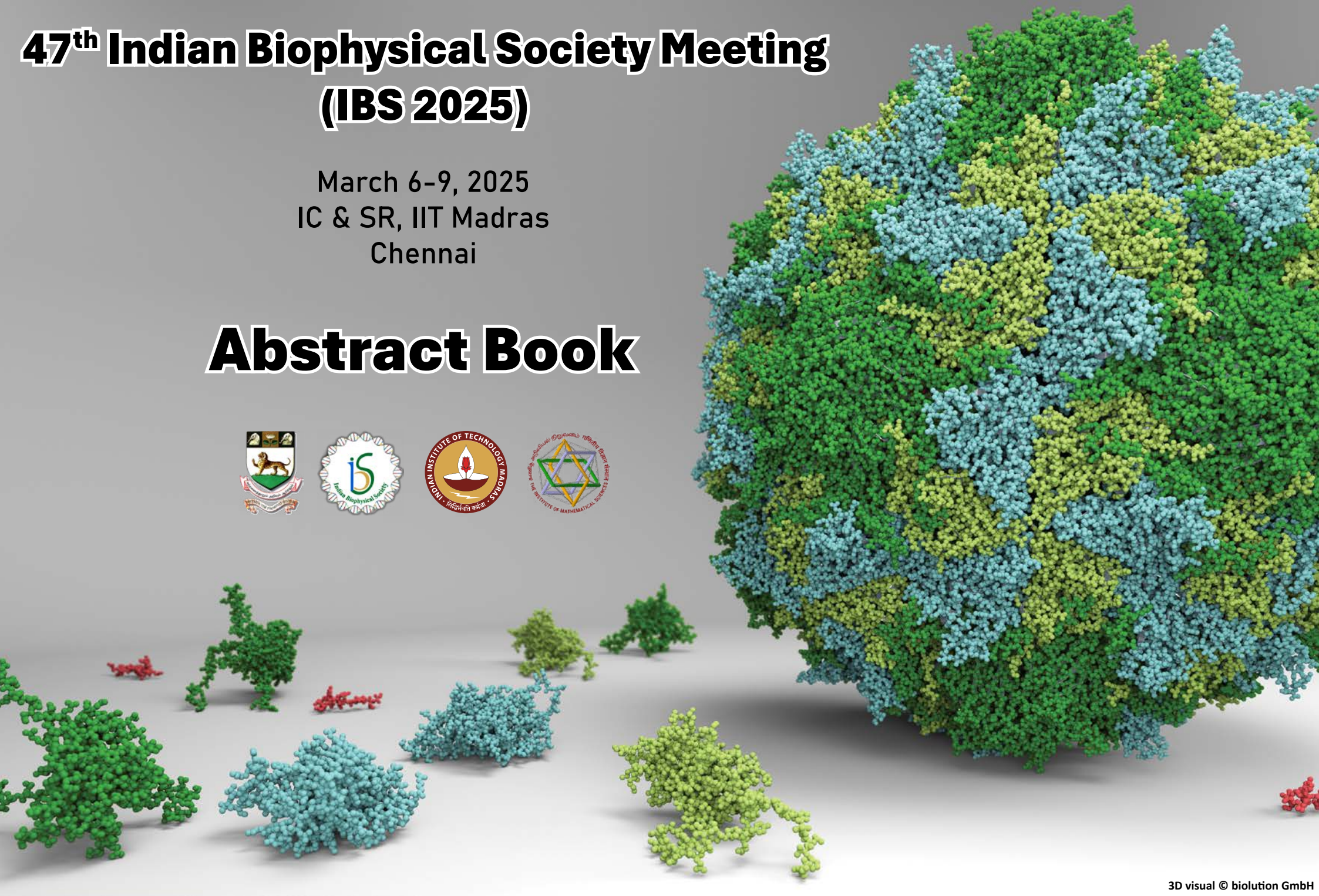
47th Indian Biophysical Society Meeting (IBS 2025)

March 6-9, 2025
IC & SR, IIT Madras
Chennai

Abstract Book



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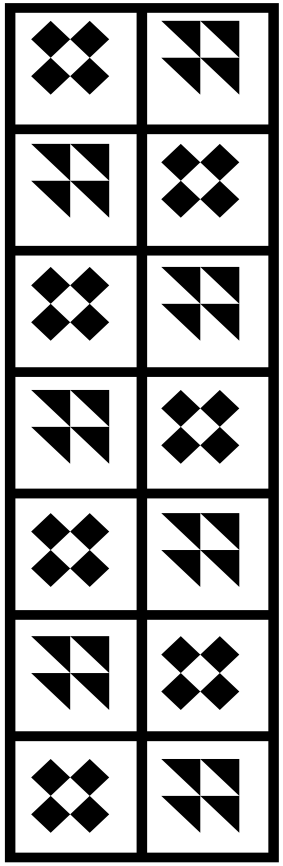

Credits

Abstract Book Design and Editing: Sharad J S and Protein Biophysics Lab members,
IIT Madras, Chennai

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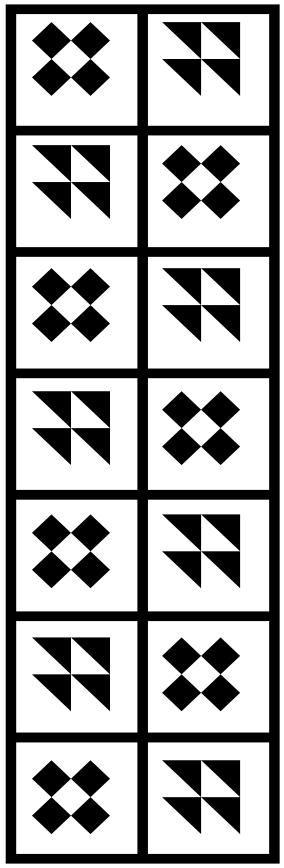
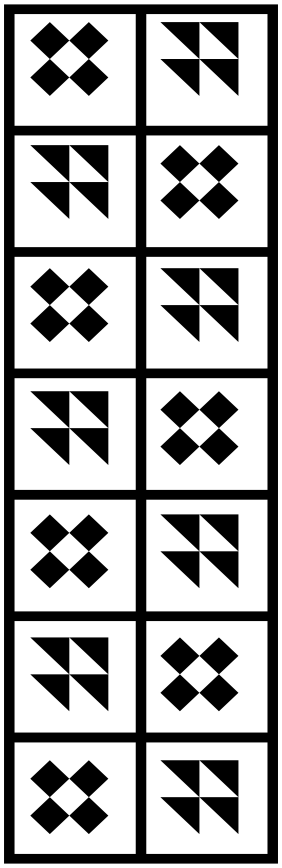


About the Meeting



The Indian Biophysical Society (IBS), founded in 1965 and registered under the Act XXVI of 1961 at Kolkata with its office at Saha Institute of Nuclear Physics (SINP), has grown over the years. Presently, it is holding over 1000 Life-members from all parts of the country. The interdisciplinary nature of the society attracted scientists from not only Phyiscs, Chemistry and Biology, but also from other related areas too, such as Biotechnology, Bioinformatics and Medicine. To brief on the historical evolution of this society, the first IBS Executive Council (EC) comprised of Dr. D. M. Bose as its first President, N. N. Dasgupta and B. Mukherjee as Vice-Presidents, N. N. Saha as the Secretary, B. D. Nagchaudhuri as the Treasurer, and A. K. Saha, M. N. Rao and S. N. Chatterjee as Members of the Council. IBS gives many awards to young and established scientists to promote biophysics in India. In addition to eight poster awards and one young scientist award, IBS gives two travel awards to cover partial expenses for attending the IUPAB International Biophysics Congress held once every three years and the Asian Biophysics Association (ABA) meeting, which is held once in two years. These awards are noted as Prof. J. C. Bose award for senior scientists above 35 years of age and Prof. G. N. Ramachandran award for younger scientists below 35 years of age.

