increase of membrane order with increasing cholesterol, as may be expected. However, in an apparent contradiction, these changes do not correlate with the lowering of the force required to indent the membrane with an AFM tip. Significantly, the AFM indentation force measurements correlate with an increase in the fusion kinetics of vesicles to artificial lipid bilayers, measured using a Total Internal Reflection Fluorescence Microscope. This suggests that membrane indentation force can act as a robust assay for vesicle fusion propensity, and membrane order measurement techniques can be misleading in this respect.

We then tested the biological significance of this effect. In a live cell, the fusion machinery may override any impact that the changes in membrane properties may have on vesicle fusion. However, on using mβCD to reduce cholesterol from cellular membranes, we observe that vesicular exocytosis indeed slows down with the reduction of cholesterol. Given the ubiquitous use of cholesterol-lowering drugs by the aging population, this may have clinical implications. We have also examined the effect of some small signaling molecules (related to serotonin) on membrane properties and vesicular fusion since serotonin has already been shown to affect the membrane (1,2). N-acetyl serotonin (NAS) and 5-hydroxy tryptophan (5HTP) are two examples. Our goal is to identify the specific characteristic of these tiny molecules that most strongly influence how they interact with membranes. Using Indentation force measurements and measurements of vesicle fusion and exocytosis using TIRF microscopy, we find that 10 mM of NAS, but not 5HTP, increases the rate of endocytosis in a neuronal cell line by 36%. This is likely a hitherto unknown accessory pathway for neuromodulation. These findings can potentially pave the way for the discovery of small molecules, which can be used to alter membrane properties and therefore influence exocytosis without interacting with any receptors.

References:

1. S. Dey et al. *Chem. Eur. J*., **2021**, *27*, 1
2. D. S. Roy et al. *bioRxiv*, **2024**.01. 20.576155

P 13

Protein function modulation via decoy peptides

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A rationally designed drug molecule has to recognize the protein of interest, bind to it with high affinity, and modulate its function. Developing such a drug is a laborious, time-consuming, and resource-intensive process. We propose a novel strategy using 'decoy-peptides'[1] which provides an efficient shortcut. Decoy-peptides are separate near-identical copies of fragments of the target protein. They can modulate protein function based on their folding topology. We have demonstrated the strategy using a fluorescent protein EGFP. The decoy peptides of EGFP showed no effect on the fluorescence activity of folded EGFP but the presence of decoy-peptides during the folding process of EGFP strongly reduced its fluorescence. FCS and TCSPC measurements indicated the binary nature of fluorescence inhibition upon decoy peptide interaction. The reduction of fluorescence was much less when the sequence of the decoy-peptide was randomized, demonstrating its specificity. The efficacy of this strategy in the biological context was evaluated using an *in vitro* translation assay. We show that the GFP fluorescence can be silenced when the decoy is present during the ribosomal synthesis of the protein. Further, we applied this strategy to inhibit a critical protein of pathogenic bacteria, ß-barrel assembly machinery A (BamA). The decoy is strongly toxic to the bacteria, showing a potential route to antibiotics[2]. Using in vitro assays, we show that BamA decoys can directly inhibit the assembly of BamA client proteins, even in the absence of BamA. In conclusion, decoy-peptides provide a promising new pathway for drug discovery.

References:

1. A. Das et al. *J. Am. Chem. Soc.* **2021**, *143*, 18766.
2. Xu et al. *Molecules.* **2023**, *28*, 3758.

P 14

Unravelling the Phototherapeutic Potential of Pristine 2D Borophene Towards Destruction of Bacteria and Biofilms

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The increasing prevalence of bacterial infections and biofilm formation on clinical wounds pose a significant global health challenge. In this regard, light-mediated phototherapy in the near-infrared (NIR) region has emerged as a promising, non-invasive solution against the surgical modalities. However, conventional dyes used in phototherapy suffer from rapid photobleaching and are primarily activated in the UV-visible range, limiting their clinical utility. Herein, we demonstrate the potential of borophene nanosheets as an exceptional phototherapeutic nanoagent with a photothermal (PTT) conversion efficiencies of 32% at 808 nm (NIR-I) and 26.3% at 1064 nm (NIR-II), along with robust photodynamic (PDT) capabilities across all three NIR windows. Notably, borophene's ability to inhibit biofilm formation and eradicate mature biofilms offers a groundbreaking approach to manage biofilm-associated infections, a critical issue in clinical settings. Our results elucidate the possible mechanisms of borophene-bacterial/biofilm interaction involved in efficient elimination. Furthermore, in vivo studies using a zebrafish model showed that borophene exhibited minimal toxicity, suggesting strong potential for safe clinical translation. This research highlights the potential for eliminating the need for surgical interventions and providing a robust, non-invasive approach to infection control, borophene could pave the way for advanced antimicrobial clinical devices. Our findings provide a strong foundation for future development of borophene composites and hybrids, positioning them as a versatile tool in combating bacterial infections in medical environments.

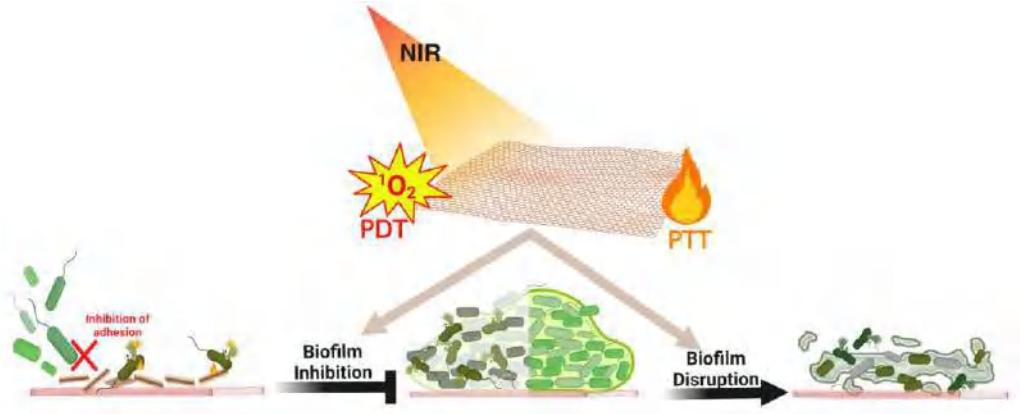

Figure 1: Schematic illustration of the phototherapeutic property of borophene for biofilm inhibition and disruption upon NIR photon irradiation.

References:

1. Ling Xiao et al. *ACS Nano* **2011,** 5,
2. Yu Cheng et al. *ACS Langmuir* **2010**, *26*, 4.
3. Allie Clinton el al. *Laboratory Medicine, 46,* 277.

P 15

Probing the Modulation in Condensate Material Properties using Fluorescence Anisotropy-based HomoFRET Imaging

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Biomolecular condensation into non-canonical, membrane-less organelles has gained prominence as a critical phenomenon in orchestrating complex cellular biochemistry and spatiotemporal organization across organisms. These regulatable supramolecular assemblies are thought to form via a multitude of transient, intermolecular forces that sequester intrinsically disordered proteins and other biomolecules into non-stoichiometric, liquid-like compartments. However, unregulated formation and abnormal maturation of these dynamic, reversible assemblies into irreversible gel-like or solid-like aggregates is implicated in the pathology of a range of neurodegenerative diseases. Thus, the pathophysiological functions of these cellular assemblies are governed by their material properties, necessitating the development of novel tools and methodologies for the detection of altered condensate properties in response to various small molecule regulators and post-translational modifications. Utilizing homo-Förster Resonance Energy Transfer (homoFRET) imaging, we capture the changes in molecular packing within the biomolecular condensates of an archetypical phase separating protein Fused in Sarcoma (FUS). Our single-droplet anisotropy-based homoFRET measurements within the condensates of fluorescently-tagged FUS shed light on the changes in the nanoscale architecture and the resulting modulation in droplet properties upon RNA, ATP, and post-translational methylation. We further extend this in vitro application of homoFRET imaging to probe intracellular phase transitions of nuclear and cytoplasmic FUS within mammalian cell lines.

References:

1. A. Joshi*, A. Walimbe* et. al. *bioRxiv*, **2024** (*Contributed equally) (Accepted in *Nat. Commun.* 2024).

P 16

Aggregation in Deep Eutectic Solvents (DESs): Formation of Polar DES-in-Nonpolar DES Microemulsions

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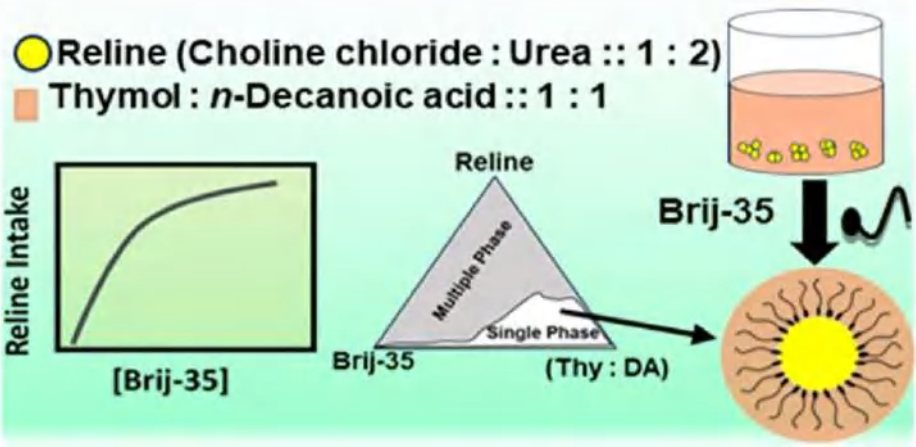
The versatility of environmentally-benign and inexpensive deep eutectic solvents (DESs) lies in their widely varying physicochemical properties. Depending on its constituents, a DES may be highly polar or highly nonpolar in nature. This offers enticing possibility of formation of novel nonaqueous microemulsions (MEs). Evidence of the presence of polar DES-in-nonpolar DES MEs is presented with reline (formed by mixing choline chloride and urea in 1 : 2 T single-phase region where MEs may be forming. Dynamic light scattering (DLS) confirms the presence of MEs of 2 - 10 nm size. Even as up to 2.5 M (ca. 0.35 mole fraction) reline, whose dynamic viscosity (η) and electrical conductivity (κ) are very high, is added to 100 mM Brij-35 solution of Thy : DA, the η and κ of the solution increases insignificantly thus conforming formation of MEs in the solution. FTIR absorbance spectra and fluorescence probe responses further indicate that reline is not dispersed in the medium, it rather forms polar pools of the MEs. These novel nonaqueous polar DES-in-nonpolar DES MEs will not only expand application potential of DESs, they also offer new class of organized media with widespread potential.



References:

1. Van Horn et. al. *J. Am. Chem. Soc.* **2009**, 8030.
2. Baker et. al. *Chem Commun* **2003**, 2932.
3. Correa et. al. *Chem. Rev.* **2012**, 4569.
4. Eastoe et. al. *J. Am. Chem. Soc.* **2005**, 7302.

P 17

Understanding Htt Protein Aggregation in Cell Mimicking Environments

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The intracellular milieu, characterized by molecular intricacy and crowding, heavily relies on proteins as fundamental biological entities. Approximately one-third of the human proteome comprises intrinsically disordered proteins (IDPs). Huntington's disease (HD) arises from the aggregation of mutant Huntington protein (HD39Q), which is one such IDP containing 39 glutamine repeats. This investigation explores the effects of macromolecular crowders—Dextran, Ficoll, and polyethylene glycol (PEG)—on the aggregation process of HD39Q. Using fluorescence-based assays, including concentration-dependent Thioflavin T (ThT) fluorescence, we analyzed the kinetics of protein aggregation, observing distinct influences of each crowder and binary mixtures of crowders on the aggregation mechanism. Nano-particle Tracking Analyzer (NTA) studies further confirmed the modulation of aggregation kinetics by different crowders, showing a reduction in smaller oligomers and a concurrent increase in larger aggregates over time. Fluorescence correlation spectroscopy (FCS) studies, performed subsequently, revealed dynamic changes in oligomer diffusion times and hydrodynamic radii, providing insights into the early stages of aggregation. Confocal microscopy confirmed that different crowders significantly affect the morphology of the resulting aggregates, with fibrillar structures forming more rapidly in crowded environments. The use of BODIPY TMR-labeled HD39Q, along with crowders in various combinations, further unveiled crucial insights into liquid-liquid phase separation (LLPS) and its role in modulating aggregation within distinct phases formed by crowder mixtures. This study highlights the critical role of macromolecular crowding in modulating both the kinetics and morphology of Huntington protein aggregation.

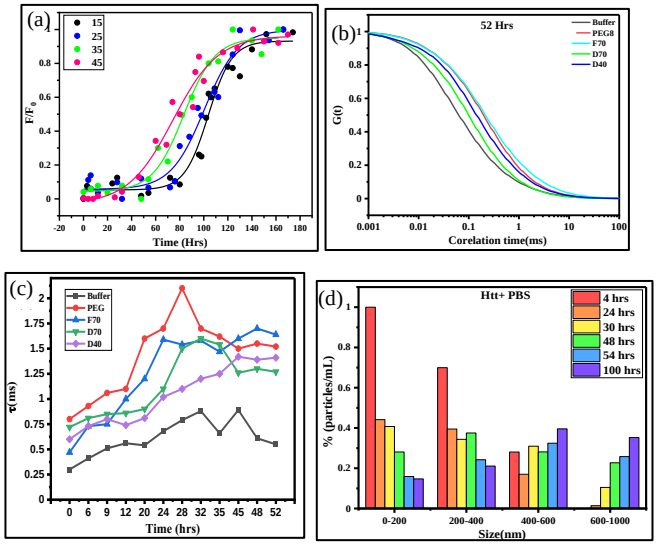

Figure: (a) Th-T intensity overlap (F/Fo) vs time for different concentrations of Htt protein (b) Fluorescence correlation curves shift to longer lag times indicating the increase in the size of the aggregates (c) Change in diffusion time monitored as a function of incubation time (d) Changes in hydrodynamic radii as observed with NTA

References:

1. Larissa A. Munishkina et. al. *Biochem*, **2008**, 47, 8993.

P 18

Quantification of forces involved in cytoplasmic streaming in plants and their temperature dependence

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Cytoplasmic streaming is a phenomenon observed in plants where the circulation of cellular components occur around the central vacuole. It is crucial for the spatio-temporal distribution of organelles in plant cells and thus has an important role in plant growth. Myosin XI, a plant motor protein has been implicated in the transport of organelles during cytoplasmic streaming. Myosins are a family of ATP-dependent motor proteins that move on actin filaments. Although cytoplasmic streaming in plants has been widely studied, the forces involved in this phenomenon remain to be quantified. We have performed in vivo optical trapping in the model organism of our choice-onion cells and measured the forces involved in transporting organelles during cytoplasmic streaming. Cytoplasmic streaming has been previously shown to increase with temperature in plants, but the cause of this dependence is not known. In our study, we have measured the forces involved in this phenomenon at different temperatures in order to study the correlation of myosin force generation and temperature dependent increase of cytoplasmic streaming. It has been found that enhanced cytoplasmic streaming yielded higher growth of plants and better foliage. Understanding how this phenomenon is regulated can help us to develop plants that are able to utilize nutrients more efficiently, thus increasing their yield and survival.

References:

1. M. Tominaga et. al. *Curr Opin Plant Biol*, **2015**, *27*, 104.
2. M. Tominaga et. al. *EMBO J*, **2003,** 22, 1263.

P 19

Elucidation of the excited state dynamics of organoboron fluorophores from ortho-substituted phenolic Schiff bases

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Conformational relaxation of the flexible molecular skeleton and photoisomerization along C=N bond as major non-radiative pathways in solutions of a family of Schiff.[1-3] Chelation of metal ions, especially Al^{3+} and Zn^{2+} by these Schiff bases hinders such relaxation, leading to significant fluorescence enhancement, which is favoured in rigid microenvionments[1,4] The better performance of Al^{3+} than Zn^{2+}in this context prompts the quest for other elements, that may be even more efficient. Boron is a natural first choice, belonging to the same group as Al. In the present work, photophysical properties of three organoboron complexes, two of 2-((2-hydroxybenzylidene)amino)phenol (HBAP) and one of 2,2′-((butane-1,4-diylbis(azaneylylidene))bis(methaneylylidene))diphenol (salbn). are investigated. The organoboron complexes of HBAP, OB1 and OB2 are hardly fluorescent in non-viscous solvents, but exhibit fluorescence enhancement in glycerol implying that the effect of chelation is offset by the introduction of new nonradiative channels by the phenyl group, which is a rotor. However, OB3, the complex of salbn, which is devoid of such a rotor group, is found to be strongly emissive and exhibit room temperature phosphorescence.

References:

1. S. Dasgupta et al. *Phys. Chem. Chem. Phys.* **2021**, *23*, 19494.
2. A. Chowdhury et al. *Chem. Phys. Impact* **2021**, *3*, 100057.
3. J. Dobkowski et al. *Chemistry - A European Journal* **2015**, *21*, 1312.
4. T. Khan et al. *J. Phys. Chem. B* **2016**, *120*, 10319.

P 20

Thermally Activated Delayed Fluorescent Probe for Simultaneous Imaging of Lipid droplets and Endoplasmic reticulum

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Organelles, the essential subunits within eukaryotic cells, are crucial for carrying out various complex biological functions to sustain life.[1,2] Multiple organelle-specific tracker dyes are used to visualize organelles and elucidate their interactions. However, issues such as significant spectral cross-talk, high cytotoxicity, and complicated operational procedures often limit their effectiveness in live-cell imaging.[3] Thus, developing single fluorescent probe capable of simultaneously and selectively visualizing two organelles and their interactions can be a unique approach reducing the overall incubation time which in turn reduces the cytotoxic effects and spectral crosstalk. In this regard, despite this necessity, only a few probes or effective design strategies have been reported so far.[4] Conventional luminescence imaging may suffer from the autofluorescence from the surroundings. In this context, organic thermally activated delayed fluorescent probes could eliminate this shortcoming due to their long-lived emission properties.[5] Herein, we have developed a delayed fluorescent probe DCBPY having diphenylamine as the donor unit and ketocoumarin as the acceptor unit. DCBPY displayed delayed fluorescence properties due to a minimal singlet-triplet energy gap, as observed from the time-resolved spectroscopic studies. The aqueous dispersions of DCBPY nanoparticles displayed enhanced delayed fluorescence properties and a higher quantum yield (15%), and was employed for intracellular imaging. The self-assembled nanoparticles localised within lipid droplets (LDs) and endoplasmic reticulum (ER) simultaneously as confirmed from the colocalization studies with the commercially available tracker dyes. Further, we aim to study the inter-organelle interaction between ER and lipid droplets in luminescence as well as in time-resolved mode due to their close association in diverse physiological pathways.

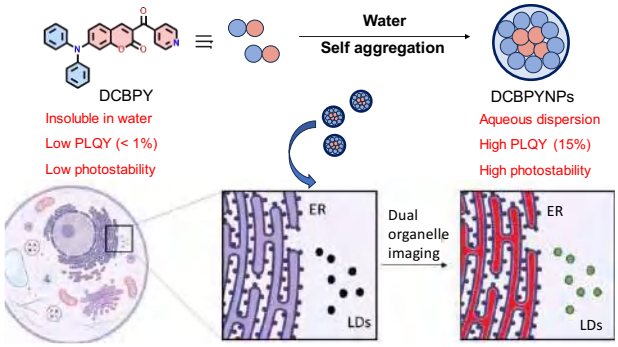

Figure 1: Schematic illustration depicting the fabrication of self-assembled nanoparticles of thermally activated delayed fluorescent probe DCBPY and its application as a dual organelle targeting probe in live cells.

References:

1. Gao et. al. *Chem. Sci*, **2019**,*10*, 6035
2. Hao Zhu et. al. *Acc. Chem. Res*, **2016**, *49*, 2115
3. Jaiswal et. al. *J. Mater. Chem. C*, **2022**, *10*, 6141
4. Guo et. al. *J. Am. Chem. Soc*, **2021**, *143*, 3169
5. Das et. al. *Chem. Sci*, **2024**, *15*, 102

P 21

Photoactivated plasmonic nanohybrid fibers with prolonged trapping of excited charge carriers for SERS analysis of biomolecules

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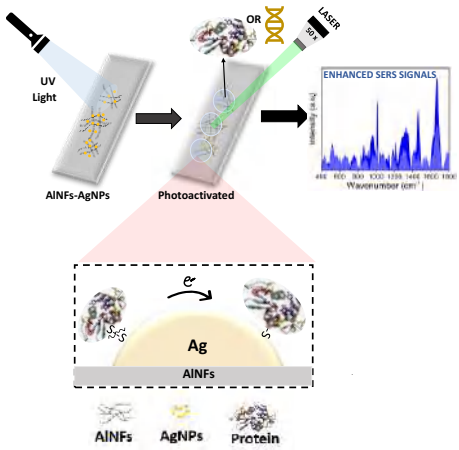
The pursuit of enhancing Raman spectroscopic signals through the strategic design of plasmonic substrates has facilitated the identification and characterization of pharmaceutically significant molecules with low-scattering cross-sections, including amino acids and proteins. This advancement has made significant strides in the realm of biomedical sciences. This study introduces a straightforward approach to fabricating silver nanoparticles-incorporated alumina nanofibers (Ag–AlNFs) using controlled microwave synthesis. The aim is to amplify the surface-enhanced Raman chemical enhancement factor by inducing photo-induced charge accumulation at the plasmonic–dielectric interface. The plasmonic–dielectric fibers effectively trap charge carriers, as evidenced by ultrafast transient absorption spectroscopy studies. Beyond chemical enhancement, the augmented electronic surface charge facilitates the capture of protein disulfide bonds by these electrons, forming a transient disulfide electron adduct radical that transforms into a free thiol radical upon dissociation. This mechanism enables protein molecules to bind to the nanoparticle's surface via a favorable silver thiol bond, resulting in enhanced surface affinity and greater SERS enhancement. The proposed Ag–AlNFs offer a cost-effective material that holds the potential for label-free probing of biological systems by photoactivating the SERS substrate to achieve higher enhancement factors.



Scheme: Schematic for the enhanced chemical enhancement in protein Raman signals after shining UV light on Ag-AlNFs substrate

References:

1. S. Ben-Jaber et. al. *Nat Commun*, **2016**, 7, 12189.
2. Q. D. Mai et. al. *ACS Appl. Nano Mater*, **2022**, 5, 15518.

P 22

Pyrediyne Quantum Dots (PDYQDs) in gel

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Quantum dots (QD) are zero-dimensional semiconductor nanocrystals that has distinct mechanical, catalytical, electrical and optical properties when compared to their corresponding bulk materials. The unique properties of the traditional semi-conductor based quantum dots were analysed in different media and exploited in practical applications such as solar cells, light-emitting devices (LEDs), computer tomography, magnetic resonance imaging, bio-imaging and etc.[1] However, cytotoxicity, low thermal stability and presence of surface defects, limits the applications of traditional semiconductor quantum dots.[2] Even though a core-shell nanostructure can reduce surface traps and consequently the blinking effect, widely used core-shell QDs contains cytotoxic moieties such as cadmium and selenide.[3] To overcome these flaws, PDYQDs, the newest member of the carbon quantum dots family was synthesized. Unlike its bulk counter-part, PDYQDs exhibited good photoluminescence properties such as appreciable quantum yield, high fluorescence lifetime and solvent sensitive emission behaviour. Photophysical properties of QDs can be enhanced further by incorporation of functional groups, structural modification and immobilization of quantum dots in a gel medium. Thus, in the current study PDYQDs (figure 1a) were incorporated into a transparent, thermo-reversible leucine based organogel (OG-leucine) (figure 1b) and the photophysical properties of PDYQDs in gel media were analysed using steady-state and time-resolved fluorescence measurements. OG-Leucine gelator and PDYQDs were synthesized according to a reported literature protocol.[4,5] As shown in the figure 1c, PDYQDs exhibits excitation wavelength dependent emission behaviour in ethanol due to the presence of QDs with varied size and surface defects.[4] The emission peaks at 410 to 430 nm are the characteristics of the monomeric precursor of the PDYQDs, tetraethynyl pyrene (TEP). The longer wavelength emission peaks are observed as a result of QDs with different sizes and formation of static dimers.[4] However, PDYQDs did not exhibit excitation wavelength dependent emission properties and the longer wavelength emission peaks are diminished in gel media (figure 1d). The emission spectra of the PDYQDs resembles the emission spectra of PDYQDs in toluene (figure 1e) which implies that the QDs resides in the toluene pool of the gel, the uniformity of QDs size is enhanced, the formation of static dimers was restricted in gel media. It is evident from the current study that incorporating pyrediyne quantum dots in gel media aided in diminishing the non-uniformity in QDs particle size. The methodology can be extended in future to obtain size-controlled defect free QDs with narrow emission bandwidth which can be used for various applications.

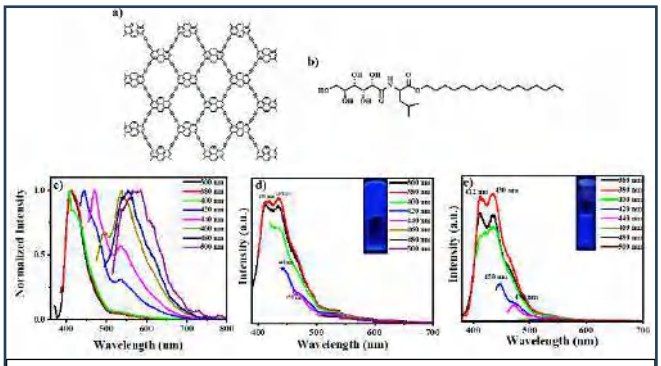

Figure 1. Structure of PDYQDs (a) and OG-leucine (b), emission spectra of PDYQDs in ethanol (c), toluene (d) and (e) at different excitation wavelengths.

References:

1. D. Bera et. al. *Mater,* **2021**, 4, 2260.
2. H. Moon et. al. *Adv. Mater*, **2019**, 34, 1804294.
3. A. M. Derfus et. al. *Nano Lett,* **2004**, 1, 11.
4. J. S. Arya et. al. *Adv. Mater.Interfaces,* **2020**, 15, 1902209.
5. N. Minakuchi et. al. *Langmuir,* **2012**, 25, 9259.

P 23

Carbon Quantum Dots: A Promising Factor for Hydrophobic Drug Delivery and Live Cell Imaging

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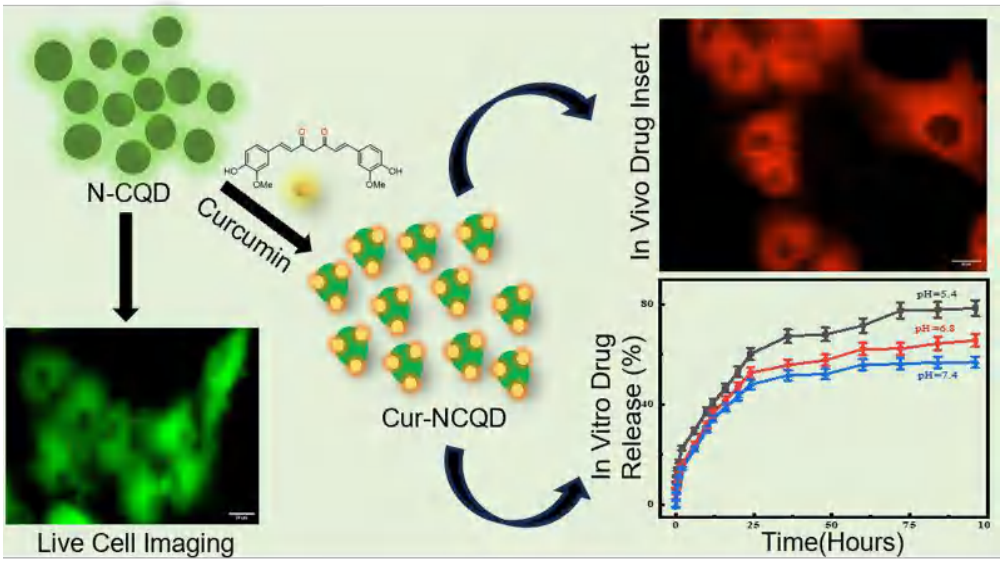
Due to unique physicochemical properties like excellent biocompatibility, photostability, and surface functionality, Carbon Quantum Dots (CQDs) nowadays emerged as a promising nanomaterial for the delivery of hydrophobic drugs and for live cell imaging.[1] My study investigates the potential of N-doped CQDs (N-CQDs) as carriers for hydrophobic drug molecules such as Curcumin, which alone often face challenges like bioavailability and efficacy due to its chemical instability, poor absorption, and rapid systematic elimination.[2] The N-CQDs produced in this study exhibit excellent water solubility, remarkable stability, and high biocompatibility. By functionalizing N-CQDs with hydrophobic drugs, the nanocarriers significantly improve drug solubility, enhance cellular uptake, and allow controlled release within targeted cells.[3] Simultaneously, the intrinsic fluorescence of N-CQDs and cur-NCQs enables high-resolution imaging of live cells. Experimental findings on live cells demonstrate the efficacy of N-CQDs in overcoming the solubility limitations of hydrophobic drugs within the live cancer cell without inducing cytotoxicity. This dual function of N-CQDs in both drug delivery and live cell imaging can make it as a versatile platform for advancing therapeutic delivery systems and diagnostics in nanomedicine.[4]

Graphical



References:

1. Jana et. al. *Mater. Today Commun*, **2022,** *32*, 104068.
2. Sohn et. al. *Pharmaceutics*, **2021,** *13* (12), 2102.
3. Guo et. al. N*anomaterials*, **2024,** *14* (13), 1088.
4. Rong et. al. *Int J Nanomedicine.*, **2021**, 16, 7195.

P 24

Effect of commercial fertilizers in the growth of microgreens: Through the Terahertz lens

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Microgreens have become increasingly popular as a nutrient-rich food source, requiring only 7-14 days to harvest, with the global market expected to grow at a CAGR of 11% to USD 2.6 billion in 2031 from 2022 [1]. With seeds readily available in household kitchens and accessible growth media such as water, tissue-paper, coir, soil, microgreens are being widely cultivated in kitchen gardens due to their short shelf lives and cost effectiveness. Terahertz (THz) radiation, spanning the 0.1 to 10 THz, has shown promise in agriculture due to its low ionization energy, which is harmless to plants, its high sensitivity to substances with unique spectral fingerprints, and its absorption properties in the presence of polar molecules like water.

This work, therefore, investigates the growth dynamics of various microgreens, with and without the application of commercially available fertilizers containing nitrogen, phosphorus, and potassium, using a THz time-domain imaging system in a transmission mode to monitor their growth in terms of girth and length of primary roots. The microgreens examined include chia (*Salvia hispanica*), mustard (brown, *Brassica juncea*; yellow, *Sinapis alba*), fenugreek (*Trigonella foenum-graecum*), and red lentils (*Lens culinaris*). Preliminary results indicate that THz imaging effectively distinguishes the growth patterns of plants in different media, proving to be an effecting tool in the growth and health monitoring of microgreens. Future studies will extend this research to explore the role of THz in evaluating the impact of nutrient variation and fertilizer dosage on the maturity and development of microgreens, aiming for a detailed analysis.

References:

1. Straits Research. *Microgreens market analysis, growth, forecast to 2030* (Report Code: SRFB1654AB). Straits Research. (**2023, December 15**). https://straitsresearch.com/report/microgreens-market (Accessed: September 8, 2024)

P 25

Deciphering effect of having different branch lengths in Gold nanostars in their ability to avoid sequestration by protein-corona

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Gold nanoparticles (AuNPs) have a strong potential to be used as nanocarriers for diagnosis and therapeutics due to their biocompatibility, stability, and robust surface chemistry [1,2]. The aptamer-loaded AuNPs, for example, have emerged as promising nanoconstructs targeting specific receptors on the cancer cell membrane. These nanoconstructs, however, need to overcome the sequestration effect caused by the non-specific adsorption of serum proteins (i.e., formation of protein-corona) on their surface to efficiently target specific receptors involving NP-surface ligands [3]. Therefore, designing AuNPs that can circumvent the effect of protein corona to target cancer cells effectively is crucial. Several recent studies have indicated that anisotropic gold nanostars (AuNS) offer benefits over spherical gold nanoconstructs in projecting out the functional moieties, even in the presence of protein corona [3-5]. Anisotropic AuNS particles have branches of different lengths that taper to form sharp tips. These sharp branches likely play a key role in reducing the impact of the protein corona. To determine the best designs, it is essential to evaluate how different branch lengths influence the receptor-targeting efficiency of AuNS. However, performing this comparative analysis is challenging as the seedless synthesis of anisotropic AuNS results in a diverse mixture of star-shaped nanoparticles with uneven branch lengths and quantities. In this study, we have successfully synthesized AuNS with precise control over their branch lengths and branch numbers. We then functionalized them with DNA aptamer molecules capable of targeting Human Epidermal growth factor Receptor 2 (HER2) on cancer cell membranes to trigger apoptosis. TEM imaging clearly showed that the longer branches in AuNS were efficiently protruding out of the protein corona. The *in vitro* cytotoxic efficacies of these AuNS were then compared with that of anisotropic AuNS and spherical AuNPs, which clearly showed that the nanoconstructs with longer branches were significantly more efficient in causing cytotoxicity in HER2 overexpressing SKOV3 cells. These AuNPs, when functionalized with a control aptamer (with no HER2 specificity), did not cause any cytotoxic effect, as expected. This research illuminates the complex interplay between the branch lengths of AuNS and their effectiveness in targeted drug delivery, providing valuable insights for developing more efficient nanomedicines in the future.

References:

1. A. E. Nel et. al. *Nat Mater*, **2009**, 8, 543.
2. P. Kumthekar et. al. *J Clin Oncol*, **2019**, 37, 3012.
3. D. Bhowmik et. al. *ACS Nano*, **2019**, 13, 13637.
4. P. Choo et. al. *J Am Chem Soc*, **2021**, 143, 4550.
5. B. Diloknawarit et. al. *ACS Nano*, **2024**, 18, 12537.

P 26

Enzyme-free Detection of Uric Acid using Fluorescent Carbon Quantum Dots

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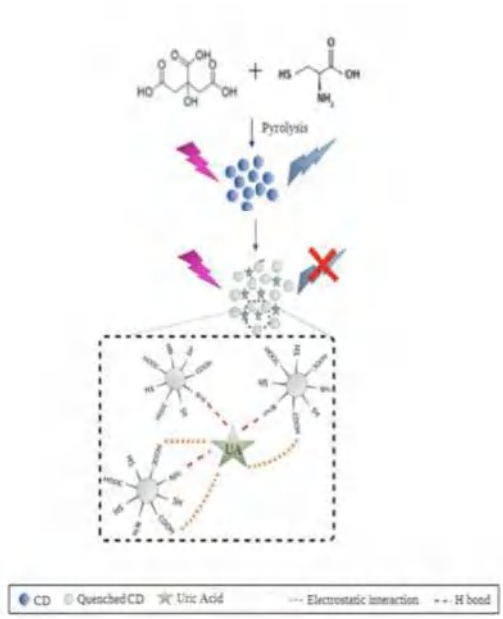
Since uric acid (UA) is involved in several metabolic pathways, abnormal levels of it in the human body is indicative of disease conditions like gout, Alzheimer's, multiple sclerosis, and renal failure, among others [1]. It also plays a role in pro-inflammatory pathways [2]. The conventional and standard methods for UA detection utilise enzymes, which poses difficulties in remote areas and point-of-care applications, due to the specific transport and storage conditions they require. This work focuses on developing carbon quantum dots (CQDs) that can detect the target analyte without enzymatic reaction through a fluorescence-based detection approach. We synthesised blue emitting fluorescent CQDs following one-pot pyrolysis method, further characterization of the CQDs were done with spectroscopy and microscopy. Upon analysis with UA-CQDs, we found an interesting fluorescent behaviour that showed fluorescence quenching at 390nm excitation wavelengths. The limit of detection (LOD) obtained was 96.32µM for UA. The observed finding suggests a potential application of the synthesised CQDs in developing an optical-based UA biosensor, without enzymes.



References:

1. Youssef M. Roman. *J. Pers. Med*, **2023**, *13*, 1409.
2. Mihai-Emil Gherghina et. al. *Int J Mol Sci*, **2022**, *23*, 3188.

P 27

Novel Lipid Corona Formation around Nanoparticles

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While protein corona formation is well-known, the "lipid corona" is relatively new and its dependency on lipid property and stability is yet to be explored. At first, we reported a novel lipid corona formation and its underlying mechanism using aromatic amino acid-functionalized gold nanoparticles (Au-AA NPs). Our study demonstrated that in the presence of high lipid concentration, the Au-AA NPs intrinsically tow the lipid molecules from the lipid vesicles and decorate themselves by lipid leading to unique lipid corona formation. In contrast, at low lipid concentration, the Au-AA NPs underwent lipid-induced aggregation. Significantly, we found that the colloidal property of these lipid-coated nanoparticles (lipid corona) was immune to resist extreme harsh conditions, that is, high acidic pH, several repetitive freeze–thaw cycles, and high salt concentration. Next, we investigated the effect of lipid phase states on lipid corona formation and lipid-induced aggregation of phenylalanine-functionalized gold NPs (Au-Phe NPs). Based on the stability, for the first time, we classify lipid corona as "hard lipid corona" and "soft lipid corona".

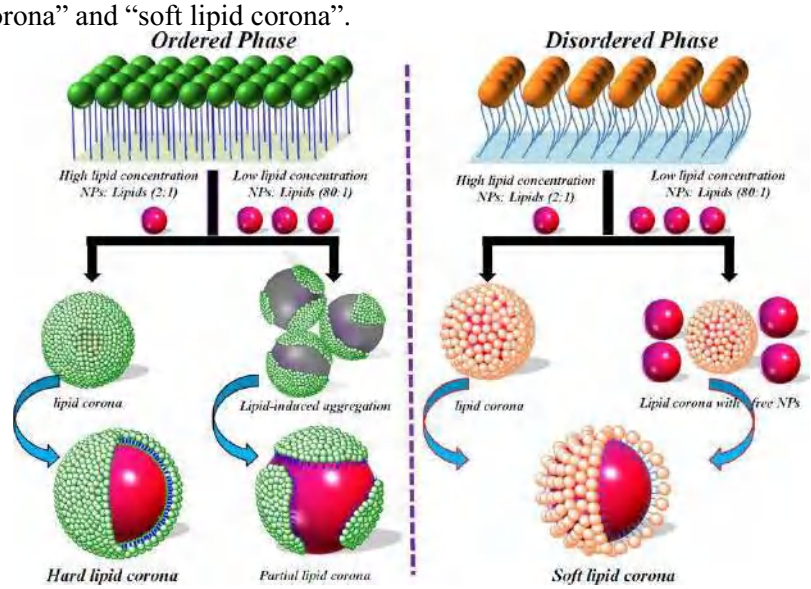

***Figure**: Schematic representation of lipid corona formation around nanoparticles in presence of different concentration and phase state lipid vesicles.*

References:

1. Avijit Maity et al. *J. Phys. Chem. B,* **2021**, *125,* 2113.
2. Avijit Maity et al. *J. Phys. Chem. B,* **2022**, *126,* 2241.

P 28

Development of A Novel Mitochondria Targeted BODIPY-Based Macrocyclic Ratiometric Fluorescence Sensor for Mn^{2+} ions

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Mn^{2+} ions play crucial roles in living systems including regulating immune response, brain function, metabolism, defence against oxidants, and photosynthesis [1]. Thus, tracking this essential metal ion is important to elucidate the *in cellulo* localization and dynamics of Mn^{2+} ions both under physiological conditions and pathological disorders. However, poor binding affinity of Mn^{2+} ions to ligand-scaffolds and fluorescence quenching by the metal ion leads to turn-off sensors that are not applicable for in vivo imaging. Datta and co-workers have reported boron-dipyrromethene (BODIPY)-based penta-aza macrocyclic "turn-on" fluorescent sensor **M1**, and a water soluble, cell permeable sensor **M4** to visualise the manganese dynamics in live mammalian cells [2,3]. The next step in Mn^{2+} sensing is taking these probes to the intra-organelle level to track the dynamics of Mn^{2+} ions within cellular compartments. We are developing a novel, water-soluble, reversible, Mn^{2+} ion selective ratiometric fluorescent sensor, **A2**, which targets the mitochondria of living cells. The design, synthesis, and characterization of the probe will be presented.

References:

1. K. Barnese et al. *Proc. Natl. Acad. Sci. U.S.A*, **2012**, *109*, 6892.
2. S. Bakthavatsalam et al. *Chem. Commun*, **2015**, *51*, 2605.
3. S. Kahali and S. K. Das et al. *Chem. Sci*, **2024**, *15*, 10753.

P 29

Photocatalytic Oxidation of Organic Sulfides inside Water-soluble Nanocages

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Aromatic sulfoxides are very important building blocks for the synthesis of fragrances, and pharmaceutical ingredients[1]. Traditionally the synthesis of sulfoxides generally requires stoichiometric use of oxidizing agents (H_2O_2), metal oxides, and photosensitizers[2]. Although they give moderate to good yield in reaction turnovers but the often associated with the formation of toxic sulfone as side product and use of transition metal-based catalyst and organic solvent generate toxic wastes. To address these challenges and to synthesize aromatic sulfoxides, we have chosen to synthesize a supramolecular cage with a capping ligand. We focused into utilizing the donor-acceptor CT interactions formed between electron-rich aromatic sulfide molecules and electron-deficient cavities. The cavity synthesis was done in three steps as per the literature protocol[3]. We optimized the reaction condition for various substrates with achiral nanocage and after optimization of the photocatalytic reactions, we found the selectivity of sulfoxide products going up to 100 % from GCMS with TON reaching up to 50. We wanted to know about excited state dynamics of this reaction for that we probed the formation of radical cations on the sulfides[4] and anion radicals on the triazine panel[5] from transient absorption measurements that suggest a single electron transfer event.

References:

1. X. Yin. *Organics*, **2023**, 4, 173
2. E. Skolia. *Chempluschem*, **2022**, 87
3. Y. Nishioka. *J. Am. Chem. Soc*, **2008**, 130, 8160
4. S. Tojo. *RSC Adv*, **2016**, 6, 109334
5. Y. Furutani. *J. Am. Chem. Soc*, **2009**, 131, 4764

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Biophysics and Spectroscopy in Tandem: Investigating Structural Stability And New Functionalities Of YedX

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Functional amyloids are a special class of amyloidogenic proteins that serve an important biological function, unlike disease-related amyloids [1]. One such bacterial functional amyloid is curli, produced by *Escherichia coli* during biofilm biogenesis. Biofilm is a complex, well-structured bacterial community that adheres to various surfaces and is encased in a self-produced extracellular matrix (ECM). ECM is majorly composed of polysaccharides, lipids, and proteinaceous heteropolymers of curli-specific proteins [2]. The precursors of curli, CsgA and CsgB are produced in cytoplasm as intrinsically disordered proteins (IDPs). CsgA and CsgB travel to periplasmic space and then secreted out in extracellular space where they polymerise and form amyloid fibrils to strengthen the biofilms [2]. A class of molecular chaperones inside the cytoplasm keeps a check on CsgA and CsgB oligomerisation inside the cell to avoid cytotoxicity [3]. In the periplasmic space, another protein belonging to csg operon, CsgC, keeps CgsA and CsgB in natively unfolded structure. CsgC has also been studied to modulate human amyloidogenic proteins such α-synuclein and reported to prevent its aggregation [3]. Recently, CsgC has been reported to possess structure similarity to human transportational protein, transthyretin (TTR) [4]. TTR also has been studied to possess anti-amyloid activity against bacterial amyloids such as CsgA and CsgB as well as human amyloidogenic proteins such as Aβ [5]. Transthyretin-related proteins (TRPs) are a group of proteins that exhibit sequence and structural similarity to TTR and exist in a wide range of species [6]. TRP from *E. coli*, also known as YedX, is also localized in the cytoplasm and function as hydrolase in purine metabolism [6]. Since TTR and YedX exhibit high structure similarity, we hypothesize that YedX may also have other biological functionalities. In this study, we have utilized various spectroscopy techniques such as UV absorbance and fluorescence spectroscopy and circular dichroism spectroscopy to evaluate the stability of YedX and its anti-amyloid activity. Our biophysical and biochemical studies suggest that YedX modulates the CsgA amyloid assembly and keeps CsgA in its native soluble form. Our findings hypothesize the unrevealed function of YedX, previously known as a hydrolase enzyme, to now we shed light on the possible chaperone-like activity of YedX, which is also present in the cytoplasm and may aid the stable protein homeostasis within the cell.

References:

1. Balistreri et al. *Microorganisms*, **2020**, *8*, 1951.
2. Hung et al. *mBio*, **2013**, *4*(5), e00645-13.
3. Evans et al. Prion, **2011**, 5, 323–334.
4. Jain et al. PNAS, **2017**, 114, 12184.
5. Costa et al. FEBS Letters, **2008**, 582 1873.
6. Eneqvist et al. Eur J Biochem, **2003**, 270, 518.

P 31

Living Biosensors for Selective and Ultrasensitive Detection of Biomarker Nitric Oxide

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Nitric oxide (NO) can be defined as an essential disease marker relevant to numerous chronic inflammatory conditions and malignancies. A fully characterized nitric indicator system is helpful for the prompt advancement of bacterial treatment and synthetic biology. In this work, we designed a group of NO-inducible biosensors with the help of the PnorV promoter and its NorR regulator in the norVW operon and characterized and optimized the circuits in Escherichia coli.

Here we have taken advantage of synthetic biology tools for generating an array of genetically engineered whole cells. Along with this, we describe a set of modular and gain-tunable genetic amplifiers constructed in Escherichia coli that can amplify transcriptional signals with a high control in the cascaded-gene networks. The devices are constructed by using hrpRS, hrpV, and PhrpL which are orthogonal genetic elements from the hrp gene regulatory network in Pseudomonas syringae.

Since whole-cell biosensors (WCBs) have great potential to specifically detect these compounds and provide information about the bioavailability and toxicity of these di-atomic compounds on human health. The combinatorial approach of synthetic and structural biology has enabled us to design “living sensors” with fluorescence outputs. However, initial testing revealed poor detection sensitivity, which was addressed by incorporating transcriptional and translational synthetic control modules. These modules significantly reduced background fluorescence and shifted the sensor response threshold to ~sub nanomolar concentrations, while augmenting the dynamic range.

Quantitative NO estimation directly from whole cells can open new avenues for detecting NO at the cellular level in situ. Through site-directed mutagenesis of the active centre, the activity of the protein can be increased leading to enhanced outputs. It is one of the methods for in-situ detection of such compounds/gases. This will be a low-cost biosensor suitable for on-site monitoring of a range of such compounds.

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The disordered C-terminal region of eukaryotic translation initiation factor 4B dynamically binds RNA

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The eukaryotic translation initiation factor 4B (eIF4B) plays an essential role in translation by enhancing the eIF4A helicase activity in unwinding the structured motifs of mRNA. It is particularly crucial for the translation of mRNAs with long and structured 5'-end untranslated regions, such as those coding for many proto-oncogenes. The eIF4B is predicted to be predominantly disordered, except the folded RRM domain. The disordered C-terminal half of eIF4B (eIF4B-CTR) is essential for RNA binding, as it possesses a non-canonical RNA binding motif, enriched in arginine residues. However, the exact mechanisms of these interactions are currently unknown.