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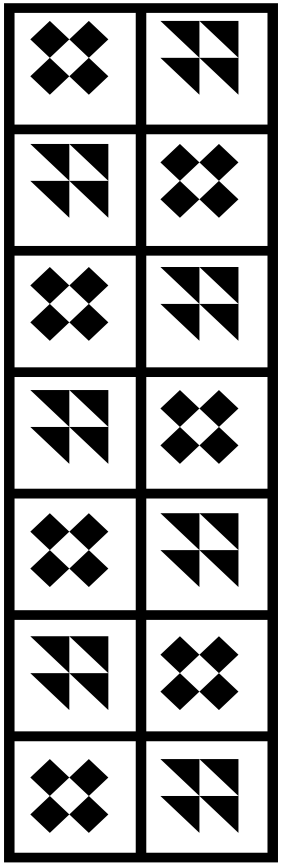

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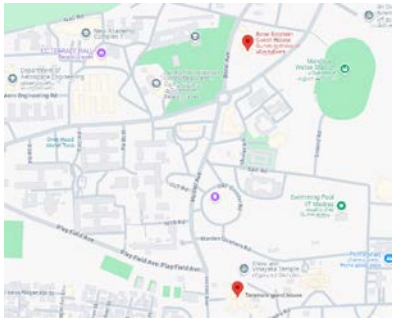
Venue

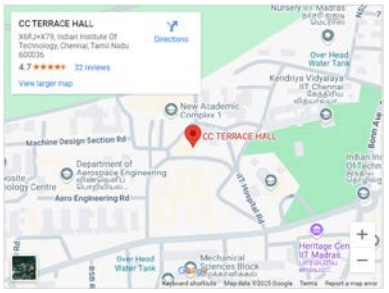
Guest Houses
BEGH & TGH

Meeting Venue
IC & SR, IITM

Poster Session
CC Terrace Hall

Student Hostels





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Schedule

March 6, 2025 (Thursday) – 'Cytiva Day'			
Time	**Chair**	**Speaker**	**Venue**
09:00 – 10:15	Registration, Coffee/Tea		TTJ Auditorium, IC & SR Building
10:15 – 10:30	Opening Remarks – **Sudipta Maiti (President IBS, BITS Hyderabad)**		
10:30 – 11:10	Ansuman Lahiri (Univ. of Calcutta)	Immunogen design to enhance stability and efficacy of viral vaccines **Raghavan Varadarajan (IISc)**	
11:15 – 11:55		Self-assembly of the Tau protein: Liquid-liquid phase separation and fibrillization **Joan-Emma Shea (UC Santa Barabara, USA)**	
12:00 – 12:20		Technical Talk - Cytiva	
12:20 – 14:00	**Lunch** (IC & SR Building Main Dining Hall, Dining Annex)		
14:00 – 14:40	P Karthe (Univ. of Madras)	β-Barrel chaperones: Multipoint assembly landscapes and lipid–regulated conformational plasticity **Mahalakshmi R (IISER Bhopal)**	TTJ Auditorium, IC & SR Building
14:45 – 15:25		Microbes and humans: A battle between membranes and pores **Thomas Gutsmann (Research Center Borstel, Germany)**	
15:30 – 15:50	Sudipta Maiti (BITS Hyderabad)	Meet the Editor: **Prof. Joan-Emma Shea** (ACS, JPC)	
15:50 – 16:30	**Tea** (IC & SR Building Main Dining Hall, Dining Annex)		
16:30 – 17:10	Hamsa Priya M (IIT Madras)	Molecular simulations as window into cellular dynamics **Gerhard Hummer (Max Planck Inst. of Biophysics, Germany)**	TTJ Auditorium, IC & SR Building
17:15 – 17:55		Modeling protein dynamics with machine learning and molecular simulation **Cecilia Clementi (Freire Universität Berlin, Germany)**	
18:00 – 18:15		Technical Talk – Zelle Biotech | Twist Biosciences	
18:20 – 19:00		Molecular dynamics simulations and machine learning to investigate protein-ligand binding in [NiFe] hydrogenases **Ariane Nunes Alves (Technical University Berlin, Germany)**	
19:05 – 19:15		Technical Talk - Leica	
19:15 – 19:25		Technical Talk – Spinco Biotech	
19:25 – 19:35		Technical Talk – BD Biosciences	
19:40 – 21:00	**Dinner** (IC & SR Building Main Dining Hall, Dining Annex)		

March 7, 2025 (Friday) – 'Swayam Prabha Day'			
Time Slot	**Chair**	**Speaker**	**Venue**
09:00 – 10:00	Sudipta Maiti (BITS Hyderabad)	**GN Ramachandran Lecture** Biophysical journeys on fitness landscapes: from atoms to populations and back **Eugene Shakhnovich (Harvard Univ., USA)**	TTJ Auditorium, IC & SR Building
10:00 – 10.20		Swayam Prabha (Prof. Arun Tangirala)	
10:25 – 10:45	**Tea** (IC & SR Building Main Dining Hall, Dining Annex)		
10:45 – 11:25	Sudipta Maiti (BITS Hyderabad)	Single-molecule FRET and FCS investigation of the interaction between SARS-CoV2 N protein and RNA **Satoshi Takahashi (Tohoku University, Japan)**	TTJ Auditorium, IC & SR Building
11:25 – 12:05		Elucidating the mechanics and structure of whole mitotic chromosomes **Gijs Wuite (Vrije Universiteit Amsterdam, Netherlands)**	
12:10 – 12:20		Technical Talk – Spinco Biotech.	
12:20 – 12.30		Technical Talk – Toshniwal Bros.	
12.30 – 13.15		IBS General Body Meeting	
13:15 – 14:30	**Lunch** (IC & SR Building Main Dining Hall, Dining Annex)		
14:30 - 16:30	**Poster Session 1** & **Tea** (Terrace Hall)		

Session 1 Microscopy and Single Molecules **Venue** TTJ Auditorium			**Session 2** Biophysics Across Length Scales **Venue** Halls 1 & 3		
Time Slot		**Speaker**	**Time**		**Speaker**
16:30 – 16:50	Sri Rama Koti Ainavarapu (TIFR Mumbai)	*Changes in global RNA-protein metabolism during ageing cause alteration in stress granule properties* **Shovamayee Maharana (IISc)**	16:30 – 16:50	Anand Srivastava (IISc)	*Insights into the architecture and channel properties of Pannexins* **Aravind Penmatsa (IISc)**
16:50 – 17:10		*Structural and functional insights into Cren7: A DNA-bending protein from extremophiles* **Soumit Mondal (IISER Tirupati)**	16:50 – 17:10		*Role of metastable conformational states in proteins: From function to drug discovery* **Suman Chakrabarty (SNBCBS)**
17:10 – 17:30		*Insights into the malarial parasite cytoskeleton using cryo-electron microscopy* **Mamata Bangera (IIT Madras)**	17:10 – 17:30		*Thermodynamics and kinetics of biomolecular recognition* **Tarak Karmakar (IIT Delhi)**
17:30 – 17:50		*Force-driven transformation of tunnel-associated chaperones: Unveiling a strain-energy-based mechanism for enhanced protein folding* **Shubhasis Haldar (SNBCBS)**	17:30 – 17:50		*Self-assembly pathway, energetics and morphology of peptide nanostructures* **M Hamsa Priya (IITM)**
17:50 – 18:10		*Replicating active transport of micro-organisms in synthetic systems* **Sabyasachi Rakshit (IISER Mohali)**	17:50 – 18:10		*F-actin architecture in the cytokinetic ring restricts E-cadherin mobility at furrow zone during cell division* **Anup Padmanabhan (Ashoka Univ.)**
18:10 – 18:30		*Sequence-dependent phase separation by Mycobacterium nucleoid-associated protein Lsr2 mediates DNA compaction* **Mahipal Ganji (IISc)**	18:10 – 18:30		*Physical confinement selectively favours bacterial growth based on cell shape* **Tapomoy Bhattacharjee (NCBS)**
18:30 – 18:50		*Determination of thermally induced cell membrane slope fluctuations using rotational optical tweezers* **Basudev Roy (IITM)**	18:30 – 18:50		*Polymer simulation study of the spatiotemporal organization of chromatin at the gene-length scale* **Ranjith Pandinhateeri (IIT Bombay)**
19:30 – 21:00	**Dinner** (Terrace Hall)				

Schedule

March 8, 2025 (Saturday)			
Time Slot	**Chair**	**Speaker**	**Venue**
09:00 – 09:40	Neelanjana Sengupta (IISER Kolkata)	Allosteric regulation of the restriction enzyme McrBC: towards deciphering the mechanism of activation of an endonuclease by a rotary motor **Saikrishnan Kayrat (IISER Pune)**	TTJ Auditorium, IC & SR Building
09:45 – 10:25		Decoding the rules of phase separation through minimalist peptide models and atomistic simulations **David de Sancho (DIPC, Spain)**	
10:25 - 10.50	**Tea** (IC & SR Building Main Dining Hall, Dining Annex)		
10:50 – 11:30	Neelanjana Sengupta (IISER Kolkata)	Physics of body axis formation **Stephan Grill (MPI-CBG, Germany)**	TTJ Auditorium, IC & SR Building
11:35 - 12:15		Combatting ribosomal methylation based antibiotic resistance: A war at the microscopic level **Ruchi Anand (IIT Bombay)**	
12:20 – 12:30		Technical Talk – GeneX	
12:30 – 13:30	**Lunch** (Terrace Hall)		
13:30 - 15:30	**Poster Session 2** & **Tea** (Terrace Hall)		

Session 3 Protein Structure and Mechanisms **Venue** TTJ Auditorium			**Session 4** Computational Biophysics **Venue** Halls 1 & 3		
Time		**Speaker**	**Time**		**Speaker**
15:30 – 15:50	Preethi Raghunathan (Univ. of Madras)	*Ion channel-mediated regulation of excitatory/inhibitory (E/I) imbalance in Alzheimer's disease* **Swagata Ghatak (NISER, Bhubaneshwar)**	15:30 – 15:50	Rajaram Swaminathan (IIT Guwahati)	*Uncovering high-resolution organization of genomic loci via multiscale polymer simulations* **Arnab Bhattacharjee (JNU)**
15:50 – 16:10		*Fate of knotted proteins during directed degradation and constrained folding conditions* **Hemachandra Kotamarthi (IITM)**	15:50 – 16:10		*Exploring the energy landscapes of intrinsically disordered proteins using coarse-grained simulations* **Debayan Chakraborty (IMSc)**
16:10 – 16:30		*A plausible mechanism of transcription activation of a subset of NF-kappaB repressed IRF3 target genes* **Sulakshana Mukherjee (IISER Berhampur)**	16:10 – 16:30		*Decoding thermosensation and associated response mechanisms in Caenorhabditis elegans* **Rati Sharma (IISER Bhopal)**
16:30 – 16:50		*Profiling functional dynamics of high molecular weight proteins using solution NMR based methods at natural abundance* **Subhabrata Majumder (SINP)**	16:30 – 16:50		*Recent developments in integrative structural modeling of macromolecular assemblies* **Shruthi Viswanath (NCBS)**
16:50 – 17:10		*Utilising simple building blocks for complex catalytic functions* **Dibyendu Das (IISER Kolkata)**	16:50 – 17:10		*Deciphering thermostability and structural dynamics in carbohydrate-active enzymes* **Ragotthaman Yenamalli (SASTRA)**
17:10 – 17:30		*Are the transmembrane domains of class I viral fusion proteins passive anchors or do they enable viral fusion?* **Shachi Gosavi (NCBS)**	17:10 – 17:30		*Dynamics of active tracer particles in complex environments: insights from computer simulations* **Rajarshi Chakrabarti (IIT Bombay)**
17:30 – 17:50		*Glycans as attachment factor of SARS-CoV-2* **Nagma Parveen (IIT Kanpur)**	17:30 – 17:50		*Allosteric regulation of β-arrestin 1 and 2 effector specificity by GPCR phosphorylations* **Rajesh Murarka (IISER Bhopal)**
18:00 – 19:00	**Flash Talks – Session 1**		18:00 – 19:00	**Flash Talks – Session 2**	
19:00 – 20:30	**Dinner** (Terrace Hall)				

March 9, 2025 (Sunday)			
Time Slot	**Chair**	**Speaker**	**Venue**
09:00 – 09:40	Sunil Kumar PB (IIT Madras)	A structure-based approach to tackle protein aggregation in Parkinson's disease **Salvador Ventura (Autonomous Univ. of Barcelona, Spain)**	TTJ Auditorium, IC & SR Building
09:45 – 10:25		Modeling DNA flexibility and its role in protein binding **Martin Zacharias (Technical University Munich, Germany)**	
10:30 – 10:40		Technical Talk - Nanotemper	
10:45 – 11:05	**Tea** (IC & SR Building Main Dining Hall, Dining Annex)		
11:05 – 11:45	Sunil Kumar PB (IIT Madras)	Unveiling the role of network stabilization in the aging dynamics of biomolecular condensates linked to neurodegenerative diseases **Nunilo Cremades (University of Zaragoza, Spain)**	TTJ Auditorium, IC & SR Building
11:50 – 12:30		Protein silencing with self-peptides **Sudipta Maiti (BITS Hyderabad)**	
12:35 – 13:15		Protein dynamics and kinetics studied by NMR spectroscopy **Christian Griesinger (MPI- Göttingen, Germany)**	
13:15 – 13:40	Student Awards, Closing Remarks (**Dr. Hemachandra Kotamarthi**, IIT Madras)		
13:00 – 15:00	**Lunch** (IC & SR Building Main Dining Hall, Dining Annex), **Departure**		

Flash Talks

Flash Talks

Session 1 (TTJ Auditorium)			
Index	**Name**	**Institute**	**Title of the Talk**
1	Gaurav Kumar	University of York	Linker mediated phase separation of Rubisco in algal pyrenoids: a tale of stickers and spacers
2	Aravind R	NCBS	Plasticity of the proteasome-targeting signal Fat10 enhances substrate degradation
3	Aruldoss Immanuel	SASTRA Deemed to be University	Computational modelling of Levan Biosynthesis pathway in *Bacillus subtilis*: Implications in strain optimization and engineering
4	Parthasarathi Sahu	National Institute of Technology Durgapur	Efficient replication and information storage capacity sets the genome length
5	Ankush Garg	Aarhus University	Oxygen partitioning into biomolecular condensates is governed by protein density
6	Arya Krishna	Rajiv Gandhi Centre for Biotechnology	Conformational Dynamics of Membrane Porins Control Cyclic Sugar Transport
7	Chandrasekaran Prabaharan	International Institute of Molecular and Cell Biology	Structural and biochemical characterization of cauliflower mosaic virus reverse transcriptase
8	Priyotosh Sil	The Institute of Mathematical Sciences (IMSc), Chennai	Biologically meaningful regulatory logic enhances the convergence rate in Boolean networks and bushiness of their state transition graph
9	Takahiro Kimura	Tohoku University	Single-molecule FRET investigation on the long-range contact formation in the folding of a gRNA granule of SARS-CoV-2
10	Aditya Shrivastava	Tata institute of fundamental research	Ionic Liquid-Induced Modulation of Ubiquitin Stability: The Dominant Role of Hydrophobic Interactions

Session 2 (Hall 1 & Hall 3)			
Index	**Name**	**Institute**	**Title of the Talk**
1	Suman Pal	IISER Pune	Phase Transitions of mammalian Prion Protein: Molecular Mechanisms, Structural Insights, and Disease Relevance
2	Harini SureshKumar	Indian Institute of Science	Emergence of soft dynamic channels in highly ordered lipid bilayers
3	Anjana Peethambaran Menon	Indian Institute of Technology Bombay	Decoding the role of mycobacterial lipid remodelling and membrane dynamics in antibiotic tolerance
4	Mahima	Indian Institute of Technology Jammu	The Dual Influence of Transcriptional and Translational Kinetics on Gene Expression Noise
5	Aarcha Radhakrishnan	Institute of Nanoscience and Technology, Punjab	Bio-nanoreactor formation using BMC shell protein by probing the role of disordered regions of enzyme cargoes
6	Anjana V M	National Institute of Technology Karnataka	What drives vesicle formation in peptide permeation through the cancerous membrane: Insights from umbrella sampling simulations
7	Saloni Goyal	Indian Institute of Technology Madras	Polyphosphate Discriminates Protein Conformational Ensembles More Efficiently than DNA Promoting Diverse Assembly and Maturation Behaviors
8	Smriti Mukherjee	CSIR-Central Leather Research Institute (CSIR-CLRI)	Engineering Collagen Mimicry with Short, Hyperstable Collagen-Mimetic Peptides via Terminal π-Capping
9	Sukanya Sadhu	Raman Research Institute	Bi-Directional DNA Translocation in Conical Nanopores