Hence, we characterized the eIF4B-CTR alone and in complex with RNA, employing a combination of single-molecule Förster resonance energy transfer (smFRET), nanosecond fluorescence correlation spectroscopy (nsFCS) and nuclear magnetic resonance (NMR) spectroscopy. Our results show that eIF4B-CTR is disordered and flexible, with significant presence of intrachain interactions [1, 2]. The RNA binding to eIF4B-CTR is highly dynamic, with fast exchange between the molecules forming the complex, and is highly sensitive to ionic strength, implying an electrostatic mechanism of interactions. Upon RNA binding, the N-terminus of eIF4B-CTR compacts, whereas the C terminus expands. After binding to RNA, eIF4B-CTR remains disordered. The interaction of eIF4B-CTR is dependent on the length and sequence of the RNA. The eIF4B-CTR has higher binding affinity for single stranded RNA than double stranded RNA suggesting binding preference to single stranded RNA over structured RNA. Overall, our study provides detailed understanding of the RNA interaction with eIF4B-CTR.

References:

1. S. Mondal et al. Biomol. NMR Assign, **2023.** 17, 199.
2. B. C. Swain et al. bioRxiv, **2024**, 2024.06.21.600094.

P 33

Ion Concentration Effects on Conformational Dynamics of DNA Holliday Junctions: Insights from Fluorescence Correlation Spectroscopy

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DNA Holliday junction (HJ) is a four-way-stranded DNA intermediate that plays a crucial role in genetic recombination. It exists in generally two configurations, i.e., "X-stacked" and "open planar". The conformational changes of HJ depend on the electrostatic interactions of negatively charged chains and salt ions.[1] To enhance the stability of these configurations, Mg^{2+} ions are utilized.[2,3] It has been already reported that at lower ion concentrations HJ exchanges its conformation faster between *iso-I* and *iso-II* whereas at higher concentrations stacked conformation is prominent.[4] In this work, we used fluorescence correlation spectroscopy with different ion concentrations of Mg^{2+} to investigate the diffusion and conformational dynamics on a millisecond time scale.[5] We have found that as the concentration of Mg^{2+} increases the diffusion of DNA HJ decreases. This decrease observed can be due to multiple factors such as increase in the hydrodynamic radius, viscosity of the solution, and ion acting as a crowding agent as it is present in very high concentration. In our study, we have mainly focused on the interactions of DNA HJ phosphate backbone with $Mg^{2+}$and after the binding how hydrodynamic radius increases resulting in slower diffusion. Also, we have observed the conformational dynamic components in µs time scale. There are three components that we assume correspond to three different isomeric conformations.

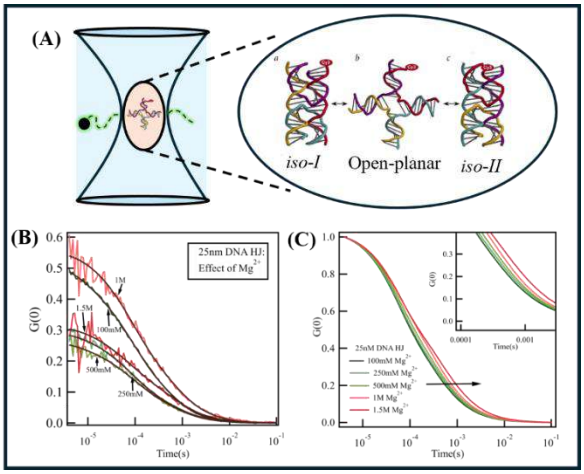

Figure: (A) Diffusion and conformational dynamics of DNA HJ through confocal volume in fluorescence correlation spectroscopy. **(B)** Unnormalized autocorrelation curves of 25 nM DNA HJ in 100 mM, 250 mM, 500 mM, 1 M and 1.5 M of Mg^{2+}. **(C)** Normalised and fitted autocorrelation lines of 25 nM DNA HJ in 100 mM, 250 mM, 500 mM, 1 M and 1.5 M of Mg^{2+}.

References:

1. Qinqin Song et al. *Int. J. Mol Sci*, **2022**, 23, 9730
2. Richard Owczarzy et al. *Biochemistry*, **2008**, 47, 5336
3. Chirlmin Joo et al. *J Mole Biol*, **2004**, 341, 739
4. Sahil Hasan Kabir et al. *bioRxiv*, https://doi.org/10.1101/2024.08.23.609282
5. Dibyendu Kumar Sasmal et al. *J. Phys. Chem. B*, **2011**, 115, 13075

P 34

Designing a bioluminescent fusion protein switch for detection of bacterial virulence factors

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Many pathogenic bacteria responsible for deadly diseases require rapid identification for timely prevention and treatment. Diverse methods have been developed, differing in sensitivity, specificity, cost, and duration, and are based on serological, microbiological, visual or optical, nucleic acid-based, or biosensor-based techniques.[1] In particular, cell-free synthetic biology approaches are highly appealing due to their compactness, low cost, portability, and sensitivity.[2] Herein, we describe the design of a cell-free protein synthesis/IVT-based platform that exploits the transcription regulator RfaH to control the expression of a small subset of genes involved in bacterial virulence across several pathogenic strains.[3] During its functional cycle, RfaH transitions from an auto-inhibited, closed state to an active, open state.[4] This conformational change, along with its DNA-binding specificity during the transcription of target genes, makes RfaH an attractive candidate for detecting genes associated with virulence factors in various pathogenic bacteria. In this study, we present a luminescent reporter construct of RfaH (NanoBiT-RfaH) capable of detecting virulent genes in bacterial species such as *E. coli*, *Salmonella*, *Shigella flexneri*, *Yersinia enterocolitica*, *Vibrio cholerae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* that exploits the open-to-closed conformational conversion reaction.

References:

1. M. Nemergut et. al. *Nat Commun*, **2023**, 14, 7864.
2. A. Shakhmin et. al. *Org. Biomol. Chem*, **2017**, 15, 8559.
3. E. Eremeeva et. al. *Int J Mol Sci*, **2020**, 21, 5446.
4. P. K. Zuber et. al. *Nat Commun*, **2019**, 10, 702.

P 35

Slow relaxation of oil film on water-air interface

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The behavior of droplets deposited on substrates is critical to a wide range of applications, including coating technologies, inkjet printing, microfluidics and phytosanitary treatments. Understanding the dynamics of oil spread on calm water is an active area of research, driven by the need for better control of oil pollution. In this work, we examine the instability that results from the deposition of a two-component oil droplet consisting of vegetable oil and a volatile alkane on a water-air interface. Presence of the volatile alkane forces the vegetable oil drop to spread into a thin film of a much larger radius than its equilibrium radius. The ensuing spatiotemporal evolution of the oil film is recorded and examined, demonstrating the existence of a radial flow that pushes the droplets towards the water bath's edge. Eventually the oil drop breaks into several smaller droplets. We study the relaxation dynamics of the oil film forced to spread on the water surface towards the equilibrium configuration consisting of tiny droplets. The boundary plays a critical role in this process, with the binary mixture exhibiting different wetting behaviors depending on the size of the water bath. After interacting with the boundary, the liquid forms a thin layer over the interface, which eventually undergoes dewetting. The system's propensity to minimize surface energy drives the formation and coalescence of holes as a result. Interestingly, the binary mixture sometimes enters a dynamic loop, oscillating between minimizing surface area and other parameters, which lengthens the time required to reach equilibrium. Analytic scaling laws provide an adequate description of the intricate relationship between fluid dynamics, wetting, and evaporation.

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Looking for Alternative to BODIPY: A Mn Selective Fluorescence Sensor Containing a Naphthalimide Dye

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Manganese, an indispensable trace metal crucial for all living organisms, plays a pivotal role in the catalytic function of multiple metalloenzymes, sustains brain function, acts as an antioxidant, and engages in various metabolic processes.[1] Excessive accumulation of Mn can lead to a neurological disorder resembling Parkinson's disease, known as Manganism.[1] Therefore, elucidating Mn ion homeostasis under physiological and pathophysiological conditions is necessary. Detecting Mn^{2+} ions within living cells requires a non-invasive technique with precise spatial (μm) and temporal (ms) resolution. Fluorescence confocal microscopy fulfils these criteria. However, designing a selective Mn^{2+} binding ligand is challenging due to the low affinities of Mn^{2+} ions for most ligands with N-, O-, and S-donor atoms. Additionally, the paramagnetic nature of Mn^{2+} ions can quench fluorescence. Our group has successfully addressed these challenges by developing Mn^{2+} ion sensors, utilizing BODIPY as a responsive unit and a PeT-based sensing modality.[2,3] Due to challenges associated with BODIPY, such as synthetic complexity and limited photostability, we are exploring the use of naphthalimide dyes as an alternative.[4] Naphthalimide offers advantages like chemical stability, easy synthesis, higher photo-stability, tuneable emission wavelength, and increased fluorescence quantum yields.[5] In this context, we have designed a Mn^{2+} responsive fluorescence sensor using Naphthalimide dye as the reporter unit. In my poster, I will present the design, synthesis, characterization, and in vitro studies of the novel sensor.

References:

1. Das, et.al. A. *Dalton Transactions* **2019**, *48* (21), 7047–7061
2. Bakthavatsalam, S.et.al. *Chemical Communications* **2015**, *51* (13), 2605–2608.
3. Kahali, S.et.al. Chemical Science **2024**, *51*, 1007–1017.
4. Wüstner, D.et.al. D *Molecules* **2014**, *19*, 11096-11130
5. Kagatikar, S.et.al. *J Mater Sci* **2022**, *57, 105–139*

Spectroscopic study of Thiamine and its detection using HPLC LED-IF

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Thiamine, also known as Vitamin B1, is a water-soluble nutrient found in various food items such as fish, meat, nuts, seeds, beans, soy products, etc. It is one of the eight essential B vitamins for the regular functioning of the central and peripheral nervous systems. It helps the body's conversion of food (fat, carbohydrates, and protein) into energy. The structural formula for thiamine is 2-methyl-4-aminopyrimidine linked to a thiazole ring by a methylene group.
The detection of thiamine is mainly done by the fluorescence. Even though thiamine is a weakly fluorescing molecule, it can be oxidized to form thiochrome, which is strongly fluorescing, using suitable derivatizing agent. Potassium ferricyanide is the most commonly using derivatizing agent. Thiochrome is unstable and degraded when exposed to light and heat. So, it has to be kept in dark and temperature-controlled environments. The stability and fluorescence intensity of thiochrome also depend on the pH [1,2].

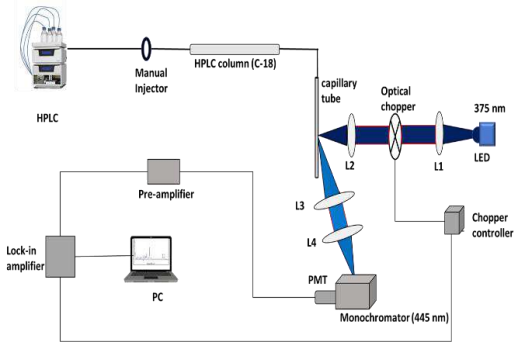

Figure 1: Schematic diagram of HPLC with fluorescence detection.

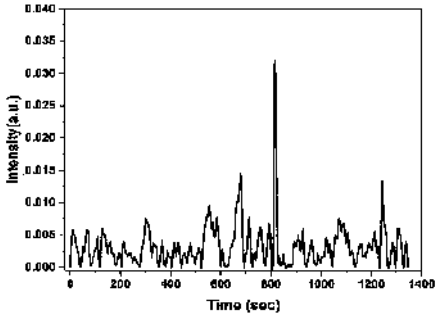

Figure 2: Chromatogram of 10 nM Thiochrome

HPLC (High Performance Liquid Chromatography) methods are generally used for the detection and quantification of metabolites including thiamine. Pre-column derivatization combined with fluorescence detection is the most common method [3]. We propose the HPLC LED-IF (LED Induced Fluorescence) technique for the quantification of thiamine.

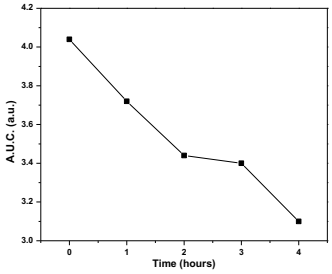

Figure 3: Plot of A.U.C. of chromatogram of 150 nM thiochrome v/s time after derivatization

We were able to detect up to 10 nM concentrations of TDP (Thiamine diphosphate) using the developed system. We have also done the stability study of thiochrome. It was observed that fluorescence intensity is decreasing even after 1 hour of derivatization.

References:

1. Edwards KA et.al. ChemistryOpen. **2017**;6(2):178-91.
2. Kraft CE, et.al. The Quarterly Review of Biology. **2017** Jun;92(2):151-168.
3. Mathew EM et.al. Biomed Chromatogr. **2019**;33(11): e4668.

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Non-Fano-Type Wavelength and Power-Dependent Raman Manifestation of Resonant Electron-Phonon Interaction

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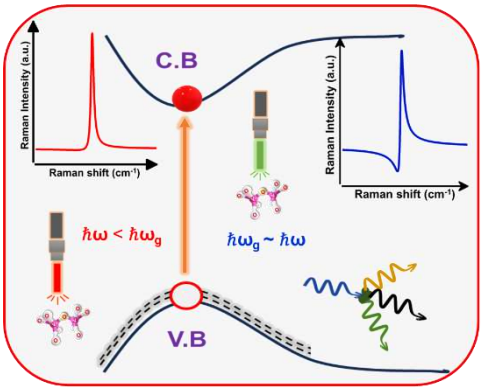
In semiconductors in particular, the existence of Fano resonance in a system frequently turns out to be highly helpful in understanding various electronic and quantum properties of materials. It is challenging to definitively detect its presence and nature because other factors/processes also affect them, even if Fano-type properties, such as asymmetry and antiresonance in spectral line shape, make them identifiable using Raman spectroscopy. When combined with the relevant theoretical analysis, a wavelength- and power-dependent Raman scattering experiment in orthorhombic V2O5 demonstrates the resonant nature of electron–phonon interaction in the Ag Raman mode (994 cm^{-1}). The presence of a Fano interaction is supported by the asymmetric Raman line shape with an antiresonance dip and an electronic Raman background. The electron-phonon coupling strength is quantified by the Fano coupling parameter (q) through theoretical fitting of experimental data. The resonant character of the Fano interaction has been identified by using the excitation wavelength-dependent Raman spectra, which seem to contradict the Fano-type behavior. Because anharmonic effects are involved, the Fano interaction becomes weaker as the excitation power increases.[1]



References:

(1) Rath, D. K. *et.al. J. Phys. Chem. C* **2024**, *128* (36), 15186–15193.

P 39

Unusual Stability of Metastable Vesicles Evolving during the Self-Assembly of Phenylalanine

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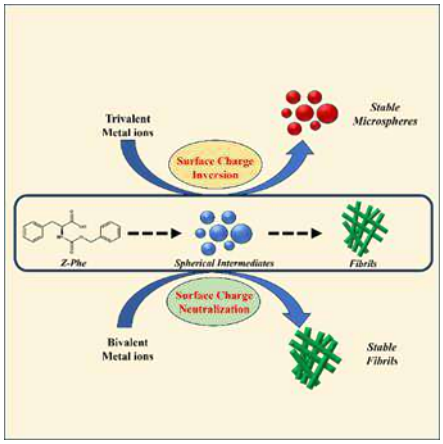
Biomolecular self-assembly is a ubiquitous process in nature, and the self-assembly of proteins and peptides has attracted immense interest due to their ability to form various nanostructures with important cellular organizations and functions. However, the underlying mechanism of the self-assembly process remains elusive despite numerous reports. In this context, a reductionist approach is targeted at understanding the self-assembly of the amino acids, as they serve as the simplest building blocks. Here, we have investigated the self-assembly formation of carboxybenzyl (Z)-protected phenylalanine (ZF). The intermediates formed during the self-assembly process can be stabilized by tuning the metal ion–amino acid interaction. Microscopic and spectroscopic investigations of the self-assembly of ZF reveal that the bivalent metal ions do not affect the self-assembly, as it eventually leads to the formation of fibrillar networks similar to blank ZF. Interestingly, the trivalent ions develop vesicle-like intermediates that do not undergo fibrillation for a prolonged time. The unusual stability of the vesicle-like intermediates in the presence of selective metal ions is well rationalized with the metal ion coordination, metal ion-specific entropy factor, and excess hydrophobicity induced by the trivalent metal ions. Furthermore, the time-lapse measurement of surface charge reveals that the surface charge of blank ZF and in the presence of bivalent metal ions changes from a negative value to zero, implying unstable intermediates leading to the fibril network. Strikingly, a prominent charge inversion from an initial negative value to a positive value in the presence of trivalent metal ions imparts unusual stability to the metastable intermediates.



References:

1. Bagchi et.al. *J. Phys. Chem. Lett.* **2024**, *15*, 4468.

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Oxidative redox intermediates of phenalenone inside water-soluble nanocages

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Redox chemistry is widely used in biological processes e.g. photosynthesis where several enzymes take part in catalytic and electron transfer reactions. Chemists have mimicked the enzymes by creating supramolecular cavities which can serve as active sites for catalysis[1]. In this regard, our group has demonstrated host-guest charge transfer (CT) mediated chemistry to generate cage-confined neutral radicals which can be exploited for catalytic events[2,3]. Hence, we thought to use this host-guest CT paradigm to get access to oxidative intermediates of phenalenone (PLY) chromophores, which have been used previously for carrying out photocatalytic reductive transformations in organic solvents[4]. For this, we have used $Pd_6L_4^{12+}$ supramolecular nanocages to demonstrate a photocatalytic route towards the generation and stabilization of radical cation and neutral radical intermediates on the PLY backbone. Steady-state absorption shows the formation of CT states after PLY encapsulation. Broadband transient absorption (fs-ns) spectroscopy was used to show sequential electron and proton transfer from N-H and O-H bonds to form the corresponding neutral radicals which were further supported by low-temperature EPR studies. We envision that this method of photo-generating oxidative intermediates on PLY derivatives in an aqueous medium will open up new routes for catalytic transformations.

References:

1. R. Breslow. et.al. Acc. Chem. Res. **1995**, 28, 146-153
2. Das, A. et.al, *Sci. Adv.* **2019**, 5, 13.
3. Roy, D. et.al, *Angew. Chem. Int. Ed.* **2023**, 62, e202312500
4. Datta P.et.al. *ACS Catalysis* **2024**, 14, 3420-3433

P 41

Membrane-mediated signalling by monoamine neurotransmitters

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The fundamental event in chemical neurotransmission involves the fusion of synaptic vesicles at the plasma membrane and the subsequent release of its neurotransmitter cargo. The neurotransmitters are known to function only through a receptor-mediated pathway after their release. However, monoamine neurotransmitters have been shown to bind and and increase the fluidity and water penetration of lipid membranes [1]. Here, we show a lipid membrane-mediated action of monoamine neurotransmitters (especially serotonin) to control cellular functions. Using Total Internal Reflection Fluorescence microscopy we find that the interaction between monoamines and lipid bilayers enhance the association of lipid bilayers, and can accelerate vesicular fusion *in vitro* [2]. Further, using a home-built three-photon microscope that enables label-free imaging of serotonin and simultaneous spectral/lifetime imaging of Nile red/FliptR (probes for vesicular membrane fluidity), we show that intra-vesicular serotonin increases the fluidity of vesicular membranes in live neurons. Upon depolarization, this increased fluidity facilitates the exocytosis of mature vesicles which contain higher serotonin concentrations. We conclude that the intra-vesicular monoamine provides 'feedback' through the vesicular membrane which allows it to modulate its own exocytosis. In addition, we also show that the interaction of monoamines with the plasma membrane can control the activity of non-cognate GPCRs [3]. Our results expand our understanding of the effects of monoamines in neurons and suggest that receptor-orthogonal serotonin analogues can be potential drug candidates.

References:

1. S. Dey et.al., Chem. Eur. J., **2021**, 27, 7533–7541.
2. D. S. Roy et.al., **2024**, bioRxiv doi: 10.1101/2024.01.20.576155.
3. D. S. Roy et.al. J. Phys. Chem. Lett., **2024**, 15, 1711–1718.

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Mechanical roles of molecular chaperones observed under Single-molecule force spectroscopic technique

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Protein folding under force invariably generates mechanical output, and while chaperones are well-known for assisting this process, their role in mechanically-tuned cellular energetics has not been thoroughly explored. In this study, we utilized single-molecule covalent magnetic tweezers to investigate the mechanical role of various molecular chaperones [1,2]. Here we mimic the physiological force environment on substrate proteins, keeping the chaperones unperturbed and investigated the role of twelve different chaperones with two structurally different proteins- talin and protein L and proposed a noble mechanism of chaperones. Our findings show that tunnel-associated chaperones (TF, DsbA, DsbC, and PpiD), which act as foldases under mechanical force, assist folding under force and generate energy to facilitate translation or translocation [3]. Conversely, cytoplasmic chaperones (PDI and thioredoxin) and the well-known foldase chaperone DnaKJE do not exhibit this mechanical folding ability. Instead, transferring chaperones (DnaJ, DnaK, SecB, Skp, and Spy) act as unfoldases, preventing misfolding of client proteins and aiding in the translocation of substrate proteins. These results provide new insights into the mechanical roles of chaperones, revealing how they can reshape the energy landscape of client proteins and modulate energy consumption in various biological processes, thus highlighting their significant impact on cellular energetics and protein folding dynamics [4,5,6].

References:

1. I. Popa et.al. *J. Am. Chem. Soc.* **2016**, *138*, 10546–10553
2. Tapia-Rojo, R. et.al. *Nat. Phys.* **2023**, *19*, 52–60
3. Eckels, E. C. et.al. *Chem. Sci.* **2021**, *12*, 11109–11120
4. Chaudhuri, D. et.al. *Biomacromolecules*, **2022**, *23*, 2951–2967
5. S. Chakraborty et.al. *Nanoscale*, **2022**, *14*, *7659–7673*
6. Chaudhuri, D. et.al. *Nanoscale*, **2024**, *16*, 2540-2551

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Anomalous Fluorescence Quenching in Fluorous Solvent-Added Media

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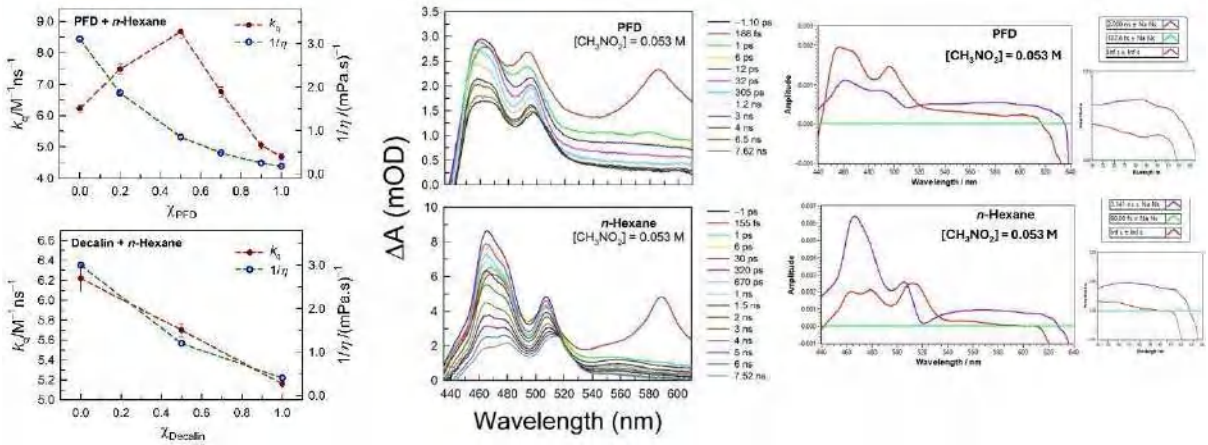
Fluorinated media have potential to exhibit unusual solvation properties in the context of photoinduced electron/charge transfer (PCT). In this study, we have employed binary mixtures of perfluorodecalin (PFD) and *n*-hexane to afford contribution of PFD specifically. Time-resolved fluorescence (TRF) quenching and ultrafast transient absorption (TA) spectroscopy are used for the quantitative interpretation of the diffusion, dynamics, and PCT mechanism. Addition of PFD to *n*-hexane results in unusual charge transfer between polycyclic aromatic hydrocarbons (PAHs) and nitromethane. As more viscous PFD is added to less viscous *n*-hexane, the dynamic viscosity (η) of the media increases. The bimolecular quenching rate constants (k_q) of the PAHs instead of decreasing, increase as PFD is added to *n*-hexane till equimolar mixture composition; k_q exhibits expected decrease only in PFD-rich region of the mixture. It is proposed that highly electronegative fluorines on PFD stabilize partial positive charge (δ^+) that develops on excited PAH during electron/charge transfer to the quencher nitromethane facilitating quenching in the process. The decay constants are similar to that observed for normal kinetic analysis of the transient absorption spectra with no clear evidence of the charge-transfer states. We infer that the initial photoinduced charge transfer is the rate determining step and the charge recombination is faster than the charge separation. Gibbs free energy change [$\Delta G^{(0)}_{PCT}(\varepsilon)$] for the PCT process is calculated using Rehm-Weller equation. k_q follows thermodynamic predictions only in case of neat *n*-hexane using nitrobenzene as quencher. In all other cases the k_q deviates significantly from the thermodynamic predictions. It can be inferred that PCT in the studied systems is kinetically-controlled and the observed charge transfer rate constant should primarily be governed by the reorganization energy of the immediate solvent molecules (outer-sphere) and solute molecules (inner-sphere).



References:

1. Zhang et.al. *Chem. Commun*. **2008**, 5686.
2. Matyus et.al. *J. Photochem. Photobiol. B* **2006**, *83*, 223.
3. Sun et.al. *Sens. Actuators, B* **2018**, *265*, 476.
4. Zhang et.al. *Photochem. Photobiol. Sci*. **2014**, *13*, 917.
5. O'Mari et.al. *Curr. Opin. Electrochem.* **2022**, *31*, 100862.

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Peptide-Based Antiviral Breakthroughs: Mutation-Resistant Drugs

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The rise of antimicrobial resistance (AMR) among viruses has become a notable concern, underscoring the necessity for effective antiviral treatments, sometimes in conjunction with vaccines. The rapid mutation of viruses, poses a significant hurdle in the pursuit of a universally effective vaccine that provides lifelong immunity. Beyond vaccines, antiviral drugs serve as the second line of defence. However, the efficacy of drugs raises concerns, ranging from notable side effects to the emergence of drug-resistant viruses with continued use. To address these issues, the idea of this work is to design antiviral strategies by envisioning potent cocktails of peptide-based virucidal drugs. The aim is to rationally design peptides to be used as targeting moieties onto the scaffold so to make new virucidal antivirals that target various proteins and various parts of proteins on two prominent enveloped viruses, (1) influenza A viruses (IAVs) and (2) severe acute respiratory syndrome coronavirus(SARS-CoV2). Such an approach offers potential therapeutic benefits superior to available approaches especially when it comes to dealing with the mutation-resistance. Through persistent efforts in optimizing and harnessing the unique properties of peptide-based drugs combining both experimental and computational approaches, significant advancements in antiviral therapeutics can be envisioned.

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Probing Brij Surfactant Vesicles Using a New Polarity Probe - AICCN

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The present work involves a comprehensive study on 2-amino-4-(1*H*-indol-3-yl)-4*H*-chromene-3-carbonitrile (AICCN) on vesicular systems. The molecule AICCN (synthesized earlier in our laboratory [1]) has been previously shown to function as an effective polarity probe [2]. Thus, it is expected that AICCN fluorescence can be an effective tool for probing organized assemblies i.e. vesicles. Two types of vesicular systems, niosomes and bilosomes have been prepared. Niosomes have been used as drug delivery vehicles [3] and in cosmetic formulations in the past [4]. The bilosomes are unique in the sense that they can withstand the harsh conditions of the gastrointestinal tract (GIT) [5], unlike the older counterparts i.e. liposomes and niosomes. Three types of non-ionic Brij surfactants have been used to prepare niosomes and bilosomes – Brij S2, Brij S10 and Brij S20. The Brij based surfactants were prudently selected so as to compare the effect of varying head group and HLB on the final vesicular structure and morphology. The fluorescence of AICCN served as an effective tool for studying these systems.

Fluorescence anisotropy, temperature effect on fluorescence, Red Edge Excitation Shift (REES) all indicated that Brij S2 forms robust vesicles with rigid bilayer. Confocal microscopy was used to assess the localization of AICCN within the vesicles. Studies are underway to study entrapment and release of AICCN from the vesicles and use of these vesicles for delivering AICCN to targeted cell lines. There are plans to study the diffusion coefficient of biologically active molecules such as AICCN within these vesicles.

References:

1. Biswas et.al. *Mater. Res. Express* 2015, 2, 065011.

2. Mandal et.al. *Phys. Chem. Chem. Phys.* 2023, 25, 18197.

3. Mondal et.al. *ACS Appl. Bio Mater.* 2022, 5, 8, 3896.

4. Handjani-vila et.al. *Int. J. Cosmet. Sci.* 1979, 1, 303.

5. Conacher et.al. *Vaccine* 2001, 19, 2965.

P 46

Engineering Bacterial Microcompartments: Insights from Shell Protein Interaction Studies

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Bacterial microcompartments (BMCs) are the polyhedral organelles that are composed of shell proteins encapsulating an enzyme core and provides metabolic advantages to bacteria. Outer shell of PduBMC is made up of eight different self- assembling shell proteins, among these PduN is the only pentameric protein that occupy the vertices. PduN is known to interact with the sheet-forming PduA shell protein, influencing the curvature and structural integrity of the microcompartment. Deletion of the pduN gene results in a disrupted, tubular BMC structure. So, here we are understanding the interaction of PduN with other shell proteins in assembling (PduBMC) and engineering them into some useful morphological architectures. We have studied the interaction between PduN with PduA, PduBB' and PduJ (Major shell proteins) using fluorescence based and optical (BLI) techniques. Interestingly, we observed that all these three protein combinations interact and yields diverse morphologies from open to closed compartments and sheets in heterologous host (*E.coli*). The resultant compartments can effectively associate and encapsulate native enzymes while preserving their functional activity. Further, we conducted *in vivo pduN* gene repositioning studies to understand why it is placed after the major shell and enzyme genes in *pdu operon*. We repositioned *pduN* before and after enzyme genes. Our *pduN* gene repositioning studies reveal that changing *pduN* position was unable to rescue the polyhedral structure of BMCs alters BMC structure and function. By integrating genetic engineering with advanced imaging techniques, we have decoded the structure-function relationship within PduBMCs, providing critical insights into microcompartment biogenesis. This approach not only enhances our understanding of BMC assembly but also offers potential for engineering protein nanocontainers and nanoreactors with wide-ranging biotechnological applications.

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Exciton-Polaron in Quasi-1D perovskite-like material-HDABiI5

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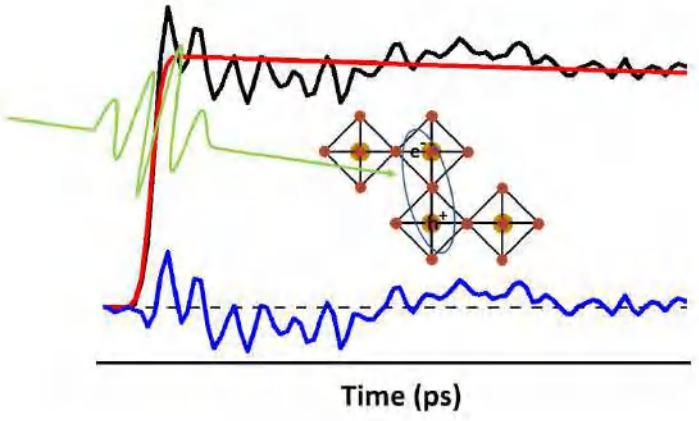
Lower dimensionality invokes properties like quantum confinement in the semiconducting materials[1]. Hybrid Perovskites are rather special in such cases as, along with reduced dimensionality, the organic cations also introduce softness in the lattice. This softness allows for strong coupling between photogenerated charge carriers and the lattice surrounding them[2]. Such interactions in these materials become the basis for next-generation devices for quantum control. A short pulse pump-probe experiment can resolve these coupled lattice modes well. Here, we use time-resolved impulsive Raman spectroscopy[3-6] to investigate the structural deformations in the lattice of organic Bismuth halide compounds on photoexcitation. We find lattice modes at 140 cm-1 & 17 cm-1 corresponding to BiI6 Octahedra stretching and tilting that strongly couples to the generation of the photo-excited states in these materials leading to the formation of Exciton Polaron.



References:

1. O.Yaffe,et.al. Phys. Rev. B, **2015**, 92, 045414.
2. K.Miyata,et.al. Sci. Adv., **2017**, 3, e1701217
3. Y.Yang, Nat. Commun., **2016**, 7, 12613.
4. Thouin, Nat. Mater., **2019**, 18, 349.
5. Ni L, ACS Nano, **2017**, 11, 10834.
6. D.Giovanni,Adv. Sci. **2018**, 5, 1800664.

P 48

TFAM-mediated dual role in DNA dynamics brings about mitochondrial genome compaction and transcriptional regulation via distinct bending states.

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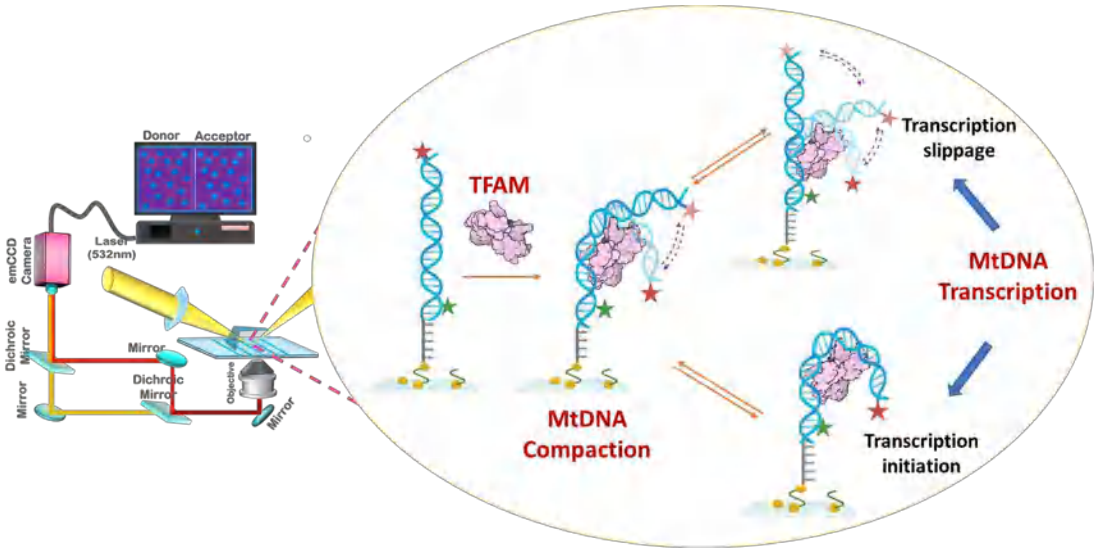
Mitochondrial transcription factor A (TFAM) plays a key role in DNA bending, crucial for both transcription initiation and genome compaction[1]. We studied the binding affinity between TFAM to its specific promoter sequences (LSP & HSP) and also non-specific sequence (NS-DNA). Circular dichroism spectroscopy revealed differential effect of TFAM on these sequences. Using single-molecule FRET, we investigated TFAM's interaction with the light strand promoter (LSP), heavy strand promoter (HSP), and non-specific (NS) DNA. In the absence of TFAM, LSP DNA displayed low FRET efficiency indicating a linear conformation. Upon TFAM binding, mostly a full (F) bent state with occasional transitions to a partial (P) bent state were observed. HSP binding showed three distinct FRET states with majority transitions between linear and partially bent conformations, suggesting TFAM does not stabilize full bending, and thereby somewhat not likely facilitating transcription initiation. In contrast, with NS DNA, TFAM stabilized the fully bent state while also keeping the DNA flexible by allowing transitions into the P-state further supporting its role in DNA compaction. These results highlight TFAM's conformational control over DNA structure, with distinct effects depending on DNA sequence, offering insights into its dual roles in transcription regulation and genome packaging.



Reference:

1. Ngo, H. et al. *Nat Commun*, **2014,** *5*, 3077.

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How robust is studying the structural dynamics of biomolecules by single molecule FRET spectroscopy?

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Biomolecules function by small and large scale structural motions spanning over nanoseconds to seconds. Single-molecule FRET (Foerster resonance energy transfer), is an established technique of single molecule fluorescence spectroscopy when combined with energy transfer between the fluorophores thereby measuring the nanometer distances between them[1]. Previous studies on variety of biomolecules, organic fluorophores, employed set-up configurations, and data analysis makes single-molecule FRET experiments hard to compare.

Recent community wide single-molecule FRET study measuring the nanometer distances between the organic fluorophores attached onto a double stranded oligonucleotides confirmed the robustness of this technique with high accuracy and precision[2]. To extend the study on a more complex biological molecule, we assesses the consistency of the technique to measure the conformational dynamics on millisecond timescale in proteins, where we measured the same protein samples together with the other 19 laboratories. The study reiterated the similar accuracy and precision in the nanometer distance measurements on proteins as on oligonucleotides. Furthermore, by identifying the potential configurations in the measurement set-ups which may introduce the variations amongst different laboratories and avoiding the fluorophore induced effects, dynamic analysis on proteins proved to be reliable in distinguishing the millisecond timescales of sub-nanometer distance changes[3].

References:

1. E. Lerner et al. *Science*, **2018**, *359*, 6373.
2. B. Hellenkamp et al. *Nat. Methods.*, **2018**, *15*, 669.
3. G. Agam et al. *Nat. Methods.*, **2023**, 1.

P 50

Modulation of ESIPT and ICT pathways in symmetrical azines through micro heterogeneous medium

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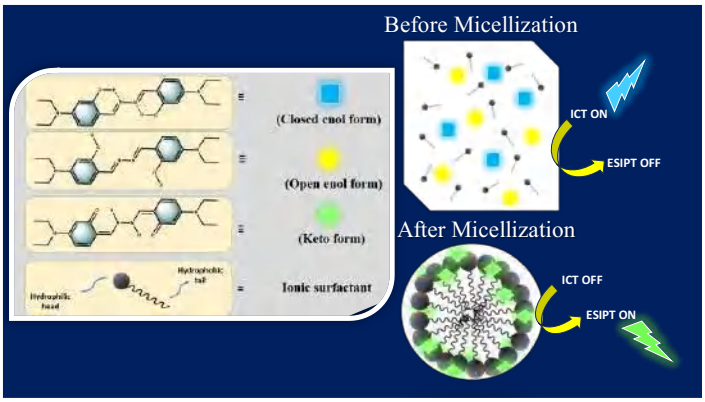
The organic luminescent molecule with tunable emission property has been widely examined due to their well-established role in analysing from single cell to large system. The distinguishable excited state exhibited by luminescent molecules play a dynamic role based on the microenvironment around them. Which are applicable in various fields such as fluorescent sensors, chemical warfare agents, OLED (Organic light emitting diode), and biomarkers (1). The photophysical property of the fluorescent probe is sensitive to the various external factors, particularly, microenvironment around them which influences the various excited state pathways such as PET(Photoinduced Electron Transfer), ICT (Intramolecular Charge Transfer) and ESIPT (Excited State Intramolecular Proton Transfer). Here, we have designed various symmetrical azine based fluorescent probes in the way they possess both ICT and ESIPT mechanisms in the same molecule (2,3,4). Salicylidene based azine molecule (DEASAD) showed dual charge transfer emission due to the presence of open enol (480 nm) and closed enol (510 nm) forms in polar protic solvents (5,6). Upon increasing the concentration of ionic surfactants, there is a significant increase in the emission intensity of both the enol forms of DEASAD until premicellar concentration. After micellization, occurrence of a new anomalous keto form emission through ESIPT was observed around 530 nm in ionic micelles and its intensity changes depend on the micellar surface charge The emission studies revealed the position and interaction of DEASAD with the charge of micellar stern layer as confirmed through interaction of metal ion with the probe and control molecules with and without ESIPT and ICT moieties.



References:

1. Fang M, et al. *Prog. Mater. Sci*., **2022,** *125*, 100914.
2. Thiagarajan V, et al. *Bull Chem Soc Jpn*., **2007**, *80*, 1307.
3. Hao G. et al. *Chem Commun*., **2023**, *59*, 2056.
4. Lu XL et al. *J. Anal Chem*., **2021**, *49*, 184.
5. Damoc M. et al. *J. Phys. Chem. C.*, **2022**, *127*, 99.
6. Suhasini R. et al. *Photochem. Photobiol*., **2024**, *00*, 1.

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SARS-CoV-2 Binding to Terminal Sialic Acid of Gangliosides Embedded in Lipid Membranes

Geetanjali Negi, Anurag Sharma, Monika Chaudhary, Divya Gupta, Krishnan H. Harshan, and Nagma Parveen*

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Multiple recent reports indicate that the S protein of SARS-CoV-2 interacts explicitly with membrane receptors and attachment factors other than ACE2. Which act as an primarily receptors i.e. play an active role in cellular attachment and entry of the virus. In this article, we examined the binding of SARS-CoV-2 virions to three different sialylated gangliosides, i.e., GD1a, GM3, and GM1 incorporated in SLBs. These gangliosides have 2, 1, and 1 SIA, respectively, bonded to a galactose unit of the glycan chain. We traced the binding kinetics of fluorescently labeled SARS-CoV-2 particles to the ganglioside-rich SLBs by recording time-lapse TIRF images of the labeled SARS-CoV-2 at the single-particle level. We observed that the virus specifically binds to sialylated (sialic acid, SIA) gangliosides, i.e., GD1a, GM3, and GM1, as determined from the acquired single-particle fluorescence images using a time-lapse total internal reflection fluorescence microscope. The data of virus binding events, the apparent binding rate constant, and the maximum virus coverage on the ganglioside-rich SLBs show that the virus particles have a higher binding toward the GD1a ganglioside. Enzymatic hydrolysis of the SIA–Gal bond of the gangliosides confirms that the SIA sugar unit of GD1a and GM3 is essential for virus attachment to the SLBs. The structural difference between GM3/GD1a, GM1 is the presence of SIA at the main or branched chain. We conclude that the number of SIA per ganglioside can weakly influence the initial binding rate of SARS-CoV-2, whereas the terminal or more exposed SIA is critical for the virus binding to the gangliosides in SLBs.

Reference:

1. Geetanjali Negi et al., *ACS Infectious Diseases*, ***2023***, *9, 1346.*

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Exploring Chemical Equilibrium Under Vibrational Strong Coupling

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Studies under the collective vibrational strong coupling of the molecules with the electromagnetic cavity modes can provide a unique window into the fundamental processes underlying chemical reactions. [1-2] By coupling the specific vibrational modes of molecules or solvent we can study the role of that specific vibrations in driving chemical transformations. This study has the potential to unlock new knowledge about reaction mechanisms, molecular dynamics and also the possibilities of new reaction pathways. [1-2] We studied the kinetics of the monomerization under the strong coupling of different vibrational modes of the molecules. We observed that this monomer-dimer equilibrium shows shift under vibrational strong coupling.

Reference:

1. Garcia-Vidal FJ et al., *Science*, **2021**, *373*, 6551.
2. Nagarajan K. et al., *JACS*, **2021**, *143*, 16877.

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Deciphering the propionylation on lysine by using a novel label-free tool: Protein Charge Transfer spectra

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Propionylation is a post-translation modification that plays a crucial role in regulating various cellular processes and is implicated in multiple diseases. This modification typically occurs on the cationic groups of proteins and it removes the positive charge of the protein. Traditionally, PTMs have been detected using spectroscopic methods such as protein labelling with external probes, NMR, and mass spectrometry (MS). However, these conventional techniques require expensive instrumentation, increasing the overall cost. Currently, there is no method available that can detect PTMs without labeling, using low-cost instruments, and in quick time. Recently, our group discovered a novel intrinsic chromophore in proteins that exhibits charge transfer transitions, producing a broad UV-Visible absorption band ranging from 250 to 800 nm [1]. This newly identified absorbance band, called **Protein Charge-Transfer Spectra (ProCharTS)** offers a new label-free approach for probing proteins and their interactions. In this study, we explore the chemical propionylation of charge-rich proteins such as α_3C and α_3W using ProCharTS. Initially, we confirmed protein propionylation through MALDI-ToF and analyzed the secondary structure by CD spectroscopy. The ProCharTS spectra of propionylated proteins were recorded, which demonstrated a decrease in intensity for α_3C and α_3W after propionylation. Our studies show that a decrease in the ProCharTS profile after propionylation is indicative removal of charge from the protein.

References:

1. Prasad, Saumya, et al., *Chemical Science*, **2017**, 8, 5416.

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Spectroscopic analysis of Potassium Bromide as host material in Terahertz range

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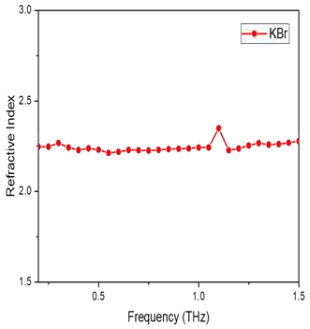
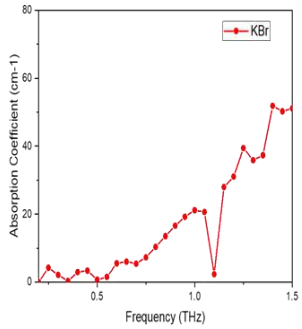
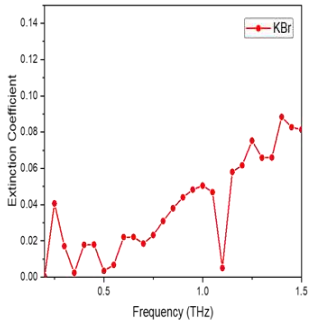
Potassium Bromide (KBr) has been widely used as a host material for various spectroscopic techniques such as Raman spectroscopy, IR spectroscopy etc. In this study, the efficiency of KBr to be used as a host material for THz Time Domain spectroscopy (THz-TDS) has been discussed. Measurements on KBr pellets were performed using THz-TDS spectroscopy. A femtosecond (fs) Ti: Sapphire laser amplifier and two zinc telluride (ZnTe) crystals were used to generate and detect Terahertz (THz) radiation using optical rectification and electro-optic sampling method, respectively. The changes in optical properties of KBr in THz range such as refractive index, absorption coefficient and extinction coefficient have been experimentally shown and discussed in this study.