Poster Presentations

March 7, 2025 (13:30 – 15:30) (Terrace Hall)			
Session 1			
Index /Poster No.	**Name**	**Institute**	**Title of the Poster**
1	Abdul Basit	Jawaharlal Nehru University	Reliability of Calcium-Binding Site Prediction with AlphaFold 3: Insights from Analysis of Crystal Structure
2	Aditi G Muddebihalkar	Indian Institute of Technology Madras	Unlocking the Puzzles of Self-Assembly and Disassembly of Viral Capsid
3	Aishani Tewari, Gayatri Tendulkar	Krea University	Benchmarking Computational Models for Relaxation Dispersion NMR: Insights into Sub-Millisecond Protein Dynamics
4	Anjana V Mathath	National Institute of Technology Surathkal	What drives vesicle formation in peptide permeation through the cancerous membrane: Insights from umbrella sampling simulations
5	Anusree Sen	S. N. Bose National Centre for Basic Sciences	Alteration at a Protein-Water interface due to changes in pH
6	Aravind R	National Center for Biological Sciences, Tata Institute of Fundamental Research (NCBS), India	Plasticity of the proteasome-targeting signal Fat10 enhances substrate degradation
7	Aruldoss Immanuel	SASTRA Deemed to be University	Computational modelling of Levan Biosynthesis pathway in *Bacillus subtilis*: Implications in strain optimization and engineering
8	Bhanu Priya	Indian Institute of Technology Madras	Investigating The Influence of Solvent on Self-Assembly Mechanism of Peptides
9	Bharath Raj P	Indian Institute of Technology Madras	Molecular Insights into CETP-Mediated Lipid Transfer and Inhibition Mechanisms
10	Bhavna Chaudhary	Indian Institute of Technology Madras	Influence of Cardiolipin on Bacterial Membrane Dynamics Under Osmotic Stress
11	Dhruv Kumar Chaurasiya	Indian Institute of Technology Madras	The thermodynamic architecture of eukaryotic protein kinase
12	Digvijay Lalwani Prakash	National Center for Biological Sciences, Tata Institute of Fundamental Research (NCBS), India	SuBMIT: A Toolkit for Facilitating Simulations of Coarse-Grained Structure-Based Models of Biomolecules.
13	Gugan Kothandan	University of Madras	Predictive modeling to forecast SARS-COV-2 variants and understanding its virulence by integrating machine learning and biophysical studies
14	Harini SureshKumar	Indian Institute of Science, Bengaluru	Emergence of soft dynamic channels in highly ordered lipid bilayers
15	Jaya Vasavi Pamidmukkala	Indian Institute of Technology Madras	An integrative computational approach to predict viral epitopes by targeting the MHC-TCR complexation
16	Kartik Majila	National Center for Biological Sciences, Tata Institute of Fundamental Research (NCBS), India	A deep learning method for predicting interactions for intrinsically disordered proteins
17	Kavana Priyadarshini Keshava	Jawaharlal Nehru University	From Contacts to Patterns: Quantifying Structural Changes in HIC data
18	Kompella V K Srinath	Aganitha.ai, Jubilee Hills, Hyderabad	Synergizing Physics-based modeling with AI/ML for BioPharma R&D
19	Koushik Ghosh	National Institute of Technology Durgapur	Non-enzymatic Kinetic Error Correction in Primordial Nucleotide Replication through Asymmetric Cooperativity
20	Madhurima Khamaru	Presidency University	An insight into the leucine zippers present in the unique blue light photoreceptor of marine alga *Ectocarpus siliculosus*
21	Mahima	Indian Institute of technology Jammu	The Dual Influence of Transcriptional and Translational Kinetics on Gene Expression Noise
22	Menaka Thambiraja	SASTRA Deemed to be University	Computation Analysis of Pathogenic Variants in the POLG2 and TWNK Mitochondrial Proteins.
23	Nisha Nandhini Shankar	SASTRA Deemed to be University	Family-level characterization of lytic polysaccharide monooxygenases using multiscale modeling
24	Parthasarathi Sah	National Institute of Technology Durgapur	Efficient replication and information storage capacity sets the genome length
25	A Dhanusha	Bharathidasan University	Graphene Hybrid Scaffolds for Laser Stimulated Neuro-regeneration
26	Aarcha Radhakrishnan	Institute of Nanoscience and Technology, Punjab	Bio-nanoreactor formation using BMC shell protein by probing the role of disordered regions of enzyme cargoes
27	Aditya Shrivastava	Tata Institute of Fundamental Research, Mumbai	Ionic Liquid-Induced Modulation of Ubiquitin Stability: The Dominant Role of Hydrophobic Interactions
28	Ahana Banerjee	CSIR-Indian Institute of Chemical Biology, Kolkata	Exploring Liquid-Liquid Phase Separation in ALS-Associated SOD1 Mutants
29	Aishi Dasgupta	IITB-Monash Research Academy	Characterization of SARS-CoV-2 Pseudoviruses: Investigating Spike Protein Interactions with Mammalian Cells at Membrane and Global Levels
30	Ajit Seth	Shiv Nadar Institution of Eminence	Hydrophobicity Dependent Deformation of Bacterial Cell Wall Induced by Imidazolium-Based Ionic Liquids
31	Akhil K Mohan	CSIR-National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum	Outer Membrane Vesicle-Derived Membrane Model for Probing the Peptide Interactions
32	Anagha C Unnikrishnan	CSIR-Central Leather Research Institute (CSIR-CLRI)	Dominance of Hydrogen Bonding over Aromatic π-π Interactions in the Efficiency of Peptide Gelators in Supramolecular Hydrogelation: Asparagine as a Key Factor
33	Anirban Mullick	National Institute of Technology Tiruchirapalli	Confirmatory detection and evaluation of acid rain stress in Neem (Azadirachta indica) using spectroscopic techniques
34	Anjali D. Patil	CSIR-National Chemical Laboratory	Functional Nucleic-Acid Binding Domain Of TAR-DNA Binding Protein-43 Forms Pathogenic Beta-Aggregates Via Monomeric Early Precursor Intermediate
35	Anjana P Menon	Indian Institute of Technology Bombay	Decoding the role of mycobacterial lipid remodelling and membrane dynamics in antibiotic tolerance
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37	Ankush Roy	Indian Institute of Science, Bengaluru	Structural Analysis of Broadly Neutralizing Antibody Interactions with Dengue Virus.
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Poster Presentations

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42	Arya Krishna	Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram	Conformational Dynamics of Membrane Porins Control Cyclic Sugar Transport
43	Avinash Chettri	University of Potsdam	2D-IR Spectroscopy Shows the Influence of Sequence Specificity on the Macrostructure of Single-Repeat GLFG Hydrogels.
44	Aysha Fahmeetha	National Center for Biological Sciences, Tata Institute of Fundamental Research (NCBS), India	Translational regulation by Huntingtin and its Rhes-mediated pathogenesis in Huntington's Disease
45	Ayushi Srivastava	Indian Institute of Science, Bengaluru	Investigation into a multi-domain transporter reveals new modes of allosteric regulation in cation: H+ antiporters
46	Bhavika Gupta	Indian Institute of Technology Bhubaneshwar	The N-Terminal domain of Mycobacterium leprae HSP18: Key to its structural integrity and chaperone activity
47	Chandrasekaran Prabaharan	International Institute of Molecular and Cell Biology in Warsaw	Structural and biochemical characterization of cauliflower mosaic virus reverse transcriptase
48	Debodyuti Sadhukhan	BRIC National Institute of Immunology	Structure-function relationship studies of a mitoribosomal protein MRPL50
49	Devasena Umai R	Indian Institute of Technology Madras	pH and chemical induced denaturation of Esterase from Clostridium acetobutylicum
50	Dimple Goel	Institute of Nanoscience and Technology, Punjab	Engineering Bacterial Microcompartments: Insights from Shell Protein Interaction Studies
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52	Gaurav Kumar	University of York	Linker mediated phase separation of Rubisco in algal pyrenoids: a tale of stickers and spacers
53	Harshita Dutta	Indian Institute of Science, Bengaluru	Role of RNA pseudoknot antitoxin in type III toxin-antitoxin complex assembly and toxin inhibition
54	Indu	Jawaharlal Nehru University	Understanding the mechanism of human gamma D-crystallin aggregation at acidic and physiological pH
55	Irawati Roy	Indian Institute of Science, Bengaluru	Unambiguous assignment of kinked beta sheets leads to insights into molecular grammar of reversibility in biomolecular condensates
56	Jayashree Ravi	University of Madras	Systematic Biophysical Approach to Study Plastic Degradation: Polycaprolactone (PCL) Degradation by Mycobacterium marinum Cutinase
57	Jayita Biswas	Indian Association for the Cultivation of Science, Jadavpur	Molecular crosstalk between ectodomains of adherens junction proteins, E-cadherin and Nectin-4
58	Judith Gracia	University of Madras	Investigation of Amlodipine Besylate and Ranitidine Hydrochloride as Bacterial RNA Polymerase inhibitors
59	Kalpaja Acharya	National Center for Biological Sciences, Tata Institute of Fundamental Research (NCBS), India	A study of aggregation kinetics of Poly-ubiquitin fibrils
60	Koushik Chalki	Tata Institute of Fundamental Research, Mumbai	Investigating the Mechanical Response of Parallelly Arranged Polyproteins: Mimicking the Mechanical Stress in Biological Systems
61	Koustav Saha	Indian Institute of Technology Guwahati	Bridging the Knowledge Gap: Investigating K18 and K19 aggregation through Protein Charge Transfer Spectra (ProCharTS)
62	Arkadeep Banerjee	NCBS-TIFR, Bengaluru	Understanding Self-Peptide Insertion During Protein Folding
63	M Abith	Bharathidasan University	Silver Incorporated Reduced Graphene Oxide-Molybdenum Disulfide (Ag-rGO-MoS2) Hybrid Material for Bio-photonic Application
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65	Mani Gupta	Jawaharlal Nehru University	Exploring the anti-fibrillating effect of the anthocyanidin Petunidin on the aggregation and amyloid fibril formation of α-synuclein involved in Parkinson's disease
66	Megha Rai	Ashoka University	ARP-2/3 regulates cell surface dynamics of CeTOCA-1 in the C. elegans zygote
67	Mona S. Kirmire	CSIR-National Chemical Laboratory	Phase Separation Dynamics of FLTDP-43: A Protective Mechanism or Pathological Driver in Neurodegeneration?
68	Muskaan Jindal	National Center for Biological Sciences, Tata Institute of Fundamental Research (NCBS), India	Integrative structure determination of sub-complexes of the mitochondrial contact site and cristae organizing system (MICOS)
69	Nafisa Siddiqui	Indian Institute of Science Education and Research (IISER) Bhopal	A first passage time study of bacterial eradication under the influence of antibacterial agents

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3	Priyanka Yadav	Indian Institute of Technology Hyderabad	Mechanistic of repeat RNA folding and its interaction with drug molecule using molecular dynamics simulations
4	Priyotosh Sil	The Institute of Mathematical Sciences (IMSc), Chennai	Biologically meaningful regulatory logic enhances the convergence rate in Boolean networks and bushiness of their state transition graph
5	Raghav Mathur	Indian Institute of Science, Bengaluru	Effect of Hydropathy Scale on Intrinsically Disordered Protein Properties Predicted using Implicit Solvent Simulations
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7	Saanya Yadav	Indian Institute of Technology Hyderabad	Structure and self-assembly of DNA nanostructure in lipid bilayer membrane
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9	Santosh Prajapati	Indian Institute of Science, Bengaluru	π - π and cation-π interactions are required in intrinsically disordered protein coarse-grained models to recapitulate experimental reality
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27	Pritam Saha	Indian Institute of Science Education and Research Mohali	Extent of stochasticity in folding dynamics determines the force-tolerance and longevity of mechanosensing proteins
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31	Rasanpreet Kaur	Indian Institute of Technology Guwahati	Decoding the Structural insights of Prothymosin-α by novel label-free approach: Protein Charge Transfer Spectra
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33	Ritu Raj	Indian Institute of Technology Madras	Impact of Nucleosome Dynamics on Condensed Chromatin Globules
34	Sakshi Priyadarsini Dutta	Indian Institute of Technology Bhubaneshwar	Impact of Multidrug Therapy Drugs on the Structure and Chaperone Function of Mycobacterium tuberculosis Hsp16.3
35	Saloni Goyal	Indian Institute of Technology Madras	Polyphosphate Discriminates Protein Conformational Ensembles More Efficiently than DNA Promoting Diverse Assembly and Maturation Behaviors
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41	Saumyadeep Goswami	Indian Institute of Technology Kharagpur	Elucidating the structural and functional aspects of the novel heterophilic interaction between E-cadherin and nectin-4
42	Sazida	BRIC National Institute of Immunology	Elucidating the Molecular Mechanism of Thioesterase 2-Acyl Carrier Protein Interaction
43	Shakya Sinha	Saha Institute of Nuclear Physics	Probing the mechanistic basis of the redox regulated chaperon Hsp33 activity
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52	Spandan Basu	Indian Institute of Science, Bengaluru	Establishing the organization of cellular condensates in aging
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60	Suganthi C	Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam	Exploring the interaction between Protein Z dependent Protease Inhibitor (ZPI) and Plasma Pre-kallikrein
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62	Suman Pal	Indian Institute of Science Education and Research Pune	Phase Transitions of mammalian Prion Protein: Molecular Mechanisms, Structural Insights, and Disease Relevance
63	Suman Tiwary	Tata Institute of Fundamental Research, Mumbai	Electrostatics dominate Au-S interaction in governing protein orientation on citrate-capped gold nanoparticles
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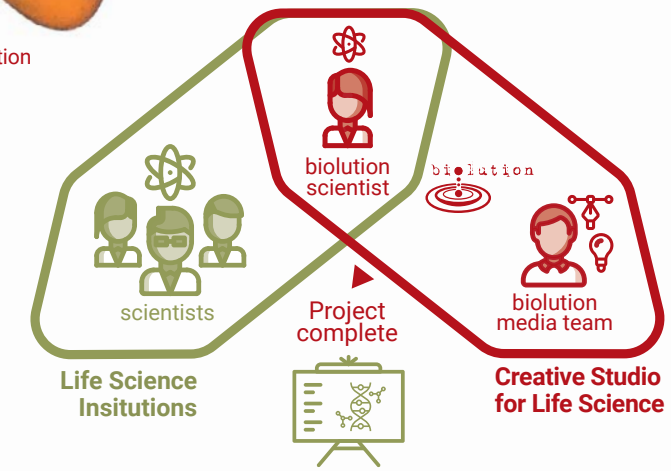

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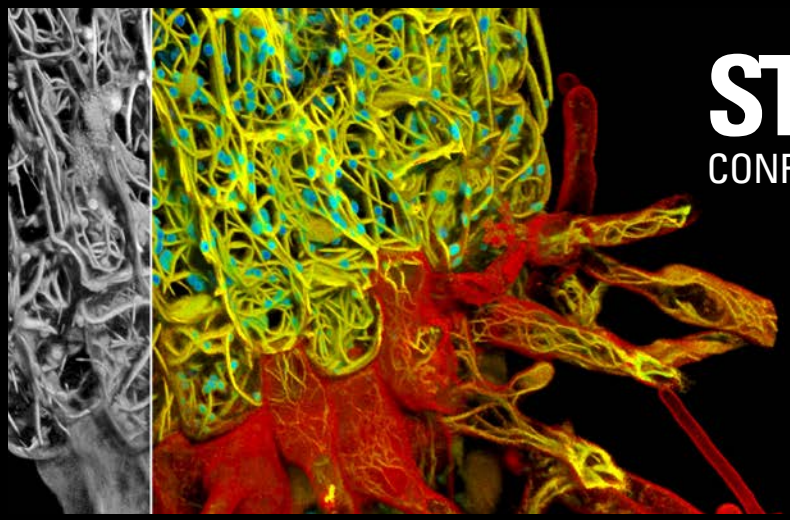