Refractive index profile of KBr



Absorption coefficient profile of KBr



Extinction coefficient profile of KBr

References:

1. Jens Neu et al., *J. Appl. Phys.* **2018**, *124*, 231101.
2. Zaira Akhvlediani et al., *Wiley Online Library* **2023.**
3. S. Biermann et al., *J. Chem. Phys.* **1996**,*105*, 9754.
4. I Kaplunov et al., *J. Phys.* **2020**: *Conf. Ser. 1697 012253*,
5. Can Koral et al., THz spectroscopy of advanced materials, *Terahertz (THz), Mid Infrared (MIR) and Near Infrared (NIR) Technologies for Protection of Critical Infrastructures Against Explosives and CBRN*, **2021**, *253–273*
6. AK Malik et al., **2010**, *107, 113105*
7. Amit C. Das et al., *Proc. SPIE 9894, Nonlinear Optics and its Applications IV*, **2016**, *98941E*
8. Yeongkon Jeong et al., *Applied Surface Science*, **2022**, *584*, 152611.
9. Emre Aytan et al., *Polymer Bulletin*, **2023.**
10. Paul D. Cunningham et al., *J. Appl. Phys.*, **2011**, *109, 043505.*,

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Designing of gold nanoparticle-cyclodextrin conjugates to assist cholesterol trafficking in Niemann Pick Disease C phenotypic cells

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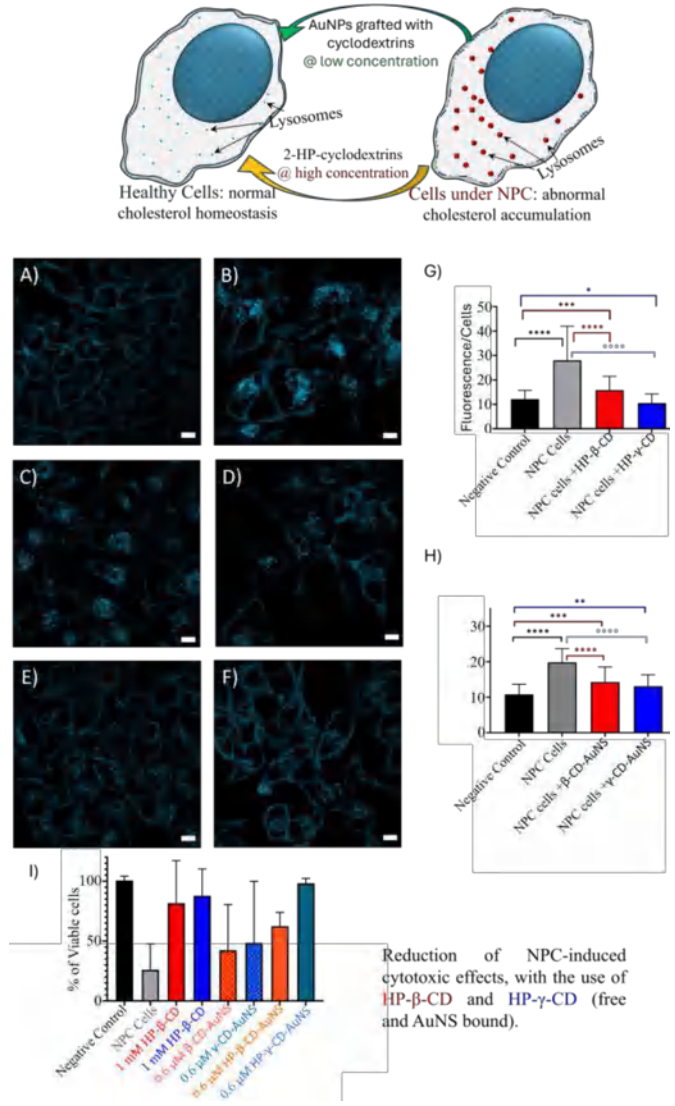
Niemann Pick disease type C (NPC) is a rare genetic disorder caused by mutation in either of the two genes NPC1 and NPC2, which results in abnormal cholesterol accumulation in late endosomes (LE) and lysosomes (LS). To date, no drug has been found with adequate effectiveness to treat NPC and current treatments merely focus on the reduction of some of the complications such as seizures, liver dysfunction, cognitive impairment, etc. Several derivatives of Cyclodextrins (CDs) [e.g., 2-hydroxypropyl β cyclodextrin (HPβCD) and 2-hydroxypropyl γ cyclodextrin (HPγCD)] have been tried against NPC, due to their ability to incorporate cholesterol molecules in their inner cavities. Despite showing early promise in cellular and animal studies, these efforts have failed during clinical trials due to poor pharmacokinetic profiles of the drugs and possible side effects.



Restoration of cholesterol distribution in the NPC-induced cells. Cholesterol distribution in A) normal cells, B) NPC-induced cells, C) and D) nanoconstruct treated NPC-induced cells, E) and F) HP-β-CD and HP-γ-CD treated NPC-induced cells. G) and H) analysis of the imaging experiment. I) Effect on NPC-induced cytotoxicity.

Our work aims to deliver the CDs by loading thiolated derivatives of βCD and γCD on top of biocompatible gold nanostars (AuNS) to improve pharmacokinetics and eliminate the need for high doses. We induced NPC mimicking conditions in SiHa cells by pharmacological inhibition of NPC1 protein. Confocal imaging performed after Filipin staining of cholesterol clearly showed that our conjugates were able to reduce the amount of cholesterol in LE and LS in NPC phenotypic SiHa cells, with the use of ~ 3 orders of magnitude lower concentrations of CDs (loaded on top of AuNS) compared to free HPβCD and HPγCD (Figure A to H). Additionally, the AuNS-conjugates effectively mitigated the cytotoxic effects associated with prolonged NPC1 blockade, requiring approximately 1,000 times lower concentrations of CDs compared to free HPβCD and HPγCD (Figure I).

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Microbial single cell Raman imaging coupled with vibrational tag for monitoring biosynthesis pathways in action

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Microbial single cells are complex, dynamic systems that require advanced multiplexing tools to image biomolecules and track their biosynthesis and turnover. Raman Imaging is a non-invasive vibrational imaging platform, enables the spatial and temporal visualization of various biomolecules—such as amino acids, nucleic acids, proteins, and lipids—simultaneously at the single-cell level. [1,2] In our study, we utilized a combination of reverse stable isotope probing methodology with Raman imaging in which carbon stable isotopes was used as the vibrational tags. The stable isotope labelled biomolecules exhibit distinct shifted peak in Raman spectra when compared to its unlabelled counterparts due to isotopic effect. $^{13}C_6$ labelled microbial cells were grown in a carbon-free culture medium with the unlabelled ($^{12}C_6$) carbon source, and single-cell Raman images were acquired at different time points. In the early incubation time points, the Raman image revealed intense signal distribution from the $^{13}C_6$-labelled biomolecules, while the Raman signal distribution from the unlabelled biomolecules were seen to be weak. As incubation duration progressed, the Raman images of the microbial cell showed diminished intensity signal of the $^{13}C_6$-labelled biomolecules, and the newly synthesized unlabelled biomolecule distribution became increasingly prominent. This approach generated spatio-temporal maps that visualized the turnover and distribution of newly synthesized biomolecules, offering insight into dynamic biosynthetic pathways. Vibrational tag Raman imaging holds significant potential for bioprocess applications in sensing and visualizing the accumulation of commercially relevant metabolites.

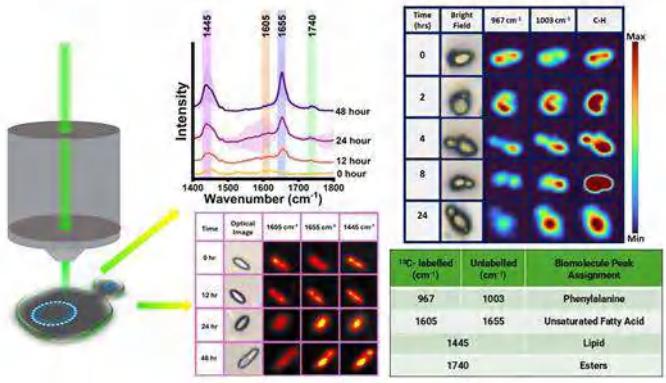

Figure: Microbial single cell Raman images showing time dependent dynamics of different metabolites in situ.

References:

1. Jiro Karlo et al., **2024**, In situ monitoring of the Shikimate pathway: A combinatorial approach of Raman reverse stable isotope probing and hyperspectral imaging. Analyst.
2. Jiro Karlo et al., **2024**, Tracking Trash to Treasure: In situ monitoring of Single Microbial Cell Oil biosynthesis from Waste Cooking Oil using Raman Reverse Isotope Probing and Imaging. ChemRxiv. (Under Review)

P 57

Insights into dynamic properties of beta-lactoglobulin dimer protein disruption by graphene oxide: Spectroscopy and fluorescence lifetime imaging microscopy

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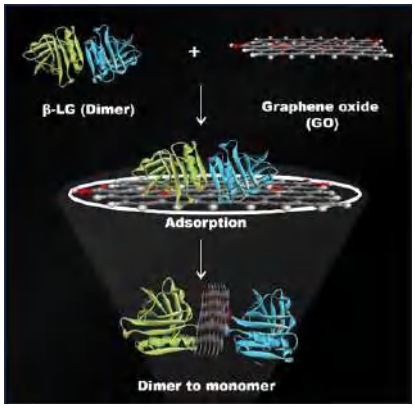
Graphene oxide (GO), a novel applied nanomaterial derived from graphene, has been employed in various biomedical applications due to its unique structure.[1] Nevertheless, their biological applications are severely limited by a lack of comprehensive understanding of GO interactions with biomacromolecules. Herein, we investigated the structure and dynamics of β-lactoglobulin (β-LG), a globular milk whey protein, on the GO surface at two different pHs, 2.5 and 6.2, in connection to the monomer and dimer forms of the protein, respectively. Molecular dynamics simulation (MD), multi-spectroscopic and imaging techniques were employed to monitor the protein interaction with GO. The steady-state results revealed the interaction is significantly stronger in a dimeric form compared to the monomeric form of protein and complexes are driven by hydrophobic forces. Interestingly, the time-resolved fluorescence measurements unveil the significant changes in average lifetime values after adsorbing on GO (from 1.79 ns to 0.20 ns at pH 2.5 and from 1.16 ns to 0.24 ns at pH 6.2). The final lifetime of both forms, with the addition of GO, explains a similar microenvironment around the tryptophan. Furthermore, to verify these changes, the secondary conformation of both forms of protein is elucidated by fourier transform infrared (FT-IR) spectra, which clearly depict the spectral profile of dimeric protein in the presence of GO are closely resembles those of monomeric β-LG at pH 2.5. In addition, comparable morphological changes of GO with protein of both forms were investigated using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Additionally, the exploration of protein interaction with GO was performed using fluorescence lifetime imaging microscopy (FLIM); both forms have a similar distribution of lifetime after adsorbing on GO, which supports the aforementioned results. The plausible reason for this change is that the GO can disrupt the dimer β-LG into a monomer.[2] In order to support the experimental results of dimer protein disruption, we have also performed the molecular dynamic simulation. The simulation results demonstrate that a GO can interfere with hydrophobic protein-protein interaction and can lead to penetrating in between the protein dimer. Hence, our findings can assimilate GO and protein interaction and help to increase the scope for potential biomedical applications.



References:

1. D. Chen et al. *Chemical reviews*, **2012**, 112, 6027.
2. B. Luan et al. *ACS nano*, **2015**, 9, 663.

P 58

Antidepressant Potential Versus Neurotoxicity of Rapidly Acting Antidepressant Drugs (RAADs)

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Rapidly acting antidepressants (RAADs) partially relieve depression in < 1 day and have sustained antidepressant effects for several days. This rapid onset is 10- to 100-fold faster than selective serotonin and norepinephrine reuptake inhibitors (SSRIs and SNRIs). RAADs have varying chemical structures: some RAADs are ketamine enantiomers (R-Ket/S-Ket), metabolites [(2R, 6R)-HNK/(2S, 6S)-HNK], or analogs such as methoxetamine (MXE), and radafaxine (Rad). Other RAADS, such as scopolamine (Scop), are best known as muscarinic antagonists and are structurally unrelated to ketamine. Ketamine (Ket) and scopolamine present something of a paradox for pharmacology, as they exhibit rapid and robust antidepressant activity but have potent neurotoxic potential. However, it is not yet clear precisely which molecular mechanisms mediate these beneficial and deleterious effects; thus, dosing is mostly a trial-and-error affair. Here we carefully dig into the therapeutic and toxic effects of ketamine and scopolamine and their metabolites, shedding light on the mechanisms, cell types, and timescales in cells and tissues.

The first goal of our research program was to develop genetically encoded fluorescent biosensors to follow the import, trafficking, and metabolism of RAADs. We developed a family of "intensity-based RAAD-Sensing Fluorescent Reporter" [iRAADSnFR] indicators. In solution, iS-KetSnFR responds to S-Ket with an EC_{50} ~ 150 nM with maximal fluorescence increase ($\Delta F_{max}/F_0$) ~ 3.2; iS-HNKSnFR responds to (2S, 6S)-HNK with an EC_{50} ~ 110 nM and $\Delta F_{max}/F_0$ ~ 3.6; iR-KetSnFR senses R-Ket with EC_{50} ~ 130 nM and $\Delta F_{max}/F_0$ ~ 4.0; iR-HNKSnFR responds to (2R, 6R)-HNK with EC_{50} ~ 500 nM and $\Delta F_{max}/F_0$ ~ 4.0; and iD-HNKSnFR responds to dehydronorketamine (DHNK) with EC_{50} ~ 160 nM and $\Delta F_{max}/F_0$ ~ 4.0. iScopSnFR senses Scop and displays EC_{50} ~ 510 nM and $\Delta F_{max}/F_0$ ~ 2.8.These sensors will be useful for the study of Ket, its metabolites, and related drugs in a variety of experimental settings. We have previously shown that many drugs unexpectedly act, at least in part, at intracellular targets in various organelles. We wished to assess the potential of RAADs and their metabolites to traffic to, and act on, such intracellular targets. Thus, we incorporated diverse organellar targeting peptides to the iRAADSnFR family and expressed them in cells. Fluorescence microscopy shows that, at the nM concentrations associated with antidepressant activity, RAADs traffic into diverse organelles within seconds, equilibrating with extracellular levels. We next sought to express the iRAADSnFRs *in vivo* in mouse, fish, and worm – the former with adeno-associated virus (AAV) transduction, the latter two with transgenesis. We have expressed the sensors in diverse locations in the central and peripheral nervous systems and subjected the animals to diverse drug regimens. We have also performed complementary experiments using proteomics, immunohistochemistry, biochemistry, and behavior tracking to profile neurotoxicity of ketamine, its metabolites, and related drugs. Together, this research program will help us unravel the complicated mechanisms of action of RAADs in diverse settings, and to design and synthesize next-generation drug analogs with preserved efficacy but minimized side effects.

References:

1. Shivange et al. *2019, 151(6), 738.*
2. Bera et al. *2019, 13, 499*.
3. Beatty et al., *2022, 12(22): e4551*.
4. Blumenfeld & Bera et al., *2024, 49, 246*.

P 59

Parametric optimization for rapid discrimination of *Acinetobacter baumannii* using Surface-Enhanced Raman Spectroscopy (SERS)

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Ubiquitously present Gram-negative bacterium *Acinetobacter baumannii* (*A. baumannii*) has evolved as a highly successful nosocomial pathogen. *A. baumannii* reported to have acquired resistance against almost all clinically important antibiotics. The WHO has listed carbapenem-resistant *A. baumannii* in the top "Critical" tier of Bacterial Priority Pathogens List (BPPL) 2024 – which requires urgent control measures. Rapid detection of clones and antimicrobial resistance (AMR) profile is important for quick decision on treatment options. Raman Spectroscopy is proven to be useful in rapid understanding of the biochemical alterations among the susceptible and resistant microorganism. The spectral fingerprint of Raman spectroscopy is unique for microbial species, however, obtaining high quality Raman spectra is challenging due to the weak signals. These signals can be enhanced to several order of magnitude by placing plasmonic metallic nanoparticles closer to that of the microorganisms and this technique is referred as Surface-Enhanced Raman Spectroscopy (SERS). The present study was initiated to determine the culture conditions, time of culture, and the use of silver nanoparticles (AgNPs) of 60-80 nm (synthesised by Lee-Meisel method) for a reliable SERS measurement that can be useful for rapid differentiation purpose. The bacteria were cultured in LB media at 37 ºC and harvested at different growth phases (6, 8, 12, and 16 hours). Following the cell harvesting, several solutions (distilled water, 1X PBS, 0.9% saline, glucose, and glycerol) were tested for an effective removal of the cellular impurities, which might interfere or shadow with the Raman signals. The samples were casted in triplicates on the aluminium foil covered with a glass slide and each dot was measured thrice in two forms, liquid and dry which resulted in stable spectra with maximum number of peaks that were matched to carbohydrates (589 cm^{-1} and 885 cm^{-1}), phospholipids (1070 cm^{-1}), protein (1202 cm^{-1} and 1654 cm^{-1}). High resolution spectra were best obtained when 1:5 ratio of AgNPs and bacterial cells were used. Both liquid and dry form resulted in comparable peaks, while maximum intensity was observed in the dry form. The results showed that cells harvested at 16 hours and washed with distilled water has given the reproducible spectra with minimum noise.

P 60

Investigating the Mechanical Response of Parallelly Arranged Polyproteins: Mimicking the Mechanical Stress in Biological Systems

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Proteins play numerous roles in cellular processes. The well-defined structure of proteins is attributed to their functional properties executed in various cellular functions.[1] Many proteins are subjected to biologically relevant mechanical forces[2] in several cellular processes, generally called elastomeric proteins. Single-molecule force spectroscopy like atomic force microscopy (AFM) has been employed to investigate the mechanical fingerprint of proteins under external force.[3] Traditionally, the mechanical response recorded from AFM is ascribed to the behaviour of individual protein domains. However, it is important to note that in many biological processes, proteins exist in parallel/antiparallel fashion to execute their biological function.[4] For example, in striated muscle sarcomere, the giant muscle protein titin exists as hexameric bundles to mediate the passive elasticity of muscles.[5,6] To understand how protein domains, arranged parallelly, respond to external forces, a special molecular biology design is necessary which can directly probe the mechanical features of proteins in parallel. In the previous attempt[7] using an alpha-helical coiled-coil domain to create a parallel dimer, it has been shown a doubling of the unfolding force of individual domains. Further study[8] using SpyCatcher-Spytag chemistry and covalent immobilization of proteins on coverslip claimed the ratio of unfolding force in parallel arrangement versus monomer to be always less than 2. Both the previous study has their drawbacks related to the design of the system. In this context, our study provides a suitable molecular biology design to study the mechanical features of proteins arranged in parallel. We used unnatural amino acids to create a junction in the polyprotein construct and kept the alpha-helical coiled-coil domain at the terminus of the construct for the heterodimerization [Fig. 1]. This leads to the formation of a conserved parallel construct to be used to directly probe the mechanical feature through AFM. Moreover, our design can be elongated from parallel dimer to parallel tetramer which can maximize the situation of parallel existence of proteins in biological systems. Our study aimed to have a clear conclusion regarding the change in the mechanical feature of proteins from monomer to parallel dimer and to provide a suitable molecular biology design to probe proteins in parallel arrangement.

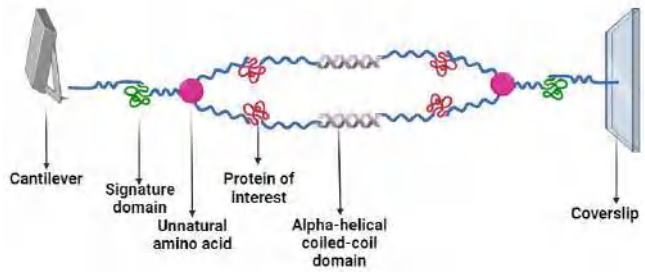

Figure 2: Molecular biology design to probe protein domains in parallel

References:

1. Kessel, A. et.al. *Introduction to Proteins: Structure, Function, and Motion, Second Edition* 2018, 1–932.
2. Halma et. al. *Biophys Rep* 2024, *9* (0), 1.
3. Scholl, Z. N et. al. *Methods in Molecular Biology* 2018, *1814*, 35–47.
4. Ohashi, T et. al. *PNAS* 1999; Vol. 96.
5. Tskhovrebova, L et. al. *Nature Reviews Molecular Cell Biology* 2003, pp 679–689.
6. Rivas-Pardo, J. A. *Emerging Topics in Life Sciences* 2018, pp 681–686.
7. Sarkar, A et. al. *Biophys J* 2007, *92*, 4.
8. Zuo, J et. al. *ACS Nano* 2023, *17* (8), 7872.

P 61

Unveiling Tau K18 and K19 Aggregation through Protein Charge Transfer Spectra (ProCharTS): Addressing Research Gaps and New Insights

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Tau is an intrinsically disordered protein (IDP) which is associated with Alzheimer's disease. Tauopathies are neurodegenerative disorders [1] characterized by its abnormal aggregation, with the K18 and K19 domains consisting of only the four and three repeats of tau protein respectively. They retain the key aggregation-prone regions and it makes them more suitable for studying the early events of aggregation which are critical for understanding the disease onset and progression as compared to the wild-type tau. Traditional spectroscopic techniques like Circular Dichroism (CD), fluorescence spectroscopy, have provided valuable insights into Tau protein aggregation but they often fall short in explaining the nuanced charge transfer dynamics underlying these processes as well as the intricate details involving the early events of aggregation [2]. In this study we introduce protein charge transfer spectra (**ProCharTS**) as a novel and label-free tool [3] to explore the aggregation behavior of Tau K18 and K19 domains thereby providing unique advantages in directly probing the charge transfer interactions within the Tau aggregates which are less accessible through conventional methods. Our investigations have unravelled the previously unrecognized aggregation intermediates and distinct signatures associated with charge transfer for both the K18 and K19 domains. The results not only enhance the resolution of tau aggregation studies but also provide a deeper understanding of the electrostatic contributions to tauopathies. By focussing on protein charge transfer spectra (**ProCharTS**) we unveil critical insights that may guide the development of novel diagnostic and therapeutic strategies. Our results therefore underscore the utility of **ProCharTS** as an intrinsic spectral probe to monitor the various events of aggregation of any protein rich in charged amino acids.

References:

1. Chong, F.P., et al., *Tau proteins and tauopathies in Alzheimer's disease.* 2018. **38**: p. 965-980.
2. Alom, S.E., et al., *Early events during the aggregation of Aβ16-22-derived switch-peptides tracked using Protein Charge Transfer Spectra.* 2024. **1297**: p. 342374.
3. Prasad, S., et al., *Near UV-Visible electronic absorption originating from charged amino acids in a monomeric protein.* 2017. **8**(8): p. 5416-5433.

P 62

The Interplay Between Protein Flexibility, and Associated Water Dynamics in osmolyte solution: Does it Affect macromolecule's thermal stability

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Small organic molecules, or osmolytes, play a crucial role in stabilizing the native conformation of biomolecules within cells, a property that has also been harnessed in biopharmaceutical formulations. Despite their significance, the mechanisms underlying protein-osmolyte interactions remain poorly understood. Water, a key participant in virtually all biomolecular processes, is thought to play a critical role in these interactions as well. In this study, we investigate how osmolytes influence the dynamics of associated water and reveal a striking correlation between modified water behaviour and the stability of proteins across different osmolyte environments. Furthermore, we explore how osmolyte-induced changes in the dynamics of biological water affect the internal flexibility of proteins. Our findings demonstrate that the internal regions of proteins are sensitive to these water dynamics, leading to modulation of their flexibility. In summary, our results suggest that osmolytes interact with proteins by altering the properties of associated water, which in turn affects protein flexibility and is reflected in the thermal stabilization or destabilization of the macromolecules.

P 63

An Activity-Based Fluorogenic Sensor for Cu ions: Deciphering the Sensing Mechanism and Detecting Cu Ions In Vivo

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Activity-based sensors exploit coordination chemistry and metal-assisted reactions for selective and sensitive detection of metal ions in the biological milieu. Activity-based fluorogenic metal ion sensors consist of a fluorophore conjugated to a metal-binding scaffold via a reactive bond.[1] Binding of a specific metal ion to the scaffold results in a metal-catalyzed reaction that leads to the release of the fluorophore thereby increasing fluorescence.[1] The release of fluorophore from the sensor leads to significant fluorescence enhancement and also overcomes fluorophore quenching due to unpaired electrons on paramagnetic metal ions. Further the detection of metal ions that are weak binders based on Irving Williams Series[2] might be feasible via this strategy as an activity-based sensing response does not solely rely on metal-binding to the scaffold. Importantly, activity-based sensors can be synthesized using a modular synthetic approach via separate synthesis of the dye and the scaffold followed by conjugation of two units, facilitating the incorporation of changes in improving the selectivity and sensitivity. Thus, activity-based sensing is an attractive strategy for the detection of weak binding metal ions with unpaired electrons. So far, most activity-based sensors have utilized pyridine donors with di-/tri-/tetra- coordinating N donor sites. These sensors had preferential selectivity towards strong binding metal ions.[1] In this backdrop, to access sensors for the weak binding metal ions, we developed a novel activity-based sensor (**N5-CP)** with 5-N donor sites. **N5-CP** was highly selective toward $Cu^{+/2+}$ ions and gave a 63 times fluorescence enhancement in the presence of Cu^{2+} ions. The sensor could detect Cu ions in both living cells and in a live zebrafish larval model.[3] Interestingly, the sensor worked exclusively in the presence of glutathione and ambient oxygen.[3] Characterisation of intermediates and products hinted towards the involvement of a Cu^{II}-hydroperoxo species in the catalytic mechanism.[3] Since the previously reported activity-based sensors for metal ions also function in the presence glutathione and ambient oxygen, we believe that our proposed catalytic mechanism via a Cu^{II}-hydroperoxo species, supported by experimental evidence, would be applicable to the class of activity-based sensors that function via metal-mediated oxidative cleavage. Finally, the results suggested the need for further fine-tuning of scaffold design to access sensors for metal ions that lie lower in the Irving-Williams series. The sensor design, sensing mechanism, and biological studies will be presented.

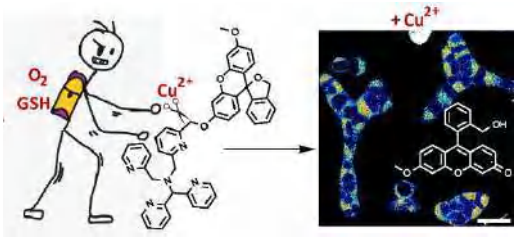

Figure 1: Scheme depicting Cu ion sensing via oxidative cleavage in the presence of glutathione and ambient oxygen

References:

1. D. A. Iovan et al. *Inorg. Chem.*, **2019**, 58, 13546.
2. H. Irving et al. *J. Am. Chem. Soc.*, **1953**, 3192.
3. K. Gupta et al. *Chem. Commun.*, **2023**, 59, 8282.

P 64

$CdTe_{0.25}Se_{0.75}$ Quantum Dots as Efficient Room Temperature Single Photon Source for Quantum Technology

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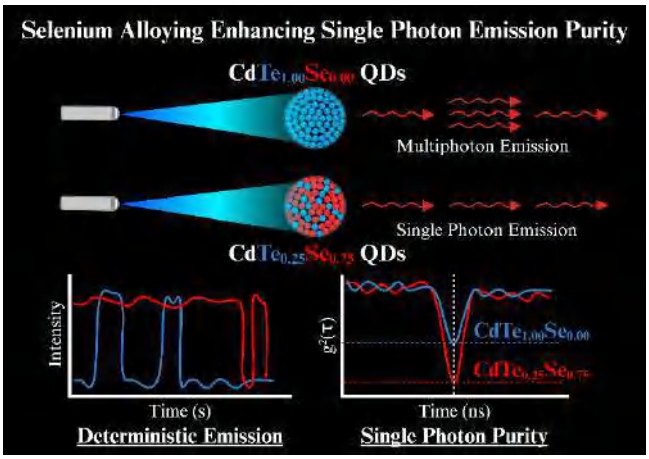
Room temperature single photon sources (SPS) are crucial for developing the next generation quantum technologies. Quantum dots (QDs), recently, have been reported as promising materials as SPS at room temperature. By optimizing the single particle optical properties of a series of water-soluble, $CdTe_xSe_{1-x}$, here we provide an efficient SPS with increased single photon purity. The data revealed that second order photon correlation, $g^2(0)$ value decreases substantially from 0.21 in CdTe to 0.02 in $CdTe_{0.25}Se_{0.75}$ QDs. They also exhibited deterministic emissions with an increase in ON time exceeding 95% of the total time. This was accompanied by an increased photon count rate, substantially reduced blinking events, and extended single particle ON-time. The increased single photon emission in $CdTe_xSe_{1-x}$ is attributed to very fast electron trapping to dense trap states, which suppresses the multiexciton recombination.



References:

1. V. Chandrasekaran et al., *Nano Lett.*, **2017**, *17*, 6104.
2. C. Zhu et al., *Nano Lett.*, **2022**, *22*, 3751.
3. H. J. Chuang et al., *Nano Lett.*, **2024**, *24*, 5529.
4. R. Uppu et al., *Nat. Commun.*, **2020**, *11*, 3782
5. J. Tan et al., *New J. Chem.*, **2015**, *39*, *4488*
6. Y. Arakawa and M. J. Holmes, *Appl. Phys. Rev.* **2020**, *7*, 021309

P 65

Cholesterol-Dependent Membrane Binding of ApoE Signal Peptide: Phosphatidylglycerol as a Switch

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A short segment of signal peptide (SP) located at the N-terminal of the apolipoprotein E (ApoE) protein directs the ApoE protein to the endoplasmic reticulum after its synthesis. Previous research has demonstrated that this peptide exhibits varying binding affinity toward lipid membranes in a manner influenced by cholesterol. However, the specific interaction mechanism is still not fully understood. In our study, we explored how the composition of adjacent lipids affects the binding of the ApoE signal peptide to the membrane. We discovered that a negatively charged lipid, such as phosphatidylglycerol, functions as a switch, reducing the peptide's binding efficiency to cholesterol-rich membranes. It was interesting to note that phosphatidylethanolamine doesn't activate the cholesterol-dependent binding of the ApoE signal peptide, but it does work in conjunction with phosphatidylglycerol to enhance cholesterol sensitivity in membranes containing phosphatidylglycerol. Thus, the binding affinity of a peptide to a membrane is influenced by neighboring lipids rather than the lipid-binding domain of the peptide. Our findings have uncovered a new role of lipid diversity in modulating the membrane binding of the ApoE signal peptide.

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Complex condensation of a prion-like protein and a J-domain protein inhibits aberrant phase transition and amyloid formation

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Eukaryotic cells organize a wide array of biochemical processes by creating membrane-bounded organelles in a spatiotemporal fashion to function properly. In addition to this, cells also contain membrane-less organelles (MLOs) or biomolecular condensates formed via phase separation that are primarily composed of intrinsically disordered proteins/regions and nucleic acids. These biomolecular condensates are involved in a myriad of critical cellular processes and are implicated in various fatal neurodegenerative diseases (1, 2). However, the phase separation of proteins inside the cell is tightly regulated to maintain the metastable state of these condensates and prevent any excessive abnormal aggregation. These MLOs consist of a wide range of J-domain proteins (JDPs), which can play an important role in modulating the sequence of events that govern the intracellular phase transition into liquid-like droplets, further preventing pathological amyloid aggregation (4). This regulation process by JDPs remains largely unexplored. I will discuss our recent results on the heterotypic condensation of a prion-like protein and a JDP into liquid droplets. Utilizing a multidisciplinary approach involving a diverse range of biophysical, molecular biology, and imaging tools, our study delineates the complex network of interactions that govern the heterotypic phase separation of these proteins, which effectively impedes the formation of fibrous amyloid aggregates (5).

References:

1. Boeynaems, S., et al. *Trends Cell Biol.* **2018**, 28.
2. Agarwal, A., et al. *Nat. Commun*. **2022**, 13.
3. Bard, J. A. M., Drummond, D. *Front. Biophys.* **2024**.
4. **Arora, L**., et al. *Biorxiv*. **2024**.

P 67

Partitioning of gentamycin and oxytetracycline in spherical and rod shaped micelles formed by using TTAB, sodium salicylate and pyridoxamine and delivery to bovine serum albumin: Thermodynamic insights

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In this study we have used TTAB micelles representing drug carrier of antibiotic drugs which are gentamicin (GM) and oxyteracycline (OTC). The CMC of TTAB was found to be around 3.5 mM and above 26 mM it was observe to be in rod like shape. We have used two additives namely sodium salicylate (NaSal) which is a hydrotrope and pyridoxamine (PYR), one form of vitamin B6 to check the effect on the premicellar (2.5 mM) and postmicellar (7 mM) concentrations of TTAB surfactant. The intermolecular interactions between cationic surfactant and aromatic salt (NaSal) or essential organic compound pyridoxamine and their subsequent evolution to worm like micelle (WLM) have been investigated. Further, the partitioning of drugs into these systems have been studied by measuring the corresponding energetics involved, using isothermal titration calorimetry (ITC)[1]. GM and OTC both showed two sequential binding profile in the presence of additive. From ITC experiment it was observed that GM is able to partition more in the micellar system having higher concentration of TTAB with addition of hydrotrope NaSal. It has been reported that NaSal promotes the change in the shape of spherical to rod like micelle of TTAB. Imae and Kohnsaka model also suggests that the insertion of salicylate observed to be in between the head groups of the micelle which stabilizes the micellar structure[2,3]. In the presence of NaSal and PYR the first set of OTC and GM molecules showed very low value of partitioning constant (K_1) while K_2 with the high affinity which suggests that the first set of drug molecules might be creating space in palisade layer of TTAB micelle to enter next set of molecules with high affinity. The Stern –Volmer and Benesi-Hildebrand equation from fluorescence and UV-visible spectroscopy method respectively has been used to determine binding constant values which were found to be in accordance with each other. The partitioning of GM into TTAB+ NaSal/PYR systems showed static type while OTC in the same systems showed dynamic type of quenching by using fluorescence and TCSPC technique. From dynamic light scattering experiment it was observed that the addition of NaSal and PYR in TTAB surfactant increases the shape of micelle which we have further confirmed by SEM analysis. It was found that higher molality of TTAB increases the stability maintaining secondary structure of BSA as compared to low molality of TTAB. This change in molality of TTAB and addition of NaSal / PYR further changes the shape of the micelle which results in formation of sphere and / rod shaped micelle. In this sphere and rod shaped micelle GM and OTC are able to partition differently. These micellar systems further can be used as a drug delivery purposes[4].

Reference

1. Judy, E.; Kishore, N. *Langmuir* **2021**.
2. Kabir-ud-Din *J. Dispers. Sci. Technol.* **2009**.
3. Pawar, K.; *J. Dispers. Sci. Technol.* **2012**.
4. Rajput, S. M et al *Colloids Interface Sci. Commun.* **2020**.

P 68

Emergence of Dynamic G-Tetraplex Scaffold: Uncovering Low Salt-Induced Conformational Heterogeneity and Folding Mechanism

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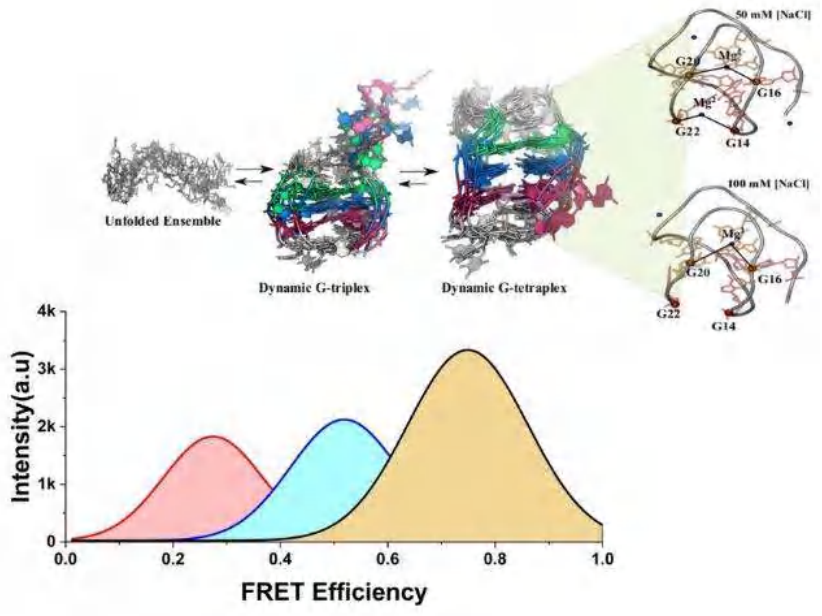
The topological diversity of human telomeric G-quadruplex structures is intrinsically related to their folding mechanisms, and is significantly modulated by ion-atmospheric conditions. Unlike previous studies that focused on higher Na+ or K+ concentrations, this study explores G-quadruplex folding and dynamics under low NaCl conditions (≤100 mM) using single-molecule FRET microscopy and advanced structure-based DNA simulation techniques. The smFRET data reveal three distinct populations; unfolded, intermediate dynamic triplex, and dynamic tetraplex structural ensemble. The broad distribution of the folded population highlights the dynamic nature of the quadruplex structure at low salt conditions. In agreement with smFRET result, free energy simulations show that with increase of NaCl concentration, the population shifts towards the folded state, and differentiates all intermediate structural ensemble. The dynamic equilibrium between the triplex and tetraplex scaffolds explain the microscopic basis of conformational heterogeneity within the folded basin. Simulations also reveal that the flexibility of dynamic tetraplex bases depends on the equilibrium distribution of ions underpinning a few ion-mediated dynamic non-native interactions in G-quadruplex structure. Contrary to the previously held belief that Na^+ induces minimal structural heterogeneity, our combined experimental and simulation approaches demonstrate and rationalize the structural variability in G-quadruplexes under low NaCl concentrations.



References:

1. Aznauryan, M.et al. *Nucleic Acids Research* **2016**, *44* (22), 11024-11032.
2. Noer, S. L et al. *Nucleic Acids Res* **2016**, *44* (1), 464-471.
3. Rajendran, A. et al *Angewandte Chemie International Edition* **2014**, *53* (16), 4107-4112.

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Utilizing coronin 1 as a template to develop peptide-based broad-spectrum entry inhibitors: the importance of tryptophan-aspartic acid repeats

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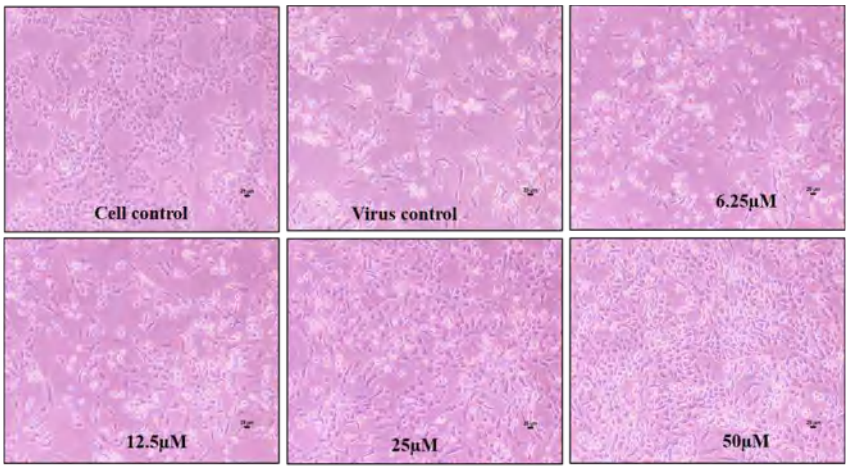
Enveloped viruses can enter the host cells by endocytosis and subsequently fuse with the endosomal membranes, or fuse with the plasma membrane at the cell surface. The crucial stage of viral infection, regardless of the route taken to enter the host cell, is membrane fusion. The present work aims to develop a peptide based fusion inhibitor that prevents membrane fusion by modifying the properties of the participating membranes, without targeting a protein. This would allow us to develop a fusion inhibitor that might work against a larger spectrum of enveloped viruses as it does not target any specific viral fusion protein. With this goal, we have designed a novel peptide by modifying a native sequence derived from coronin-1, a phagosomal protein, that helps to avoid lysosomal degradation of mycobacterium-loaded phagosomes. The designed peptide, mTG-23, inhibits ~30-40% fusion between small unilamellar vesicles containing varying amount of cholesterol by modulating the biophysical properties of the participating bilayers. As a proof of principle, we have further demonstrated that mTG-23 inhibits Influenza A infection in A549 and MDCK cells (with ~EC50 of 20.45 µM and 21.45 µM, respectively), where viral envelop and endosomal membrane fusion is a crucial step. Through a gamut of biophysical and biochemical methods, we surmise that mTG-23 inhibits viral infection by inhibiting viral envelop and endosomal membrane fusion. We envisage that the proposed antiviral strategy can be extended to other viruses that employ a similar modus operandi, providing a novel pan-antiviral approach.



References:

1. G. P. Pattnaik et al. *Chem. Phys. Lipids*, **2018,** 217, 35
2. G. P. Pattnaik et al. *Biochim. Biophys. Acta, Biomembr.*, **2019**, 1861, 183056.
3. S. Pandia et al. *J. Phys. Chem. B*, **2024**, 128, 4986.

P 70

Toward Development of Peptide-based Multi-analyte Fluorescent Sensors and Theranostic Agents

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Biological analytes like phospholipids, reactive oxygen species, H^+ ions, proteins, and metal ions play critical roles in survival and growth of a living system [1-3]. Dynamic changes in the localization and distribution of these bio-analytes transmit and regulate cellular signals, thereby driving ongoing life processes. Furthermore, homeostasis of one analyte is related to that of other analytes present in the biological systems. Disruptions in the correlated distribution of the bio-analytes lead to pathophysiological conditions such as neuro-degenerative diseases [4] and cancers [5]. Hence, it is important to detect multiple analytes simultaneously in living cells to understand their comprehensive role in executing a particular biological process and the effect of dysregulation in their correlated distributions leading to disease conditions. Moreover, the drugs used to treat these diseased conditions can also modulate the levels of one or more bio-analytes in the living system. Owing to regional or genetic variations, concentration and distribution of a particular analyte maybe different in different biological systems as well. This can lead to personalized variations in symptoms and response to treatments. Therefore, it is also important to track the activity of drugs via sensing the levels of the affected bio-analytes in real time. In order to ensure that the drug and the sensing moiety reach the same intracellular localization at the same time, it is necessary to combine the drug and the sensing unit into a theranostic agent [6]. Both the discussed issues involve combining more than one sensing or therapeutic units into a single molecule. To achieve this, we have used peptides as the common platform for attaching different moieties due to their biocompatibility and ease of synthetic modulation [7]. In this backdrop, I will detail the design, synthesis and other studies of a peptide-based multi-analyte sensor and a peptide-based theranostic agent in my poster.

References:

1. G. R. V. Hammond et al. *Curr. Opin. Cell. Biol.* **2020**, *63*, 57.
2. H. J. Forman et al. *Biochemistry* **2010**, *49*, 835.
3. S. Conus et al. *Biochem. Pharmacol.* **2008**, *76*, 1374.
4. K. Jomova et al. *Mol. Cell. Biochem.* **2010**, *345*, 91.
5. V. Ruiz-Rodado et al. *Nat. Methods.* **2022**, *19*, 1048.
6. S. S. Kelkar et al. *Bioconjugate Chem.* **2011**, *22*, 1879.
7. C. Wynne et al. *Sens. Diagn.* **2024**, *3*, 987.

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Investigating Nanoscale Polarity in Thin Polymer Films Using Nile Red Dye and Single Molecule Spectroscopy

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Understanding the polarity of nanoscale environments within thin polymer films is critical for optimizing their performance in various applications, such as in sensors, coatings, and electronic devices.[1] However, the heterogeneity of these environments poses a significant challenge in characterizing local polarity.[2,3] In this study, we use Nile Red,[4] a polarity-sensitive fluorescent dye, as a molecular probe to investigate these nanoscale variations. By employing single-molecule spectroscopy, we gain insights into the distinct nanoenvironments present within the polymer matrix. Our approach enables the detection of variations in polarity at the nanoscale, revealing the complex interplay between polymer morphology and environmental conditions. Preliminary results demonstrate significant heterogeneity in local polarity across different polymer matrices, including PMMA, PVP, PVDF, and Zeonex. These findings highlight the need for single-molecule level analysis to fully understand the heterogeneity within each polymer environment.

References:

1. Palanichamy, K. et al. *Mater Res Express* **2023**, *10* (2)
2. Hess, C. M.et al. *Journal of Physical Chemistry B* **2014**, *118* (29), 8905–8913.
3. Hou, Y. et al. *Journal of Physical Chemistry B* **2000**, *104* (2), 212–219.
4. Freidzon, A. Y et al. *Int J Quantum Chem* **2012**, *112* (18), 3059–3067.

P 72

Elucidating the Aggregation Behaviour of Human β-Synuclein and Its Pathological Mutants :Modulation by Neurotransmitter Interactions

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The misfolding and aggregation of proteins, particularly within the synuclein family, are critical processes underlying the pathology of several neurodegenerative diseases, including Parkinson's disease. Among the synucleins, β-synuclein (β-syn) has been understudied despite its significant role in modulating α-synuclein aggregation and its emerging association with neurotoxicity. This study explores the aggregation properties of wild-type and pathological mutants (P123H and V70M) of recombinant human β-syn under physiologically relevant conditions, focusing on the modulatory effects of various neurotransmitters. It is known that β-syn is resistant to fibrillation under normal cytoplasmic conditions, yet small changes in pH, which frequently occur in cellular environment, can induce fibril formation. Through a series of Thioflavin T (ThT) assays, we observed that catecholamines such as dopamine and epinephrine significantly suppress β-syn fibrillation, a phenomenon further corroborated by ANS binding assays that demonstrated decreased surface hydrophobicity in the presence of these neurotransmitters. Structural and morphological analyses using techniques like Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) provided additional insights into the nature of these fibrils and their modulation by neurotransmitters.

This research highlights the complex role of β-syn in synucleinopathies and underscores the potential of targeting neurotransmitter interactions as a therapeutic strategy. Future work will focus on the differential effects of wild-type and mutant β-syn species on neuronal cell lines, with an emphasis on the chaperonic effects exhibited by these proteins.

Keyword : β-synuclein, Neurotransmitters, Neurodegenerative diseases

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Folate-targeted delivery of doxorubicin in HeLa cells using water soluble ZnS-shelled $CuInS_2$ quantum dots

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Quantum dots have emerged as promising nanocarriers for targeted drug delivery due to their unique optical and electronic properties. The present work explores the prospect of quantum dots for the delivery of a widely used chemotherapeutic agent, doxorubicin (DOX) via a folate receptor mediated pathway. The folate receptor, which is overexpressed in many cancer cells, offers a strategic target for improving the specificity of drug delivery, reducing systemic toxicity, and enhancing therapeutic efficacy. Copper indium sulfide quantum dots with a ZnS shell are functionalized with folic acid to selectively target cancer cells that overexpress folate receptors. DOX added to this system binds to the surface of the quantum dot electrostatically. Fluorescence lifetime imaging microscopy (FLIM) and Fluorescence correlation spectroscopy (FCS) are employed to investigate the intracellular kinetics and interaction of quantum dots and DOX release, providing real-time visualization and quantification of drug accumulation within the cancer cells [1]

References:

1. Suarasan, S. *et al. ACS Appl Mater. Interfac.* **2019**,.11, 7812–7822

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Effect of Vibrational Strong Coupling on Glass Transition Temperature of Polyvinyl acetate

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Vibrational strong coupling (VSC) is mediated by the interaction of the vacuum field in resonance with molecular vibrations. The phenomenon of VSC gives rise to two vibro-polaritonic states that are hybrid of light-matter states.[1,2] This strong light-matter interaction can alter the properties of a molecule such as conductivity[3] and crystallization[4]. It can also modulate the product selectivity and rates of chemical reactions.[5] One such property we would like to study is phase transition such as the glass transition temperature of a polymer (polyvinyl acetate). The experimental results are complemented with transfer matrix method (TMM) simulations. Phase transition is a process that reveals the changes in the physical properties when external conditions such as temperature vary. Studying glass transition with VSC can give insights into inter- and intra-molecular interactions. Here, we are investigating the influence of VSC on the molecular properties like refractive index of the molecules that are undergoing glass transition. Additionally, we are probing if the Fabry-Perot cavity can be used as a tool to study the phase transition of the material. This study would contribute to understanding the mechanism of VSC and how VSC can influence molecular interactions.