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Plenary Talks

Eugene Shakhnovich

Chemistry and Chemical Biology, Harvard University, Cambridge, USA

GN Ramachandran Lecture

Biophysical Journeys on Fitness Landscapes: from Atoms to Populations and Back

PS

I will present recent theoretical and experimental developments aimed at understanding the two way link between protein biophysics and protein evolution. The novel multiscale models integrate the molecular effects of mutations on physical properties of proteins, most notably their stability, into physically intuitive yet detailed genotype-phenotype relationship (GPR) assumptions. I will present a range of models from simple analytical diffusion-based model on biophysical fitness landscapes to more sophisticated computational models of populations of model cells where genetic changes are mapped into molecular effects using biophysical modeling of proteins and ensuing fitness changes determine the fate of mutations in realistic population dynamics. Examples of insights derived from biophysics-based multiscale models include parameter-free prediction of distribution of protein stabilities in natural proteomes that explains the observation of "marginal stability" of proteins without resorting to unproven stability-activity tradeoffs, the fundamental limit on mutation rates in living organisms, physics of thermal adaptation, co-evolution of protein interactions and abundances in cytoplasm and related insights. Next, I will describe experimental efforts to establish the relationship between biophysical properties of proteins (stability, activity, interactions with other proteins) and fitness. We focused on two genes encoding essential metabolic enzymes in *E. coli* – folA and adk and derived direct relationship between protein biophysics and fitness providing new insights into several exciting eras of research such as evolutionary dynamics of antibiotic resistance, evolutionary dynamics of viral escape leading to pandemics and other fields on the interface of biophysics and evolutionary analysis.

Raghavan Varadarajan

Molecular Biophysics Unit
IISc, India

Immunogen Design to Enhance Stability and Efficacy of Viral Vaccines

PS

Most current viral vaccine formulations, including for COVID-19, require low temperature storage. This is a major impediment to widespread deployment, and contributed to the highly skewed distribution of vaccines, worldwide. Despite recent advances in the use of AI/ML for protein structure prediction and design, prediction of stabilizing mutations for complex, hetero-oligomeric proteins remains challenging. We have developed general methodology to both rapidly isolate stabilized protein variants and map their corresponding conformational epitopes in polyclonal sera. The workflow has been applied to identify thermostable Receptor Binding Protein (RBD) derivatives of the Spike protein of SARS-CoV-2 and other sarbecoviruses. These display enhanced yield and immunogenicity relative to both the corresponding wild-type RBD and Spike ectodomains. Several of these formulations can be stored at 37°C for several weeks without loss of antigenicity or protective efficacy. Using related approaches, we have developed multiple influenza vaccine formulations to protect against seasonal and pandemic influenza. One of these has been shown to be safe and well tolerated in a Phase 1 clinical trial in Australia and further clinical development in India is ongoing.

Joan-Emma Shea

Department of Chemistry and Biochemistry , Department of Physics, University of California, Santa Barbara, USA

Self-Assembly of the Tau Protein: Liquid-Liquid Phase Separation and Fibrillization

PS

Tau is an intrinsically disordered protein that plays an important role in stabilizing microtubules. Under pathological conditions, this protein can also self-assemble into fibrillar structures, a process that has been associated with a class of neurodegenerative diseases known as Tauopathies. Interestingly, this protein is also capable of assembling into liquid droplets through a process of liquid-liquid phase separation (LLPS). Using a combination of fieldtheoretic simulations, coarse-grained models, and atomistic simulations, we present an investigation of the mechanisms of fibrillization and phase separation of this protein. We investigate fragments of Tau that have a propensity to either phase separate or form fibrils, enabling us to shed light into the sequence characteristics linked with these two modes of assembly. Finally, we introduce a 19-residue fragment of Tau that is capable of seeding the fibrillization of full-length Tau, and we discuss the effect of point mutations in modulating aggregation in familial forms of Tauopathies.

Radhakrishnan Mahalakshmi

Molecular Biophysics Laboratory, Department of Biological Sciences, IISER Bhopal, India

β-Barrel chaperones: Multipoint Assembly Landscapes and Lipid–Regulated Conformational Plasticity

PS

β-Barrel outer membrane proteins (OMPs) are unique to Gram-negative bacteria and mitochondria. All OMPs require the Barrel Assembly Machinery (BAM) (bacteria), and Sorting and Assembly Machinery (SAM) (mitochondria), with BamA and Sam50 being their core chaperones.[1] Despite their indispensable role, we know little about both proteins. Here, we obtain a complete molecular map of regulatory elements for both BamA and Sam50, by combining ultrafast measurements of their stepwise molecular (un)folding in native-like membranes, with end-state thermodynamics, and single-molecule functional studies.[2] We establish how both BamA and Sam50 evolved conformational buffering of sequence–guided structure–function handles for superior OMP biogenesis and BAM/SAM interactome *in vivo*. Our findings open avenues for developing designed peptidomimetics as highly effective nextgeneration therapeutics for disease states.

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Thomas Gutsmann

Research Center Borstel, Leibniz Lung Center, Borstel, Germany

Centre for Structural Systems Biology, Hamburg, Germany

Microbes and Humans: a Battle between Membranes and Pores

PS

In the era of antibiotic therapy, the development of new active substances seemed less important, but the emergence of antimicrobial resistance (AMR) has become an increasing problem in recent years. The WHO estimates that around 10 million deaths in 2050 will be due to resistant bacteria. Mycobacteria are already one of the leading causes of death worldwide, accounting for 1.3 million deaths. Between microbes and humans there is a permanent battle in which pore-forming molecules play a decisive role. On the one hand microbes attack other microbes, and the cytoplasmic or the phagosome membrane of mammal cells by pore forming proteins or peptides. On the other hand mammals use pore-forming peptides of the innate immune system, the so called Host Defense Peptides (HDP) or Antimicrobial Peptides (AMP), to kill microbes. All these membrane active peptides and proteins follow a general interaction pathway: binding, intercalation, aggregation, modification of membrane properties, pore formation, and disruption of membranes. However, the specificity to lipid composition, pH, ions, transmembrane potential and other factors is crucial for the respective function. Our goal is to elucidate the molecular interaction mechanisms in order to identify targets to prevent exit of microbes and to improve drug design.Using various membrane reconstitution systems (liposomes, planar bilayers, solid-supported bilayers and multilayers) composed of phospholipids and bacterial glycolipids we analyzed the basic structure of these membranes, e.g. supramolecular organization of the lipids, phase separation and orientation of fatty acids, and the influence of specific membrane active peptides and proteins.

Gerhard Hummer

Department of Theoretical Biophysics, Max Planck Institute of Biophysics

Institute for Biophysics, Goethe University Frankfurt, Germany

Molecular Simulations as Window into Cellular Dynamics

PS

Molecular dynamics simulations perfectly complement electron and light microscopy to capture the dynamics and function of living cells at the molecular scale. By adding dynamics in atomic detail and on a sound physical basis, simulations link structure to function, and in turn help us to identify sites targetable for therapeutic interventions. An explosion in raw computational power, the development of powerful simulation algorithms, and sophisticated artificial intelligence methods now make it possible to tackle biological systems and processes of significant size and complexity. In my presentation, I will highlight our work on autophagy and nuclear transport. As programs critical to cell function and the defense of pathogens, both are subject to rich regulatory control. Molecular simulations combined with experiments by our collaborators have allowed us to identify some of the molecular interactions ensuring precise targeting and tight control over autophagy initiation, a process to remove dysfunctional cellular components and intruding pathogens. With simulations and our collaborators' experiments, we have also started to characterize the nuclear pore complex as the permeability barrier controlling the entire molecular traffic in and out of the nucleus, up to the passage of entire viral capsids of the HIV-1 retrovirus. By revealing the molecular interactions regulating these complex cellular programs, molecular simulations point to new therapeutic targets.