References:

1. Garcia-Vidal et. al *Science* **2021**, *373*(6551), eabd0336.
2. Hertzog, M. et al. *Chem. Soc. Rev.* **2019**, *48*(3), 937.
3. Kumar et al. *J. Am. Chem. Soc.* **2024**, *146*(28), 18781.
4. Hirai, K., et. al *Chem. Sci.* **2021,** *12*(36), 11986.
5. Thomas, A. et al. *Science* **2019**, *363*(6427), 615.

P 75

Femtosecond pulse intensity impact on optical nonlinearity of a carbazole-picric acid complex for photonic applications

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The present research article highlights to unveil the nonlinear optical properties of the Carbazole-Picric Acid (Cz-PA) Complexd confirmed through various structural analyses like X-ray crystallography, Infrare (IR) and NMR spectroscopy with UV-Vis absorption spectroscopy. Herein we performed Z-scan experiments (closed as well as open aperture schemes) [1,2] under femtosecond laser pulse excitation (100 fs, 1 kHz) to explore the third-order optical nonlinearity of the complex. The optical nonlinear measurements were performed with the variation of pulse intensities (37-85 GW/cm^2) at 520 nm and the intensity effect on cubic nonlinearity is highlighted. Within the given range of pulse intensity, the material shows the positive absorptive nonlinearity ($\beta > 0$) generated by the two-photon absorption (2PA) process, as well as the electronic Kerr-induced refractive nonlinearity ($n_2 > 0$). We calculated hyperpolarizability (γ) in the order of 10^{-31} esu with the nonlinear susceptibility $\chi^{(3)}$ in the order of 10^{-13} esu. These values increased with intensity. Furthermore, the obtained figures of merit and 2PA cross-section values demonstrate the potential uses of Cz-PA in photonic devices such as ultrafast switching.

References:

1 M. Sheik-Bahae et al. *IEEE J. Quantum Electron.*, 1990, **26**, 760.

2 M. Sheik-bahae et al. *Opt. Lett.*, 1989, **14**, 955.

P 76

Loop Kinematics of Dengue Protease and its role in Allosteric Regulation.

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The Dengue virus (DENV) is the causative agent of dengue fever, and currently, no direct-acting antiviral drugs or approved vaccines exist to combat infections. The NS3 protease, along with its cofactor NS2B, plays a pivotal role in viral replication within human host cells, making them critical drug targets. Previous attempts to design active-site inhibitors have faced challenges due to the relatively shallow active site and surrounding charged residues. To overcome these limitations, the design of allosteric inhibitors is essential for protease inhibition. Our findings emphasize the importance of Finger II motion in opening the claw, which allows the C-terminal region of NS2B to enter, thereby transforming the protease into its active conformation. Further it was established that some known allosteric inhibitors disrupt this motion, effectively inhibiting NS2B/NS3 protease activity and presenting a promising strategy for the development of targeted dengue virus therapeutics.

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Spectroscopic Insights into Beryl Structural Dynamics: A Molecular Approach

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The optical properties of Beryl make it useful in biomedical imaging systems, providing high transparency and durability. Its crystal structure is also explored for potential applications in drug delivery systems and tissue engineering due to its stability and biocompatibility. The optical properties are also utilized in imaging devices, contributing to advancements in medical diagnostics. Hence, molecular orientations and structural distortions are essential for comprehending molecules' responses to variations in temperature (*T*) and pressure (*P*). The features mentioned are also crucial in disciplines such as geology and materials science, where understanding the behavior of materials under different thermodynamic situations is highly significant.

This research comprehensively examines the mineral Beryl utilizing Terahertz (THz), infrared (IR), and Raman spectroscopy techniques at elevated temperatures. These sophisticated spectroscopic techniques enable us to analyze the vibrational modes of Beryl, offering valuable information on its stability and structural features when subjected to temperature stress. Our studies reveal that Beryl retains its structural integrity up to temperatures of 600°C. The vibrational modes of the mineral, which are essential to its behavior in metamorphic rocks, are intimately related to its stability. It is vital to comprehend these vibrational modes to optimize the material's qualities in various geological and industrial applications. Our work adds significant information to geology and materials science disciplines by thoroughly examining its behavior at high temperatures. It clarifies how minerals like Beryl can resist harsh environments without losing their essential characteristics.

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Inhibitory Effect of Diosgenin on α-Synuclein Aggregation: An in-Silico Study

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Parkinson's Disease (PD) is characterized by the accumulation of α-synuclein protein, particularly in its mutated (A30P, A53T, E46K) forms [1, 2]. This aggregation leads to neuronal cell death. To identify the most promising drug candidate, we conducted a high-throughput molecular docking screen of 2000 natural molecules isolated from Indian traditional medicinal plants. Diosgenin emerged as the top candidate, exhibiting the highest binding affinity of -7.5 kcal/mol. Therefore, this study explores the potential of Diosgenin, a phytoconstituent identified through ADMET predictions, to inhibit A30P, A53T, and E46K mutants of α-synuclein aggregation. The temporal and conformational stability of the Diosgenin-protein complex was further validated through extensive molecular dynamics (MD) simulations for 500 ns. MD simulation results at both extracellular (0.145 M) and intracellular (0.015 M) salt concentrations [3] revealed that Diosgenin stabilizes α-synuclein mutants by inducing conformational changes that reduce β-sheet content, a key factor in aggregation. The salt concentration influenced the structural dynamics, with higher salt levels generally promoting more compact and stable conformations. Principal component Analysis (PCA) and free energy calculations further supported the idea that Diosgenin successfully inhibits the α-synuclein mutants investigated here. These results suggest that Diosgenin could be a promising drug for treating PD by preventing α-synuclein aggregation. Further validation can be obtained through wet-lab experiments.

References:

1. Choi, W et al. *FEBS Letters*, 2004. **576**(3): p. 363.
2. Lemkau, L.R., et al. *Journal of Biological Chemistry*, 2012. **287**(14): p. 11526.
3. *Neuroscience*, D.F. Dale Purves , George J. Augustine, Editor. 2018, Sinauer Associates: Oxford University Press, United States.

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Tuning Protein Bioconjugation in Liquid Droplet State

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Understanding diversified role of different form of phosphate-linkage in maintaining biological processes is a topic of interest both in chemistry and biology.[1,2] In this context, we showed here role of nucleotides and phosphates in protein (Bovine Serum Albumin) modification in condensed state.[3] Lysine-specific modification gets inhibited in presence of ATP, but significantly enhanced in presence of monophosphates which allows temporal control over dynamic change in protein functionalization via enzymatic ATP hydrolysis. We believe this temporal modulation of nucleotide-mediated chemoselective behavior in liquid droplet state of protein will find immense application in bioconjugation strategies, protein engineering and also in deciphering altered protein modification behavior in diverse condensed phases.

References:

1. Knouse, K. W. et al. *ACS Cent. Sci.* **2021**, *7 (9)*, 1473.
2. Westheimer, F. H. *Science* **1987**, *235 (4793)*, 1173.
3. Küchler, A. et al. *Nat. Nanotechnol.* **2016**, *11 (5)*, 409–420.

P 80

Enzymatic Dissociation of DNA-Histone Condensates in an Electrophoretic Setting: Modulating DNA Patterning and Hydrogel Viscoelasticity

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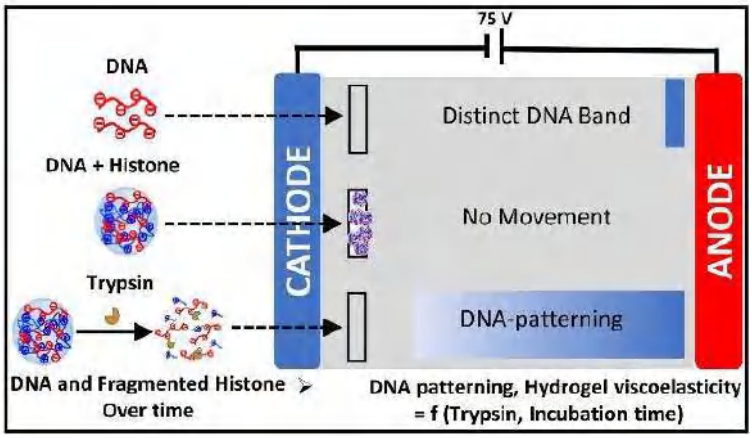
Development of an energy-driven self-assembly process is a matter of interest for understanding and mimicking diverse ranges of biological and environmental patterns in a synthetic system. In this article, first we demonstrate transient and temporally controlled self-assembly of a DNA-histone condensate where trypsin (already present in the system) hydrolyzes histone, resulting in disassembly. Upon performing this dynamic self-assembly process in a gel matrix under an electric field, we observe diverse kinds of DNA patterning across the gel matrix depending on the amount of trypsin, incubation time of the reaction mixture, and gel porosity. Notably, here, the micrometer-sized DNA-histone condensate does not move through the gel and only free DNA can pass; therefore, transport and accumulation of DNA at different zones depend on the release rate of DNA by trypsin. Furthermore, we show that the viscoelasticity of the native gel increases in the presence of DNA and a pattern over gel viscoelasticity at different zones can be achieved by tuning the amount of enzyme, i.e., the dissociation rate of the DNA-histone condensate. We believe enabling spatiotemporally controlled DNA patterning by applying an electric field will be potentially important in designing different kinds of spatiotemporally distinct dynamic materials.



References:

1. Neetu Sivoria et al. *Langmuir* **2024**, *40*, 13505.

P 81

Photo-responsive and shape-switchable MoS_2-Peptide-Hybrid Nano-systems for Enacting Photo-Chemo and siRNA-Mediated Gene Therapy in Glioma

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Photonic nanomedicine includes utilization of a comprehensive range of optical nanomaterials, which upon absorption of photon beam enacts their therapeutic potential. Implementation of light to activate optical materials at the desired site of action has enabled development of successful deep tumor therapeutics. The combination of photochemotherapy with 2D Molybdenum disulfide (MoS_2) nanostructures has attracted attention due to its high surface area and photothermal properties. Here, a novel optically active material-based approach is introduced to induce photothermal anti-glioma effect. Bulk MoS_2 is exfoliated to produce photo-active nanostructures using shape-tunable aqueous peptide scaffolds. Nanofiber and nanosheet like morphology of our self-assembled peptide nanostructures could exfoliate the bulk MoS_2 differentially, altering its ability to generate heat upon NIR photon absorption. Interestingly, exfoliated MoS_2-peptide nanosheets here proves to be an excellent photothermal agent by inducing a temperature elevation up to ~51 ˚C upon 808 nm NIR absorption. Furthermore, ligation of folic acid is carried out to promote tumor-targetability of this NIR-responsive delivery system, that is loaded with anticancer siRNA/drug for treating glioma. Enhanced siRNA/Dox loading onto the 2D flat morphology of MoS_2-peptide nanosheets demonstrates ~90 % cancer cell death in C6 glioma cells under NIR exposure. Expression of an oncogene, galectin-1 also appears to be suppressed following the treatment. Thereafter, *in vivo* analysis in C6 glioma syngeneic rat model demonstrates a remarkable ~11.9 times decrease in the tumor volume with siRNA/Dox loaded nanosystem + NIR as compared to the PBS control group. Taken together, our findings promote the utility of MoS_2-based opto-nanomaterials in conjecture with bio-compatible peptide scaffold as a tri-modal chemo, gene, and phototherapeutic effect in glioma treatment.

References:

1. Wang, Jianling, et al. *Bioactive Materials* 6.11 (2021): 4209.
2. Zhou, Zhan, et al. *Advanced NanoBiomed Research* 2.11 (2022): 2200065.
3. Pang, Bo, et al. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 608 (2021): 125506.
4. Yin, Wenyan, et al. *ACS nano* 8.7 (2014): 6922.
5. Chibh, Sonika, et al. *Nanoscale* 14.3 (2022): 715.

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Tuning the fluorescence emission of DADQ based molecular solids by dielectric environment variation [1]

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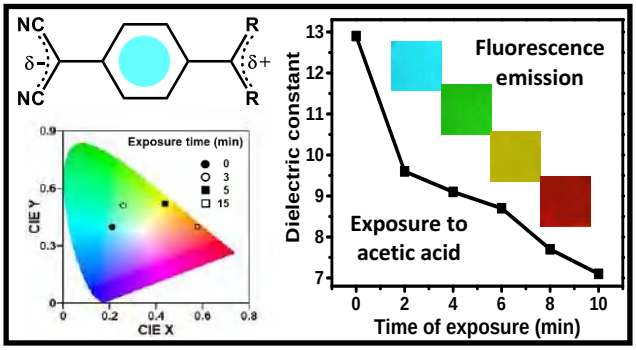
Fluorophores which show enhanced light emission in aggregated or solid-state have been an active area of research due to their applications in fields like optoelectronics [2], information encryption [3], bioimaging [4] and sensing applications [5]. Fluorescence response tuning can be achieved by modification in molecular structure or supramolecular assembly patterns. We show that the fluorescence emission in DADQ based molecular solids can be fine-tuned by short exposure to acetic acid vapors and the effects can be reversed. Contrary to the earlier observation in similar context, there was no structural change observed. Studies based on NMR, mass spectrometry and optical spectroscopy reveal variation results from weak protonation which leads to subtle change in the dielectric environment of molecules. This idea is also supported by computational modelling of electronic ground state and excited state of a DADQ molecule including the standard solvation effect. Dielectric measurement by impedance spectroscopy and surface potential measurement by Kelvin probe force microscopy provide experimental verification of this general concept. The model developed can be useful for broader understanding of fluorescence emission tuning in molecular solids under mild perturbations.



References:

1. Nikita Mahajan et. al. *J. mater. Chem. C* **2024,** 12, 13430.
2. Canbin Oyuyang et. al. *ACS Appl. Mater. Interfaces* **2017**, 9, 10366.
3. Wei Wang.et. al. *ACS Appl. Mater. Interfaces* **2024**, 16, 15096.
4. Senthilnathan N. et. al. *Sci. Rep*. **2017**, *7*(1), 10583.
5. Tianhong Chen. et. al. *J Mater. Chem. A* **2020**, 8, 24695.

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Reduction of membrane cholesterol inhibits neuronal exocytosis

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Exocytosis is essential for neuronal communication and involves significant membrane remodelling. Cholesterol is a strong modulator of membrane properties and can in principle affect exocytosis. However, there are conflicting reports about its effect on exocytosis. This study investigates this puzzle using multiple techniques on both well-characterized artificial bilayers and on living cells.

We measure various physical properties of a lipid membrane (whose composition mimics the synaptic vesicle membrane) as a function of its cholesterol content (from 0% to 50%). As expected, fluorescence lifetime and spectral shift measurements of indicator dyes (e.g., Flip-tR, Nile Red, and Prodan) show that membrane order increases with cholesterol. Surprisingly, the membrane indentation force, as measured by Atomic Force Microscopy, decreases with increasing cholesterol. Importantly, the indentation force correlates with enhanced fusion kinetics involving artificial vesicles and bilayers, as assessed by Total Internal Reflection Fluorescence Microscopy. This indicates that indentation force is a reliable measure of vesicle fusion propensity, while dye-based membrane order measurements may be misleading.

To explore the biological significance, we examined the effect of reducing cholesterol in live cells using mβCD. We found that vesicular exocytosis slows down with decreasing cholesterol, as observed with artificial bilayers. We also investigated whether serotonin-derivatives, such as N-acetyl serotonin (NAS) and 5-hydroxytryptophan (5HTP), can rescue the effect of reducing cholesterol, since serotonin is known to do so[1]. Notably, 10 mM NAS, but not 5HTP, increased the rate of endocytosis in a neuronal cell line by 36%. These insights could aid in identifying small molecules that modulate membrane properties and influence exocytosis without receptor interactions. Though these results are obtained only from *in vitro* cellular systems, they raise potential clinical concerns given that widely used cholesterol lowering drugs cross the blood brain barrier.

Reference and notes:

1. Saha Roy et.al. *J. Phys. Chem. B* 2024, 128, 20, 4975

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Symmetry-Breaking Charge Separation in a Chiral Bis(perylenediimide) Probed at Ensemble and Single-Molecule Levels

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Chiral molecular assemblies exhibiting symmetry-breaking charge separation (SB-CS) are potential candidates for developing chiral organic semiconductors. Herein, we explore the excited state dynamics of a helically chiral perylenediimide bichromophore (Cy-PDI_2) exhibiting SB-CS at the ensemble and single molecule levels (Figure 1). Solvent polarity tunable interchromophoric excitonic coupling in chiral Cy-PDI_2 facilitates the interplay of SB-CS and excimer formation in the ensemble domain.[1] Analogous to the excited state dynamics of Cy-PDI_2 at the ensemble level, single-molecule fluorescence lifetime traces of Cy-PDI_2 depicted long-lived off-states characteristic of the radical ion-pair mediated dark states.[2,3] The discrete electron transfer and charge separation dynamics in Cy-PDI_2 at the single molecule level are governed by the distinct influence of the local environment.[3,4] The present study aims at understanding the fundamental excited state dynamics in chiral organic bichromophores,[5] for designing efficient chiral organic semiconductors and applications towards charge transport materials.

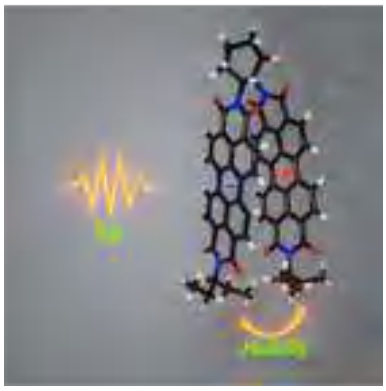

Figure 1: Elucidation of symmetry-breaking charge separation in helically chiral perylenediimide dimer

References:

1. Sebastian et al. ACS *Energy Lett.* **2022**, *7*, 696.
2. Ernst et al. *Chem. Phys. Lett.* **2009**, *482*, 93.
3. Yeow et al. *J. Phys. Chem. A* **2006**, *110*, 1726.
4. Whitcomb et al. *J. Phys. Chem. B* **2015**, *119*, 9020.
5. Haase et al. *Phys. Chem. Chem. Phys.* **2011**, *13*, 1776.
6. Maret et al. *J. Phys. Chem. Lett.* **2023**, *14*, 8667–8675.

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Rapid and sensitive detection of Rhodamine B in cotton candy using surface-enhanced Raman spectroscopy

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Rhodamine B (RhB), a synthetic organic dye, is widely employed in various industries, including textiles, cosmetics, and food coloring. However, its unauthorized use in food products, such as cotton candy, poses significant health risks due to its potential carcinogenic effects. At low concentrations (10^{-7} M), RhB becomes colorless, making its detection challenging. Therefore, developing rapid and sensitive methods for Rhodamine B detection in food products is essential for safeguarding consumer health.

In this study, a cylindrical copper (Cu) substrate combined with silver colloid was utilized for the detection of Rhodamine B in cotton candy using surface-enhanced Raman scattering (SERS) spectroscopy. Various experimental parameters were optimized, including the concentration of the colloid, the colloid-to-analyte ratio, and the volume of the analyte-colloid mixture used for analysis. UV-Visible spectroscopy was employed to characterize the morphology of the SERS substrate. Under optimal conditions, the SERS substrate demonstrated the ability to detect RhB concentrations as low as micromolar levels in an aqueous solution. At an excitation wavelength of 785 nm, the SERS intensity of RhB at 619 cm^{-1} exhibited a linear correlation with the logarithm of RhB concentration, ranging from 10^{-3} to 10^{-9} M, with high reliability ($R^2 = 0.98$). The detection limit (LOD) reached 1×10^{-9} M. Additionally, the RhB spectra at a concentration of 10^{-7} M closely matched that of the real sample.

This study highlights the successful application of SERS for Rhodamine B detection in cotton candy, presenting significant implications for food safety. It contributes to the advancement of rapid, non-destructive analytical techniques for detecting synthetic dyes in food products, ultimately promoting consumer health and safety.

Keywords: Surface Enhanced Raman spectroscopy (SERS); Rhodamine B; Cotton candy;

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Molecular Probe to Image the Intracellular Modulation of NADH Concentration in the Presence of Glycolytic Inhibitor

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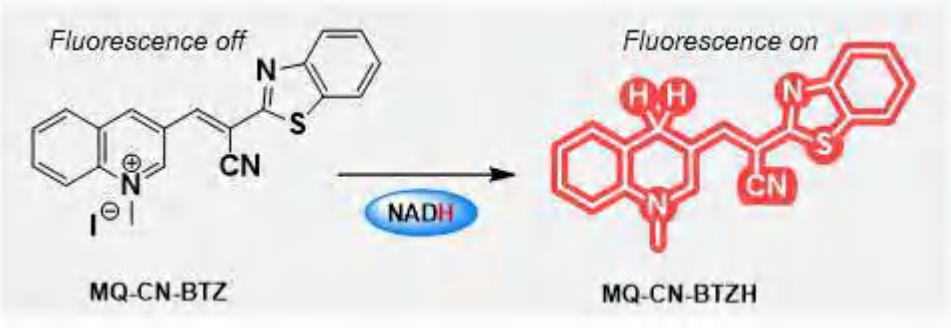
NADH is one of the essential coenzyme participates in various biological processes including maintaining the redox states. To diagnose various pathological conditions at early stages, it is crucial to develop understanding on the intracellular NADH levels. Here, we report the application of a dual channel probe **MQ-CN-BTZ** for fluorescence imaging of intracellular NADH levels. Interestingly, it was observed that by varying the ratio between probe and NADH concentration in solution phase, the probe showed dual emission at ~530 nm and ~660 nm when excited at 475 nm. Also, very large Stokes shift of ~180 nm was observed with respect to the longer emission wavelength. Furthermore, on the basis of good optical response, the probe was explored to image the crucial event of glycolysis pathway by employing glycolytic inhibitor 3-bromopyruvic acid (3-BrPA) that inhibits the activity of glyceraldehyde phosphate dehydrogenase (GAPDH) enzyme. As the depletion of the NADH levels corresponds to the inactivity of GADPH upon treatment with inhibitor, we attempted imaging of the modulation of NADH concentration in cellular system in the presence of 3-BrPA inhibitor indicating the importance of glycolysis step in elevating NADH levels. Overall, the dual channel optical probe was used to image the modulation of NADH concentration in in the presence of glycolytic inhibitor during the glycolysis step.



References:

1. Li, M, et al. *Journal of Materials Chemistry B*, **2021**, *9*, 9547.
2. Pan, X, et al. *Chemical Science*, **2019**, *10*, 8179.
3. Zhao, Y, et al. Analytical chemistry, **2018**, *91*, 1368.
4. Fomin, A, M, et al. *ACS Sensors*, **2016**, *1*, 702.
5. Magesh, K, et al. *ACS Applied Bio Materials*, **2024**, *7*,5679.
6. Wang, X, et al. *Chemical Engineering Journal*, **2024**, *495*, 153652.

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Divalent Cations Modulate Depth-Dependent Polarity and Hydration at Lipid/Water Interface

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Ion binding at the lipid/water interface can significantly impact the structural, functional, and dynamic properties of cell membranes [1-3]. Although ion-lipid interactions have been widely studied, the precise influence of ion binding on the depth-dependent polarity and hydration of the lipid/water interface is still not well understood because of the differential binding of the divalent cations [4,5]. This work investigates the impact of three biologically significant divalent cations Mg^{2+}, Ca^{2+}, and Zn^{2+} on the depth-resolved interfacial polarity and hydration of zwitterionic DPPC lipids in its gel-phase at ambient temperature. To quantify these depth-specific characteristics, a set of solvatochromic fluorescent probes derived from 4-Aminophthalamide, with varying alkyl chain lengths (4AP-Cn; n = 5, 7, 9), was employed. Using steady-state fluorescence measurement and extensive molecular dynamics (MD) simulations, we quantify the minimal or maximal changes in interfacial polarity and hydration caused by divalent cation binding at the lipid/water interface. Our observations show that Zn^{2+} induces a significant blue shift in the steady-state fluorescence spectra of all the 4AP-Cn dyes, indicating a marked reduction in local polarity ($E_T^N \leq 0.05$) at the lipid/water interface compared to Mg^{2+} and Ca^{2+}, which leads to higher polarity ($E_T^N \geq 0.2$) in Richardts' polarity scale. The penetration-dependent steady-state fluorescence spectra of the 4AP-Cn dyes in the presence of Mg^{2+} and Ca^{2+} are similar to those observed for without cations, with only a slight red shift for Mg^{2+}, suggesting a minor hydration at the lipid interface. The MD simulations reveal that cations primarily bind to the phosphate group and glycerol regions of the lipids head groups. Our MD simulations also indicate that Zn^{2+} binds near the phosphate and glycerol group, and causes significant dehydration at the lipid/water interface, as detected by the 4AP-Cn dyes, whereas Mg^{2+} and Ca^{2+} have a much milder impact, with Mg^{2+} inducing slight hydration. Our current study unfolds the unique effects of divalent cations as probed by 4AP-Cn probes at the lipid/water interface, highlighting the potential of these dyes to track penetration-dependent changes in membrane properties induced by external agents or local environmental conditions.

References:

1. Friedman, R *J Membr Biol* **2018**, *251*, 453.
2. Yang, J et al. *J Chem Theory Comput* **2015**, *11*, 4495.
3. Kanti De, S et al. *Physical Chemistry Chemical Physics* **2018**, *20*, 14796.
4. Alsop, R. J et al. *Soft Matter* **2016**, *12*, 6737.
5. Hossain, S. I et al. *Biophys J* **2022**, *121*, 3520.

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NusG displaces σ^{70} from mature transcription elongation complexes

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The *E. Coli* housekeeping sigma factor (σ^{70}) - a crucial component of the RNA polymerase (RNAP) holoenzyme is essential for promoter recognition and transcription initiation. Previous studies revealed that in addition to its classical role in initiation, σ^{70} can continue to influence transcription during later stages by remaining associated with the elongation complex (RDe). Additionally, structural studies revealed that an important transcription factor - NusG – can bind to a site in RDe closely overlapping with the s^{70} binding site in RNAP. Here we used fluorescence correlation spectroscopy (FCS) to develop an assay quantifying the fraction of elongation complexes which retained σ^{70} in presence and absence of varying amounts of NusG and found that NusG enabled release of σ^{70} from stalled RDe in a concentration dependent manner. Further, Single molecule TIRF experiments revealed that the energy barrier for σ^{70} release was greater in case of early elongation complexes (RDe14) in comparison to mature elongation complexes (RDe27). Our results suggest that σ^{70} plays a more prominent role in early transcription elongation, while NusG facilitates its release in later stages.

References:

1. Mazumder et al. *Journal of Molecular Biology*,**2019**, *Vol 431, Issue20.*
2. Kapanidis et al. *Science*, **2006,** *vol 314, Issue 5802.*

P 89

Ultrafast photoinduced electron transfer from Cu-deficient CuInS quantum dots to methyl viologen in water

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Increase in Cu-deficiency leads to the enhancement in photoluminescence (PL) intensity and lifetime in aqueous L-glutathione capped CuInS/ZnS quantum dots (QDs) [1] Such compositional variation is found to affect the rate of ultrafast photoinduced electron transfer (PET) through the ZnS shell from the QDs to methyl viologen (MV^{+2}). The extent of PL quenching by MV^{+2} gradually decreases with increasing Cu deficiency.in QDs. Stern-Volmer plots constructed using PL intensities exhibit upward curvature for all cases and PL lifetime remain unaffected. This observation can be explained by assuming the adsorption of Poisson distributed quencher molecules on the surface of negatively charged QDs. and PET happening in ultrafast regime, post excitation. Femtosecond Transient Absorption Spectroscopy (TAS) confirms the occurrence of ultrafast PET from QDs to quencher as the magnitude of ground state bleach (GSB) of the QDs gradually decrease with increase in concentration of quencher.[2] The dynamics of PET is estimated from the rate of GSB recovery, which becomes faster with decrease in Cu deficiency. As the PET happens through trap states closer to the conduction band edge, the density of these trap states decreases with increasing Cu deficiency due to more incorporation of Zn^{+2} ions in lattice of QDs by ZnS shelling. This observation forms the basis for future experiments on photosensitization of molecular catalysts by these QDs.

References:

1. A. Fuhr et al. *ACS Nano* **2020**, 14, 2212.
2. N. Eliasson et al. *J Phys Chem C* **2021**, 125, 14751.

P 90

Direct visualizing three intermediate conformation in single SARS-CoV-2 spike trimer during entry

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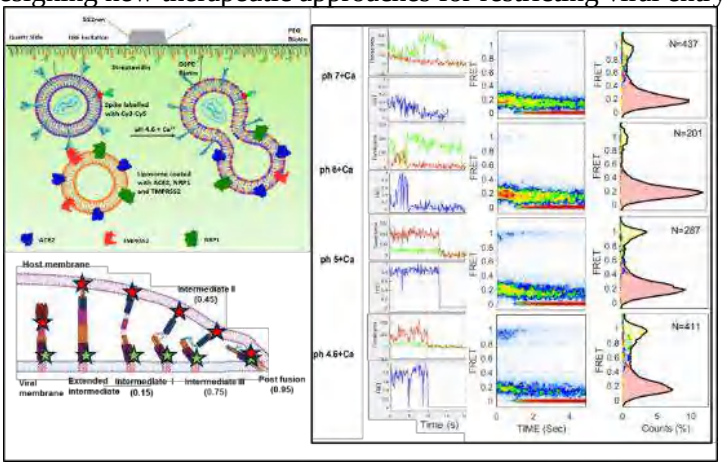
The SARS-CoV-2 is the causative agent for COVID-19 disease. Several variants of SARS-CoV-2 emerged during COVID pandemic. Out of all the variants, the omicron variant (B.1.529.1) was the latest variant of concern and has highest transmissibility owing to its enhanced immune evasion ability [1]. Therefore, understanding the molecular mechanism of cellular entry of SARS-CoV-2 omicron is of huge importance. The entry of omicron virus depends on the trimeric spike glycoprotein, which mediates the membrane fusion between the viral membrane and cellular membrane. The membrane fusion is a multi-step process [2], in which the spike first binds with the receptor protein ACE2 (Angiotensin convertase enzyme 2). Binding of ACE2 with the S1 domain of the spike glycoprotein triggers the spike fusion domain (S2) [3]. Structural study of both the pre and post fusion spike protein suggests a large-scale conformational rearrangement of the S2 domain [4]. Here, using Single molecule foster resonance energy transfer (smFRET) imaging technique, I have directly visualized the conformational dynamics in the S2 domain of individual spike glycoprotein trimers in the surface of the SARS-CoV-2 omicron virion during entry. I discovered that at neutral pH condition, prefusion structure of omicron spike samples three new conformations, which were previously unknown, in reversible manner. Lowering of pH shifts the equilibrium towards a high-FRET intermediate state on pathway to fusion. Finally, addition of Ca^{2+} at low pH shifts the conformational changes of spike to the post-fusion coiled-coil state. I have found that target membrane also allosterically regulates the dynamic intermediates of S2 on the pathway to fusion. My single molecule analysis has determined at least three possible intermediate states of omicron S2 domain on pathway to fusion. My study distinctively depicts the specific interaction between the membrane proximal region (MPR) and fusion peptide proximal region (FPPR) of S2 domain for forming the post-fusion structure. My single molecule assay developed will be utilized for designing new therapeutic approaches for restricting viral entry.



References:

1. Zhang et. al. *Cell Rep*. **2022** ,*39:* 110729.
2. Marcink et. al. *Sci Adv*. **2022**, *8(33)*
3. Singh et.al. *Cell Rep*. **2022**, *39(3):*110694
4. W Shi, et al. *Nature* **2023** *619*,403.

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Probing the oligomerization/de-oligomerization process of *Mtb*'s ATPase, Mpa

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Protein degradation is a naturally occurring cellular process that plays an important role in post-translational protein quality control and maintaining protein homeostasis. Enzyme complexes known as proteases and proteasomes carry out this process[1]. *Mycobacterium tuberculosis* is a type of actinobacteria that causes tuberculosis and has both bacteria-like proteases and eukarya-like proteasomes. Similar to other proteasomal complexes, *Mtb*'s proteasomal complex is made up of two independent compartments: a hexameric ATPase compartment called mycobacterial proteasomal activator (Mpa) and a peptidase compartment called 20SCP. *Mtb*'s ATPase, Mpa, is a homohexameric complex that is a class of AAA+ (ATPase Associated with various cellular Activities) ATPase superfamily. It recognises, mechanically unfolds, and translocates the substrate protein having a degron tag called Pup (prokaryotic ubiquitin-like protein) using ATP as the energy source[2,3]. In contrast to other ATPases of proteases, Mpa can hexamerise spontaneously even in the absence of the nucleotide because of the strong non-covalent interactions (H-bonding, electrostatic interactions, and hydrophobic interactions) between its OB domain[4]. However, the oligomerisation process of Mpa hexamer is not well studied yet. To probe the oligomerisation/de-oligomerisation process of Mpa hexamer, we have used both chemical and thermal denaturation methods. Secondary structure analysis using CD indicates a two-state process from a hexameric state to the unfolded state, while the tertiary structure analysis monitoring the tryptophan fluorescence indicates a transition from the hexameric state to the unfolded state via an intermediate state, plausibly a monomeric state. This transition from hexamer to monomer before unfolding is also supported by the DSC measurements. Interestingly, the chemically refolded state retains its structure and can hexamerize spontaneously but looses its ability to hydrolyse ATP. These studies indicate that Mpa deoligomerizes into only one state, plausibly a monomer, before unfolding but can spontaneously oligomerize upon refolding, although loosing its functional ability.

References

1. De Mot, et al. *Trends Microbiol* **1999**, *7*, 88.
2. M Kavalchuk et al. *Nat Commun* **2022**, *13*, 276.
3. F Striebel et al. *EMBO J* **2010**, *29*, 1262.
4. T Wang et al. *Structure* **2009**, *17*, 1377.

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Evaluating the stability of FtsZ polymers using chemical and mechanical disrupters

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The cell division process is fundamental to all life forms. FtsZ, Filamenting temperature-sensitive Z-mutant, is a conserved protein in all bacterial species, and the importance of its polymers during the earliest stage of cell division has recently been realized [1]. The polymers of FtsZ form when the GTP-binding pocket in the N-terminal of one subunit is in contact with the GTP hydrolyzing domain in the C-terminal of another subunit[2]. These FtsZ polymers further assemble at the mid-cell in a ring called "Z-ring"[3] to initiate the division. One of the proposed mechanical models of cytokinesis indicates the bending of the FtsZ polymers upon GTP-hydrolysis, which induces the inward constriction force in the cell wall[4,5] to form the septum (*figure 1*). Inhibition of Z-ring formation will enlarge the cell into filamentous form, making it more susceptible to lysis/death. In this regard, molecular features that can serve as "chemical disrupters" of FtsZ polymers can be beneficial pharmacophores for antibiotic discovery. In our study, we are testing a hybrid of "Combretastatin" and "Discoipyrrole" that have previously shown good anti-polymerization activity against tubulin (a eukaryotic homolog of FtsZ).
Considering the role of FtsZ as the force generator, It is essential to comprehend the mechanical stability of FtsZ monomers and polymers to construct a qualitative model of the constriction mechanism. We will employ Atomic Force Microscopy (AFM)--based SMFS to understand the mechanical properties of FtsZ by determining the stability of FtsZ monomer and filaments along the direction of polymerization.
I will present our recent results on the chemical and mechanical disruption of Ftsz polymers.

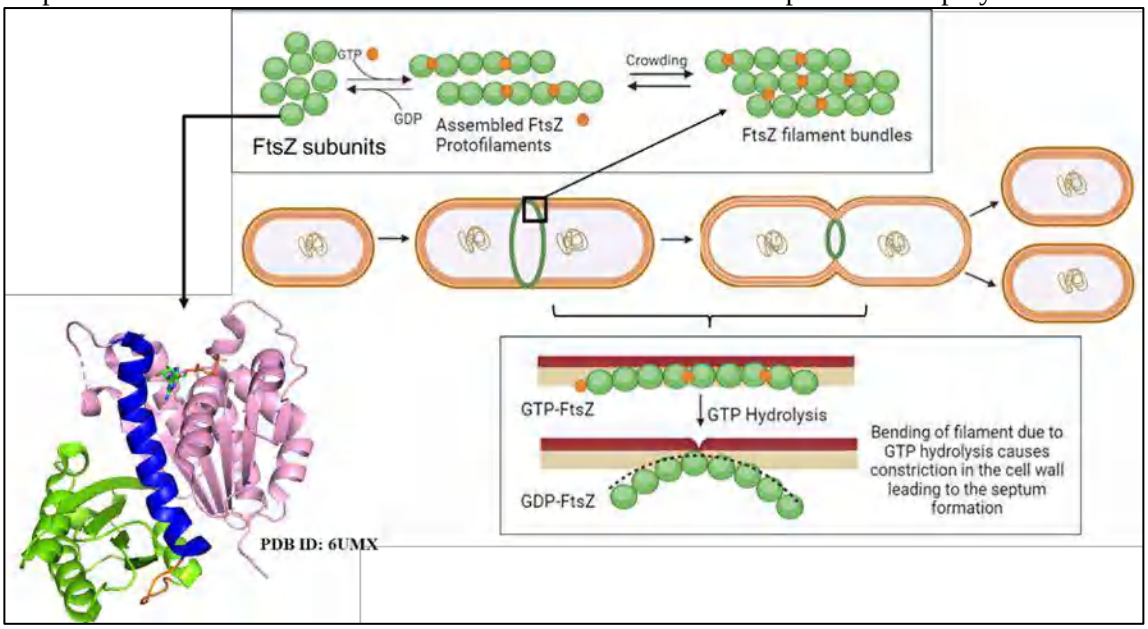

Figure 1. Role of FtsZ in Bacterial Cell wall Constriction

References:

[1] Barrows JM, et al. *Cell Biol* **2021**, *68*, 163.
[2] Löwe J et al. *Nature* **1998**, *391*, 203.
[3] Du S et al. *Trends Microbiol* **2019**, *27*, 781.
[4] Levin PA et al. *EcoSal Plus.* **2021,** *9*, 0022.
[5] Li Z et al. *EMBO Journal* **2007**, *26*, 4694.

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Exploring the Specific Role of Iron Center on the Catalytic Activity of Human Serum Transferrin: CTAB-induced Conformational Changes and Sequestration by Mixed Micelles

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Understanding the fundamentals of conformational changes in a protein and their associated biological activities is an indispensable research topic.[1] This concept becomes more relevant in the context of metalloproteins, owing to the formation of specific conformation(s) induced by internal perturbations (like change in pH, ligand binding, or receptor binding), which may carry out the binding and release of the metal ion/ions from the metal binding center of the protein.[2] Herein, we have investigated the conformational changes of an iron-binding protein, monoferric human serum transferrin (Fe-hTF), using several spectroscopic approaches. We could reversibly tune the cetyltrimethylammonium bromide (CTAB)-induced conformation of the protein, exploiting the concept of mixed micelles, formed by three sequestrating agents: (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) hydrate (CHAPS), and two bile salts namely, sodium cholate (NaC), sodium deoxycholate (NaDC).[3]

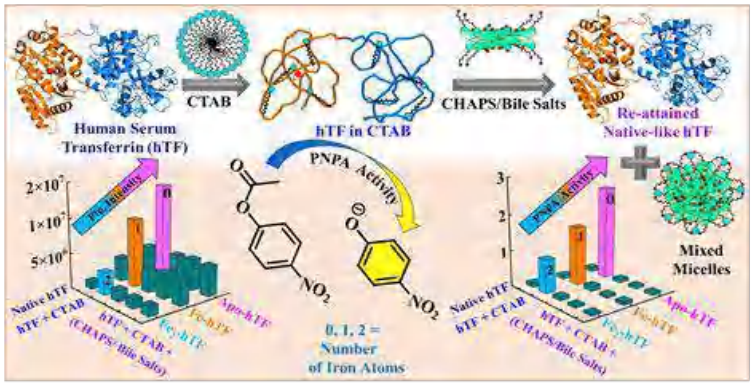

Figure: Pictorial representation of conformational dynamics of human serum transferrin (hTF) induced by CTAB and subsequent sequestration by CHAPS/Bile salts mixed micelles.

The formation of mixed micelles between CTAB and these reagents (CHAPS/NaC/NaDC), results in the sequestration of CTAB molecules from the protein environment, and aids the protein to re-attain its native-like structure. However, the guanidinium hydrochloride-induced denatured Fe-hTF did not acquire its native-like structure using these sequestrating agents, which substantiates the exclusive role of mixed micelles in the present study. Apart from this, we found that the conformation of transferrin (adopted in the presence of CTAB) displays pronounced esterase-like activity toward *para*-nitrophenylacetate (PNPA) substrate as compared to native transferrin. We also outlined the impact of the iron center and amino acids surrounding the iron center for the effective catalytic activity in the CTAB medium. We estimated ~3 times higher specific catalytic efficiency for the iron-depleted Apo-hTF compared to the fully iron-saturated Fe_2-hTF in the presence of CTAB.[3]

References:

1. Harroun et al. *Nat. Methods* **2022**, *19*, 71.
2. Joy et al. *J. Phys. Chem. B* **2023,** *127*, 4374.
3. Yadav et al. *Langmuir* **2024,** *40*, 6172.

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Visualizing Biomolecular Conformational Dynamics using Single-Molecule FRET

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Förster Resonance Energy Transfer (FRET) has been extensively utilized to determine proximity between fluorophore tagged with biomolecules and probe their structural changes or interaction dynamics. However, solution FRET measurements are often inadequate to unveil mechanisms or real-time dynamics owing to ensemble averaging effect. In contrast, single-molecule FRET (smFRET) on a large number of individual donor-acceptor fluorophores attached to surface immobilized biomolecule(s) can reveal intricacies of both intra and inter molecular conformational dynamics, thereby providing new mechanistic insights.[1]

We have assembled a smFRET setup in our lab which involves a home-built total internal reflection fluorescence microscope (TIRFM) equipped with multiple laser lines and a dual-color detection system using an image splitter and an CCD camera. This setup allows us to visualize and track the dynamics a large number dual-dye labelled biomolecules one at a time, when these are either immobilized on a surface or diffuse within the evanescent field. Here, we discuss two projects which utilize inter- and intra-molecular smFRET via labelling distinct subdomains of proteins, with our inferences relying on the analyses of FRET efficiency histograms from several hundred trajectories. First, our studies elucidate interactions between a ribosomal methyl transferase enzyme (Erm) and a precursor (immature) bacterial ribosome during the methylation process of a ribosomal RNA, which forms the basis for antibiotic (erythromyocin) resistance in certain bacteria.[2] Second, our analyses reveals allosteric fluctuations of subdomains within an enzyme involved in the purine biosynthetic pathway (PurL), during the transient formation of a transport tunnel via which the product of one catalytic reaction (ammonia) migrate to the second active site, where ammonia acts as a substrate.[3]

References:

1. Lerner E. et al. *Science*. **2018**, *19*, 359.
2. Schaenzer, A.J. et al.*Trends in molecular medicine*, **2020**, *26(8)*, 768.
3. Sharma, N. et al., *ACS Catalysis*, **2022**, *12(3)*, 1930.

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Engineered MXene Based Photocatalyst for the Selective Photoreduction of CO_2 to Ethanol

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Photocatalytic CO_2 reduction has emerged as a promising strategy, capitalizing on solar energy to drive the conversion of CO_2 into valuable products.[1,2] **CeO_2**, a metal oxide is a promising material for designing highly active catalyst for the photoreduction of carbon dioxide (CO_2RR). It is redox-active in nature and can switch between the oxidation states from Ce^{3+} to Ce^{4+} while exposed to light. Herein, **CeO_2/Ti_3C_2 MXene** hetero-structured photocatalysts have been synthesized hydrothermally that exhibit superior CO_2 reduction to ethanol and methane. The intimate interfacial interactions between **CeO_2** and **Ti_3C_2MXene**, can lead to improved charge separation, faster charge transfer kinetics, and an increased degree of surface sites for CO_2 absorption and activation, in the **Ti_3C_2 MXene**/**CeO_2** heterostructure photocatalysts. The charge separation efficiency and the electron−hole pair recombination process of the photocatalysts can be understood from the photoluminescence (PL) spectra and the charge transfer kinetics can be understood from the time-resolved photoluminescence (TRPL) analysis. In order to optimize the photocatalytic CO_2 reduction efficiency, **Ti_3C_2 MXene** loading amount has been varied and the optimized **5-TC/Ce** shows CO_2 reduction with a drastically enhanced yield of ethanol of the order of 6127 μmolg^{-1} at 5h with 98% selectivity and 7.54% apparent quantum efficiency, which is 6 folds higher than that of ethanol produced by the bare **CeO_2**.[3]

References:

1. K. Niharika et al. *Journal of Materials Chemistry A*. **2023**, *11*, 20839.
2. B. Mishra et al. *Chem Su sChem*, **2019**, *12(14)*, 3383.
3. R. P. Mishra et al. *Langmuir*, **2023**, *39*, 14189.

P 96

Unravelling the LLPS behaviour of SUMO1 protein

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Liquid-liquid phase separation of intrinsically disordered proteins and RNA molecules plays a very important role in many regulatory cellular functions. Our group recently reported small ubiquitin-like modifier (SUMO1) shows LLPS in its native state in the presence of artificial crowders[1]. What are the underlying reasons for its LLPS in its native state are yet to be studied. By confocal and scattering studies, we observed that SUMO1 LLPS is pH-dependent and in a specific pH window, indicating that some residues play a major role in condensate formation. The minimum saturation concentration at which protein starts to show LLPS in the presence of crowders is significantly less for multimeric SUMO1 than SUMO1 monomer. It is biologically relevant because SUMO1 also forms polySUMO1 chains during post-translational modification of various proteins[2,3].

References:

1. S. Arora et al. *Journal of Physical Chemistry Letters* **2023**, *14* (40), 9060.
2. P. Krumova et al. *Journal of Cell Biology* **2011**, *194* (1), 49.
3. A. Princz et al. *Gerontology* **2020**, *66* (2), 122.

P 97

Investigating Kinesin 1 role as a Tether at Lipid droplet and Endoplasmic Reticulum Membrane contact site

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Lipid Droplets (LD) are dynamic organelles which play a crucial role in lipid metabolism and storage. They are known to interact with various organelles, at specific sites known as Membrane contact sites (MCS). LD interacts with endoplasmic reticulum network at specialized domains to carry out various cellular processes such as synthesis, transport, and breakdown of lipids, and facilitates exchange of proteins and lipids across these membranes. Cellular trafficking of LD by motor proteins like Kinesin or Dynein is required for such site-specific interaction. Previously, our lab has elucidated the mechanism by which kinesin motor protein is recruited on LD and drives the LD metabolism towards secretion of VLDL particles at Hepatocytes periphery. In this study, we investigate the role of Kinesin 1 motor protein to convert into a tether via ER-resident protein, Kinectin1 at LD-ER membrane contact site (MCS). Using high spatiotemporal resolution imaging techniques, we assessed the motion of LDs and their distribution in the absence/presence of Kinesin 1 by live cell imaging in COS7 cells and characterize its colocalization with Kinectin 1 at ER-LD MCSs. We hypothesize that the interplay between Kinectin 1, Rab18, and Kinesin 1 is essential for mediating ER-LD tethering and affects the subsequent exchange of lipids and protein between these organelles. To better understand this ternary interaction, we exploit invitro reconstitution assay, biochemical assay, and proximity labelling strategy. Additionally, we will examine the impact of Kinesin1-Kinectin1 complex on lipid droplet biogenesis, growth, and lipolysis. Our findings will contribute to a better understanding of the molecular mechanisms underlying ER-LD interactions and may have potential applications in the treatment of lipid metabolism disorders.

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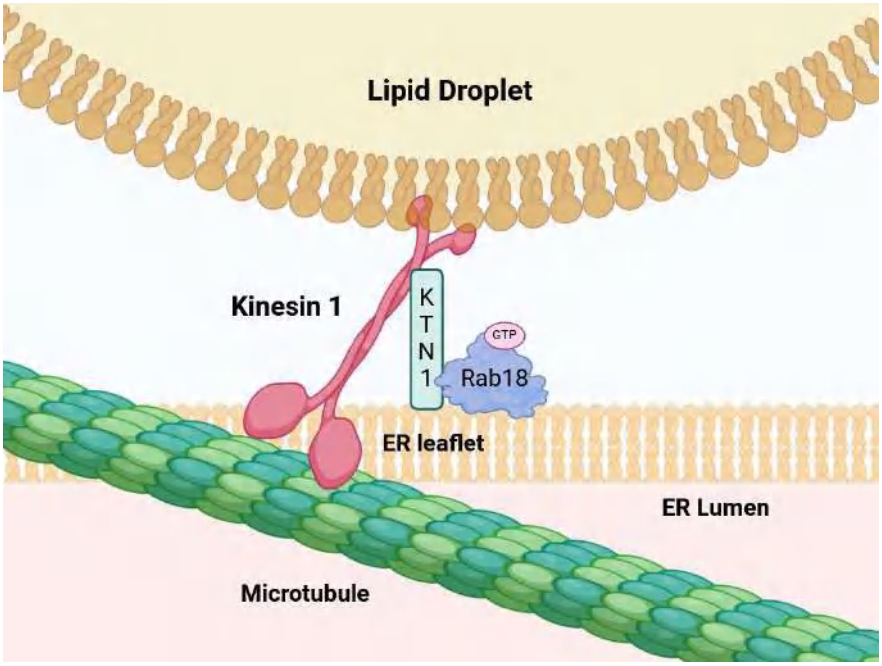

P 98

Designing ECM inspired Bioactive 3D Hydrogel based Biomaterial to fabricate Advanced Tissue Mimics

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In recent times, the main focus of tissue engineering and regenerative medicines has been to obtain advanced biomaterials that can reliably propagate the functionality and properties of the damaged tissues. Precisely, peptide hydrogels owing to their biocompatibility and their ability to mimic the structural and functional complexity of native Extracellular Matrix (ECM) are gaining utmost attention. Mimicking ECM by using biomolecular scaffolds is extremely crucial for facilitating the cellular proliferation, differentiation, and other molecular processes. In this regard, utilization of minimalistic bioactive peptide sequences for the creation of tissue engineering scaffolds offers a significant advantage over traditional design methods, which focused mostly on peptide sequences, far from bearing any suitable biological relevance. In our work, we are mainly focusing on mimicking various proteins present in the native ECM like, laminin and collagen. To this end, we have designed a novel minimalistic peptide hydrogelator to develop scaffolds based on laminin-511 which shows a solvent mediated self-assembly. The secondary structure formed by the self-assembled hydrogels was confirmed using CD, fluorescence and FTIR spectroscopy. These designed hydrogels displayed a nanofibrillar morphology that may create a suitable interface to support cellular growth and proliferation as confirmed using confocal microscopy. Interestingly, these bioactive hydrogels showed optimal mechanical properties which was reflected in significant cellular viability. Furthermore, we adapted a non-equilibrium self-assembly approach to create diverse nanostructures based on a single gelator domain. The rationale behind this strategy stems from the fact that self-assembly of peptides is highly susceptible towards environmental conditions like temperature, pH, ionic concentrations, which leads to diverse nanostructures. Here, we have checked the interaction between collagen mimetic positively charged peptide with biologically relevant anions. The electrostatic interaction between peptide and ions mediate the self-assembly and form distinctive secondary structure which was confirmed by fluorescence, CD, UV-Vis and FTIR spectroscopy. These ion-responsive gels can mediate cellular adhesion and proliferation. We believe the incorporation of short bioactive peptide can effectively mimic ECM proteins to fabricate innovative biomaterials, essential in the field of tissue engineering.

References:

1. R. Jain et al. *Materials Science and Engineering: C* **2020**, *108*, 110483.
2. V.K. Pal et al. *Macromolecular Bioscience* **2022**, *22*, 2100462.
3. V.K. Pal et al. *Langmuir* **2020**, *36*, 1003.
4. R. Jain et al. *ACS Biomaterials Science & Engineering* **2020**, *6*, 2832.

P 99

Effect of solvents on morphological anisotropy of aromatic amino acid-based Aggregation-induced emission (AIE) active system

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The development of fluorogenic materials from amino acids is challenging, but it can be addressed by using derivatives of these amino acids in synthetic protocols. Herein, an aromatic amino acid-derived Schiff base of 1-pyrene carboxaldehyde has been synthesized, exhibiting aggregation-induced emission (AIE). The AIEgen molecule follows a photoinduced electron transfer (PET) pathway. It also demonstrates morphological anisotropy in solvent mixtures, likely due to noncovalent interactions and solute-solvent interactions (e.g., hydrophobicity). This phenomenon has been analyzed through thermodynamic aspects to understand the nucleation-elongation process, as evidenced by variable temperature absorption spectra and the roles of secondary nucleation and cooperative growth, observed through variable temperature circular dichroism. Moreover, the influence of organic solvents such as protic MeOH and the cosolvent H2O on morphological anisotropy has been evaluated using the polar organic solvent DMSO and solvent isotope effects, respectively. Morphological anisotropy can arise from molecular arrangements leading to both amorphous and crystalline phases. These synthesized AIE-active molecules have potential applications in bioimaging, biosensing, OLEDs, and more

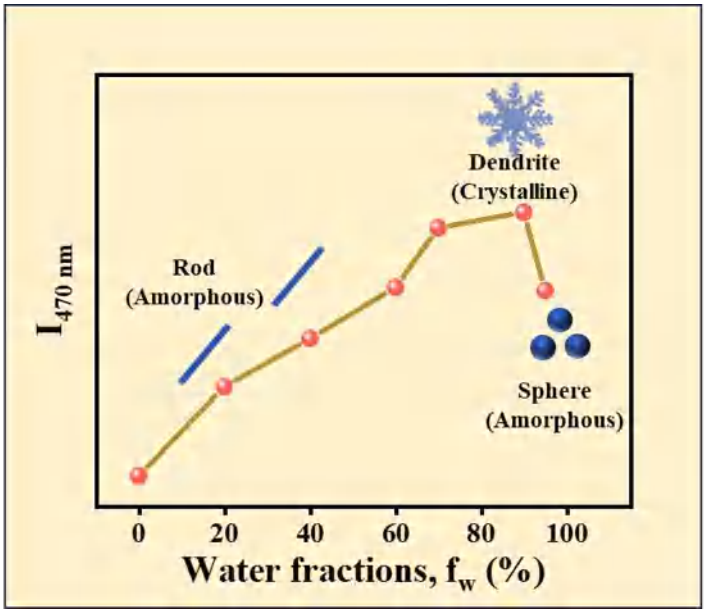

Fig. Aggregation-induced emission and the morphological anisotropy of synthesized Schiff base.

References:

1. Panigrahi et al. *ACS Appl. Mater. Interfaces* **2020**, *12*, 5389−5402
2. Pal et al. *J. Am. Chem. Soc.* **2023**, *145*, 2532−2543

P 100

Lanthanides driven microphase separation of proteins: ions hydration matters.

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Lanthanides, well-known rare earth elements (REE), acquire a major position in the modern world. Their growing application have prompted researchers to study their effects on human life. In presence of Lanthanide (III) cations, proteins exhibit intriguing phase behaviours such as liquid liquid phase separation (LLPS), reentrant condensation, etc which are highly dependent on concentration. In our study, we choose La3+, Nd3+, Gd3+, Ho3+ and Lu3+ ions and BSA protein to observe the phase changes upon the addition of salt to the protein solution. We illustrate the phase diagram which is classified in three regimes; regime I (R1) and regime III (R3) are visually transparent whereas regime II signifies the turbid phase with two critical concentrations C* and C** and the middle point depicts as Cm. The phases are characterized by turbidity, zeta-potential and optical microscopy measurements. Finally, we explore the role of ion hydration in these processes using THz-FTIR (0.2-22.5 THz) measurements. Our experimental results clearly demonstrate that considering only the charge (III) is insufficient to explain the observed phases; ion hydration must also be taken into account.

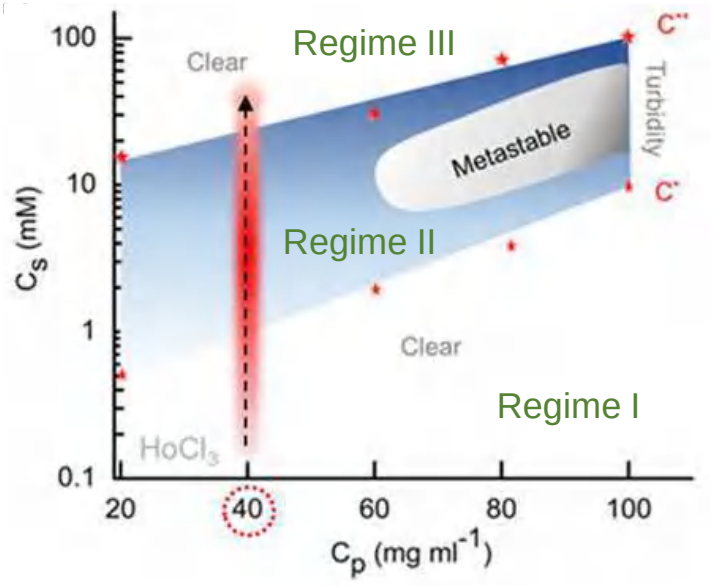

Figure. Representative phase diagram of BSA protein against $HoCl_3$ salt concentration where three regions are mentioned as regime I, II and III depending on appearance and disappearance of turbidity.

References:

1. O. Matsarskaia et al. *Chem Phys Chem* **2020**, *20*, 1742.
2. R. Saha et al. *Phys. Chem. Chem. Phys.* **2022**, *24*, 23661.
3. R. Saha et al. *Langmuir* **2023**, *39*, 4601.

P 101

Encapsulation of Thioflavin T (ThT) by Different Bile Salt Aggregates: Spectroscopic Investigation

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Bile salts are amphiphilic molecules, but their structure is quite different from that of other conventional surfactant molecules. They have a hydrophobic convex side and a hydrophilic concave side. Above Critical micellar concentration, they form primary aggregates via hydrophobic interaction between monomers. At very high concentrations, these primary aggregates form secondary aggregates via hydrogen bonding and hydrophobic interaction. These aggregates are quite unique as they have both hydrophilic and hydrophobic pockets, which can bind different types of guest molecules depending on their nature. In this work, we investigated the binding efficiency of amphiphilic probe Thioflavin-T (ThT) with three different bile salts aggregates, namely Sodium Cholate (NaCh), Sodium Taurocholate (NaTC) and Sodium Deoxycholate (NaDC). The changes in absorption and emission properties of probe molecule (ThT) were found to be sensitive to increasing bile salt concentration. Due to the encapsulation by bile salts aggregates, the emission intensity and emission lifetime of ThT increase significantly. Moreover, we have also investigated the effect of change in the ionic strength of the medium on the spectroscopic properties of ThT inside bile salt aggregates by adding sodium chloride (NaCl). In the presence of NaCl, the fluorescence lifetime of ThT in bile salts increases significantly due to the formation of more rigid aggregates. The encapsulation efficiency of ThT in bile salt aggregates has been assessed by using iodide (I^-) as an external ionic quencher. We found that NaDC aggregates are more efficient in the modulation of the photophysical properties of ThT than the other two bile salt aggregates.

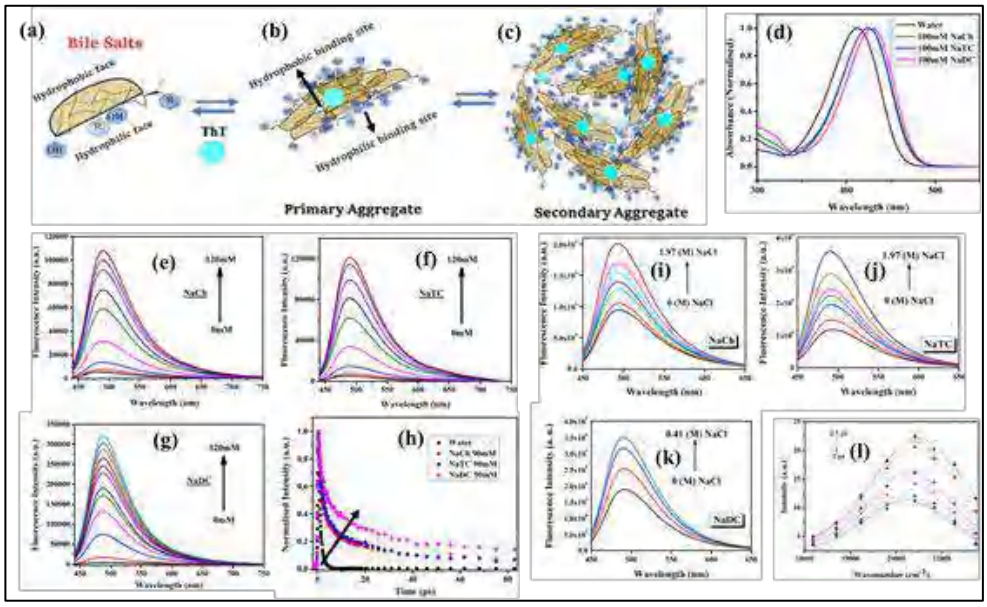

Figure: a,b,c are general structures of monomers and different aggregates of bile salts. d-l represents various spectroscopic properties of ThT inside different bile salt solutions.

References:

1. Ritwik Hazra et al. *Langmuir* **2024**, *40*, 16272.
2. Miguel Gomez-Mendoza et al. *J. Phys. Chem. Lett.* **2011**, *2*, 782.

P 102

Fluorescent Nanothermometer with Exceptional Sensitivity for Intracellular Temperature Sensing

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Abstract

Precise temperature sensing and measurement at the nanoscale level within biological systems is vital for understanding cellular processes such as gene expression, metabolism, and enzymatic activity [1]. In this study, we design and present luminescent niosomes as a novel nanothermometer with exceptional figure of merits. The change in the fluorescence intensity and wavelength shift for common fluorophores are used as indicators for temperature sensing when the fluorophores are entrapped in niosomes, and the niosomes undergo gel-to-liquid phase transition with an increase in temperature. When Coumarin 153 (C153) loaded in Span60-Pluronic L64 (hybrid) niosomes, is excited at 420 nm, the system exhibits a 20 nm blue shift in fluorescence (from 540 nm to 520 nm) with more than double enhancement in its fluorescence intensity at 50 °C, with increasing temperature (20–50 °C), enabling temperature sensing in the very relevant temperature range for biological imaging. The sensor shows excellent reversibility, environmental stability, high relative sensitivity (7.4% $°C^{-1}$), and temperature resolution (0.087 °C) under varying ionic strength and pH conditions [2]. Further, when Nile Red-loaded hybrid niosomes are excited at 520 nm, the system shows an 11-fold increase in fluorescence (highest, 618 nm) with the best temperature sensitivity of 19%$°C^{-1}$ at 42 °C. While Nile Red-loaded pure L64 niosomes exhibit a staggering ~100-fold enhancement (highest, 618 nm) under the same experimental condition with 36.4%$°C^{-1}$ sensitivity at 40 °C. Due to their excellent stability, reversibility, biocompatibility, high-temperature sensitivity, and resolution, the sensors are capable of temperature sensing when loaded inside FaDu cells (Fig. 1). These results highlight the potential of niosome-based fluorescent nanothermometers for biomedical research, allowing precise detection of minute temperature variations associated with cellular processes and diseases such as cancer.

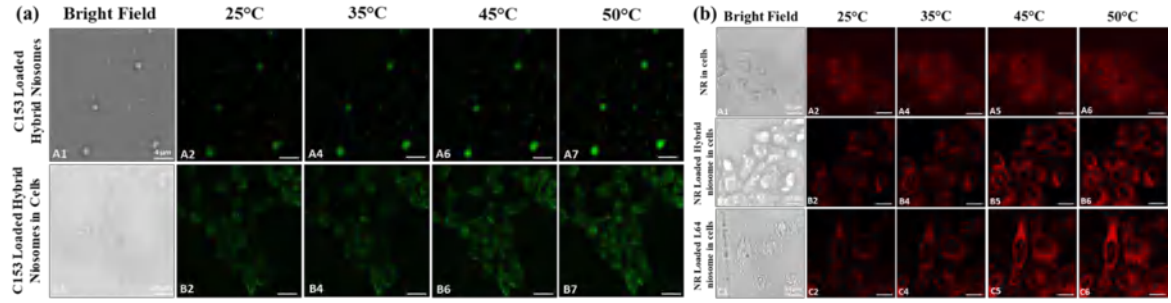

Figure 1. Laser scanning confocal microscopic images at different temperatures: (a) Only C153- loaded niosomes (A1–A7) (scale bar = 4 μm) and (B1–B7) FaDu cells incubated with C153-loaded niosomes (scale bar = 25 μm); λ_{ex} = 405 nm, λ_{em} = 510–530 nm; (b) FaDu cells loaded with only Nile Red (NR) (A1–A6), NR loaded Hybrid Niosomes (B1-B6), NR loaded in L64 niosomes (C1-C6), respectively. (scale bar = 25 μm and 10 μm) (λ_{ex} = 514 nm; λ_{em} = 610–650 nm).

References

1. Albenc Nexha et al. *Nanoscale*, **2021**, 13, 7913.
2. Ronak Lazarus et al. *Analyst*, **2023**, 148, 3169.

P 103

Esterase-responsive Fluorogenic Prodrugs of Aldose Reductase Inhibitor Epalrestat: An Innovative Strategy towards Enhanced Anticancer Activity

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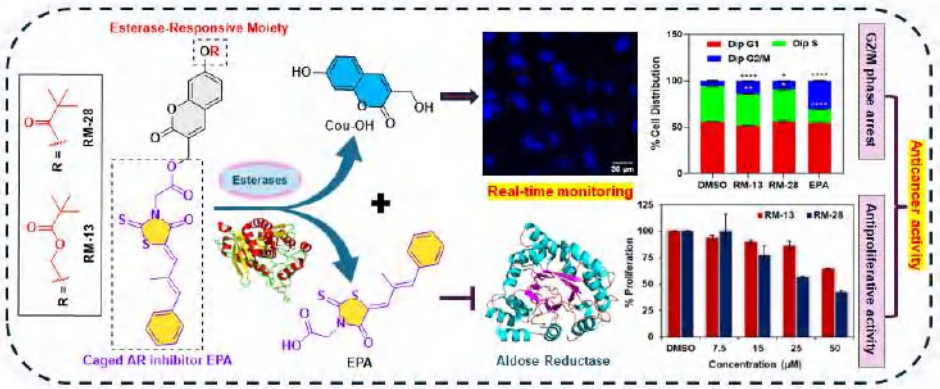
In addition to the conventional chemotherapeutic drugs, potent inhibitors of key enzymes that are differentially overexpressed in cancer cells and associated with its progression are often considered as drugs of choice for treating cancer. Aldose reductase (AR), which is primarily associated with the complications of diabetes, is known to be closely related to the development of cancer and drug resistance.[1] Epalrestat (EPA), an FDA-approved drug, is a potent inhibitor of AR and exhibits anticancer activity.[2] However, its poor pharmacokinetic properties limit its bioavailability and therapeutic benefits.[3] We report herein the first examples of the esterase-responsive turn-on fluorogenic prodrugs **RM-13** and **RM-28** for the sustained release of EPA to the cancer cells with turn-on fluorescence readout.[4] The prodrugs could be activated in the presence of esterases, which are overexpressed in cancer cells. Spectroscopic and HPLC studies revealed a simultaneous release of both the active drug and the fluorophore from the prodrugs over time. While the inhibitory potential of EPA released from the prodrugs towards the enzyme AR was validated in the aqueous medium, the anticancer activity of the prodrugs was studied in a representative cervical cancer cell line (HeLa). Interestingly, our results revealed that the development of the prodrugs can significantly enhance the anticancer potential of EPA. Finally, the drug uncaging process from the prodrugs by the intracellular esterases was studied in the cellular medium by measuring the turn-on fluorescence using fluorescence microscopy. Therefore, the present study highlights the rational development of the fluorogenic prodrugs of EPA, which will help enhance its anticancer potential of EPA with better therapeutic potential.



References:

1. Srivastava et al. *Chemico-Biological Interactions* **2011**, *191*, 330.
2. Bailly et al. *European Journal of Pharmacology* **2022**, *931*, 175191.
3. Banala et al. *Biomaterials Science* **2019**, *7*, 2889
4. Misra et al. *ACS Applied Bio Materials* **2024**, ASAP Article

P 104

Plasmon-driven surface reactions and enzyme catalysis probed using surface-enhanced Raman spectroscopy and 2DCOS

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A circular economy and a more sustainable future depend heavily on catalysis. At the forefront of photocatalysis right now is plasmonic catalysis, which allows one to get beyond the drawbacks of "classical" broad bandgap semiconductors for solar-driven chemistry. Because plasmonic catalysis uses localized surface plasmon resonance (LSPR) stimulation, it can accelerate and control a wide range of chemical reactions [1]. The utility of these plasmonic probes has been restricted at times due to the lack of understanding and control of the reactions which can lead to a diverse set of products. Our work is focused on creating multifunctional plasmonic nanostructures for plasmon-driven catalysis by engineering nanostructured probes and adjusting their optical characteristics. Our proposal in this work is to use in-situ surface-enhanced vibrational 2D correlation spectroscopy (2DCOS) to monitor these reactions in a completely new way [2] [3]. Interestingly, we see a synergistic effect of the reaction time, presence of protic solvents, material of the surface and the laser exposure duration on the nanoparticle surface, and this determines whether the reaction stops at the intermediate or proceed towards the completely reduced product. We have explored the mechanistic underpinnings of intermediate formation on nanoparticle surfaces using a combined spectroscopic and 2DCOS visualization techniques. This spectroscopic visualization technique provided real-time information about the mechanism of the reaction which could otherwise be obtained by a combination of data analytical techniques such as thorough spectral processing and the use of advanced chemometric techniques. We have tried to monitor and control enzyme function through irradiation of plasmonic nanoparticles using UV and visible light as stimuli. We propose an entirely new approach to monitor these reactions which could shed light on the underlying mechanisms and thus help in tuning the conditions to remotely monitor plasmon induced surface reactions and enzyme catalysis at the molecular level.

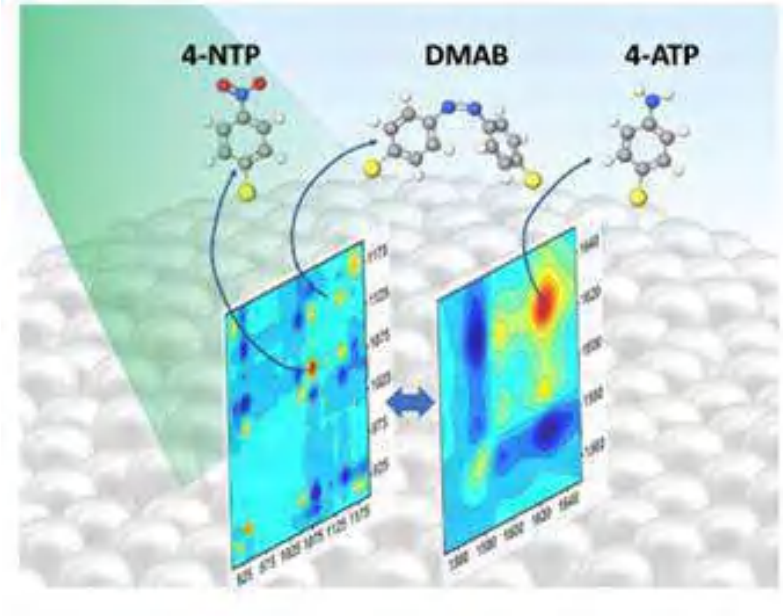

Figure 1: Visualization of plasmon induced surface catalytic reactions using 2DCOS

References:

[1] Z. Yang et al. *Journal of Raman Spectroscopy* **2009**, *40 (10)*, 1343.
[2] I. Noda *Vibrational Spectroscopy* **2012**, *60*, 146.
[3] R. Singh et al.. *Physical Chemistry Chemical Physics*, **2023** *25(8)*, 6032.

P 105

In-situ visualization of growth kinetics of silver nanorods

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Metal nanostructures find extensive applications in diverse fields, such as bioimaging, photothermal therapy, targeted drug delivery, and optoelectronics [1]. As a consequence of their extensive utility, there have been multiple attempts to understand the growth kinetics of these nanostructures. Understanding growth mechanisms allows the control of their size, shape, and morphology, resulting in customising their properties for specific applications [1].

To investigate nanostructure growth mechanisms, we have developed interferometric scattering (iSCAT) [2] based microscopy and correlation spectroscopy, which is a non-invasive, label-free technique often used for applications like single particle tracking and imaging nanoscale entities such as proteins, other biomolecules, metal nanoparticles, semiconductor quantum dots, and single organic molecules [3]. Here, we present a novel approach by employing this technique for real-time, in-situ visualization of growth kinetics of seed-mediated growth of silver nanorods [4]. Using laser light, we promote uniform growth and tailor the aspect ratios of the nanorods, enhancing both their yield and quality. Our observations reveal a more complex growth process than the conventional one-step transformation from spherical seeds to elongated rods. Instead, the growth involves forming larger structures from the seeds, which disintegrate into smaller fragments that reassemble into nanorods. This novel approach offers unprecedented insights into the kinetics of nanostructure formation, which provides significant potential for rational design and tailored synthesis of nanostructures.

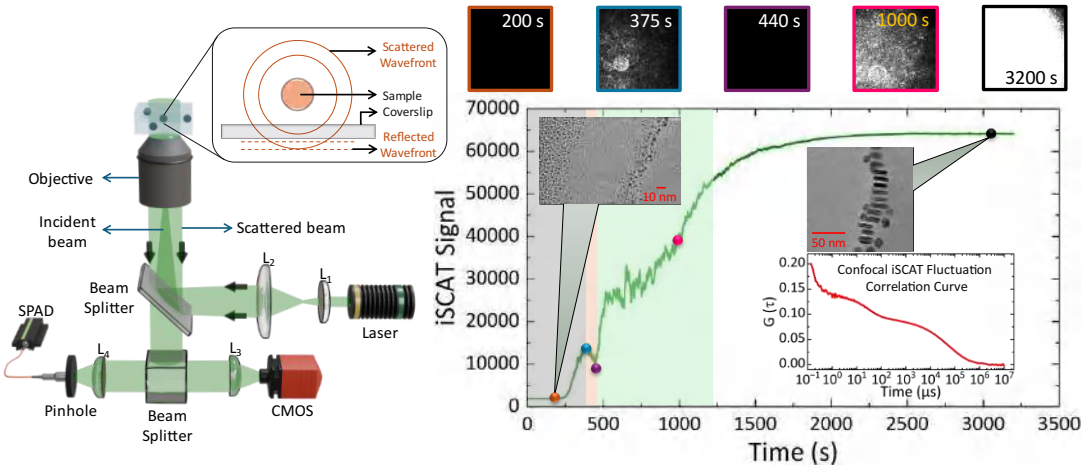

Figure 1: (Left) Schematics of the custom-built Interferometric Scattering (iSCAT) Microscope. (Right top panel) Image frames acquired as a function of time during the nanoparticle growth phase. (Right bottom panel) Frame averaged intensity signals used in visualizing the growth kinetics of silver nanorods. (Right bottom inset) 1D-Fluctuation correlation curve corresponding to the nanostructure growth.

References:

(1) N. Thanh et al. *Chem. Rev.* **2014**, *114* (15), 7610.
(2) J. Ortega Arroyo et al. *Nat. Protoc.* **2016**, *11* (4), 617.
(3) G. Young et al. *Annu. Rev. Phys. Chem.* **2019**, *70*, 301.
(4) N. R. Jana et al. *Chem. Commun.* **2001**, *7*, 617.

P 106

Catalytic Dynamics in Phase-Separated Environments: Contributions of Micro and Macromolecules

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Transient non-covalent interactions between biopolymers (protein-protein or protein-nucleic acid) can induce liquid-liquid phase separation (LLPS) resulting in the formation of distinct dense and dilute phases. The dense phase resulting from the LLPS of catalysts, called the catalytic condensates, can effectively concentrate the catalyst and substrate, thereby significantly accelerating the reaction rate[1]. The functionality of these condensates can be influenced by changes in the local environment which can be modulated by both macro- and micro-molecules[2]. In our current study, we utilize a combination of microscopic and spectroscopic techniques to examine how macro- and micro- molecules can alter the reaction rates by modulating the phase behaviour of these condensates. We have observed that the substrate (a micro-molecule) can induce phase transition within the liquid condensate on increasing its concentration above a critical concentration. Due to this phase transition, the overall reaction rate gets decreased in condensates. In addition to this, macromolecules can induce further phase separation within single phasic condensate leading the formation of multiphasic condensates. We aim to further investigate the effect of such multiphasic condensates, on the reaction kinetics, in the presence of an additional dense phase of macromolecules.

References:

1. Bedi et al. *Chemical Communication.* **2022**, *58*, 8634.
2. Babinchak et al. *Nature communications*. **2020**, *11*, 5574.

P 107

Interaction of human BRAC1 protein with Holliday Junction: Preference for an open X-like conformation

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BRCA1 is a multifaceted tumor suppressor protein involved in key cellular processes such as DNA double-strand break repair and cell cycle checkpoints [1]. Cells deficient in BRCA1 show reduced homologous recombination (HR) activity, leading to an increased reliance on error-prone non-homologous end joining for DNA repair [2–5]. The Holliday junction (HJ) is a crucial intermediate in HR, and while BRCA1 exhibits a strong affinity for HJ and recruits various proteins to DNA damage sites, its binding mode with HJ remains unclear [6,7]. Using single-molecule Fluorescence Correlation Spectroscopy (FCS), we have demonstrated that BRCA1 preferentially binds to an open X-shaped conformation of HJ and has a lower affinity for stacked HJ. Additionally, molecular docking and all-atom molecular dynamics simulations revealed that charged and polar amino acids in BRCA1's DNA-binding region (aa340-554) form a complex with HJ, many of which are reported as crucial sites of missense mutations [8].

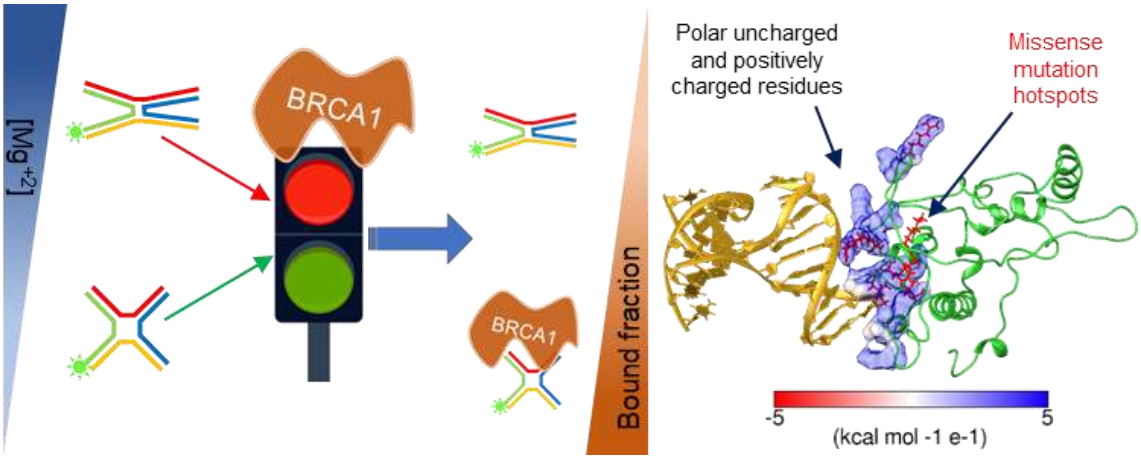

Fig 1: (a) Schematic representation of BRCA1 protein's preference for an open X-like conformation of HJ, (b) Crucial polar uncharged and positively charged amino acids from DNA binding region of BRCA1 protein interacting with a HJ.

References:

1. Li X et al. *Cell Res* **2008**; *18*, 99.
2. Tarsounas M et al. *Nat Rev Mol Cell Biol* **2020**; *21*, 284.
3. Zhao W et al. *Annu Rev Biochem* **2024**, *28*, 221.
4. Venkitaraman AR et al. *Cell* **2002**, *108*:171.
5. Mylavarapu S et al. *Front Oncol* **2018**, *8*, 16.
6. Brázda V et al. *BMC Mol Biol* **2016** *17*, 1.
7. Paull TT et al. *Direct DNA Binding by Brca1* **2001**, *98.11*, 6086.
8. Ramirez CJ *et al. Oncogene* **2004,** *23:1780*,8.

P 108

Effect of Plasma Membrane Cortical Architecture and Protein Crowding on the Transport Properties of Membrane Proteins

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The plasma membrane is a complex mixture of proteins and lipids that serves as an organizing centre for a variety of sorting and signalling processes. It is also heterogeneous and has a composite nature with a variety of barriers to protein and lipid mobility. Understanding sorting and signalling processes at the membrane require an understanding of these barriers. These barriers emerge at the least as a result of protein crowding in the plane of the plasma membrane and the effect of the underlying cortical actin meshwork. They influence the transport properties and as a consequence, the interaction kinetics of all the molecules on the membrane. To understand the effect of protein crowding in the plane of the plasma membrane and the effect of the actin meshwork-based corralling and picketing on the transport properties of membrane bound protein molecules, we have developed an in-vitro, minimal model of the plasma membrane to systematically tune protein crowding and actin meshwork-based picketing. We probe the effect of these mobile and immobile barriers to study the diffusion of membrane molecules by FRAP and Fluorescence Correlation Spectroscopy (FCS) based methods. Further, we have developed a method to correlate these changes in transport properties of a tracer with the spatiotemporal maps of pickets and crowders in live cell membranes.

P 109

Evaluating the inclusion complex of triazolium-based ionic liquid in β-cyclodextrin media by Nile blue fluorescence

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Ionic liquids (ILs) are remarkable chemical compounds with applications in many fields of modern science. The majority of attention has been focused on imidazolium-based ILs and their biological applications [1]. While only a few 1,2,4-triazolium-based ILs have been synthesized and systematically studied. β-cyclodextrin (β-CD) is a type of oligosaccharide composed of glycopyranose units. Encapsulation within β-C alters the chemical reactivity of guest molecules, stabilizes oxygen and light-sensitive substances, and improves solubility [2]. To evaluate the inclusion complexation of triazolium-based IL in β-CD media, the current work will provide an account of the photophysical response of Nile blue chloride (NBC) in presence of both the media. NBC is a versatile, nontoxic visible Oxazine dye that has demonstrated potential efficacy as a photosensitizer for photodynamic therapy of malignant tumors. NBC is also a well-known DNA probe. Understanding how NBC interacts with biological and biomimicking systems is crucial.

Here, we have synthesized 1,2,4-triazolium-based IL, 1-propyl-1,2,4-triazolium trifluoroacetate (1-prop3HTTFA), by simple neutralization method, and structurally characterized by NMR spectroscopy. A comparative analysis of the effect of different concentrations of IL and β-CD on the photophysical properties of NBC was investigated. For NBC in an aqueous solution, emission wavelength is noted at 670 nm. When IL is added to NBC, fluorescence intensity of NBC is increased with a slight red shift from 670 nm to 674 nm. Both IL cationic and anionic parts may interact electrostatically and hydrophobically with NBC. IL and NBC molecules are encapsulated in the β-CD cavity, where they experience a hydrophobic environment. The increased fluorescence intensity of NBC in the IL-β-CD system is due to its solubility of NBC molecules in the hydrophobic cavity, which is protected from quenching by bulk water through proton transfer. Observing changes in NBC absorbances and fluorescence in aqueous IL media with increasing concentrations of β-CD revealed that β-CD and IL form inclusion complexes with 1:1 stoichiometry. The binding constant for inclusion complex formation has been derived using the Benesi-Hildebrand method.

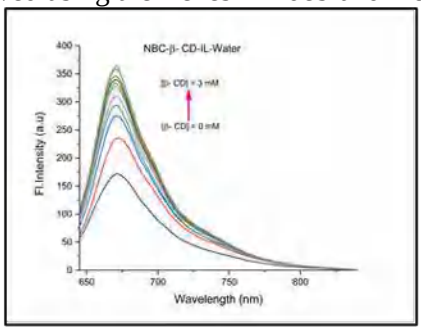

Fig.1 Emission spectra (λex = 635 nm) of NBC in β-CD-IL system

References:

1. Buettner, C.S. et al., *J. Mol. Liq.*, **2022**, *347*, 118160.
2. Sahu, K.M. et al., *Int. J. Biol. Macromol.*, **2023**, *240*, 124338.

P 110

Exploring the Impact of Chromophore Environment on Excited-State Dynamics Leading to Large Stokes Shift in Red Fluorescent Proteins

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Red fluorescent proteins (RFPs) are gaining popularity as genetically encodable bio-probes and biomarkers, with the potential to drive significant advancements in imaging and life sciences. Designing RFPs with enhanced functions or versatility necessitates a deep understanding of their mechanisms. Since fluorescence happens on an ultrafast timescale, the use of a toolkit that includes steady-state and time-resolved spectroscopic methods, as well as computational studies is essential for uncovering the crucial species and dynamic processes that govern large Stokes shift (LSS) RFPs [1]. Among the diverse LSS-RFPs created, a monomeric RFP mKeima is notable for exhibiting one of the largest Stokes shifts (~180 nm) and a unique "*reverse protonation*" effect. The substantial Stokes shift observed in mKeima arises from an ultrafast sequential process involving excited state isomerization followed by proton transfer (ESPT) between the *trans*-protonated to *cis*-deprotonated forms of the chromophore through *cis*-protonated leaving *trans*-deprotonated as a bystander [2,3]. In the recent work we investigate the intriguing photophysics of a site-directed mutant of monomeric RFP mKate (extracted from *Entacmaea quadricolor*) known as mBeRFP (monomeric blue light-excited red fluorescent protein) is noteworthy among other LSS-RFPs for its improved photostability, brightness, and responsiveness to chlorides [4,5,6]. Unlike mKeima, mBeRFP exhibits "*reverse protonation*" at higher pH and remarkable triple fluorescence at ~ 77 K which is the first report on triple fluorescence from a chromophore. In summary, we proposed a schematic of potential energy surface based on our studies by employing steady-state and time-resolved spectroscopic tools and computational calculation and discuss how local environment play a crucial role for distinct photophysical phenomenon with change of temperature and pH [7].

References:

1. Taylor D. Krueger et al. *Chemistry- An Asian Journal*. **2023**, *18*, e202300668
2. Garima Bhutani et al. *The Journal of Physical Chemistry B* **2023,** *3197-3207*, 127.
3. Garima Bhutani et al. *Photochemistry and Photobiology* **2024,** 1.
4. Jie Yang et al. *PLoS One* **2013**, *8*, e64849.
5. Rafael Salto et al. *ACS Sensor*. **2021,** *6*, 2563.
6. Rafael Salto et al. *Sensors and Actuators B: Chemical* **2024**, *410*, 135673.
7. Garima Bhutani et al. (under review)

P 111

Molecular effects behind Drug-induced liver steatosis and mitochondrial dysfunction by lipophilic anti-cancer drugs.

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Drug-induced liver steatosis and hepato-toxicity are a major cause of retraction of anti-cancer drugs from the market. Steatosis is caused by an increase in Lipid Droplets (LDs), LDs being neutral-lipid-containing organelles that can partition away lipophilic anti-cancer drugs. Among many other orally administered lipophilic multi-tyrosine kinase inhibitors, Ponatinib was reportedly partitioned inside LDs due to its hydrophobic nature. In tandem with this, Ponatinib also increases the amount of lipids inside cells and gets sequestered even more creating a vicious cycle for the fate of the drug. Though there are significant studies on the lipid biogenesis pathway being upregulated, little has been reported on lipolysis being affected in the presence of Ponatinib. Lipolysis is largely regulated by the transfer of lipids into the mitochondria from cytosol or through a direct linkage with LDs. LD-mitochondria contacts are modulated by the phosphorylation of Perilipin 5 by Protein Kinase A (PKA) during lipolysis. We observed that PKA activity is inhibited in the presence of Ponatinib and LD-mitochondria contacts are significantly reduced. This may be a previously unknown molecular mechanism at play whereby multi-tyrosine kinase inhibitors like Ponatinib inhibit PKA and block the lipolytic flux of the liver causing drug-induced liver steatosis and hepatic failure. It would thus be interesting to explore the possible interventions to mitigate the effect of Ponatinib whereby co-administration of drugs inducing lipolysis in the context of PKA might alleviate the adverse effects of hepato-toxicity.

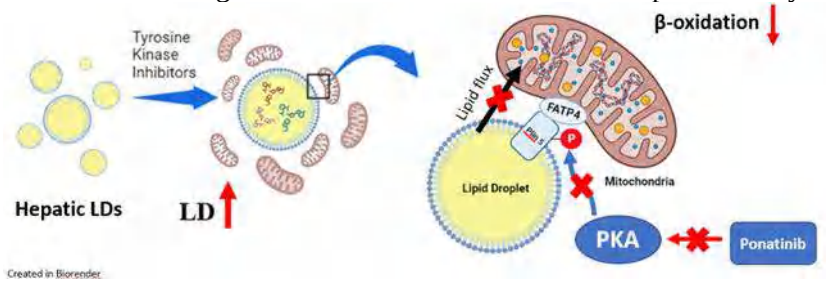

Figure 3: Proposed pathway for disruption of LD-Mitochondria linkage in the presence of lipophilic tyrosine kinase inhibitors like Ponatinib.

References:

1. Englinger, Bernhard et al. *International journal of cancer* **2020**, *147*, 1680.
2. Petan, Toni. et.al. *Reviews of physiology, biochemistry and pharmacology* **2023**, *185*, 53.
3. Yan, Gege et al. *Circulation research* **2024**, *134*, 482.
4. Talari, Noble K., et al. *Nature Communications* **2023**, *14*, 1.
5. Benador, Ilan Y et al. *Cell metabolism* **2018**, *27*, 869.
6. Keenan, Stacey N et al. *Journal of Lipid Research* **2021**, *62*.
7. Najt, Charles P et al. *Molecular Cell* **2020**, *77*, 810.
8. Miner, Gregory E et al. *Developmental cell* **2023**, *58*, 1250.

P 112

Host-Guest Charge Transfer Mediated Disequilibration of Stilbenes inside Water Soluble Nanocage

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Cis-trans photoisomerization is a basic photochemical reaction that is vital to the functioning of many biological processes, including light sensing, ion pumping and vision.[1] Thus far, photoisomerization has been exploited in backbones of molecular switches to activate many artificial light-energy conversion systems and regulate in-vivo biochemical pathways. The secret is in the reaction timescales which are usually ultrafast affecting substantial global conformational change. Although isomerization has been used in diverse applicatons, one fundamental bottleneck is producing 100% yield of one of the isomer after light-activation for any photoswitch. The problem lies in overlap of the absorption spectra of cis-trans forms of the photoswitch.[2] To solve this major challenge, we have developed a new method to control the outcome of cis to trans isomerisation in stilbene derivatives using visible light and water with high efficiency inside confinement. Furthermore, with the help of time resolved spectroscopy, we have tracked the reaction pathway and assigned the intermediates formed to be radical cations of cis and trans stilbene molecules.[3]

References:

1. Christophe D. et al. *Chem. Rev.* **2003**, *103*, 2475.
2. Jolanta K. et al. *Dyes and Pigments* **2023**, *219*, 111558.
3. Yasunao K. et al. *Bulletin of the Chemical Society of Japan* **1993**, *66(6)*, 1852.

P 113

Smartphone based portable devices for cervical and oral precancer diagnosis in clinical conditions

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In recent years, a rapid increase in cervical and oral cancer cases worldwide has been observed. In India, the International Agency for Research on Cancer (IARC) 2022 report estimated 127,000 new cases (9%) and 80,000 deaths (8.7%) due to cervical cancer. For oral cancer, 143,000 new cases (10.2%) and 79,000 deaths (8.7%) were reported [1]. Conventional techniques often fail to predict the severity of the disease at an early stage due to low sensitivity, low specificity, and the lack of real-time prediction. Early diagnosis and effective treatment can enhance patient survival rates. Optical techniques, especially fluorescence spectroscopy and imaging, have the potential to monitor subtle morphological and biochemical changes occurring in tissues with disease progression [2]. These techniques capture the spectral response of various fluorophores (NADH, FAD, collagen, and porphyrin) present in the layered tissue structure [3,4].

We have designed and developed smartphone-based, 3D-printed portable devices for the early diagnosis of cervical and oral cancers using fluorescence spectroscopy and imaging techniques. The device for cervical precancer detection, shown in Figure 1, is based on extracting intrinsic fluorescence from polarized fluorescence and elastic scattering spectra collected from the cervix [5,6]. It utilizes a mutual information and long short-term memory (MI-LSTM) based algorithm to classify different grades of cervical cancer [7-9]. The oral precancer detection device, shown in Figure 2, is a bimodal tool that captures both fluorescence spectra and images on a single platform using a smartphone as the detector [10-11]. Preliminary results from clinical testing suggest that a ratio ($\frac{I_{Porphyrin}}{I_{FAD}}$) based analysis, combined with AI/ML techniques, can effectively discriminate between normal and cancerous oral patients [12].

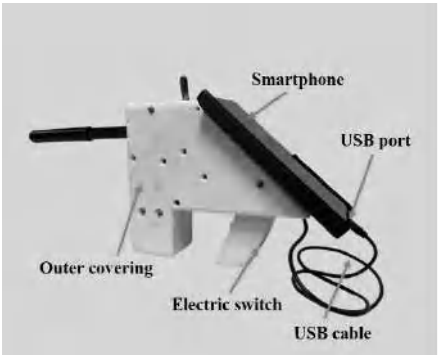

Figure 1: Photograph of the smartphone based cervical precancer detection device

Figure 2: Photograph of the smartphone based bi-modal device for oral precancer detection

References:

1. Ferlay, J. et al. **(2024).** Global Cancer Observatory: Cancer Today (version 1.1). *Lyon, France: International Agency for Research on Cancer. Available from: https://gco. iarc. who. int/today*.
2. Alfano, R. R. et al. **(1989).** Optical spectroscopic diagnosis of cancer and normal breast tissues. *JOSA B*, *6*(5), 1015-1023.
3. Ramanujam, N. et al. **(1996).** Development of a multivariate statistical algorithm to analyze human cervical tissue fluorescence spectra acquired in vivo. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, *19*(1), 46-62.