Cecilia Clementi
Department of Physics,
Freie Universität Berlin, Germany

Modeling Protein Dynamics with Machine Learning and Molecular Simulation

PS

The last years have seen an immense increase in high-throughput and high-resolution technologies for experimental observation as well as high-performance techniques to simulate molecular systems at a microscopic level, resulting in vast and ever-increasing amounts of high-dimensional data. However, experiments provide only a partial view of macromolecular processes and are limited in their temporal and spatial resolution. On the other hand, atomistic simulations are still not able to sample the conformation space of large complexes, thus leaving significant gaps in our ability to study molecular processes at a biologically relevant scale. We present our efforts to bridge these gaps, by exploiting the available data and using state-of-the-art machine-learning methods to design multiscale models for complex macromolecular systems. We show that it is possible to define simplified molecular models to reproduce the essential information contained both in microscopic simulation and experimental measurements.

Ariane Nunes-Alves

Department of Chemistry, Technical University Berlin, Germany

Molecular Dynamics Simulations and Machine Learning to Investigate Protein–Ligand Binding in [NiFe] Hydrogenases

PS

Hydrogenases are important enzymes in biotechnology due to their ability to act as efficient catalysts for hydrogen oxidation and biofuel production. However, some [NiFe] hydrogenases are inhibited by O_2 and CO. A possible strategy to obtain resistant enzymes is to block the access of inhibitors to the catalytic site by mutation. Using the [NiFe] hydrogenase from Desulfovibrio fructosovorans and 10 different mutants as a model system, we employed molecular dynamics (MD) simulations and the enhanced sampling method τRAMD (τ-Random Accelerated Molecular Dynamics) to investigate substrate (H_2) and inhibitor (O_2 and CO) unbinding.[1] We computed pathway probabilities for the unbinding of different gas molecules from the wild type and mutant forms of [NiFe] hydrogenase and we observed that, while the most probable pathways are the same for different gas molecules and different mutants, the secondary pathways are different. Additionally, we created PathInHydro, a set of machine learning models developed to facilitate data analysis.[2] It classifies unbinding pathways in MD simulations using protein-ligand contacts as features. The new methods and physical insights could be exploited to engineer O_2- or CO-tolerant [NiFe] hydrogenases.

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Satoshi Takahashi

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Japan

Single-molecule FRET and FCS Investigation of the Interaction between SARS-CoV-2 N Protein and RNA

PS

N is a structural protein of SARS-CoV2 and forms the ribonucleoprotein granules having a diameter of ~16 nm upon the association of ~12 molecules of N and ~800-base regions of viral genomic RNA (gRNA). N also participates in the selective packaging of gRNA inside of virus. In addition, many in vitro experiments demonstrated the formation of liquid-like droplets of N upon the association with short RNA fragments. However, the structural properties and the folding mechanism of the ribonucleoprotein granules are largely unknown. We used single molecule FRET and FCS spectroscopies to understand the interaction between N and different secondary structures of RNA. We used poly-adenylate bases with different lengths and labeled their 3' and 5' termini with donor and acceptor fluorophores. We also labeled the two termini of 50-base stem loop with the donor and acceptor pair. We found that the N protein started to bind to the single-stranded RNAs at the concentrations between 10 and 100 nM. The binding of the N protein to the stem loop occurred at the concentration less than 10 nM without melting the stem loop. For all the samples, the binding of multiple molecules of the RNA fragments to a single dimer of the N protein was observed. These results demonstrate that the N protein acts as a non-specific binder to both single-stranded and stem-loop units of RNA, and that the N protein might contract a long RNA chain by bridging its multiple segments. Our recent trials to observe the compaction process of long RNA samples having ~800 bases will also be presented.

Gijs J.L. Wuite

Vrije Universiteit Amsterdam, The Netherlands

Elucidating the Mechanics and Structure of Whole Mitotic Chromosomes

PS

The DNA in eukaryotes is organised into linear chromosomes. These structures are highly dynamic throughout the cell cycle, and undergo dramatic compaction during mitosis to adopt the characteristic "X-shape". This metamorphosis is driven mainly by the combined action of condensins and topoisomerase IIα. Yet there is little known about the structural organization and mechanics of a mitotic chromosome. Here we introduce a workflow to investigate the organization of human chromosomes based on optical trapping and manipulation. This allows high-resolution force measurements and fluorescence visualization of native metaphase chromosomes to be conducted under tightly controlled experimental conditions. We have used this method to extensively characterize chromosome mechanics and structure. Next, we employ AFM to study the structure of human metaphase chromosomes in a liquid environment that closely resembles their physiological conditions, without the use of fixation agents and enzymatic treatments. Our analysis reveals the finer details of the unperturbed chromosome structure, shedding light on the organization of the chromatin fiber network and testing its stability against changes in the environment ionic conditions. With AFM-based Force Spectroscopy we also probe the response of mitotic chromosomes to nanoscale deformations in the presence and absence of specific SMC proteins thereby providing new insights into their contribution to the chromosome structural stability.The methods described here open the door to a wide array of investigations into the structure and dynamics of both normal and disease-associated chromosomes.

Saikrishnan Kayarat
Department of Biology, Indian Institute of Science Education and Research Pune, India

Allosteric Regulation of the Restriction Enzyme McrBC: Towards Deciphering the Mechanism of Activation of an Endonuclease by a Rotary Motor

PS

Bacteria have an arsenal of defense systems of diverse mechanisms to protect them from their viruses (bacteriophages). These include the restriction-modification enzymes, the CRISPR-Cas systems, toxin-antitoxin systems etc. McrBC is a GTP-dependent restriction endonuclease in *Escherichia coli* that cleaves incoming foreign DNA having methylated cytosine (^{m}C). The target site of McrBC is two base pairs long containing a purine followed by ^{m}C. The nuclease activity of the subunit McrC is coupled to the GTPase activity of McrB oligomers belonging to the AAA+ family. The GTPase activity of McrB is stimulated on complexation with the endonuclease McrC. Detailed structural studies using cryo-electron microscopy and other biophysical techniques have revealed the architecture of the McrBC assembly in near-atomic detail. Based on the structure and biochemical studies, a sequential mode of GTP hydrolysis by the hexameric McrB is proposed. Structure-directed mutagenesis combined with biochemical assays and computational studies have provided a model for the mode of GTP-hydrolysis. The study has also revealed insights into the allosteric regulation of the GTPase activity of McrB by McrC, and also the activation of the McrC nuclease by McrB. McrBC, thus, serves as a paradigm for regulation of multifunctional enzymes driven by AAA+ proteins.

David De Sancho
UPV-EHU / Donostia International Physics Center, Spain

Decoding the Rules of Phase Separation through Minimalist Peptide Models and Atomistic Simulations

PS

Short peptides have been instrumental as models for more complex systems. Studying short peptides, we have gained understanding of fundamental folding events like alpha helix nucleation or hairpin folding, and in the determination of the microscopic origin of internal friction. Short peptides are also challenging tests for the calibration of modern force fields, and hence have been extensively used in recent optimization efforts. In our laboratory, we have recently investigated these peptides as simplified model systems to study intrinsically disordered proteins (IDPs). Examples of our recent work include probing how metal ions influence their conformational dynamics and exploring the potential of cyclic peptides to serve as molecular binders. In my talk I will focus on our recent work on phase separation. Using peptide models, we test the hypothesis that phase separation is an emergent property determined by composition, even in the absence of a polypeptide chain, multivalency and patterning effects. For this, I use atomistic molecular dynamics (MD) simulations of saturated solutions of individual amino acids and mixtures thereof in stoichiometries comparable to those of phase-separating low complexity domains. Additionally, I disentangle the hierarchy of interaction strength between the two most dominant types of aromatics in condensates, phenylalanine and tyrosine. Our results are broadly consistent with trends observed in experiments and in atomistic simulations of full-length IDPs and reconcile findings from decades of work in physical chemistry and protein biophysics.