P 114

Triphenylamine/Phenothiazine-linked 2,4,6-Trisstyrylpyrylium based Probes for Detecting and Differentiating Biologically Relevant Amines

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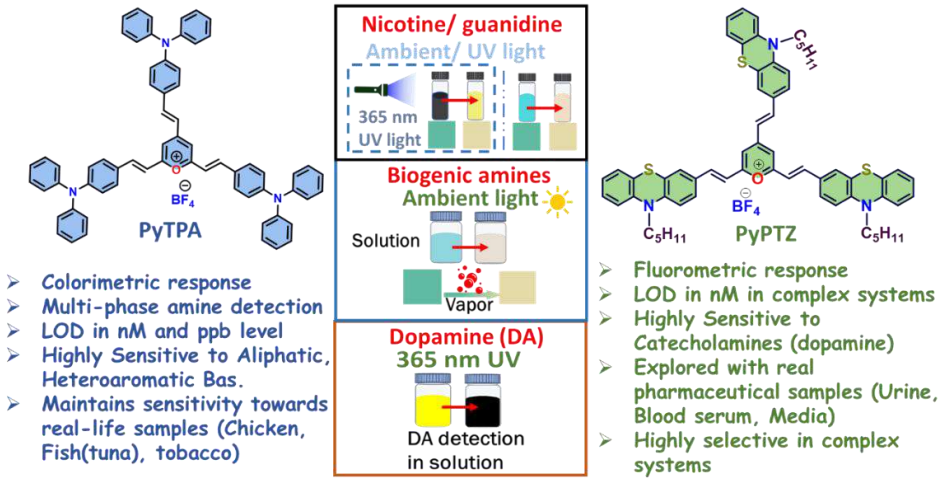
A class of amines, classified as small biomolecules, is generated through the microbial decarboxylation of amino acids in fermented foods and beverages. These amines often play crucial roles in neurological functions. They are categorized as biogenic amines (BAs) and catecholamines, depending on their origin. Given their potential health risks at high concentrations, BAs are considered food hazards, highlighting the importance of monitoring their levels in food samples [1-3]. Herein, we develop two conformationally twisted colorimetric/fluorometric probes, **PyTPA** and **PyPTZ**, where triphenylamine or phenothiazine is linked to 2,4,6-tristyrylpyrylium motif. These probes are designed to detect biogenic amines and catecholamines (dopamine, serotonin) at nanomolar concentrations. This novel and previously unexplored styrylpyrylium probe design provide unique electronic conjugations, steric and geometric constraints, along with good thermal and photostability. These features enable the detection of various amines in distinct and specific ways. The deep-violet **PyTPA** or deep-green **PyPTZ** in solution and solid exhibited a rapid and significant discoloration in response to various aliphatic biogenic amines i.e. putrescine, cadaverine, spermidine, spermine, and also aromatic BAs i.e. histamine, serotonin, 2-phenylethylamine, and dopamine. Notably serotonin and dopamine belong to catecholamine family. The detection of these important amines is remarkable across different phases and has been applied for on-site testing of fresh chicken and fish (tuna). Additionally, **PyPTZ** was utilized for detecting dopamine in human urine and plasma samples. Mass spectrometry played a key role to analyze the products formed from the reactions between amine and pyrylium salts. Further, ^{1}H-NMR, FT-IR, SEM, PXRD, and XPS studies provided a detail insights into the mechanism of this colorimetric/fluorometric detection. We notice the formation of mono- or bis-pyridinium salts after the amine treatment. This newly designed pyrylium salts represents a significant advancement in this class of molecules, offering great potential for detecting and differentiating various biologically important amines.



References:

1. Oguri, S. et.al. *Anal. Chim. Acta.* **2006**, *558*, 326.
2. Patange. S.B. et al. *Food Control*. **2005**, *16*, 465.
3. Munzi, G. et al. *Analyst* **2021**, *146*, 2144.

P 115

Investigating Urea's Role in Protein Denaturation: Insights from Water Dynamics via Two-Dimensional Infrared Spectroscopy

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Osmolytes play a crucial role in cellular function by stabilizing proteins, adjusting osmotic pressure, and maintaining cell volume. However, urea, a unique osmolyte, denatures proteins and counteracts the effects of stabilizing osmolytes like trimethylamine N-oxide. The molecular mechanism behind urea's denaturing ability remains debated, centered around two opposing mechanisms: direct and indirect. The direct mechanism proposes that urea disrupts hydrogen bonds and hydrophobic interactions within proteins, while the indirect mechanism suggests that urea modifies water properties, thereby disrupting protein-protein interactions.[1-3] A key question is whether urea acts as a "structure-maker" or "structure-breaker," akin to how salts are classified by the Hofmeister series. To explore these mechanisms, we investigated water dynamics through the spectral diffusion of the OD stretch in isotopically diluted water in the presence of urea and other osmolytes using two-dimensional infrared (2D IR) spectroscopy. By comparing water dynamics in the presence of urea and other osmolytes to those of neat water, our spectroscopic analysis provides new insights into the water-urea system, shedding light on the intricate mechanisms of protein denaturation by urea.

References and Notes

1. Ding, B. *et al. The Journal of Physical Chemistry Letters* **2018,** *9* (11), 2933.
2. Carr, J. K. *et al. The Journal of Physical Chemistry B* **2013,** *117* (42), 13291.
3. Borkowski, A. K. *et al. The Journal of Chemical Physics* **2023,** *158* (6).

P 116

Development of a Fluorescent Biosensor for Sensitive Trypsin Detection Using a Tetraphenyl Ethylene-Histone Complex

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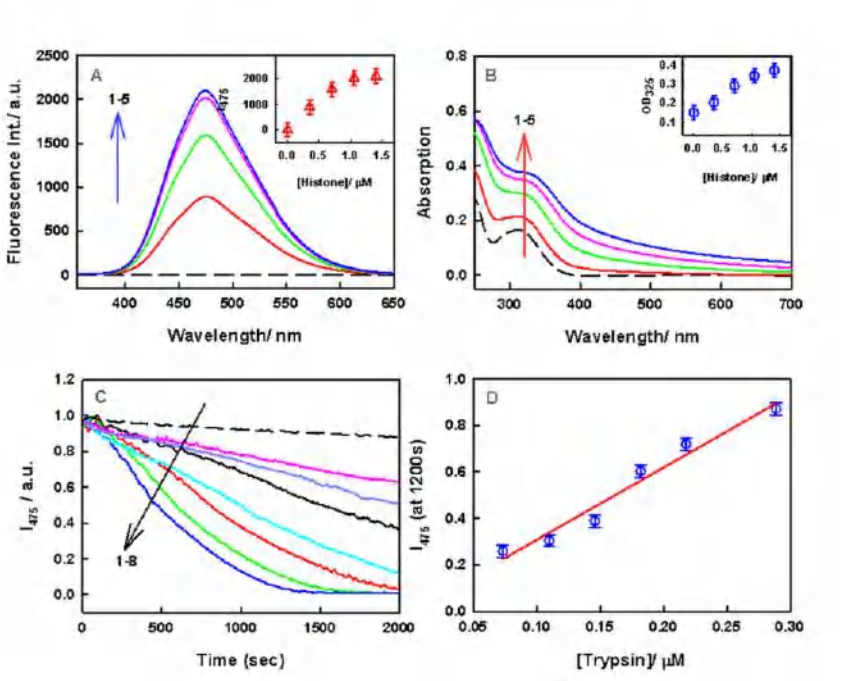
Trypsin is a digestive serine protease that hydrolyzes dietary proteins in many eukaryotic and prokaryotic organisms. It has numerous applications in various fields and is a potential biomarker for diseases, such as pancreatitis, cystic fibrosis, etc [1,2]. The present contribution describes a fluorescence turn- off sensing scheme for Trypsin, which utilizes the phenomenon of aggregation-induced emission (AIE). The sensing scheme of the probing system involves the interaction of a water-soluble Tetraphenylethylene (TPE) fluorophore, sodium 1,2-bis[4-(3-sulfonatopropoxyl)phenyl]-1,2-diphenylethene (BSPOTPE) with Histone. The sensing scheme for Trypsin detection is based on the principle of trypsin-dependent Histone hydrolysis. The interaction between the components of BSPOTPE-Histone-Trypsin system has been thoroughly investigated using various photophysical techniques such as ground-state absorption, steady-state, and time-resolved emission. Besides being simple and selective, the present sensor system is highly sensitive to Trypsin. Moreover, BSPOTPE is a commercially available probe molecule that abstains from time-consuming protocols and provides potential utility for real-life applications. The application for the present sensor system has been demonstrated in real urine matrix also.



References:

1. Kaur Jet al. *J Mol Liq.* **2021**, *333*, 115980.
2. Wang et al. Talanta. **2023**, *259*, 124562.

P 117

Unraveling Lipid Droplet Dynamics during Ferroptosis using a Novel D-A-D Fluorescent Probe

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Lipid droplets (LDs) are crucial cellular organelles involved in lipid metabolism, energy homeostasis, and signal transduction. [1] This work investigates the dynamics and polarity changes of LDs during ferroptosis, a regulated form of cell death marked by iron-dependent lipid peroxidation. [2] Utilizing the terephthalate group based Donor-Acceptor-Donor (D-A-D) fluorescent probe R1(figure 1) , we have conducted real-time visualization of LD behavior in HeLa and A549 cells under various conditions using confocal laser scanning microscopy (CLSM) and Fluorescence Lifetime Imaging Microscopy (FLIM). The results demonstrate that R1 selectively localizes to LDs, providing a robust and sensitive means to monitor LD dynamics. Upon inducing ferroptosis with erastin, a significant decrease in R1 fluorescence intensity as well as lifetime is observed within LDs, indicating an increase in polarity, which is a hallmark of ferroptosis. The probe also effectively tracks the fusion and migration of LDs over time, revealing their dynamic nature. These findings highlight the potential of R1 as a powerful tool for studying LD-related processes, particularly in understanding ferroptosis mechanisms and lipid metabolism disorders. This work establishes the use of R1 for advancing lipidomics research and offers potential applications in disease diagnostics and the development of targeted therapeutic strategies.

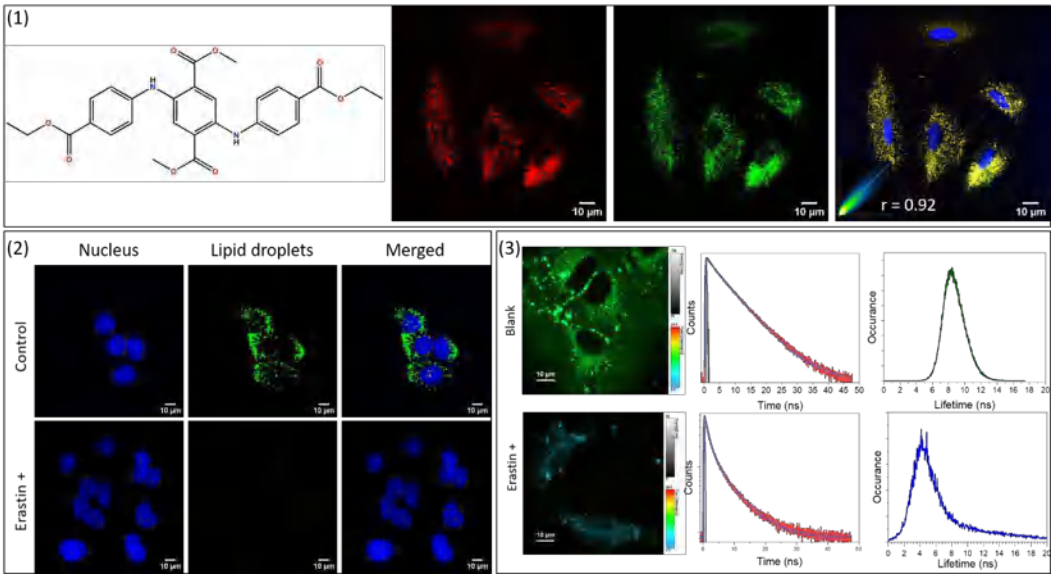

Figure: (1) Structure of fluorophore R1 followed by CLSM images of A549 cells treated with Nile Red, and R1. Inset in the merged image of DAPI, Nile red and R1 shows Pearson's correlation plot (r) of Nile Red and R1 fluorescence intensities, indicating co-localization of lipid droplets. (2) CLSM images of A549 cells. Top: Control cells with normal distribution of nuclei (DAPI) and lipid droplets (R1). Bottom: Cells treated with erastin (10 µM, 6 hours) showing altered lipid droplet distribution and nuclei morphology. (3) FLIM images of A549 cells. Top: Control cells showing normal lipid droplet distribution with corresponding fluorescence decay trace and lifetime distribution. Bottom: Erastin-treated cells (10 µM, 6 hours) displaying altered lipid droplet distribution with corresponding fluorescence decay trace and a shifted lifetime distribution, indicating changes in the cellular environment.

References

1. Beckman, M. *Science* **2006**, *311*, 1232–1234.
2. Yang, W. S. et al. *Trends Cell Biol.* **2016**, *26*, 165–176.

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Elucidation of Origin of Electroluminescence Intermittency in Hybrid Lead Halide Perovskite Crystal

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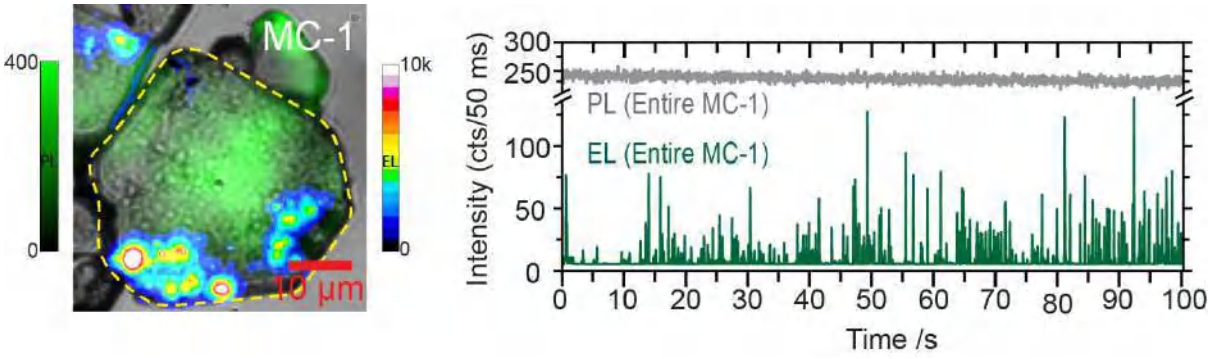
Over the past decades, metal halide perovskites (MHPs) have come up as the most promising materials for both photovoltaic and optoelectronic devices. There are numerous reports on the photoluminescence (PL) intermittency (blinking) of MHP nano/micro-crystals. Reports of MHPs displaying electroluminescence (EL) intermittency, however, are quite rare [1-3]. Herein we report bulk crystals of $MAPbBr_3$ exhibiting intermittent EL, while having temporally stable emission under photoexcitation. Single-particle wide-field imaging allows to probe the temporally unstable EL emission emanating from stochastically changing local emission centers within an individual crystal. The stochastic EL helps to understand the carrier recombination dynamics in an MHP single-crystal on carrier injection and provide a plausible EL blinking mechanism for the observed peculiar behaviour by invoking the concept of ion migration in MHPs under externally applied bias. EL intermittency is detrimental for the working of perovskite light emitting devices as it reduces the EQE. Hence, our study may provide ways to mitigate the consequences leading to EL blinking.



References:

1. D. K. Sharma et. al. *Nat. Commun.* **2019**, *10* (1), 1.
2. S. B. Bhagyalakshmi et. al. *J. Phys. Chem. C* **2022**, *126* (42), 17826.
3. P. Andričević et. al. *ACS Photonics* **2019**, *6*, 967.

P 119

Multifunctional Pyridine-functionalized Coumarin-fused Imidazole Derivative: Ratiometric Sensing and pH Profiling

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Coumarin is a notable fluorophore with many advantages, such as high fluorescence quantum yield, large Stokes shift, good photostability, and low toxicity [1]. The development of chemosensor which is capable of recognizing specific ion in selective manner will be the challenging task for many Researchers. In this study, the pyridine-functionalized coumarin-fused imidazole derivative was successfully synthesized, characterized, and evaluated for its sensing of metal ion capabilities. The probe selectively detects Zn^{2+}, Cu^{2+} and Ni^{2+} with metal ion specific optical output. The binding mechanism of the probe with metal ion was investigated through Job's plot, Benesi-Hildebrand plot, DFT studies and mass spectrometry [2]. Additionally, the probe-Zn^{2+} ensemble exhibited intriguing ratiometric spectral reversibility in the presence of Adenosine Triphosphate (ATP), functioning as a fluorescence "on-off" sensor [3]. The quenching mechanism for Cu^{2+} and Ni^{2+} was confirmed using the time resolved measurement. Additionally, the probe exhibited specific pH sensing in the acidic region, displaying a color change from pale yellow to intense yellow under UV light, attributed to a charge transfer process. This study highlights the potential of combining coumarin with heterocyclic derivatives to create a multifunctional chemosensors having high sensitivity, selectivity, and practical applicability.

References:

1. Asim Mansha et al. *springer* **2022**, *76*, 3303.
2. Sinan Bayindir et al. *Spectrochemical Acta part A* **2019**, *213*, 6.
3. X. Zhang et al. *J. Photochem. Photobiol. B, Biol* **2021**, *223*, 112279.

P 120

Light-Induced Electron Transfer in BMC Shell Proteins: Towards Self-Powered UV Devices

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Specialized protein structures, known as bacterial microcompartments (BMCs), are primarily composed of shell proteins that self-assemble into planar sheets, forming the microcompartment's outer shell. These protein sheets display distinct patterns of electron-dense and electron-sparse regions, which have potential applications in electron conduction. In our study, we investigated PduA and PduBB', key shell proteins from the 1,2-propanediol utilization microcompartment in *Salmonella enterica*. We found that these proteins exhibit low work function and non-linear I-V characteristics, suggesting semiconducting properties. Notably, UV light exposure can induce rapid electron transfer within the shell protein sheets even without an external voltage. Our findings position PduBB' as a promising candidate for bio-photodetectors, with faster rise (0.35s) and decay (0.74s) times. The strategic arrangement of amino acids in shell proteins facilitates the Proton Coupled Electron Transfer reaction, the underlying mechanism behind the photocurrent generation. Thus, these proteins' sensitivity to UV light, coupled with high figures of merit such as responsivity, detectivity, and enhanced quantum efficiency indicates their potential as effective self-powered UV photodetectors.

P 121

Detecting early stages of glycation in Human Serum Albumin using Protein Charge Transfer Spectra.

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Protein Glycation is a spontaneous Post-Translational Modification involving the non-enzymatic covalent attachment of a reducing sugar or sugar derivative to a protein and is prevalent in biological system. Glucose initially reacts with amino groups to form glycosylamine, which dehydrates to a Schiff's base that undergoes Amadori rearrangement to yield fructosamine, classified as early glycation adducts. In later stages fructosamine changes into a stable advance glycation end products (AGEs).[1] There are various method for the detection of AGEs, Such as Immunohistochemistry, ELISA, Western blotting, MALDI-ToF, LC-MS/MS.[2] However only a few detection techniques are available for early stages of glycation, like MALDI-ToF and NBT assay. These conventional techniques either requires expensive instrumentation or external labelling which may changes the characteristic features in the protein. However, detecting glycation using a label-free intrinsic probe is limited. Recently our group discovered a new intrinsic non-aromatic chromophore in a monomeric charged rich protein. The charged residues (Lysine, Arginine, Glutamate, Histidine and Aspartate) participate in photoinduced electron transfer with the peptide backbone or among themselves. This gives rise to broad UV- Vis electronic absorption ranging from 250 to 800 nm called as Protein Charge Transfer Spectra (ProCharTS).[3] Herein we use Human Serum Albumin (HSA) to track the initial glycation stages by using ProCharTS. HSA is known to undergo glycation modification upon prolong presence of Glucose in the blood, a characteristic feature among diabetic patients. Firstly, we confirmed glycation of HSA using MALDI-ToF with the change in the mass of glycated form. We observed an increase in ProCharTS intensity after glycation in HSA signifying similar trends observed in MALDI-ToF. We further analysed the structural changes by using CD spectroscopy and local changes using Tryptophan fluorescence. The other physiochemical parameter are compared by using anisotropy measurement, DLS, Zeta potential.

References:

1. Rabbani N et al. *Amino Acids.* **2012**, *42(4)*, 1087.
2. Rabbani N et al. *Glycoconj J.* **2016**, *33(4)*, 553.
3. Singh S, et al. Chem Sci. **2017**, *8(8)*, 5416.

P 122

Role of SUMO1 in Phase Separation: More Than a Recognition Tag

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Liquid–liquid phase separation (LLPS) plays a crucial role in cellular organization, primarily driven by intrinsically disordered proteins (IDPs) leading to the formation of biomolecular condensates. [1–4] A post-translational modifier protein, SUMO, has recently emerged as a regulator of LLPS.[5–7] Given its compact structure and limited flexibility, the precise role of SUMO in condensate formation remains to be investigated. Here, we ask how SUMO protein having globular folded structure gets recruited into the liquid-like assemblies? Is this recruitment driven by their IDP substrates? Or do they possess an independent tendency to undergo weak interactions required for LLPS? Towards this, we study the phase-separation of SUMO1 protein in controlled crowded environment. We find that SUMO1 can rapidly and independently form micrometer-sized liquid-like condensates in the absence of its IDP substrates or any SUMO-interacting motifs. The liquid condensates undergo subsequent time-dependent conformational changes and aggregation which are probed by label-free methods (tryptophan fluorescence and Raman spectroscopy). Remarkably, experiments on a SUMO1 variant lacking the N-terminal disordered region further corroborate the role of its structured part in phase transitions. Our findings highlight the potential of folded proteins to engage in LLPS and emphasize further investigation into the influence of the SUMO tag on IDPs associated with membrane-less assemblies in cells.

References:

1. Feric M et al. *Cell* **2016**, 65, 1686.
2. Brangwynne CP et al. *Science* **2009**, *324*, 1729.
3. Hyman AA et al. *Cell* **2011**, 21, 14.
4. Hyman AA et al. *Annu Rev Cell Dev Biol* **2014**, *30*, 39.
5. Pountney DL et al. *Exp Neurol* **2003**, *184*, 436.
6. Kirk KE et al. Modification of Huntingtin and Huntington's Disease Pathology **1997**.
7. Dorval V et al. *Journal of Biological Chemistry* **2006**, *281*, 9919–9924.

P 123

Mechanism of Conformational Selection of tRNAArg2 by Bacterial Deaminase TadA

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Base editing is a common mechanism by which organisms expand their genetic repertoire to access new functions. Here, we explore the mechanism of tRNA recognition in the bacterial deaminase TadA which exclusively recognizes $tRNA^{Arg2}$ and converts the wobble base adenosine (A34) to inosine. We quantitatively evaluate the dynamics of tRNA binding by incorporating the fluorescent adenine analogue, 2-aminopurine (2-AP) at position 34 in the wobble base of the anticodon loop. Time-resolved fluorescence lifetime and anisotropy studies revealed that the recognition process is finely tuned. While mutations in residues that partake in assisting deamination (E55A, N42A) does not exhibit a major effect on binding dynamics, the residues that stabilize the region above the active site 'capping residues', and are exclusive to prokaryotic TadAs, such as R149, have a marked effect on binding dynamics and catalytic activity. Moreover, for effective catalysis, peripheral positively charged residues (R70, R94) that are part of the adjacent subunit in the dimeric assembly, are important to splay out the tRNA, assisting in A34 attaining a flipped-out conformation. Perturbations in these extended regions, although 15-20 Å away from the active site, disrupt the binding dynamics and consequently the function, emphasizing the fine regulation of the tRNA recognition process. Analysis reveals that tip of the extended helix where R149 is positioned, acts as a selectivity filter imparting exclusivity towards the deamination of $tRNA^{Arg2}$ by TadA.

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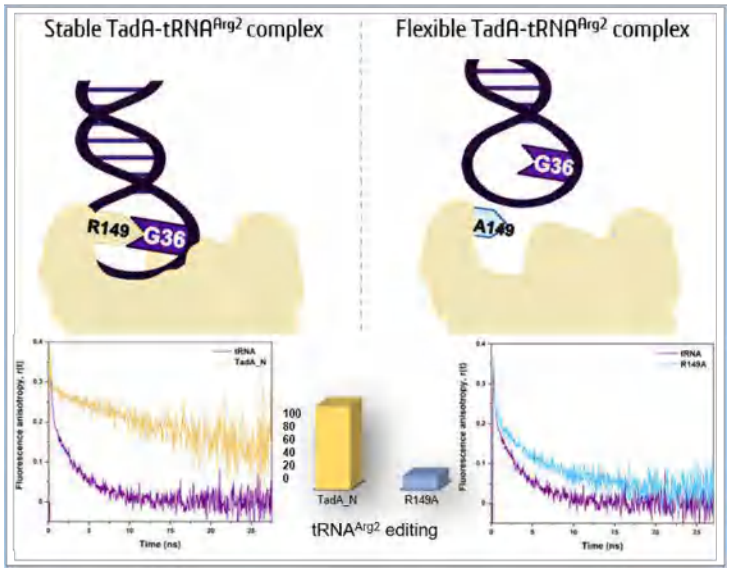

References:

1. Wolf, J., et.al. *EMBO J*, **2002**, *21*, 3841.
2. Losey, H. C., et.al. *Nat. Struct. Mol. Biol*, **2006,** *13*, 153.
3. Elias, Y., and Huang, R. H. *Biochemistry*, **2005**, *44*, 12057.
4. Luciano G. Dolce, et.al. *Nat Commun*, **2022**, *13*,6737.

P 124

Tracking Mn^{2+} Dynamics and Quantifying Labile Mn^{2+} in Living Mammalian Cells Using a Water-soluble, Cell-permeable Fluorescent Sensor

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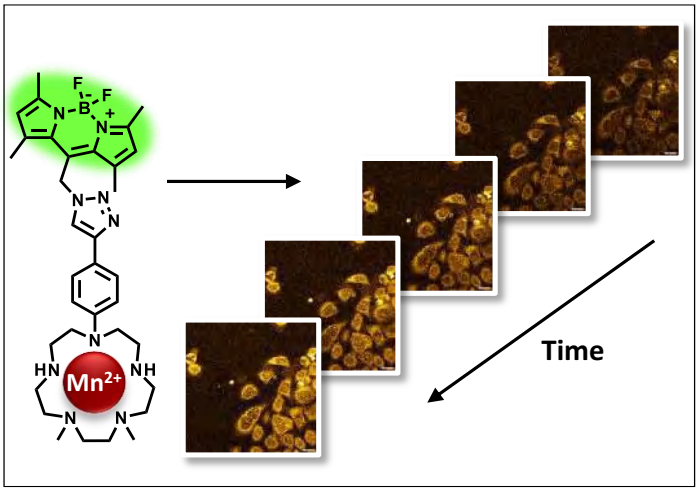
Manganese (Mn^{2+}) ions are indispensable for proper functioning of life, given their crucial roles in brain function, immunity against pathogens as well as photosynthesis.[1] Disruptions in Mn^{2+} ion homeostasis have been linked to neurodegenerations, microbial infection and have also been implicated in cancers.[2-4] This entails the need to not only detect the localization of this metal ion at a specific time, but also track Mn^{2+} dynamics in real time in living cells. For that, fluorescent sensors selective to Mn^{2+}, sensitive to physiologically relevant concentrations of Mn^{2+} ions, and responsive in aqueous media are required. So far, no Mn^{2+} sensor had a combination of all the aforementioned properties. This is because designing fluorescent sensors for Mn^{2+} is challenging. Mn^{2+} is weak-binder to ligand-scaffolds and quenches the fluorescence of fluorophores resulting in turn-off sensors, not conducive for live-cell imaging. To address these challenges, we have developed a novel water-soluble, cell-permeable fluorescent turn-on Mn^{2+}-selective sensor, sensitive to the physiological levels of Mn^{2+}.[6] We have employed penta-aza macrocycle as the Mn^{2+} -binding scaffold, aligning with the native or reported Mn^{2+} binding preferences.[7-8] Photo-induced electron transfer (PeT) was chosen as the mechanism of sensing and a density functional theory (DFT) and time-dependent DFT (TD-DFT) based computational workflow guided the design of the molecule. The computationally designed, PeT-based water-soluble, cell-permeable Mn^{2+} sensor could detect endogenous levels of Mn^{2+} in living cells in both fluorescence intensity and lifetime-based setups. The sensor enabled the visualization of disease-relevant differences in Mn^{2+} uptake dynamics and provided the first ever estimate of labile Mn^{2+} ion concentration in living mammalian cells. I will detail the design, computational studies, in vitro and in cell results achieved with this novel sensor in my poster.



References:

1. S. Das et al. *Dalton Trans.* **2019**, *48*, 7047.
2. S. M. Damo et al. *Proc. Natl. Acad.* **2013**, *110*, 3841.
3. K. Tuschl et al. *Nat. Commun.* **2016**, 7, 11601.
4. P. A. Doble et al. *Metallomics.* **2018**, *10*, 1191.
5. H. Irving et al. *J. Am. Chem. Soc.* **1953**, 3192.
6. S. Kahali et al. *Chem Sci* **2024**, *15*, 10753.
7. A.M. McGuire et al. *Biochemistry* **2013**, *52 (4)*, 701.
8. D. Salvemini et al. *Nat. Rev. Drug Discov.* **2002**, *1 (5)*, 367.

P 125

Bio-waste derived Mg-doped carbon dots: A Fluorescent Probe of Y^{3+} Metal ion and Bio imaging Application

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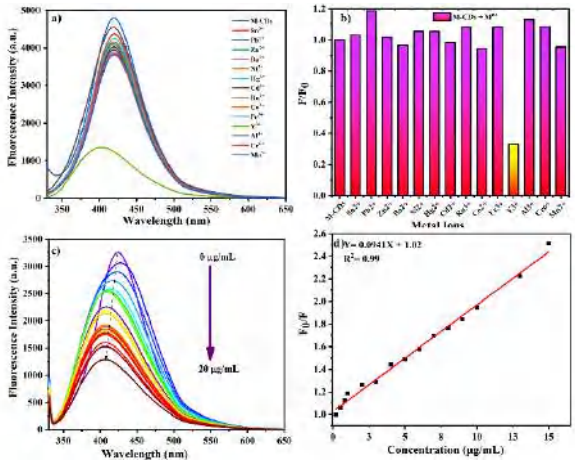
In present study, waste valorisation approach was utilizing for synthesis of Mg-doped carbon dots form cow dung using hydrothermal method. The Mg doped fluorescent carbon dots (M-CDs) having maximum emission at 420 nm upon 310 nm excitation with 20 % quantum yield. The M-CDs synthesis was confirmed using different analytical a characterization technique as well as stable in different pH as well as ionic strength solutions. The M-CDs was highly selective towards Y^{3+} ions with significant blue shift. The LOD of developed probe toward Y^{3+} was 0.019 µg/mL. The study indicates quenching of Y^{3+} was result of dynamic and IFE quenching effect [1] which was analysed by TCSPC (Time-Correlated Single Photon Count) and UV-Visible spectroscopy measurement. Further the interaction of CDs with Y^{3+} ion was investigated. The oxygen containing groups of CDs was responsible for Y^{3+}-CDs bonds [2-3]. The healthy growth of blood vessels in angiogenesis study demonstrated the cytotoxicity of CDs. further the CDs employed for MCF-7 breast cancer cell imaging [4]. The CDs were enable to interact with MCF-7 cell and brighten the fluorescence signal.



References:

1. Panpan Zhu et al. ACS Appl. Nano Mater **2023**, 6, 21194.
2. Xuelian Hu et al. Sen. Actuators B. Chem. **2021**, 338, 129809.
3. Mingxi Yang et al. Part. Part. Syst. Charact. **2019**, 36, 1800315
4. Cintya D'Angelis do E. S. Barbosa et al. Chem. Eur. J. **2015**, 21, 5055 – 5060

P 126

Untangling the degradation mechanism of knotted proteins by ATP-dependent proteases and proteasome

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Knotted protein is a special class of protein where self-entanglement of the poly peptide chain results in a folded protein with a knot. Depending upon the knot topology knotted proteins are classified as *trefoil knotted (3_1), figure of eight knotted (4_1), gordian knotted (5_2), and stevedore knotted (6_1) proteins*[1].

These proteins currently populate almost 1% of known protein structures in the PDB[2]. However, how a protein folds with a knot and what is the functional significance of a knot in a protein remains elusive. Following initial reports of knots providing proteins mechanical stability against cellular degradation by ATP-dependent proteases and proteasomes[3], we have tried to understand the degradation mechanism of model knotted protein substrates (miRFP709 and human UCHL1) by proteases ClpXP and ClpAP and proteasomal system Mpa-20S CP, using steady-state fluorescence loss assay and SDS-PAGE densitometry assays.

ClpAP and ClpXP are known to be a comparable in terms of their protein degradation activity[4]. But, to our surprise, when degraded from C to N terminus, ClpAP fails to degrade knotted protein miRFP, while ClpXP degrades it very easily. To reconcile such unanticipated behavior, we further investigated the role of knots in degradation by using fusion GFP-knotted proteins as substrate. On the other hand, the knotted protein substrate UCHL1 is seen to be resistant to degradation by both ClpXP and ClpAP when degraded from C to N terminal, whereas the Mpa-20S CP proteasome system quite easily degrades UCHL1 from N to terminal.

Thus, our study suggests that knots may contribute to protein stability against certain degradation systems, such as ClpAP. However, knotted proteins are still susceptible to degradation by other systems, including ClpXP and Mpa-20S CP. This duality could reflect evolutionary selection, where knots provide protection from some quality control mechanisms while retaining alternative pathways for maintaining protein homeostasis within the cell.

References:

1. Faisca et al. *Computational and Structural Biotechnology Journal. Elsevier B.V.* **2015**, *459*.
2. Dabrowski-Tumanski et al,. *Proc Natl Acad Sci U S A* **2017**, *114* (13), 3415
3. Xu et al. *Biophys J* **2018**, *115* (9), 1681.
4. Olivares et al., *Nat Struct Mol Biol*. **2014**, *21(10)*, 871
5. Lim et al. *J Mol Biol* **2015**, *427 (2)*, 248

P 127

Structural and functional basis of Erythromycin Resistance Methyltransferase mediated antibiotic resistance

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Antimicrobial resistance is a silent pandemic rapidly engulfing the world, driven by the misuse and overuse of antibiotics[1]. Among the many methods employed by pathogens to evade the effects of antibiotics, target modification is both efficient and straightforward. Erythromycin-resistance methyltransferases (Erms) site-specifically mono-/di-methylate A2058 (*E. coli* numbering), a base located deep within the nascent peptide exit tunnel (NPET) of the ribosome[2]. This modification confers resistance to the macrolide, lincosamide, and streptogramin B classes of antibiotics, which also bind in the NPET to disrupt protein translation. While it is established that Erms act on ribosomal precursors, the true in vivo substrate of these enzymes has remained elusive[3]. Using cryogenic electron microscopy (Cryo-EM) and single-molecule Förster resonance energy transfer (sm-FRET), we have not only captured this elusive substrate but also revealed the complex dynamic nature of this enzyme when interacting with its substrate. Our results highlight key structural determinants, located away from the enzyme's catalytic domain, that govern substrate specificity. Additionally, we observe a unique molecular motion displayed by these enzymes, which facilitates the methylation of the target base. Overall, our study provides a comprehensive understanding, from substrate identification to the elucidation of the enzyme's dynamic nature, which paves the way for drug development against these pathogenic proteins.

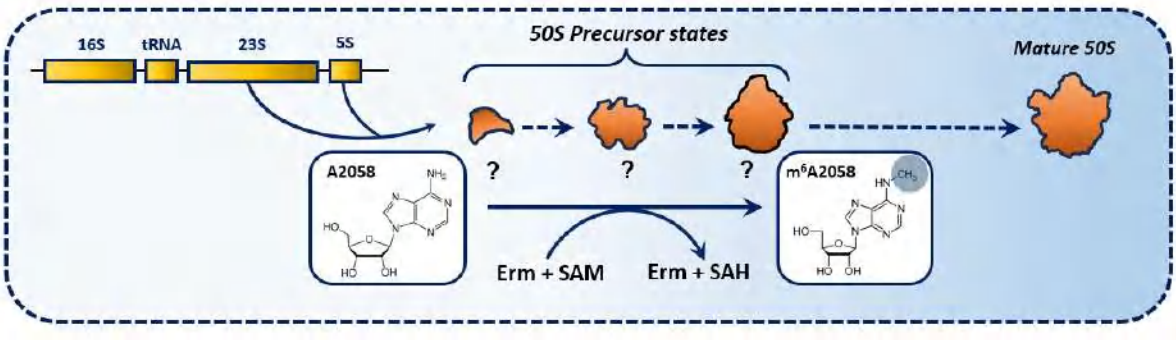

Figure 1: Schematic representing the action of Erms *in vivo*. The true substrate of Erms have remained elusive. Erms methylate an early ribosomal particle and induces drug resistance.

References:

1. Okeke, I. N. et al. The Lancet **2024,** *403*, 2426.
2. Bhujbalrao et al. *J Am Chem Soc* **2019**, *141*, 1425.
3. Pokkunuri, I. & Champney, W. S. *RNA Biol* **2007,** *4*, 147.

P 128

Drug-assisted white light generation *via* self-assembly of a styryl dye

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Fluorescence spectroscopy and microscopy stand as pivotal methodologies in the realm of scientific inquiry, offering profound insights into the intricate molecular properties of both organic and inorganic entities.[1-2] With unmatched sensitivity, fluorescence spectroscopy enables the clarification of molecular structures, interactions, and dynamics through the absorption and subsequent emission of photons by fluorophores. In this investigation, we focused on white light generation *via* the combination of a polarity-sensitive red emitting Styryl chromone 1 (**SC1**)[3] **(Fig.)** and a blue-emitting anticancer and psycotherapeutic drug Norharmane (**NHM**) [4] in a self-assembled neutral adjuvant Triton X-100 (TX-100). Incorporation of both emitters inside the micellar system results an improved fluorescent behaviour and resulting in white light emission due to complementary wavelength overlap. Further spectroscopic investigation allows us to understand the premicellar aggregation process [5] of three different type of surfactants with varying charges using the **SC1** dye. This investigation highlights the significance of co-assembly of **SC1** dye and NHM drug for the generation of a highly stable white light.

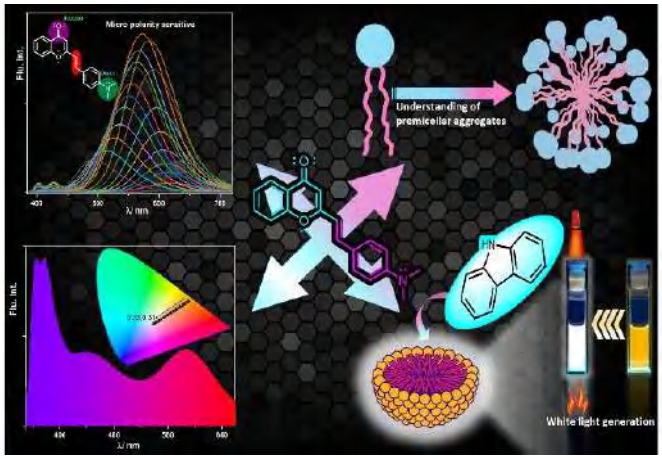

Fig. White light generation using a co-assembly of a micro-polarity sensitivity dye and anticancer drug

References:

(1) Engelborghs, Y. *et al.*, Book on: Fluorescence spectroscopy and microscopy. In *Methods in molecular biology*. Springer, **2014**.
(2) Chatterjee, O., *et al.*, *Adv. Opt. Mater.*, **2022**, *10*, 2201187.
(3) Kavyashree P. *et al.*, *J. Mater. Chem, B* **2022**, *10*, 5071.
(4) Chakrabarty, A. *et al.*, *Langmuir*, **2007**, *23*, 4842.
(5) Niu, X. *et al.*, *Analyst*, **2019**, *144*, 6866.

P 129

Scaling Principles in Chemistry: Elucidating the Reaction Kernel

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Abstract :

The visualization of atomic motion at its fundamental length scale and time scale has long been a 'Gedanken'experiment to develop the conceptual understanding of chemical reactions in condensed matter systems[1,2]. In this regard, an electrocyclic ring-closing reaction or a spin crossover reaction are prototypically classic examples of a bond-making process or an electron transfer reaction with the change of electron spin and density. Hence following the electron with its spin while bonds are being formed, is the ultimate magnum opus of chemistry. In this regard, the conquest of comprehending the reaction mechanism through the barrier-crossing process becomes imperative and even so because of the enormous reduction of dimensionality during a chemical reaction, which still alludes to chemists and physicists all around the world. The idea of only a few key reactive modes out of 3N-6 degrees of freedom (N: number of atoms) driving a chemical reaction through the transition state is still an open quest yet fully to conquer. Observation of these key reactive modes defines the reaction kernel and gives a detailed understanding of the reaction coordinate and the competition among myriads of quantum vibrations or orthogonal coordinates. In this context, we have determined the reaction kernel for the ring-closing reaction in a prototypical molecule called fulgide with three key reactive modes and, also elucidated the reaction kernel for the spin crossover process in a Fe (III) based inorganic molecule consisting of two key reactive modes. For the ring-closing reaction in fulgide, we have been able to disentangle the excited state dynamics of 70 fs followed by a ~140 fs timescale for the ring-closing reaction highlighting the ultrafast nature of the reaction. In the spin crossover process, we have discovered the participation of an intermediate quartet spin state which is populated in ~50 fs limited by the instrument response. In 96 fs, the system depopulates the intermediate state to form the high spin sextate state starting from the initially photoexcited low spin state (Ligand-to-metal charge transfer doublet state). In both systems, we observe a structural reorganization occurring in the sub-ps timescale because of the constrained crystalline environment since molecular motion is restricted. This results in the activation of molecular vibrations dephasing in a sub-ps timescale optimizing the molecule in the product potential energy surface after the ultrafast photoinduced chemical reaction. This present work has also unearthed a new method of reaction mechanism, which involves the non-linear mixing of molecular vibrations giving rise to transient vibrations on ultrafast timescales that light up as coherent photoinduced motions directing the chemical transformation. The molecular vibrations dephasing in the sub-ps timescale undergo non-linear mixing in the molecular framework to generate transient vibrations with growing amplitude. Mechanism of this kind is well known in non-linear optics but is a surprising observation in the molecular framework highlighting the strong anharmonicity within the product potential energy surface. This discovery could open new ways of perceiving chemical reactions, with yet another mechanism collapsing the system onto a few highly nonlinearly coupled coordinates. This new insight is more clearly observable in solid crystalline environments where the initial conditions are well-defined. The elucidation of the structural dynamics and the key reactive modes defining the reaction kernel in the single crystal environment will open new avenues in carrying out reactions in the crystalline environment and inspire new design strategies for better and more improved chemical reactions in comparison to the solution phase. This is possible because of conserved spatial correlations in crystals, inspiring a new field of research in open quantum systems. Current research is underway in analyzing ultrafast electron diffraction (UED) data to unearth the atomic motion leading up to the reactive kernel and visualize the serendipitous discovery of the mixing of molecular vibrations in the crystalline domain. These discoveries can be further expanded to extended systems in biology where proteins (N>1000) effectively carry out chemical transformations to drive function in living systems and our research promises to uncover the secrets of anharmonic couplings and localized motions defining the reaction kernel driving biological functions[1,2].

References

1. Ischenko, *et. al*., *Chem. Rev*. **2017**, *117*, 11066.

2. Miller, R., J., D, *Annu. Rev. Phys. Chem.*, **2014**, *65*, 583.

Complex Interplay of domains in the co-condensation of α-Synuclein and SARS-CoV-2 Nucleocapsid Protein

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Recent studies suggest that SARS-CoV-2's impact extends beyond respiratory tract infections to neurological symptoms including Parkinsonism [1, 2]. Recently, it was reported that the SARS-CoV-2 nucleocapsid protein (N-protein) accelerates the aggregation of α-Synuclein (α-Syn), a protein associated with Parkinson's disease [3]. Liquid-Liquid Phase Separation (LLPS) is key for organizing biomolecules in cells but can also lead to toxic aggregate formation in condensates [4]. α-Syn undergoes nucleation and aggregation via condensation through LLPS [5]. In this study, combining single molecule spectroscopy, microscopy and simulation we tried to understand the underlying molecular mechanism of N-protein-facilitated aggregation of α-Syn. We observed that N-protein promotes LLPS of α-Syn via co-condensate formation. Fluorescence Correlation Spectroscopy (FCS) inside droplets and Fluorescence Recovery After Photobleaching showed hindered dynamics of α-Syn in the co-condensate. Time-domain fluorescence Lifetime Imaging further confirmed the enhanced ageing of the co-condensates. The increased partitioning of amyloid reporter- Thioflavin T inside co-condensates suggested the temporal maturation of liquid droplets into β-sheet rich structures. Confocal microscopy, co-existence coarse-grained simulations and binding affinity measurements elucidated that electrostatic heterotypic interactions through oppositely charged blocks in these proteins can dictate the architecture of the co-condensates. Overall, this study reveals how α-syn and N-protein domains interact to drive co-condensation, shedding light on α-syn amyloid formation and possible reason for COVID-induced Parkinsonism.

References:

1. Cohen, Mikhal E et al. *The Lancet Neurology* **2020**, *19*, 804.
2. Lee, Bina et al. *Cell Reports Medicine* **2024**, *5*, 101570.
3. Semerdzhiev, Slav A et al. *ACS Chemical Neuroscience* **2021**, *13*, 143.
4. Boeynaems, Steven et al. *Trends in Cell Biology* **2018**, *28*, 420.
5. Ray, Soumik et al., *Nature Chemistry* **2020**, *12*, 705.

P 131

Intracellular active forces and out of plane rotation due to motor activity revealed in passive microrheology

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Optical tweezers are a common tool to investigate biological systems such as single cells, applying very low, non-invasive forces. It uses a tightly focusses laser beam to confine a micro particle and simultaneously track its position and orientation. A phagocytosed bead inside a cell is affected by its local environment, cytoskeletal dynamics as well as molecular motor activity which is responsible for transport of cargo inside cells [1-3]. Out of plane rotational motion of a trapped phagosome happens due to molecular motor activity. However, calibrating the data inside cells is a challenge [4]. We show a method to track and calibrate the out of plane rotation of a particle in intracellular environment using passive microrheology. We record Power Spectral Density in the frequency range from 0.5Hz to 1kHz. The lower frequency domain is dominated by active processes whereas thermal fluctuations prevail at higher frequency domain [5,6]. The crossover frequency from active to thermal domain has been a matter of conflict since long [5,6]. Complicacies are increased by the fact that the active processes are random and stochastic, and the cellular environment is highly complex and heterogeneous [7]. We combine the generalized Maxwell model for viscoelasticity with Active Ornstein Uhlenbeck process to derive a model to describe the complete behavior of an optically trapped bead inside a cell at all frequency regimes [8]. This model proves useful to simultaneously extract local viscoelastic parameters along with effective active force and torque exerted on the cargo by teams of molecular motors and activity timescale. The out of plane rotational motion yields interesting information about multiple motor activity elusive to conventional translational tracking.

References:

1. F. C. MacKintosh et. al. *Curr. Opin. Cell Biol*. **2010,** *22(1),* 29.
2. W. W. Ahmed et al. *Biophys. J* **2018**, *114(7),* 1667.
3. C. Leidel, et al. *Biophys. J*. **2012**, *103(3),* 492.
4. Y. Jun et al. *Biophys. J*. **2014**, *107(6),* 1474.
5. H. Turlier, et al. *Nature physics*, **2016**, *12(5),* 513.
6. D. Mizuno et al. *Macromolecules* **2008**, *41(19),* 7194.
7. K. Luby-Phelps et al. *Int. Rev. Cytol*. **1999**, *192,* 189.
8. U. Nakul, et al. *Biomedical optics express* **2023**, *14(10),*5440.

P 132

Probing SARS-CoV-2 spike mediated nascent fusion pore open-close dynamics during single membrane fusion events

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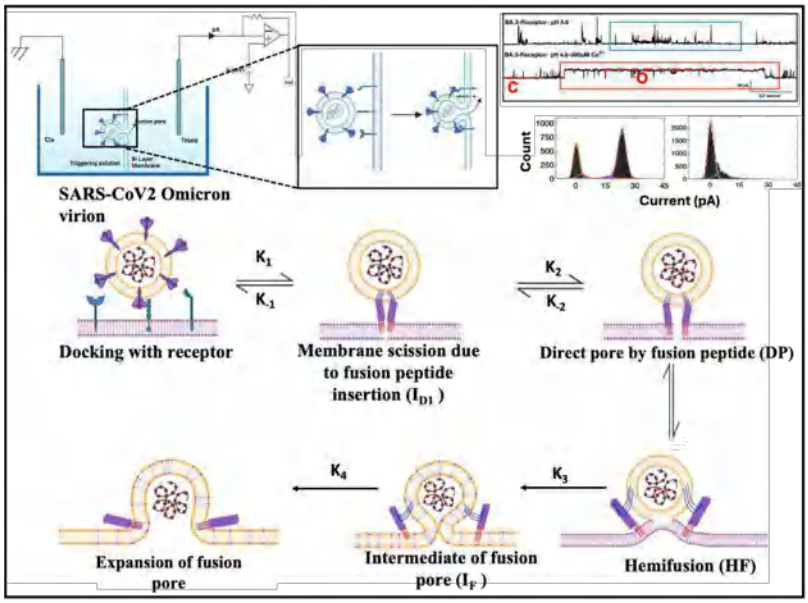
SARS-CoV-2 is the causative agent of the COVID-19 infection that has resulted public health concern worldwide. Cellular entry of SARS-CoV-2 is mediated by the spike glycoprotein fusion machinery[1,2]. During entry, trimeric spike protein facilitates fusion pore formation between viral and cellular membrane to release the viral genetic materials into the host cell[3]. Still, the mechanism of pore formation during membrane fusion by the spike protein is not properly understood. Here, using planar lipid bilayer electrophysiology and fluorescence-based lipid mixing, we directly detected the Omicron spike mediated fusion pore formation in real time at single fusion events. We found that fusion pore has a complex dynamics and it follows distinct open-close states depending on the cellular environment and spike protein localization. We also observed that the pore formation reaction proceeds through sequential intermediates on its pathway to complete fusion. Moreover, our findings also highlight the synergistic effect of lysosomal pH and presence of Ca^{2+} ion in the regulation of pore stability, dwell time and overall pore opening kinetics.



References:

1. Fan et al., *Nat Commun* **2020**, *11*, 3618
2. Yongfei Cai *et al.*, *Science* **2020**, *369*, 1586-1592
3. Singh, P et al., *Cell Rep* **2022**, *39*, 110694

P 133

Interplay of Excited State Lifetime and Redox Potentials for Intracellular Superoxide Anion Radical Generation

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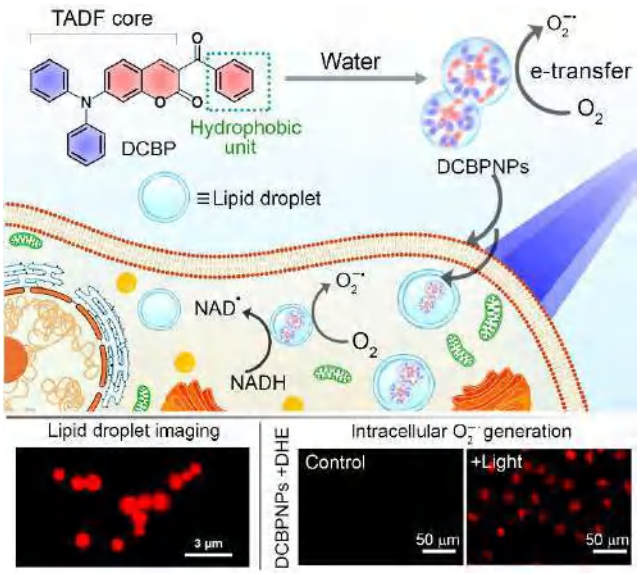
All-organic thermally activated delayed fluorescence (TADF) emitters with long-lived emission properties are emerging materials for time-resolved bioimaging and photodynamic therapy.[1-4] The triplet harvesting phenomenon of TADF materials is promising to generate intracellular reactive oxygen species (ROS) via either electron transfer (ET, Type-I) or energy transfer (EnT, Type-II) pathways for cancer cell ablation.[2,3] However, elucidating the design strategy to develop Type-I photosensitizer with tunable optical and biological properties remains a formidable challenge. Herein, employing keto-coumarin derivatives, ECBP and DCBP, we demonstrate that the presence of a long-lived excited state along with suitable excited state redox potential of the photosensitizer are the two key parameters resulting in the Type-I mode in organic delayed fluorescent materials.[5] Among the two derivatives, DCBP and its self-assembled nanoparticles (DCBPNPs) exhibit triplet harvesting properties and long-lived emission via thermally activated delayed fluorescence due to the presence of a small singlet-triplet energy gap and show promising scope for superoxide anion radical ($O_2^{-\cdot}$) generation (Scheme 1). Electrochemical and time-resolved spectroscopic studies reveal the facile electron transfer from the excited state as a driving force for the photoinduced electron transfer. DCBPNPs also showed electron transfer ability in the presence of biologically relevant analytes like NADH, which is favourable for generating oxidative stress in cells. Thereafter, the high quantum yield, photostability coupled with the inherent hydrophobicity of DCBPNPs, low dark toxicity and high phototoxicity enabled the successful imaging of lipid droplets and targeted generation of intracellular $O_2^{-\cdot}$ for cancer cell ablation. Thus, the present work presents a broad scope for the development of Type-I thermally activated delayed fluorescent photosensitizers in photodynamic therapy.



Scheme 4: Schematic illustration depicting the specific localization in lipid droplets and intracellular superoxide radical anion generation by self-assembled nanoparticles of DCBP up on light irradiation.

References:

1. F. Ni *et. al*. *Adv. Opt. Mater*. **2020**, *8*, 1902187.
2. T. C. Pham *et. al*. *Chem. Rev*. **2021**, *121*, 13454.
3. F. Fang *et. al*. *Adv. Sci*. **2021**, *8*, 2102970.
4. S. Das *et. al*. *Chem. Sci*. **2024**, *15*, 102.
5. S. Das *et. al*. (*manuscript under preparation*).

P 134

Breaking the Refractive Index Barrier: Imaging High Refractive Index Media with a Polarized TIRF Microscopic Approach

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Preferential alignment of nanostructures leads to various optoelectronic properties, which may differ significantly while probing near the interface in comparison to their bulk [1,2]. Total Internal Reflection Fluorescence Microscopy (TIRF) is a technique used to probe areas at interfaces using evanescent wave induced fluorescence, achieving surface selective excitation. [3]. However, the major difficulty arises when attempting to image objects with a very high refractive index. When the refractive index of the material is much higher than that of the substrate, total internal reflection cannot occur, and hence evanescent wave excitation is not possible. In our previous work, we used simulations to show that by introducing an intermediate thin layer with a low refractive index, we can achieve total internal reflection and generate an evanescent wave across the interface [4]. In this work, we prepared a multilayered surface consisting of an optically transparent, low refractive index thin film polymer sandwiched between the glass and the high refractive index $CsPbBr_3$ nanocrystals. These nanocrystals were excited with a 405 nm CW laser beam, and a structural similarity analysis of the widefield and TIRF images was also carried out to identify areas exhibiting significant changes in widefield mode and evanescent field excitation. In addition, excitation polarization was varied, and fluorescence anisotropic imaging was performed in widefield and TIRF modes to measure anisotropy at the interface.

References:

1. Fei Zheng et. al. *ACS Applied Materials & Interfaces* **2022**, *14*(46), 51910.
2. Heyou Zhang, et. al. *ACS nano* **2018**, 12(8) 7529.
3. Daniel Axelrod et. al. *Methods in cell biology* **1989**, *12*(8), 245.
4. Subhajit Chakraborty et. al. *Physica Scripta* **2022**, 98(1), 015014.

P 135

Development of an ^{19}F MRI Probe for Redox Sensing

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Reactive oxygen species (ROS) are free radical entities produced majorly by mitochondria during cellular metabolism and include hydrogen peroxide (H_2O_2), hydroxyl ($HO^{\bullet}$) and hydroperoxy radicals ($HO_2^{\bullet}$), and singlet oxygen ($^1O_2$). ROS play several important roles in biological systems such as providing defence against pathogens, modulating cell signalling, and preservation of cellular homeostasis[1,2]. Overproduction of ROS contributes to oxidative stress which is associated with several pathophysiological conditions such as cancers, cardiovascular diseases, and neurodegenerative disorders[3]. Hence, tracking ROS in living systems can provide both mechanistic insights into pathophysiological conditions and afford diagnostic routes. Fluorescence-based ROS responsive probes have been reported previously but have limited applicability because of the low tissue penetration of fluorescence microscopy[4]. Magnetic Resonance Imaging (MRI) is a popular technique for clinical imaging. MRI is suitable for soft tissue imaging in living systems owing to its non-invasive nature and deep tissue penetration[4,5]. Favourable magnetic resonance properties of the ^{19}F nuclei (I=1/2 Same as ^1H) and low bioavailability of fluorine (< 10^{-6} M) lead to low background signals making it an apt choice for MRI [6]. Transition metal ions like Fe^{2+}, Co^{2+} are oxidized by cellular ROS. This property has been leveraged to develop ROS responsive ^{19}F-MRI probes where oxidation of a metal ion complex containing a fluorine atom leads to modulation of relaxation properties of the fluorine nuclei [3,4]. We have attempted the development of a novel macrocyclic mixed N, O-donor based fluorine substituted metal-binding ligand toward an ^{19}F-MRI based ROS probe. The details of the synthesis, characterization, and initial relaxation measurements will be presented.

References:

1. A Abdal Dayem et.al. *Int. J. Mol. Sci*. **2017**, 18, 120.
2. C Lennicke et.al. *Molecular Cell.* **2021**, 81, 3691.
3. M Yu et.al. *Chem. Commun*. **2016**, 52, 13885
4. R Kadakia et.al. *Chem. Sci.* **2023**, 14, 5099
5. M. L James et.al. *Physiol. Rev*. **2012**, 92, 897.
6. Da Xie et.al. *Acc. Chem. Res.* **2020**, 53, 2.
7. M Yu et.al. *Inorg. Chem*. **2012**, 51, 9153.

P 136

Ultrafast dynamics of nonrigid Ni porphyrin dimer

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Photosynthesis is one of the fundamental processes in nature that efficiently converts light energy to chemical energy. Chlorophylls are arranged in a dimeric structure, called special pair which initiates a multistep electron transfer reaction. Porphyrins have almost similar macrocyclic framework as chlorophylls. Therefore, much effort has been put into mimicking the dimer structure of the special pair.[1]

Here, the photophysical properties of ethane-bridged Nickel octa ethyl porphyrin dimer has been explored and compared with its monomer analogue. Initial photoexcitation is delocalized over porphyrin π ring. However, intramolecular vibrational relaxation is hampered by the presence of intermediate states involving metal d orbitals.[2] Excitonically coupled porphyrin dimer causes an ultrafast deactivation which limits the excitonic lifetime of the dimer.[3] Axial ligand binding and excitation wavelength play a role in vibrational relaxation pathways that give us a better understanding of excess energy dissipation of highly excited Nickel (II) porphyrins into surrounding solvent molecules.[4]

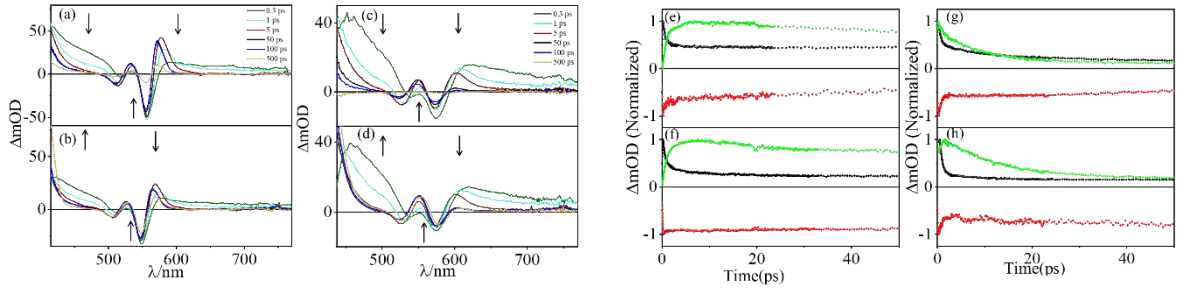

Fig 1. (a) and (b) TA spectra of Ni porphyrin monomer in chloroform and pyridine respectively. (c) and (d) TA spectra of Ni porphyrin dimer in chloroform and pyridine respectively with λ_{pump} = 400 nm. (e) and (f) Kinetics of GSB (red) at 550 nm, ESA (green, black) signal at 450 nm, 565 nm of monomer in chloroform and pyridine respectively. (g) and (h) Kinetics of GSB (red) at 575 nm, ESA (green, black) signal at 470 nm, 600 nm of dimer in chloroform and pyridine respectively.

References:

1. J. Baek et. al. *J. Phys. Chem. C* **2018**, *122*, *13285*
2. M. A. Naumova et. al. *J. Phys. Chem. Lett.* **2024**, *15*, 3627
3. L. Moretti et. al. *J. Phys. Chem. Lett.* **2020**, *11*, 3443
4. H. S. Eom et. al. *J. Phys. Chem A.* **1997**, *101*, 3661

P 137

Surface-enhanced Raman spectroscopy of atomically precise nanocluster

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Noble metal nanoclusters (NCs) are a class of nanomaterials that exhibit unique properties due to their ultra-small size (diameter <2.2 nm) and high surface-to-volume ratio[1]. In recent years, NCs have been shown to be promising candidates for various applications, including catalysis, sensing, and imaging. However, their molecular-like properties have not been fully explored. The molecular nature and atomic preciseness of nanoclusters have been unequivocally affirmed through a comprehensive suite of characterisation techniques, including UV-visible spectroscopy, photoluminescence spectroscopy, and mass spectrometry[2]. To delve further into their properties and unlock their full potential for real-life applications, additional sophisticated methods are imperative. Surface-enhanced Raman spectroscopy (SERS) emerges as a versatile and powerful tool for probing molecular vibrational levels within nanoclusters when perched atop nanoparticles. This symbiotic relationship between nanoclusters and nanoparticles not only enhances the SERS effect but also paves the way for a myriad of applications. Notably, SERS facilitates the observation of minute changes in nanoclusters even within harsh environments, offering unprecedented insights. The Raman features exhibited by clusters such as Cu_4(m-CBT)$_4$ and $[Ag_{17}(o\text{-CBT})_{12}]^{3-}$ are informative, rendering them ideal candidates as Raman reporters in SERS. The synergistic assemblies of anisotropic nanoparticles and nanoclusters, showcasing enhanced Raman capabilities, hold immense promise for sensor applications. The resulting assemblies exhibit remarkable Raman features, opening new avenues for sensor applications, particularly in the realm of biosensors. This research marks a significant stride towards harnessing the unique molecular precision of nanoclusters for practical, real-world implementations.

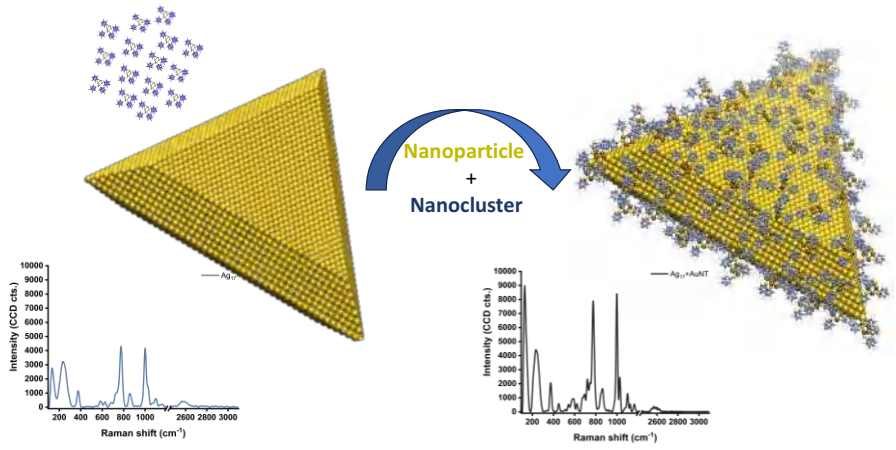

Figure. A representative diagram showing the process and generation of the SERS.

References:

1. Chakraborty, I. et al. *Chem. Rev.*, **2017**, *117*, 12, 8208.
2. Negishi, Y. Et al. J. Am. Chem. Soc., **2005**, *127*, 14, 5261.

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Activity Based Alkyne Tagged Raman (ABATaR) Probes for Detection of Bio-analytes

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Metal ions are essential for biological function.[1] However, mis-regulation of metal ions can lead to severe pathophysiological consequences. The regulation of metal ions within living cells is closely linked to the redox state of a cell. Hence, elucidating the correlated regulation of metal ions and reactive oxygen species[2] can afford key insights into the redox balance within cells under both physiological and pathophysiological conditions. This necessitates multi-analyte imaging. Fluorescent molecular probes in conjunction with fluorescence microscopy have been widely been used to image bio-analytes. Despite providing very high spatio-temporal resolution, the fluorescence imaging platform suffers from signal overlap due to the inherently broad nature of fluorescence spectra of molecular probes which significantly restricts multiplexed imaging of bio-analytes. Raman microscopy on the other hand depends on molecular vibration and hence provides ~50-100[3] times narrower spectral width compared fluorescence which can allow facile multiplexing. In recent years, bio-orthogonal Raman tags like alkyne, nitrile, C/^{13}C-H/D have been developed to visualize bio-molecule localization and distribution inside living cells.[4] However, these efforts are mostly focused on Raman tags which are non-responsive. The development of responsive Raman probes will provide the key handle to multi-plexed imaging and tracking of bio-analytes. Toward this goal we have designed a generalized platform to develop "Activity based" alkyne tagged Raman (**ABATaR**) probes for detection of bio-molecules in a multiplexed approach. Here we have leveraged pH dependent changes in the protonation state of an alkyne tagged phenol to develop **ABATaR** probes for detection of pH, ROS and Cu^{2+} ions in a multimodal fluorescence-Raman imaging platform. In my poster, I will be presenting the design principle, synthesis, in-vitro, and in-cell studies of our novel **ABATaR** probes.

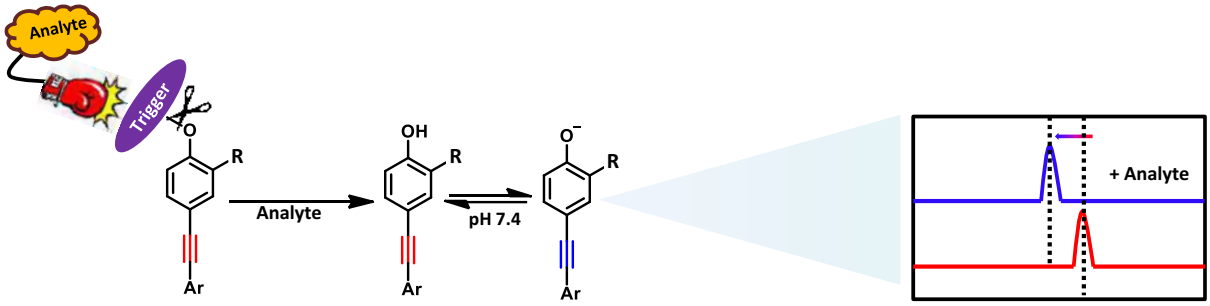

Figure 5: Scheme depicting the working principle of ABATaR .

References:

1. Emily L. Que et. al. *Chem. Rev.* **2008**, *108*, 1517.
2. Helmut Sies et. al. *Redox Biol.* **2017**, *11*, 613.
3. Hanqing Xiong et. al. *Light Sci Appl.* **2021**, 10.
4. Hiroyuki Yamakoshi et. al. *J. Am. Chem. Soc.* **2012**, *134*, 20681.

P 139

Position-Dependent Modulation of Intersystem Crossing in Iodo-Functionalized Salicylideneimine-Boron Compounds

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Intersystem crossing (ISC) is the key factor to generate the molecular triplets, which can be significantly enhanced by the introduction of heavy atoms due to their ability to strengthen spin-orbit coupling (SOC) [1]. However, the influence of the heavy atom's position within a molecule on ISC efficiency remains largely unexplored [2,3]. In this study, we address this gap by investigating the ISC process in three positional isomers of an iodine-substituted Salicylideneimine-Boron compounds: 3ISB, 4ISB, and 5ISB. Using a combination of steady-state and time-resolved absorption and fluorescence spectroscopy, we observed that ISC efficiency varies notably between the isomers. The 3ISB isomer exhibited the most efficient ISC and triplet formation, followed by moderate efficiency in 5ISB and the least efficiency in 4ISB. Quantum chemical calculations suggest that the iodine atom plays a critical role in the electronic transition process. In 3ISB and 5ISB, the HOMO to LUMO excitation effectively redistributes electron density from the iodine atom to other parts of the molecule, enhancing spin-orbit coupling. However, in 4ISB, the electron density remains largely localized on the iodine atom, which significantly reduces the overall spin-orbit coupling within the chromophore, leading to the least efficient ISC in this isomer. Additionally, our theoretical analysis suggests that SOC is not the only parameter governing the ISC process; C–X bond vibrations may also play a role in influencing ISC in such heavy-atom systems. These findings provide a deeper understanding of the positional dependency of ISC in heavy atom-modified molecules and offer valuable insights for the design of highly efficient triplet-state materials, which have broad applications in various fields [4] such as organic electronics and photodynamic therapy.

References:

1. M. A. El-Sayed *J. Chem. Phys.* **1965**, *43*, 2864.
2. Jack T. Ly et. al., *Phys. Chem. Chem. Phys.* **2021**, *23*, 12033.
3. A Das et. al. *J. Phys. Chem. A* **2022**, 126, *9*, 1475.
4. Xiao et. al. *Chem. Soc. Rev.*, **202**1, *50*, 9686.

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Exploring Iridium (III) Complex Nanoparticles Photocytotoxicity Against Hypoxic Cancer

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Near-infrared (NIR) emitters Iridium (III) complexes are an important genre of photosensitizers (PSs) for photodynamic therapy (PDT). [1][2] The current research explores nanoparticles (NPs) encapsulating the NIR-emitting Iridium (III) complex. Initially, RM2 was found to show high singlet oxygen ($^1O_2$) generation efficiency (73% in the solution phase), making it promising for PDT. [3] During the in-vitro study, it was found to have low dark toxicity but significant cell toxicity once exposed to light. Mechanistically, light-irradiated RM2-treated cancer cells exhibited a substantial increase in the intracellular reactive oxygen species (ROS) level. Neutralizing ROS by Vitamin C (Vc) pretreatment inhibited the cytotoxic ability, demonstrating that ROS is an indispensable effector of cell cytotoxicity. After nanoformulations via soluplus micelles [4], RM2 showed good biocompatibility and a significant decrease in cell viability under light irradiation, even under low oxygen conditions with a half-maximal inhibitory concentration (IC_{50}) of 100 nM. This work emphasizes the importance of the Iridium (III) complex as an important class of PS for PDT against hypoxic cancer.

References:

1. Zhijun Zhang et. al. *Chemical Society Reviews* **2022**, *51*, 1983.
2. Shengnan Liu et. al. *Chemical Communications* **2024**, *60*, 9938.
3. Thanh Chung Pham et. al. *Chemical Reviews* **2021**, *121*,13454.
4. Sumit et. al. *ACS Applied Bio Materials* **2023**, *6*, 1445.

P 141

Competition between the hydrogen bond and the halogen bond in a [ROH-CCl_4] (R = CH_3 and C_2H_5) complexes

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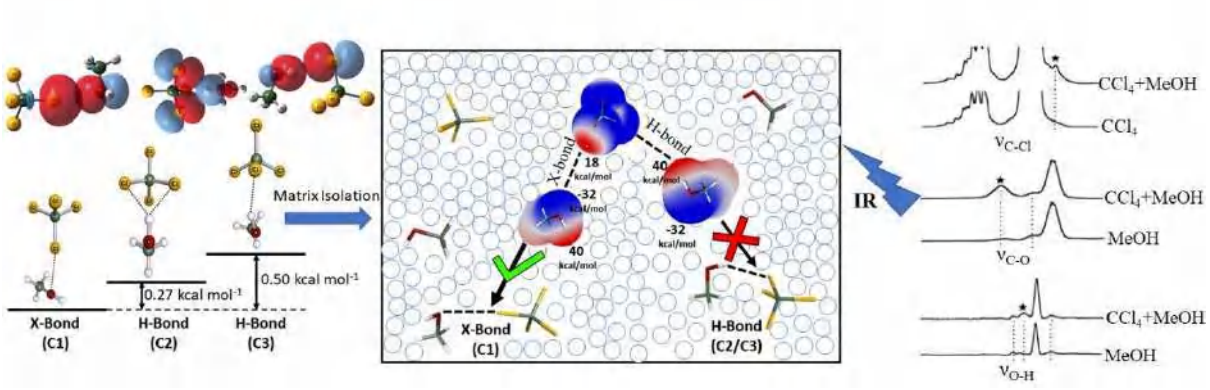
Methanol (CH_3OH) is the simplest alcohol, while carbon tetrachloride (CCl_4) is a widely used solvent in the chemical industry. Both CH_3OH and CCl_4 are significant volatile substances in the atmosphere, with CCl_4 playing a critical role as a precursor in atmospheric ozone depletion. Additionally, mixtures of CH_3OH and CCl_4 are notable as non-aqueous systems due to their significant deviations from Raoult's law [1]. At the molecular level, CH_3OH and CCl_4 can interact in two primary ways: through hydrogen bonding (O-H···Cl) and halogen bonding (C-Cl···O) [2]. The general impression of weakly bound clusters is revolved around H-Bond interaction [3].

We characterized the 1:1 [CH_3OH-CCl_4] complex using matrix-isolation infrared spectroscopy combined with electronic structure calculations. Vibrational spectra were recorded in the C-Cl, C-O, and O-H stretching regions. Our findings confirm the exclusive formation of halogen-bonded 1:1 complex in both argon and nitrogen matrices.

This study is extended to investigate the conformer-specific complexation between ethanol (C_2H_5OH) and CCl_4. Characterizing the [CCl_4-C_2H_5OH] complex is more challenging due to the presence of two conformers of C_2H_5OH, namely anti-C_2H_5OH (A-C_2H_5OH) and gauche-C_2H_5OH (G-C_2H_5OH), each capable of binding to CCl_4.



References:

1. Musso, Maurizio, et. al., *The Journal of Physical Chemistry A*, **2002**, *106(43)*, 10152.
2. Torii, Hajime, *Chemical physics letters*, **2004**, *393(1-3)*, 153.
3. Arunan, Elangannan, et. al., *Pure and applied chemistry*, **2011**, *83(8)*, 1637.

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Achieving High Temporal Resolution in Single-Molecule Fluorescence Techniques Using Plasmonic Nanoantennas

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Single-molecule fluorescence techniques are vital for studying molecular mechanisms in biological processes, but achieving sub-millisecond temporal resolution for fast molecular dynamics remains challenging. Fluorescence brightness, which determines temporal resolution, is limited in conventional microscopes and standard fluorescent emitters. Plasmonic nanoantennas have been proposed to address this, but even with significant fluorescence enhancement, brightness typically stays below 1 million photons/s/molecule, limiting temporal resolution improvements. Here we present a method to enhance temporal resolution using plasmonic nanoantennas, specifically optical horn antennas[1-3]. The study achieves around 90% light collection efficiency and a fluorescence brightness of 2 million photons/s/molecule in the saturation regime, enabling single-molecule observations with microsecond binning times and rapid fluorescence correlation spectroscopy. This work extends the application of plasmonic antennas and zero-mode waveguides in the fluorescence saturation regime, leading to brighter signals, faster temporal resolutions, and improved detection rates, advancing fluorescence sensing, DNA sequencing, and dynamic molecular studies.

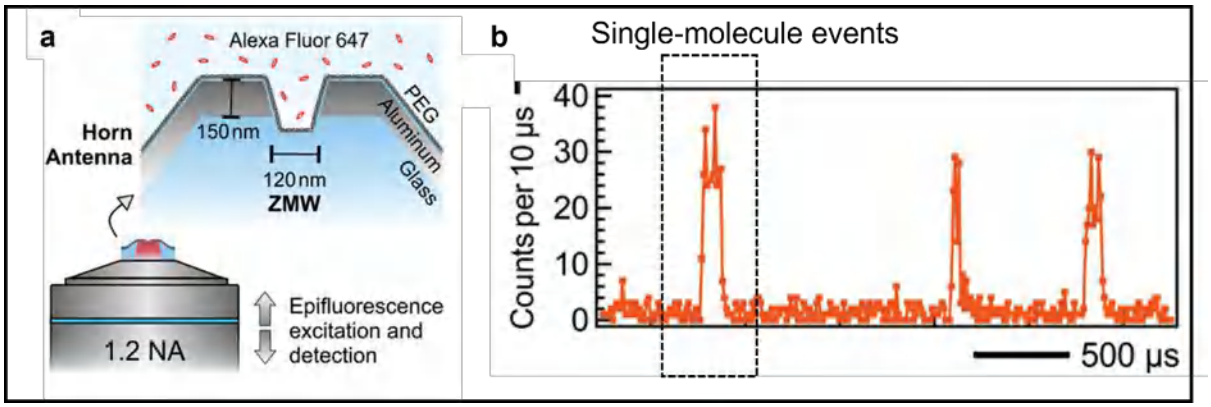

Figure 1: Optical horn antennas to achieve 90% fluorescence collection efficiency from single emitters. a) Scheme of the experimental configuration. b) Single molecules events at a fast-binning rate.

References:

1. Tiwari S, et. al., *Advanced Optical Materials*, **2023**, *11*(13), 2300168.
2. Barulin A, et. al., *Nature Communications*, **2022**, *13*(1), 1842.
3. Roy P et. al., *Nano Letters*, **2023**, *23*(2), 497.

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Mechanistic Insights into the c-MYC G-Quadruplex and Berberine Binding inside an Aqueous Two-Phase System Mimicking Biomolecular Condensates

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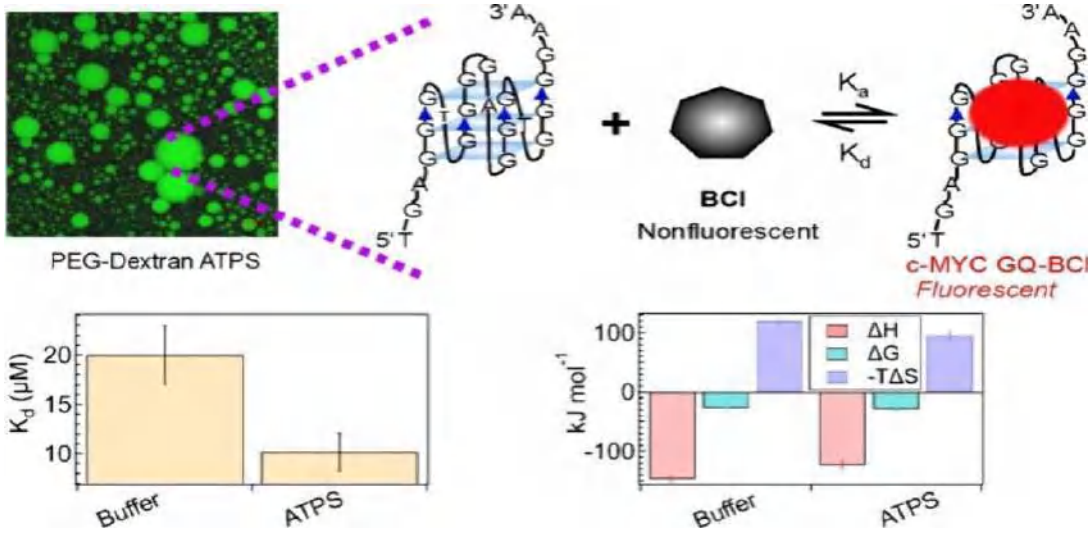
We investigated the binding between the c-MYC G-quadruplex (GQ) and berberine chloride (BCl) in an aqueous two-phase system (ATPS) with 12.3 wt % polyethylene glycol and 5.6wt % dextran, mimicking the highly crowded intracellular biomolecular condensates formed via liquid−liquid phase separation. We found that in the ATPS, complex formation is significantly altered, leading to an increase in affinity and a change in the stoichiometry of the complex with respect to neat buffer conditions. Thermodynamic studies reveal that binding becomes more thermodynamically favorable in the ATPS due to entropic effects, as the strong excluded volume effect inside ATPS droplets reduces the entropic penalty associated with binding. Finally, the binding affinity of BCl for the c-MYC GQ is higher than those for other DNA structures, indicating potential specific interactions. Overall, these findings will be helpful in the design of potential drugs targeting the c-MYC GQ structures in cancer-related biocondensates. [1]



References:

(1) S Pradhan et. al., *Journal of Physical Chemistry Letters*, **2024**, *15*, 8706.

P 144

Single-Molecule Detection of a Terrylenediimide-Based Near-Infrared Emitter

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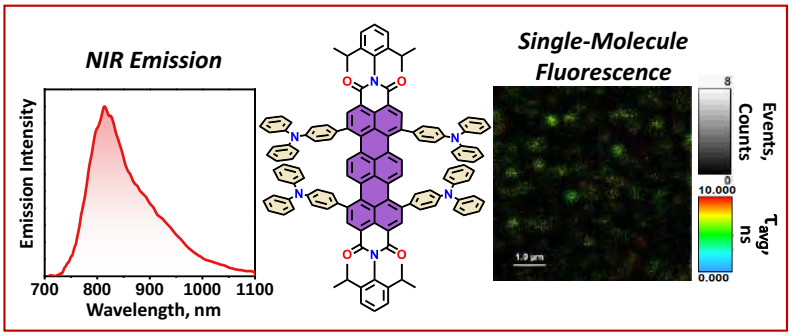
Near-infrared single-photon emitters are an emerging class of compounds for quantum information science, including computing, sensing, and communication.[1, 2] Single-molecule level scrutiny of organic near-infrared (NIR) emitting molecules has recently gained much attention.[3] The efficiency of the NIR organic emitters is limited by the energy gap law; the intrinsic forbidden nature of emission from states with low energy gap results in negligible fluorescence quantum yield.[4] The donor-acceptor systems with charge transfer (CT) character are better alternatives to circumvent the limitations possessed by conventional π-conjugated NIR molecules by narrowing the energy gap for NIR emission.[5] We report a novel NIR absorbing (λ_{max}^{Abs} = 735 nm) and emitting (λ_{max}^{Fl}= 814 nm) terrylenediimide (TDI) based donor-acceptor chromophore (TDI-TPA$_4$), exhibiting polarity-sensitive single-photon emission. By virtue of the charge transfer (CT) character, ensemble-level measurements revealed solvatochromism and NIR emission (ϕ_{Fl}= 26.2%), overcoming the energy gap law. To mimic the polarity conditions at the single-molecule level, TDI-TPA$_4$ was immobilized in polystyrene (PS; low polar) and poly(vinyl alcohol) (PVA; high polar) matrices, which enables tuning of the energy levels of the locally excited state and charge-separated (CS) state. Minimal blinking and prolonged survival time of the TDI-TPA$_4$ molecule in the PS matrix, in contrast to the PVA matrix, possibly confirms the implication of the energy gap law and polarity sensitivity of TDI-TPA$_4$. The existence of the CT state in nonpolar and CS state in polar solvents was confirmed by transient absorption measurements in the femtosecond regime. The current work sheds light on the design principle for NIR single-photon emitting organic chromophores for deep tissue imaging and probing the nanoscale heterogeneity.



References:

1. M. R. Wasielewski et. al. *Nat. Rev. Chem.*, **2020**, 4, 490.
2. F. Fuchs et. al. *Nat. Commun.*, **2015**, 6, 7578.
3. M. Musavinezhad et. al. *J. Phys. Chem. B*, **2023**, 127, 5353.
4. S. J. Jang et. al. *J. Chem. Phys.*, **2021**, 155, 164106.
5. Y.C. Wei et. al. *Nat. Photonics*, **2020**, 14, 570.

P 145

Strategic design of tryptophan-aspartic acid containing peptide inhibitors using coronin 1 as a template: Inhibition of fusion by modulating membrane organisation and dynamics

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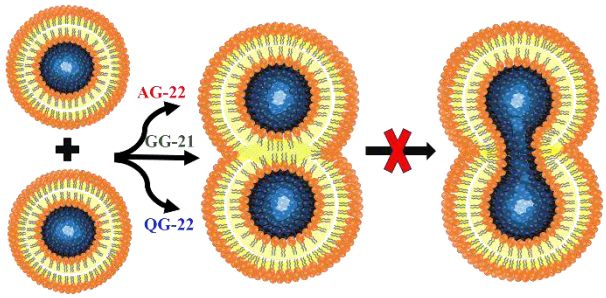
Enveloped viruses enter the host cell by fusing at the cell membrane or by entering the cell by endocytosis and fusing at the endosome. Conventional inhibitors target the viral fusion protein to inactivate it for inducing fusion. These target-specific vis-à-vis virus-specific inhibitors fail to display their inhibitory efficacy against emerging and remerging viral infections. This necessitates the need to develop broad-spectrum entry inhibitors that will be effective against a broad spectrum of viruses. The proposed fusion inhibitors modify the physical characteristics of the viral membrane in such a way that the membrane would be less susceptible to fusion. Earlier it was shown that TG-23, a tryptophan-aspartate-containing peptide from coronin 1, a phagosomal protein, demonstrated inhibition of fusion between small unilamellar vesicles (SUVs) by modulating the membrane physical properties. However, its inhibitory efficacy reduces with increasing concentration of membrane cholesterol. The present work aims to develop fusion inhibitors whose efficacy would be unaltered in the presence of membrane cholesterol. We have designed a tryptophan-aspartic acid (WD)-containing peptide, GG-21, having similar hydrophobic profile to that of TG-23. Interestingly, GG-21 displays inhibitory efficacy by modulating membrane organization and dynamics in a wide variety of lipid compositions despite having a similar secondary structure and physical properties to TG-23. These results advocate that the secondary structure and physical properties of the peptide may not be sufficient to predict its inhibitory efficacy. We have further designed two WD-containing hydrophilic peptides, QG-22 and AG-22, from coronin 1, and evaluated their fusion inhibitory efficacies in the absence and presence of membrane cholesterol. Our results demonstrate that QG-22 and AG-22 inhibit membrane fusion irrespective of the concentration of membrane cholesterol. Our measurements of depth-dependent membrane organization and dynamics reveal that they impede fusion by enhancing acyl chain order. Overall, our results validate the hypothesis of designing fusion inhibitors by modulating the membrane's physical properties.



References:

1. Pandia S., et. al. *J. Phys. Chem. B.* **2024**, *128*, 4986.
2. Pandia S., et. al. *Eur Phys J Spec Top*, 2024.
3. Chakraborty, H., et. al. *Biophys. J.* **2012**, *102*, 2751.
4. Pattnaik, G. P., et. al. *Biochim. Biophys. Acta Biomembr*. **2019**, *1861*, 183056.

P 146

Supramolecular Peptide Hydrogels with Diverse Functionalities for Directing Cellular Behavior

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Supramolecular hydrogels are emerging as advanced functional materials fabricated via classical molecular self-assembly strategy. Particularly, peptide-based hydrogels received exclusive attention due to their inherent biocompatibility and biodegradability. The well-defined fibrillar structures of these hydrogels provide an excellent three-dimensional environment for cellular growth and proliferation. In our work, we attempted to design suitable peptide scaffolds to effectively mimic the structural and functional aspects of the native extracellular matrix (ECM). Herein, we have employed a co-assembly approach to fabricate composite nanostructures mimicking the ECM by using pathway-dependent self-assembly. Our study includes co-assembly of different ratios of a gelator and a surfactant to fabricate diverse hydrogels. The gelation was triggered by utilizing distinct self-assembling pathways that resulted in differential fiber morphology as well as variable stiffness of the fabricated hydrogels which further proved the diversity of the hydrogels. Additionally, the secondary structures of the hydrogels were characterized by using CD, fluorescence, and FTIR spectroscopy. The designed hydrogels demonstrated enhanced cellular proliferation as assessed by confocal microscopy. Thus, these diverse supramolecular structures play a major role in governing cellular behavior in the co-assembled system. Such observation led us to further envisage that the differential self-assembly pathways would be a superior strategy and could eliminate the synthetic challenges associated with developing new materials. In this direction, we further explored metal-ligand coordination by employing cations of variable valency to trigger gelation within a pentapeptide derived from one of the major ECM proteins, i.e., N-cadherin, which mediates cell-cell adhesion, migration, and differentiation. Interestingly, the induction of hydrogels via the incorporation of cations at different concentrations followed the Hofmeister series. This was further confirmed by CD and fluorescence spectroscopy. The hydrogels showed tuneable mechanical stiffness which governed their cellular behavior. These highly tunable multi-component hydrogels attained from diverse designing approaches can be crucial in expanding their potential in various biomedical applications.

References:

1. S. Mohanty et. al. *Biomacromolecules* **2024**, *25*, 3271.
2. H. Kaur et. al. *Appl. Mater. Interfaces* **2020**, *12*, 52445.
3. H. Kaur et. al. *J. Mater. Chem. B* **2021**, *9*, 5898.
4. V.K. Pal et. al. *Macromol. Biosci.* **2022**, *22*, 2100462.

P 147

Role of Associated Water Dynamics on Protein Stability and Activity in Crowded Milieu

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Macromolecular crowding bridges *in vivo* and *in vitro* studies by simulating cellular complexities such as high viscosity and limited space while maintaining the experimental feasibility. Over the last two decades, the impact of macromolecular crowding on protein stability and activity has been a significant topic of study and discussion, though still lacking a thorough mechanistic understanding. This article investigates the role of associated water dynamics on protein stability and activity within crowded environments, using bromelain and Ficoll-70 as the model systems. Traditional crowding theory primarily attributes protein stability to entropic effects (excluded volume) and enthalpic interactions. However, our recent findings suggest that associated water structure modulation plays a crucial role in a crowded environment. In this report, we strengthen the conclusion of our previous study, i.e., rigid-associated water stabilizes proteins via entropy and destabilizes them via enthalpy, while flexible water has the opposite effect.[1] In the process, we addressed previous shortcomings with a systematic concentration-dependent study using a single-domain protein and component analysis of solvation dynamics. More importantly, we analyze bromelain's hydrolytic activity using the Michaelis–Menten model to understand kinetic parameters like maximum velocity (V_{max}) achieved by the system and the Michaelis–Menten coefficient (K_M).[2] Results indicate that microviscosity (not the bulk viscosity) controls the enzyme–substrate (*ES*) complex formation, where an increase in the microviscosity makes the ES complex formation less favorable. On the other hand, flexible associated water dynamics were found to favor the rate of product formation significantly from the ES complex, while rigid associated water hinders it.[2] This study improves our understanding of protein stability and activity in crowded environments, highlighting the critical role of associated water dynamics.

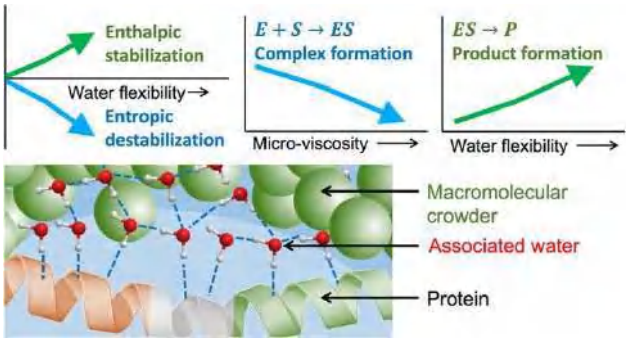

Figure 1: A flexible associated water stabilizes protein by enthalpy and destabilizes through entropy, whereas rigid water has the opposite effect. Micro-viscosity of the medium controls the formation of the enzyme-substrate complex; however, the modulation of associated water controls the product formation from the enzyme-substrate complex, where the flexibility of associated water favors the process.

References:

1. Das et al. *J. Phys. Chem. B* **2023,** *127***,** 3151.
2. Khan et al. *J. Phys. Chem. B* **2024**, *Just published*.

P 148

Mg doping induced modulation of ultrafast exciton dynamics in $FAPbBr_3$ nanocrystals

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Introducing dopants into perovskite nanocrystals has proven to be an effective method for modifying their optical and electronic properties for advanced optoelectronic applications. [1] This study explores the synthesis, characterization, and photophysical properties of magnesium (Mg)-doped $FAPbBr_3$ perovskite nanocrystals. By embedding Mg ions into the $FAPbBr_3$ lattice, we aim to boost photoluminescence efficiency through radiative decay engineering. Morphological analysis via PXRD, XPS, and FETEM reveals that doping causes lattice contraction (from 12 nm to 10 nm), resulting in increased confinement. Mg doping (4.8%) enhances photoluminescence intensity and quantum yield (increasing from 60±3% to 75±3%), along with a higher radiative recombination rate (2.35 times that of undoped nanocrystals). In femtosecond transient absorption experiments, excitation at a wavelength (λ_{ex} = 400 nm) much higher than the nanocrystals' band gap (520 nm) generates hot excitons. [2] The hot-carrier cooling time in undoped nanocrystals is 1094 ± 45 fs, while in the doped system, it is significantly faster (710 ± 26 fs) due to the enhanced coupling of conduction band states with the dopant states (Figure 1). The reduction of trap states and increased trapping-detrapping rate with doping contribute to a faster ground-state bleaching (GSB) recovery. [3] These results offer new insights into doping strategies for perovskite nanocrystals and pave the way for developing high-performance optoelectronic materials.

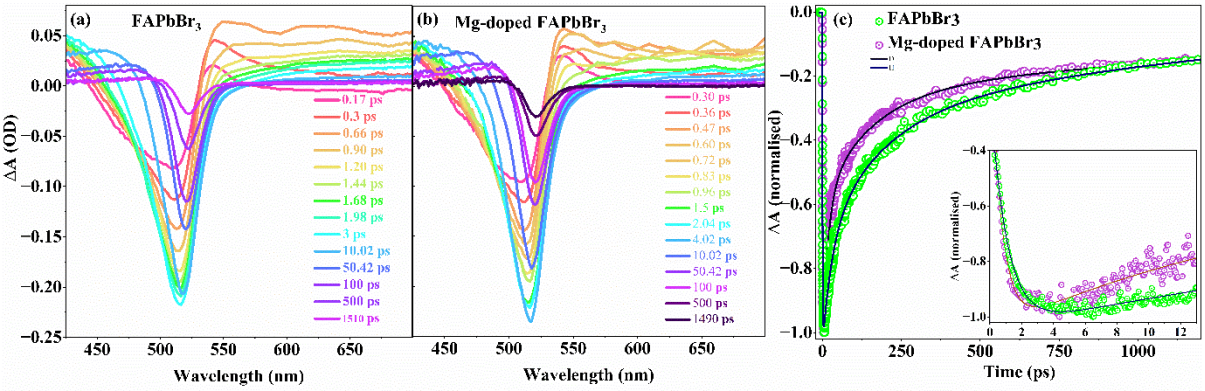

Figure 1. TAS of (a) $FAPbBr_3$ and (b) Mg-doped $FAPbBr_3$. (c) Comparison of GSB recovery kinetics of doped and undoped $FAPbBr_3$, inset depicts the zoomed shorter timescale.

References

1. C. H. A. Li et al. *Chem. Mater.*, **2019**, *31*, 6003.
2. P. K. Singha et al. *J. Chem. Phys.*, **2024**, *161*.
3. S. Das et al. *J. Phys. Chem. Lett.*, **2020**, *11*, 1178.

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An overview of the measurement of elastic moduli of RBCs using microfluidics

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Measurements of the mechanical properties of red blood cells (RBC) are very crucial because changes in these properties can be closely associated with diseases. RBCs are very deformable compared to most other cells in the human body, with their Young's modulus ranging between 2 and 8 kPa [1] [2]. When RBCs become rigid, they can block blood flow in capillaries, which may result in excessive destruction of these cells and this may result in the failure of organs. RBCs lose their flexibility after extended storage [3]. Changes in RBC deformability can be indicative of various diseases, such as sickle cell disease [4], peripheral vascular disease [5], diabetes [6], and malaria [7]. Consequently, measuring the elastic properties of RBCs could be a useful diagnostic tool for detecting and understanding these conditions.

There are several conventional methods by which the deformability of RBC populations can be measured at the level of single cells, such as micropipette aspirations [8], atomic force microscopy (AFM) [9], membrane fluctuations [10], optical tweezers [11], optical stretcher [12], and magnetic bead-based rheology [13], etc. These traditional methods require expensive, specialized equipment and are often limited by low throughput and time-consuming data analysis. To overcome these challenges, researchers have developed microfluidic techniques that improve throughput by utilizing either physical constriction [14] [15] or the hydrodynamic shear stress from a channel [16], cross-sections [17], or T-junctions [18]. For evaluating RBC deformability, typically one monitors real-time shape changes as RBCs navigate constrictions, rather than directly measuring elastic constants. These methods often require high-speed cameras (2,000–4,000 fps) to capture rapid deformations or measure the pressure required for passing through narrow constrictions [19]. Zhu and others [20] developed a numerical approach for microfluidic devices inspired by Rutherford scattering, utilizing a semi-cylindrical obstacle in a funnel to sort spherical capsules based on deformability. Later another group [21] validated this approach, showing its effectiveness in sorting artificial vesicles by size and deformability and introducing a critical capillary number for sorting regimes.

A recent study from our research group [2] modified the design reported by Zhu and others to obtain Young's modulus of healthy and artificially stiffened RBCs. This device has a single channel that opens into a funnel. There is a semi-circular obstacle where the funnel begins. As RBCs move past this obstacle, stiffer RBCs deviate more from their original path compared to softer RBCs. As a result, one needs to monitor only the path taken by RBCs, and not their shape change, which is done using a regular microscope camera at 25–30 fps. The authors also generate a one-time calibration curve by correlating the deflection trajectories of RBCs with Young's modulus values obtained from independent AFM measurements. This calibration curve allows obtaining Young's modulus of unknown RBC populations from only microfluidics measurements.

References:

1.Fay, M. E. et.al. 2016, Proc Natl Acad Sci USA 113, 1987–1992
2.Kumari, S. et.al. 2024, Cell Reports Physical Science 5, 102052
3.Park, H. et.al. 2016, Sci. Rep. 6, 34257
4.Barabino, G. A. et.al., 2010, Annu. Rev. Biomed. Eng. 12, 345–367
5.Reid, H. L. et.al., 1976, Lancet 1, 666–668
6.Tsukada, K. et.al., 2001, Microvasc. Res. 61, 231–239
7.Park, Y. et.al., 2008, Proc Natl Acad Sci USA 105, 13730–13735

P 150

Impact of post-translational modifications on LLPS in intrinsically disordered proteins

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Membraneless organelles like stress granules, p bodies, have drawn the interest of scientists in recent times. These membraneless organelles are referred to as biomolecular condensates which are mostly formed via liquid-liquid phase separation of proteins and RNAs. Liquid-liquid phase separation (Llps) plays a vital role cellular functioning like sequestration, signalling, cellular interactions. In addition to their contribution in the normal functioning of cells, they are also associated with various pathological conditions like Alzheimer's, Parkinson's Disease.

In previous studies, it has been seen that neurodegenerative diseases are the outcome of protein aggregation. But in recent studies it has been determined that they pass through Liquid-liquid phase separated state prior to aggregation. There are several factors that aggravates protein aggregation like post translational modifications. Even though they are considered to be important, their impact on phase separation remains elusive. Alpha-synuclein plays an important role in Parkinson's disease and it has been observed that they do undergo PTMs like sumoylation, phosphorylation which can either enhance [1] or inhibit their aggregation. In addition to this, alpha-synuclein has sumoylation sites and it is found in modified form in aggregates. Moreover, sumoylation targets proteins towards nucleus thereby enhancing its nuclear accumulation [2]. Although alpha-synuclein is present at the nerve terminals, biochemical analysis has identified it in the nucleus of the neurons. Intriguingly, both alpha synuclein [3] and SUMO1[4] exhibit Llps under different conditions but the nature of their interaction is unknown. Thus, the main aim of the study is to determine the effect of PTMs on alpha-synuclein, which is the major aggregating protein in Parkinson's Disease.

References:

1. Abeywardana et.al *Biochemistry* **2015**, 54, 959.
2. Pinho et. al *Hum. Mol. Genet***. 2019**, 28, 31.
3. Ray, S. et al. *Nat. Chem***. 2020,** 12, 705.
4. Arora et. al *J. Phys. Chem. Lett*. **2023**, 14, 9060.

P 151

Understanding mechano-regulation by myomerger in cell -cell fusion

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Myomerger is a small membrane protein – a fusogen known to impart positive curvature to the outer leaflet of lipid bilayers of liposomes[1]. It is crucial for cell-cell fusion during myogenesis[2] but there is little direct evidence of mechano-regulatory mechanisms processes[3] operational in cells. Local curvature induction or other membrane mechano-regulation by myomerger remain poorly explored for differentiating myoblasts. Although myomerger is believed to stress the plasma membrane post hemi-fusion, for fusion pore formation, recent work from the lab has shown that its surface levels is correlated to the surface tension even at early timepoints such that when the surface tension decreases as myomerger surface levels rise[4]. Here, we first present data to demonstrate how myomerger not only correlated but directly affected the cell surface tension. Further, imaging relative height of the basal membrane and myomerger clustering in same cells revealed that the shallow membrane remodelling by myomerger clusters are mostly exvaginating bulges. Other agents that similarly affect the membrane curvature were tested to understand if curvature remodelling affects surface tension. Connecting the mechanical changes imparted by myomerger to cell-cell fusion, we report that as the probability of hemi-fusion increases, a clear tension difference develops between the hemi-fused pair of cells underscoring how myomerger's direct impact of surface tension can tune fusion probability.

Keywords: Myomerger, Myogenesis, hemi-fusion,mechano-regulation, surface tension.

References:

1. Golani G, Leikina E, Melikov K, et. al. *Nat Commun.* **2021**, *12(1*
2. Quinn ME, Goh Q, Kurosaka M, et. al. *Nat. Commun*. **2017**, 8, 15665
3. Mukhopadhyay U, Mandal T, Chakraborty M, Sinha B. *ACS Omega*. **2024**, *9(20),* 21780.
4. Chakraborty M, Sivan A, Biswas A, Sinha B. *Front Physiol*. **2022**, *13*

P 152

Generation of autonomous spinning and gyrating spherical micro-vesicles

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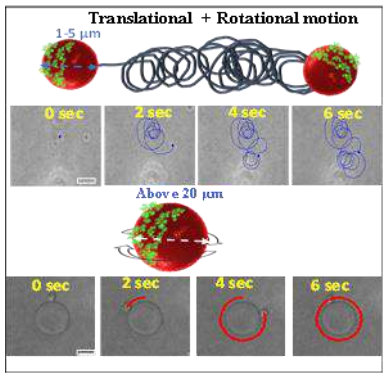
Looking into chiral active particles and rotors is an intriguing and quickly expanding field. Active particles have the potential to be useful tools in the treatment of complex in vivo issues like tissue absorption, wound healing, drug delivery, and cell sensing. In addition to eliminating drawbacks like systemic toxicity and reliance on passive diffusion for transport, active motion delivers cargo more quickly than passive diffusion. Due to the field's rapid development, an increasing number of review articles focusing on the design and construction of different active micro/nano rotors are being published. "Micro/nanomotors" (Gao and Wang, 2014), "micro/nano swimmers" (T. Li et al., 2016), "micro/nano engines" (Sanchez et al., 2011), "micro/nanomachines" (Wang and Putera, 2018), "micro/nano propellers" (Ghost and Fischer, 2009), "micro/nano pumps" (Wong et al., 2016), "micro/nano rockets" (J. Li et al., 2016a), etc. are some of the terms used in the literature to describe these small-scale rotors. In order to power these, scientists either use Janus particles as engines catalyzed by fuels like H_2O_2 or they use symmetry breaking of the chassis material, such as by creating star- or dumble-shaped rotors. However, finding practical propulsion methods for these micro/nanorobotic devices that can be used for biomedical applications is still very difficult. Their primary constraints are their size, poor biocompatibility of the fuels for active motion and the materials used for fabrication, and their mobility in biological fluids. Enzymes are excellent candidates for use as catalysts in biomedical applications because of their high turnover numbers, excellent selectivity in physiological settings, and biocompatibility. Additionally, research revealed that every functioning enzyme can produce enough mechanical force to move itself during catalysis. Here, we create chiral active particles with isotropic symmetry that are coated with enzymes. For the chassis of these active particles, we employed lipid vesicles. Internal energy is created through the transformation of chemical energy. Translating, rotating, or both, these active particles can propel themselves by using their internal energy. Vesicles ranging in size from 1 to 5 µm exhibit both translational and rotational motion, while those larger than 20 µm primarily manifest spinning behaviour.



Keywords: 1; Chiral active particles 2; autonomous rotors 3; Lipid vesicles

Fig. 1 Active enzyme coated GUVs. Upper panel shows the translation and rotational motion of 2 µm sized vesicles. Lower panel showing spinning of 20 µm sized vesicles after catalyzing enzymatic reaction (scale bar: 10µm)

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Understanding stem cell differentiation into neurons using fluorescence microscopy

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Neural differentiation from mouse embryonic stem cells is an excellent model to elucidate key mechanisms in neurogenesis, study disease mechanisms, and screen new drug compounds. For the differentiation of mouse embryonic stem cells, first, the hanging drop method is used for the formation of embryoid bodies; followed by differentiation into neurons using the neuronal differentiation media. The metabolic changes during the differentiation of stem cells into neurons are investigated by measuring the autofluorescence of coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). The ratio of FAD/ NADH provides insight into the metabolic state of a cell and is referred to as the redox ratio. The fluorescence images of NADH and FAD are acquired before induction of differentiation and on the subsequent days using confocal microscopy and two-photon microscopy.

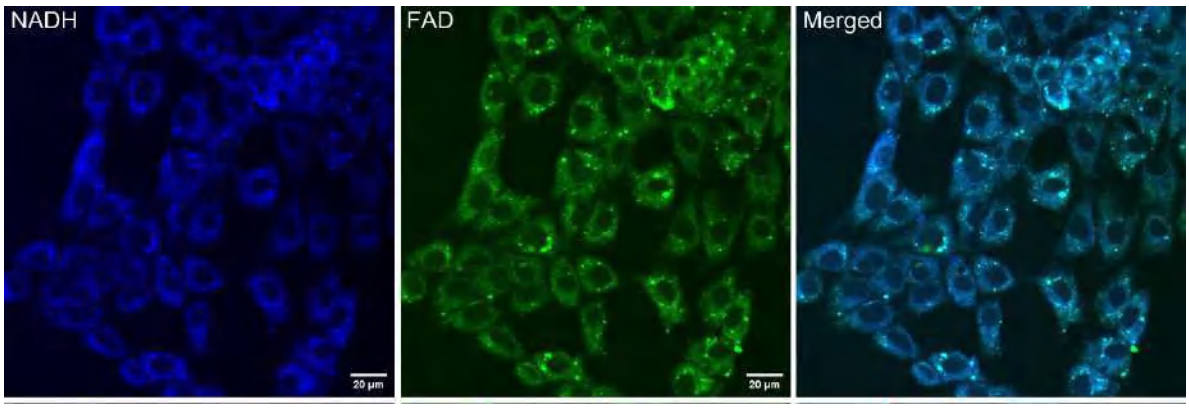

Fig 1: NADH, FAD fluorescence images captured using two photon microscopy in mouse embryonic stem cells.

References:

1. O. I. Kolenc et al. Antioxidants & Redox Signaling, 2019, 30(6):875-889.
2. R. J. Burgess et al., J Intern Med, 2014, 276(1),12–24.

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Detecting labile heme and ferroptosis through 'turn-on' fluorescence and lipid droplet localization post Fe(II) sensing

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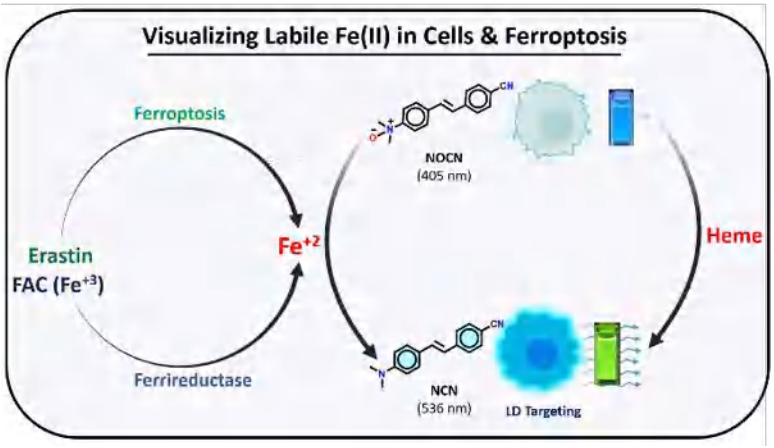
Iron, a crucial biologically active ion, is essential for metabolic processes in living organisms and plays a vital role in biological functions. The World Health Organization (WHO) has set the limit for iron in drinking water at 5.3 µM. However, many groundwater sources are contaminated with Fe(II), and imbalances in iron levels can lead to various diseases. In this study, We have designed two easily accessible, water soluble *N*-oxide based probes (NOPy and NOCN) for the selective detection of Fe(II) in presence of other interfering ion, with clearcolour demarcation. Both the probes exhibit large stoke shifts with an enhancement in fluorescence intensity post Fe(II) detection, with NOCN localizing inside lipid droplets in live*COS-7* cells. NOPy and NOCN are biocompatible and can detect Fe(II) at concentration as lowas 35nM and 42nM respectively. Additionally, NOCN is capable of detecting labile heme inside live cells, generated by aminolevulinic acid and ferric ammonium citrate. NOCN can also be used to visualize ferroptosis process, which is an iron-based apoptosis utilized in cancertherapy.



Keywords: Photophysical Chemistry, Turn-on emission, Bioimaging in live cells, Analyte Detection, Labile Heme, Ferroptosis

Reference:

1. Yogesh Dubey et. al., *Journal of Materials Chemistry B*, **2024**, *12*(20), 4962-4974.
2. T. Hirayama et. al., *Journal of clinical biochemistry and nutrition*, **2017**, *60*(1), 39-48
3. Yogesh Dubey et.al., *Organic and Biomolecular Chemistry*, **2024.**

P 155

Red-edge effects in proteins rich in charged amino acids: A Computational Study

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Protein Charge Transfer Spectra (ProCharTS) is a new and unexplored label-free UV-Vis spectral window suitable for probing proteins rich in charged amino acid content. A convolution of charge transfer transitions within charged amino acid clusters contributes to the ProCharTS spectral window. Previously, our group has proposed a computational framework to investigate the dynamics and optical properties of charged amino acid chromophores within the alpha-3c protein. The studies employed classical atomistic molecular dynamics (MD) simulations to extract the active chromophores possibly contributing to the optical charge transfer transitions. Time-dependent density functional theory was employed to analyse the spectra of a statistical ensemble of charged amino acid chromophores to construct the ProCharTS profile of the alpha-3c protein. [1]

The heterogeneities in charged amino acid clusters lead to red-edge effects. Here, I present a mathematical framework to model REES in multichromophoric charged amino acid clusters considering a two-state model.

References

1. Mandal, I., Manna, S., & Venkatramani, R. *Journal of Physical Chemistry B* **2019** 123(51), 10967–10979.
2. J. Doe, F-Q. Chen and B. White, *J. Chem. Phys.* **2026**, *193*, 34924.

P 156

Elucidating the Role of Chlorophyll-*a* in Cytochrome b_6f Complex

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Cytochrome b_6f (cyt b_6f) complex plays a key role in the electron transfer process from photosystem II (PSII) to photosystem I (PSI) by catalyzing the oxidation of plastoquinol and reduction of plastocyanin or cytochrome c_6 in oxygenic photosynthetic systems.[1,2] Electron transfer through cyt b_6f is coupled to proton transfer, this contributes to the transmembrane proton gradient (ΔpH) utilised for ATP synthesis and photosynthetic regulation. A curious feature of the cyt b_6f complex is the presence of a chlorophyll (chl) *a* molecule which does not directly participate in electron or proton transfer and is of unknown function. Chl molecules are potentially dangerous since they can transfer excitation energy from their excited triplet state to the ground state oxygen, forming singlet oxygen a potent reactive oxygen species which can damage the complex. It has been proposed that the local protein structure may act to quench the singlet excited state lifetime of the chl molecule,[3] although the exact mechanism is not clear. Herein using femtosecond transient absorption spectroscopy, we probed a mutant of cyt b_6f complex (L108W) in the cyanobacterium synechocystis and compared to its wild type. Our primary observations suggest that replacing a leucine, at 4.2 Å distance to the chl, with an aromatic amino acid (tryptophan), quenches the singlet excited state lifetime of the chl molecule from 160 ps to 90 ps. We will discuss our results in the context of the effect of local environment on the chl lifetime in cyt b_6f complex.

References:

1. W. A. Cramer et. al. Annu. rev. plant physiol. plant mol. Boil, **1996**, 47, 477.
2. L. A. Malone et. al. *BBA-Bioenergetics,* **2021**, 1862, 148380.
3. Y. Jiusheng et. al. *Biochem,* **2008**, 47, 3654.

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P 157

Effect of system-bath interaction to control spin-vibronic coupling in intersystem Crossing reaction of a di-platinum complex

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Insights into the fundamental mechanism of intersystem crossing lie at the heart of strategizing design principles to control structure-function paradigm in vitro. In this work, using transient absorption spectroscopic measurements, we have experimentally shown how crystal packing and ligand sphere affect the rate of an intersystem crossing reaction by changing the strength of spin-vibronic coupling in a di-platinum complex. We have proposed a model to describe our observation where an intermediate triplet plays a crucial role to modulate the strength of intersystem coupling via modulation the strength of spin-vibronic coupling in this complex. We found that depending on whether the energy of the intermediate state is tuned in and out of resonance with the singlet state, spin-vibronic coupling and thus the rate of intersystem crossing is strongly affected.

P 158

Conformational Heterogeneity in Intramolecular Singlet Fission

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Singlet fission (SF) is a process of multiexciton generation in which a molecule in singlet excited state shares its excitation energy with a neighbouring electronically coupled chromophore to generate two excited molecules in their triplet state, via a correlated triplet pair intermediate state.[1] This whole event is guided by optimal electronic coupling between two chromophores as well as a thermodynamic requirement of E (S_1) ≥ 2E (T_1). However, triplets can also be generated via ISC, although in much slower timescale. So, to harvest the triplets, it's very important to know the exact origin of the triplets. Herein, we probe into two covalently linked TIPS-pentacene dimers, one of which has been substituted with chlorine atoms to enhance the rate of ISC, and we wanted to see whether the enhanced rate of ISC can compete with the SF process. For that we took the help of both steady state and time resolved spectroscopy to probe the dynamics of these dimers. From the transient absorption spectroscopy, we could see a slower SF dynamic in case of the chlorinated dimer. But, apart from this we got a very interesting result of excimer formation in the chlorinated dimer due to conformational heterogeneity. For the SF vs ISC dynamics, we are taking the help of trEPR which actually shows a different dynamic for both the dimers.

References

1. Miyata, K.; Conrad-Burton, F.S.; Geyer, F.L.; Zhu, X.-Y.; *Chem. Rev.*, **2019**, 119, 4261
2. Smith, M.B.; Michl, J.; *Chem. Rev.*, **2010**, 110, 6891.
3. Feng, X., Casanova, D., Krylov, A.I., *J. Phys. Chem. C*, **2016**, 120, 19070−19077
4. Busby, E., Xia, J., Wu, Q., Low, J.Z., Song, R., Miller, J.R., Zhu, X-Y., Campos, L.M.; Sfeir, M.Y., *Nature Materials*, **2015, 14**, 426–433

P 159

Copper Chelators as Novel Theranostic Agent for Generating ROS in Prostate Cancer Inducing Fenton Reaction

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Theranostic agents, which combine therapeutic and diagnostic functionalities, represent a significant advancement in cancer treatment, offering precise targeting and real-time monitoring. This dual capability enhances treatment efficacy and provides valuable insights into disease progression and response to therapy, addressing the limitations of traditional approaches that often suffer from non-specificity and significant off-target toxicities.

Prostate cancer (PC), a major cause of cancer-related deaths, frequently exhibits resistance to conventional therapies, underscoring the need for innovative treatment strategies. PC cells are characterized by an inherent misregulation of metal ions, particularly copper (Cu), and elevated levels of hydrogen peroxide (H_2O_2)[1]. This dysregulation facilitates the Fenton reaction, which generates reactive oxygen species (ROS) and induces oxidative stress. We propose a novel theranostic agent which integrates copper chelation for inducing ROS generation and a probe that facilitates intracellular tracking, enabling real-time visualization and monitoring of the drug's distribution and efficacy within living systems as diagnostic agent. Additionally, it incorporates a prostate-specific membrane antigen (PSMA)[2] targeting unit ensures selective delivery to prostate cancer cells.

By combining therapeutic and diagnostic capabilities, this theranostic approach will enhance both treatment efficacy and diagnostic accuracy, may provide a optimistic solution for drug delivery system that improves patient outcomes and facilitates more effective management of prostate cancer.

References:

1. Lovejoy, D. B. et al. *Inorg. Chem.* 2012, *51*, 5966.
2. Kularatne, S. A. et al. *Mol. Pharmaceutics* 2009, *6*(3), 780

P 160

A Fluorescent Peptide-Based Sensor for Imaging Signal-Mediating Lipids

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Phosphatidylinositol 3,4,5-trisphosphate (PIP3) is a phospholipid found in the inner leaflet of the cell membrane. It plays a key role in cell signalling by recruiting and activating proteins involved in cell growth, survival, and metabolism. Dysregulation of PIP3 levels is associated with diseases such as cancer, neurodegenerative diseases, and diabetes due to its direct role in the PI3K/AKT pathway, which controls cell proliferation and survival.[1,2] Detecting PIP3 is essential for elucidating its dynamics and regulation within living cells which can aid in the study of disease mechanisms and the development of therapeutic interventions. Our group has recently developed peptide-based sensors that can sense and track PIP3 within living system.[3] The sensors afford ~10-fold higher selectivity toward PIP3 over other structurally similar lipids which is sufficient to detect pathophysiological levels of PIP3. However, under physiological conditions the levels of PIP3 are around 100 times lower than that of other signal-mediating lipids necessitating probes with higher selectivity. Toward this goal, we are developing PIP3 sensors with improved selectivity. We have developed a novel, reversible, ratiometric fluorescent sensor for PIP3 which affords ~40-fold higher selectivity toward PIP3 over other biologically essential phospholipids. I will present the design, synthesis, characterization, and evaluation of the probe.

References:

1. Lemmon, M. A. *Nat. Rev. Mol. Cell Biol*. **2008**, *9(2)*,99.
2. Posor et al. *Nat. Rev. Mol. Cell Biol*. **2022**, *23(12)*, 797.
3. Kundu, R. et al. *JACS Au*. **2024**. *13;4(3)*,1004.

P 161

Efficient, cleaner and automated approach towards single-molecule TIRF assays for investigating DNA replication on flow-stretched DNA substrates.

Daniel Ramirez Montero, Vincent Kruit, Francisco Palmero Moya, **Saurabh Talele**, Nynke Dekker

Single-molecule TIRF microscopy on flow stretched DNA is a powerful technique to probe the dynamics of the DNA replication machinery in real time at high-throughput. One can visualize and track the motion of fluorescently labelled proteins onto the DNA substrate which is tethered at both ends on the surface of the flow cell. The primary limitation for using this technique is protein aggregation either onto the surface of the flow cell or onto the DNA and the inability to wash away the unbound proteins in the solution. Additionally, in context of data analysis, manual labelling of ~ 1000 DNAs may be required for one experiment which is a laborious process. Here we highlight two key advancements in this methodology enabling a clean and efficient measurement along with automated and user-friendly data analysis platform. We demonstrate a hybrid ensemble and single-molecule assay where first, we perform bulk biochemical reactions such as loading, or activation of various replication associated components using desthiobiotin functionalized DNAs and streptavidin coated magnetic beads. This allows us to perform sequence of reactions followed by washing steps to remove non-specifically interacting proteins and finally elute the DNA at high concentration using biotin. In our python-based data analysis pipeline, first, we a use segmentation-based approach that allows us to detect the DNA molecules and determine their length in an automated fashion. For every DNA segment, we quantify the tension and the lateral fluctuations in the DNA molecule to provide mechanical information. To localize fluorescently labelled proteins on the DNA, we have implemented a gradient based spot detection algorithm. Additionally, we use the LAP tracker to track the motion of proteins along the DNA. The analysis pipeline is packaged with a user-friendly GUI using Napari viewer for easy access and implementation

P 162

Bio-waste derived Mg-doped carbon dots: A Fluorescent Probe of Y^{3+} Metal ion and Bio Imaging Application

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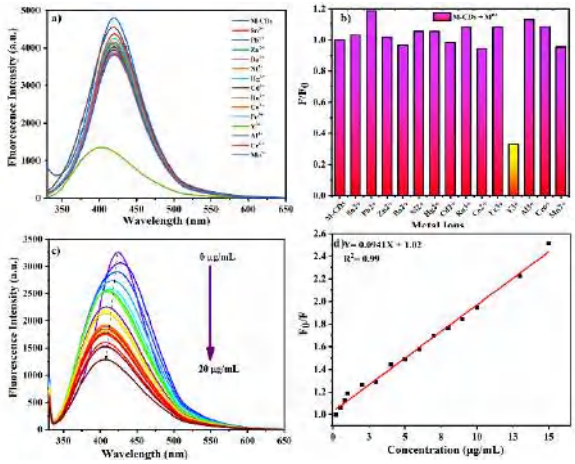
In present study, waste valorisation approach was utilizing for synthesis of Mg-doped carbon dots form cow dung using hydrothermal method. The Mg doped fluorescent carbon dots (M-CDs) having maximum emission at 420 nm upon 310 nm excitation with 20 % quantum yield. The M-CDs synthesis was confirmed using different analytical a characterization technique as well as stable in different pH as well as ionic strength solutions. The M-CDs was highly selective towards Y^{3+} ions with significant blue shift. The LOD of developed probe toward Y^{3+} was 0.019 µg/mL. The study indicates quenching of Y^{3+} was result of dynamic and IFE quenching effect [1] which was analysed by TCSPC (Time-Correlated Single Photon Count) and UV-Visible spectroscopy measurement. Further the interaction of CDs with Y^{3+} ion was investigated. The oxygen containing groups of CDs was responsible for Y^{3+}-CDs bonds [2-3]. The healthy growth of blood vessels in angiogenesis study demonstrated the cytotoxicity of CDs. further the CDs employed for MCF-7 breast cancer cell imaging [4]. The CDs were enable to interact with MCF-7 cell and brighten the fluorescence signal.



References:

1. Panpan Zhu et al. ACS Appl. Nano Mater **2023**, 6, 21194.
2. Xuelian Hu et al. Sen. Actuators B. Chem. **2021**, 338, 129809.
3. Mingxi Yang et al. Part. Part. Syst. Charact. **2019**, 36, 1800315
4. Cintya D'Angelis do E. S. Barbosa et al. Chem. Eur. J. **2015**, 21, 5055 – 5060

P 163

Post Synthetic Solvent Studies on Organic Inorganic Hybrid Perovskites

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Hybrid organic-inorganic perovskites (HOIPs) have been emerged as a superior class of materials for the optoelectronic applications due to their extremely high absorption coefficients, tunable bandgap and several other properties etc.[1,2] In this work, a bigger cation phenylethyl ammonium (PEA) has been used as A site cation to get a layered perovskite structure. The structural configuration is $(PEA)_2PbBr_4$ which is formed due to the interaction between the layered inorganic octahedra and the organic layers.[3,4] We have studied the behaviour of the $(PEA)_2PbBr_4$ in presence of different solvent environments (DMF, CLB, Hex, CAN, IPA etc). After addition of 5% of DMF with high Guttman Donor Number (DN) 26.6, the crystal gets completely disintegrated whereas after adding 22.5% Cyclohexane with 0 DN no. solvent, the crystal structure remains intact and stabilize the structure as well, shown in figure below. This will give a new insight in revealing the interaction and the long-term stability of these materials in their colloidal forms and will also help us to ponder over the interactions between the Pb complexes and the A site cations. [5,6]

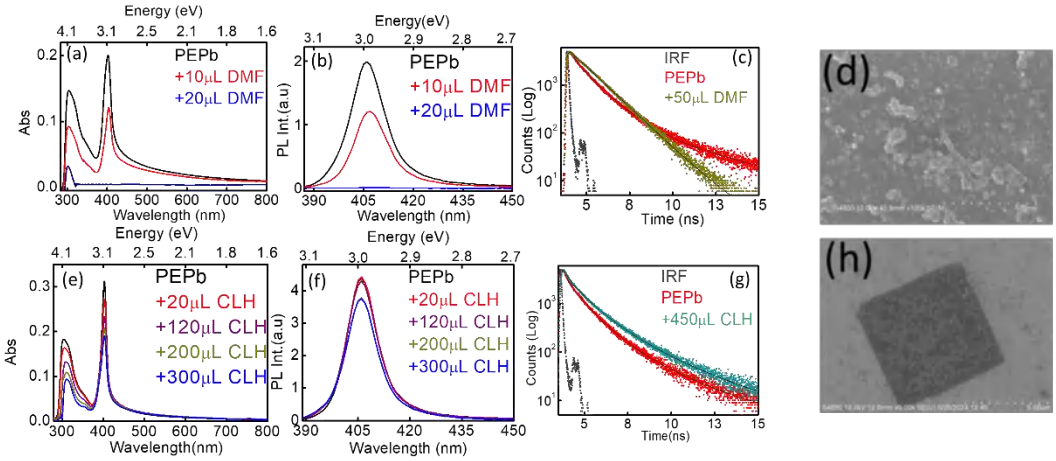

Figure 1: Uv-vis,PL and Lifetime after addition of DMF in a) ,b) and c) respectively, and in Cyclohexane in e),f), g) respectively. d) and h) is the corresponding FESEM images in these solvents

References:

1. Ke-zhao Du et al Inorg. Chem. 2017, 56, 9291.
2. Yu-Tao Li et al ACS Appl. Electron. Mater. 2022, 4, 547.
3. Dong Liang et al ACS Nano 2016, 10, 6897.
4. David B. Mitzi et al J. Chem. Soc., Dalton Trans., 2001, 1.
5. J. Clay Hamill, Jr. et al ACS Energy Lett. 2018, 3, 92.
6. Andrei S. Tutantsev et al J. Phys. Chem. C 2020, 124, 11117

P 164

The Role of Charge Transfer in CsPbBr3 Perovskite Nanocrystals for Cutting-Edge Optoelectronic Technologies

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The success of lead halide perovskite nanocrystals (P-NCs) based optoelectronic devices hinges on the swift and seamless interfacial charge transfer (CT), which dramatically boosts energy conversion efficiency. In this study, we thoroughly explore the CT process in $CsPbBr_3$ P-NCs when paired with ZnO and TiO_2 nanoparticles (NPs), which serve as excellent electron acceptors due to their optimal energy level alignment with the $CsPbBr_3$ P-NCs. By utilizing spectroscopic (steady state and time resolved) and electrical (current sensing atomic force microscopy) measurements, we observe a facile electron transfer (ET) from P-NCs to both these acceptors. Further, to highlight the crucial role of ET in the P-NC based photovoltaic devices, we have conducted simulations comparing the performance of three devices, FTO/$CsPbBr_3$/PEDOT:PSS/Au (control), FTO/ZnO/$CsPbBr_3$/PEDOT:PSS/Au (device with ZnO), FTO/TiO_2/$CsPbBr_3$/PEDOT:PSS/Au (device with TiO_2). We have seen a striking improvement in device performance (efficiency, current density, open circuit voltage and fill factor) with the incorporation of ZnO and TiO_2, surpassing the results from the control device. However, the ET rate, boost in electrical conductivity, and device performance all show marked improvements with TiO_2 over ZnO, thanks to TiO_2's energy levels being more favourably aligned with P-NCs. This study underscores the critical role of tuning interlayer charge transfer in optimizing the performance of photovoltaic devices.

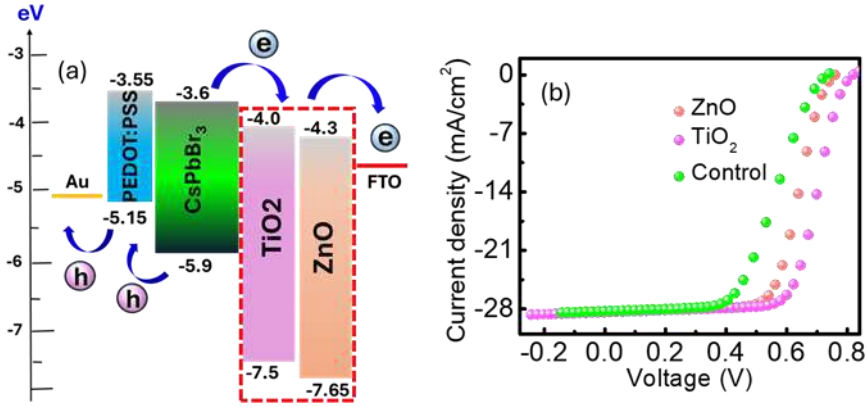

Figure: Energy band alignment of the photovoltaic device (a) and the corresponding current-voltage curve for control device, device with ZnO and TiO_2 (b).

References:

1. QA Akkerman et al. *Nature materials* **2018**, *17*, 394.
2. H Zhang et al. *Nature reviews chemistry* **2023**, *7*, 632.
3. NG park et al. *Nature energy* **2016**, *1*, 16152

P 165

Computational Simulations of Protein Charge Transfer Spectra

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Recent experimental studies have revealed that monomeric proteins rich in charged amino acids exhibit broad absorption profiles spanning the entire UV-Visible spectral band (200-800 nm). Computational investigations attribute this phenomenon to photoinduced charge transfer (CT) transitions involving charged amino acids, such as lysine (Lys), glutamate (Glu), Arginine (Arg), Aspartate (Asp), and protonated Histidines (His) [**1,2**]. This unusual absorption arising from charged amino acid rich proteins is termed Protein Charge Transfer Spectra (ProCharTS). Here, we describe a computational strategy to simulate ProCharTS profiles of entire proteins, combining classical molecular dynamics sampling and time-dependent density functional theory-based spectra calculations. We demonstrate an application of the strategy by investigation of the ProCharTS sensitivity to post-translational modifications (PTMs), specifically acetylation, which quenches the charge of Lys residues in two helical model proteins α3C (no aromatic amino acids) and α3W (wherein the CYS34 residue in alpha3C is mutated to tryptophan). We show that the statistical distribution of charged amino acid clusters changes with the degree of acetylation, resulting in a corresponding decrease in simulated ProCharTS profiles. We will discuss the potential of combining experimental measurements and computational analysis to quantify PTMs in a label-free scheme and establish the resolution and sensitivity limits of this novel approach.

References:

1. Prasad, S., Mandal, I., Singh, S., Paul, A., Mandal, B., Venkatramani, R., & Swaminathan, R. (2017). Near UV-Visible electronic absorption originating from charged amino acids in a monomeric protein. *Chemical science*, *8*(8), 5416-5433.
2. Mandal, I., Paul, S., & Venkatramani, R. (2018). Optical backbone-sidechain charge transfer transitions in proteins sensitive to secondary structure and modifications. Faraday discussions, 207, 115-135.

P 166

Novel Role of Sphingolipids in the Organization of the Actin Cytoskeleton

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Sphingolipids are essential components of eukaryotic cell membranes and constitute 10-20% of total membrane lipids. They are recognized as dynamic regulators of a multitude of cellular processes [1]. Various pathological conditions such as Niemann-Pick disease, Gaucher's disease, Tay-Sachs disease are associated with abnormalities in sphingolipid metabolism [2]. Sphingolipids are distributed heterogeneously in the plasma membrane, majorly contributing to the formation of laterally segregated membrane microdomains (sometimes referred as "rafts") along with cholesterol [3]. These microdomains are believed to serve crucial functions in regulating lateral diffusion of membrane lipids and proteins, which dictates diverse cellular signaling events [4]. Analyzing the spatiotemporal resolution of these domains remains very challenging. An interesting source of heterogeneity in cell membranes exists due to the dynamic confinement created by the underlying actin cytoskeleton [5]. The mobility of membrane proteins such as GPCRs and many microdomains-associated proteins is often regulated by the dynamic actin cytoskeletal meshwork [6,7]. This has given rise to an emerging model regarding the synergistic existence of membrane microdomains and confinement due to the actin cytoskeleton. In this context, we previously explored the role of chronic cholesterol depletion in regulating the organization of actin cytoskeleton [8]. Due to the close interaction of cholesterol and sphingolipids in membrane microdomains, we examined whether sphingolipids play any role in regulating actin organization. In this work, we explored the effect of sphingolipid depletion on cellular actin cytoskeleton organization by using a quantitative confocal microscopy based approach previously developed by us [9,10]. For this, we inhibited cellular biosynthesis of sphingolipids using metabolic inhibitors such as fumonisin B_1 (FB_1) and myriocin in CHO-K1 cells and explored the actin cytoskeleton organization using the assay. Our results show that there was a significant increase in actin polymerization upon inhibition of sphingolipid biosynthesis using FB_1 and myriocin. Notably, the process was not reversed upon sphingolipid replenishment, which could be due to the non-specific effects of the inhibitors. To test this, and to overcome any effect of accumulation of metabolic intermediates, we monitored the organization of the actin cytoskeleton in LY-B cells [11], which are CHO-K1 cells with sphingolipid-auxotrophic mutants defective in the first committed step in sphingolipid biosynthesis. Interestingly, we observed that the increase in F-actin content was reversible upon sphingolipid replenishment in LY-B cells. To the best of our knowledge, our results constitute the first report on the role of sphingolipids in actin polymerization. These results assume relevance in the context of remodeling of the actin cytoskeleton in pathological conditions with altered sphingolipid content.

References:

1. Holthuis JC et al. *Physiol. Rev*. **2001**, *81*, 1689.
2. Rabbo MA et al. *Lipids Health Dis*. **2021**, 20, 44.
3. Brown RE, *J. Cell Sci.* **1998**, *111*, 1.
4. Goñi FM, *Chem. Phys. Lipids* **2019**, *218*, 34.
5. Fujiwara TK et al. *Mol. Biol. Cell.* **2016**, *27*, 1101.
6. Shrivastava S. et al. *Biophys. J*. 2020, *118*, 944.
7. Ganguly, S. et al. *Biophys. J*. 2010, *99*, 1397.
8. Sarkar P et al. *J. Lipid Res*. **2022**, *63*, 100206.
9. Ganguly S et al. *Biochim. Biophys. Acta* **2011**, *1808*, 1921.
10. Sahu SS et al. *Methods* **2024**, *230*, 44.
11. Jafurulla M et al. *Biochim. Biophys. Acta* **2017**, *1859*, 598.

P 167

Structure-function mapping of Salmonella and host cytoskeleton interactions.

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The cytoskeletal network of a cell is key to maintenance of cell shape, uptake and transport of material, cell migration and cell division. The central nature of the cytoskeletal network makes it a key target for pathogens that hijack it for cellular entry, pathogenic replication and spread. Salmonella is a model example of an enteropathogenic bacterium that hijacks cytoskeletal remodeling and transport processes for cellular invasion. Salmonella achieves this feat by secreting pathogenic factors that interact with and modulate the function of cytoskeleton and its associated proteins. These pathogenic factors exhibit "molecular mimicry" wherein they harbor motifs like native cytoskeletal regulatory proteins that allows them to switch cytoskeletal processes towards pathogen invasion. Although several key Salmonella pathogenic factors such as SopB, SopE and SifA have been identified as cytoskeletal regulators, the structural and functional mechanisms of molecular mimicry for these factors is poorly understood. The research being done in our group aims to bridge this gap by an interdisciplinary approach combining reconstitution biology, DNA nanotechnology and FRET-based conformational biosensors to probe the structure-function mechanisms of Salmonella pathogenic factors and identify key protein-protein interactions utilised by these factors for hijacking the cytoskeletal network.

P 168

Inverse adaptation to force modalities in multidomain proteins: The role of interdomain linkers

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The mechano-biome of biological systems exhibits remarkable diversity and dynamism, as complex cellular and tissue processes are orchestrated through intricate mechanical interactions. Central to this mechano-biome are mechanical proteins, which function as both sensors and responders to a broad range of mechanical cues, thereby actively modulating cellular architecture, signalling pathways, and tissue-level mechanics. Architecturally, these proteins are often elongated, modular structures with multiple domains arranged in series, where each domain acts as a mechanically responsive element or "spring."

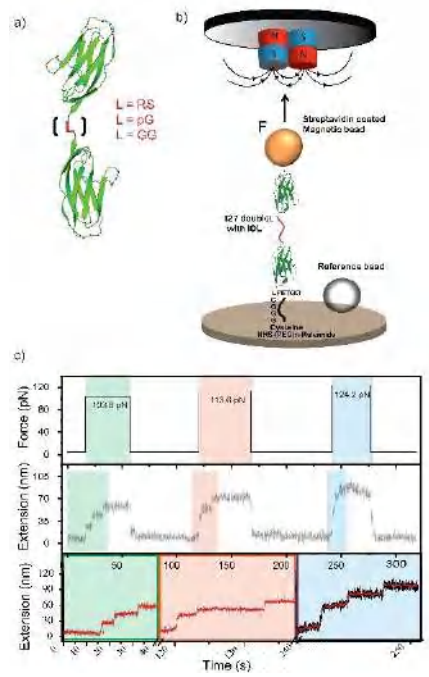

Figure: a) A cartoon representation of three protein models. b) Schematic representation of attachment setup of protein dimer variants in magnetic tweezers. c) A representative extension versus time trace of three protein models at three clamping forces.

Despite significant advancement in our understanding, the adaptive mechanisms by which these domains respond to diverse mechanical stimuli remain poorly understood. In this study, we focus on interdomain linkers (IDLs) and elucidate how chemically distinct linkers modulate the mechanical behaviour of domains within a representative muscle protein under both constant and oscillatory force conditions. Specifically, we investigate the impact of these linkers on the stability, unfolding kinetics, and interdomain interactions of repeat domains, uncovering unique mechano-adaptive roles.

Our findings reveal that IDLs differentially tune domain interactions, thereby regulating the stability and folding kinetics under sustained tension. Notably, IDLs that enhance mechano-stability under constant forces, paradoxically diminish the ability of domains to retain power under oscillatory forces, making them more susceptible to mechanical fatigue. This inverse response to force modalities suggests that linker properties are finely tuned to either sustain constant loading or to adapt to cyclic forces, but not both simultaneously.

Through these insights, we propose that strategic modifications of linker composition offer a means to engineer mechanical protein variants with tailored force responses, providing a potential framework for designing proteins optimized for specific mechanical environments. These findings underscore the critical role of interdomain linkers in the mechano-adaptation of modular proteins and present foundational principles for advancing the design of biomimetic materials like hydrogels and therapeutics.

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Unravelling the Role of Membrane Lipid Constituents in Dengue Virus Membrane Fusion

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Dengue fever, a prevalent vector-borne viral illness, is caused by the Dengue virus. Characterized by its compact, icosahedrally symmetric structure, this flavivirus is enveloped in ninety dimers of the envelope protein (E), crucial for viral attachment and membrane fusion. In this study, we systematically investigate the membrane fusion step, essential for virus entry into host cells, focusing on several key aspects including the influence of the target membrane's lipid composition, the effect of anionic lipids or other oxysterols on fusion, and the potential for direct inhibitors binding to the virus. Furthermore, our findings exhibit that 25-Hydroxycholesterol (25-HC) can reduce viral load in cell culture in a dose-established way. We also look at the mechanism of Dengue virus inhibition with the aid of 25-HC through Time of Addition assay (TOA), function of certain ISGs such as cholesterol 25-hydroxylase (CH25H) in different cell line, and also calculation of synergistic combination with some recognized anti-virals, (Chloroquine, Remdesivir, Picolinic acid, K-22), in the context to decide if the combination of compounds can enhance the effectiveness compared to the usage of them in alone. Utilizing actual-time fluorescence membrane assays, cell based dye de quenching assay, our studies uncovers new insights into how lipid compositions have an impact on dengue virus binding and membrane fusion. This study offers a nuanced understanding of the virus's entry mechanisms and lays the groundwork for potential therapeutic interventions targeting the viral fusion process.

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Heterogeneous Micropatterns of Biological Macromolecules Using Microbubble Lithography

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The recent pandemic highlights the importance of adopting high-throughput and rapid diagnostic techniques for accurate disease identification and optimal healthcare management. Developing diagnostic tools necessitates patterning biomolecules while preserving their biological activity. In contrast, the concept of bio-patterning largely relies on two methods: top-down and bottom-up. Although the top-down approach is advantageous in terms of scalability, it often exposes the biomaterial to high laser energy (in the case of photolithography), high voltage (electron beam lithography), or requires additional chemicals, such as developer-stripper solutions which are very likely to compromise the bioactivity and functionality of the patterned biospecimen seriously.

Microbubble lithography (MBL) is a comparatively recent (about a decade-old) bottom-up method that uses laser-generated and translated microbubbles to self-assemble and pattern materials of choice in real–time. Microbubbles are formed inside optical tweezers due to the high absorption of tightly focused lasers by dispersed particles immersed in or in contact with a fluid [1]. As the microbubble is manipulated by translating the laser, continuous self-assembly of the materials occurs along the direction of translation, forming continuous linear patterns. Changing experimental parameters, including laser intensity and power, translation speed, and sample concentration in liquid dispersion, allows easy control over pattern width. However, despite of its success in patterning mesoscopic entities - to the best of our knowledge – MBL has not yet been employed to develop continuous patterns of living organisms [2, 3]. This is principally due to temperature, adhesion of biospecimens (patterning efficiency) and low signal to noise ratio. Here, we outline a method for printing living matter or biospecimens using self-assembly mediated by microbubbles and report the successful creation of a strategy for immobilizing diverse biospecimens on transparent substrates. Our deployment of fast laser translation speeds ensure no temperature-induced damage to the biospecimens since their contact time with the microbubbles is less than a hundred milliseconds (ms). Overall, this study sets the groundwork for manufacturing multilayer heterostructures of living matter using MBL, and may open a new paradigm in designing bioelectronic chips.

References

1. S. Ghosh et al. *Nano Letters* **2021**, *21*, 10–25.
2. A. D. Ranjan et al, *JMCC*, **2023**, *11*, 17061.
3. A. D. Ranjan et al. *Small*, **2024**, 2401127.

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