Stephan Grill
MPI-CBG Dresden,
Germany

Physics of Body Axis Formation

PS

One of the most remarkable examples of self-organized structure formation is the development of a complex organism from a single fertilized egg. With the identification of many molecules that participate in this process, attention has now turned to capturing the physical principles that govern the emergence of biological form. Living systems are special in the sense that they structure themselves through processes that convert chemical energy into mechanical work. In this talk I will present how the Physics of Active Matter can serve as a framework to unravel how the surface of a cell can generate an active stresses that can drive its reshaping, or the reshaping of many cells that are collectively organized into a tissue. I will report on of our efforts of combining active matter theory with experiments in both worms and birds to understand symmetry breaking, pattern formation, and body axis formation in early organismal development.

Ruchi Anand

Department of Chemistry, Indian Institute of Technology Bombay, Powai, India

Combatting Ribosomal Methylation Based Antibiotic Resistance: A War at the Microscopic Level

PS

Antibiotic resistance is slowly progressing towards becoming a global pandemic and has emerged as a silent killer. Bacteria have harnessed several mechanisms to evade the effect of antibiotics with drug target modification being a highly efficient strategy utilized by pathogenic systems to render themselves resistant to antibiotics. The ribosome owing to its integral role as the protein synthesis machinery of the cell is a prime target for several antibiotics. Here, we unravel the mechanism of post-transcriptional ribosomal methylation which renders the macrolide lincosamide and streptogramin B ((MLS_B) class of antibiotics ineffective. The enzyme Erythromycin-resistance methyltransferases (Erms)[1], exclusively harboured by several multi-drug resistant (MDR) pathogens can site specifically methylate a ribosomal base (A2058, *E.coli* numbering) in the nascent peptide exit tunnel of the 50S ribosomal subunit which then renders the MDRs resistant to MLS_B class of drugs. Interestingly, we show that Erm is an opportunistic enzyme that exclusively targets ribosomal precursors. Using Cryogenic electron microscopy (Cryo-EM) we have trapped the Erm-precursor complex and showed how in a complex environment, during ribosomal biogenesis, Erm can methylate its substrate selectively. Moreover, corroborating single molecule FRET measurements were performed to understand the dynamic nature of these interactions and decipher states that the enzyme charters to achieve catalysis. The findings help in the identification of allosteric sites distal from the catalytic site of Erm which can serve as druggable targets.[2,3] Overall, we draw a holistic picture of Erm's action and delineate methods of curbing its pathogenic function.

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A Structure-Based Approach to Tackle Protein Aggregation in Parkinson's Disease

PS

Salvador Ventura

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a-Synuclein aggregation is a key driver of neurodegeneration in Parkinson's disease (PD). We have exploited the differential structural properties of toxic oligomers and amyloid fibrils to identify a family of peptides that bind to these a-synuclein species with low nanomolar affinity without interfering with the monomeric functional protein. This activity is translated into a remarkable anti-aggregation potency and the ability to abrogate oligomer-induced cell damage. With a structure-function relationship in hand, we identified human candidates expressed in the brain with remarkable binding, anti-aggregation, and detoxifying properties. The best in vitro candidate was administered to a model animal of Parkinson's disease, significantly alleviating the motor symptoms. Using a combination of structural methods, including cryo-EM, ssNMR, SAXS, XL-MS, and HXD-MS, we have been able to delineate the region of interaction between these peptides and aSyn in the context of oligomers. Likewise, we have shown that when this region of aSyn is deleted or mutated, the aggregation and toxicity of this protein are abolished. This provided the foundation for generating conformation-specific nanobodies and IgGs that, by targeting this critical region in the oligomers, abrogate aSyn amyloid formation. Overall, the novel chemical entities we present here constitute a new therapeutic paradigm in PD and are promising tools for diagnosing this devastating disease.

Martin Zacharias
Technical University of Munich, Physics Department and Center of Protein Assemblies, Germany

Modeling DNA Flexibility and its Role in Protein Binding

PS

Many cellular processes such as DNA replication, transcription and packing involve protein DNA binding. The sequence-dependent structure and deformability of DNA play major roles for binding of proteins and regulation of gene expression. So far, most efforts to model DNA flexibility are based on unimodal harmonic stiffness models at base-pair resolution. However, multimodal behavior due to distinct conformational substates also contributes significantly to the conformational flexibility of DNA, further complicated by correlations to nearest-neighbor substates. We solve this challenge by combining a multivariate harmonic approximation with an Ising model for the substates and demonstrate the performance of the model on applications to DNA fluctuations and protein–DNA complexes. Our approach offers a wide range of applications to determine sequence-dependent deformation energies of DNA and to investigate indirect readout contributions to protein–DNA binding and DNA repair. In a second part all-atom Molecular Dynamics and advanced sampling applications will be presented to follow a protein-DNA binding process and to investigate the role of peptide backbone conformational transitions for folding and binding.

Nunilo Cremades

Institute for Biocomputation and Physics of Complex Systems (BIFI) and Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Spain

Unveiling the Role of Network Stabilization in the Aging Dynamics of Biomolecular Condensates Linked to Neurodegenerative Diseases

PS

Emerging evidence supports that aberrant transitions of protein-rich biomolecular condensates into solid-like structures may play a critical role in the formation of toxic amyloid aggregates, which are a hallmark of several neurodegenerative diseases. Experimental studies have highlighted the flexible, typically disordered, and multivalent nature of the proteins in these liquid-like condensates. However, less is known about the specific molecular factors that govern the stabilization and eventual transition of these condensates into more solid-like states - a process commonly referred to as physical aging. Our lab has been investigating the electrostatic complex coacervation of alpha-synuclein,the protein linked to Parkinson´s disease, with positively charged polypeptides like Tau, which is associated with Alzheimer´s disease.[1] Our recent experimental findings reveal a system-dependent slow aging process associated with the stabilization of the electrostatic network within the condensates, which is very much in line with the theoretical model proposed by Ranganathan and Shakhnovich in 2020.[2] Our results highlight the role of network stabilization dynamics within biomolecular condensates formed by electrostatically-driven interactions on the formation of toxic protein aggregates.

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Protein Silencing with Self-Peptides

PS

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India

Designing functional molecules which can recognize and modify the activity of a specific protein is a frequently encountered challenge in biology and pharmaceutical chemistry, and requires major effort for each specific protein target. Here we demonstrate that 'self-peptides', parts of folded proteins which by their nature are recognizable by the rest of the protein [1], provide a generic route to developing such molecules. Such synthetic peptides with chemically pre-stabilized conformation can incorporate into the target protein during its folding, and can potentially displace its native counterpart to modify the protein conformation to cause functional deficits. This strategy is especially promising for proteins with ß- barrel topology, as the seam of the barrel provides a vulnerable target. We demonstrate this strategy using green fluorescent protein (EGFP) as a model, as its fluorescence is a direct reporter of its conformation and function. Refolding in presence of 33 μM of a disulphide-stabilized 22-residue self-peptide (which resembles a seam, strands 3&11, of the beta barrel) quenches the fluorescence by 97%. A peptide with the same composition but a different sequence is only 40% as effective, demonstrating that silencing is relatively specific. Fluorescence correlation spectroscopy and time resolved fluorescence lifetime measurements show that the peptide leads to near-complete long-term fluorescence silencing of the EGFP molecules it incorporates into. This result can be translated into a biological application if the self-peptide incorporates into a protein during its synthesis, before the nascent protein folds. We show that the self-peptide can indeed silence nascent sfGFP (closely related to EGFP) during its ribosomal synthesis in an in vitro translation system. Therefore, self-peptides present a potentially general strategy for developing protein-specific silencers for physiological applications.

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Christian Griesinger

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Protein Dynamics and Kinetics Studied by NMR Spectroscopy

PS

NMR spectroscopy is a powerful tool to study dynamics and kinetics of conformational ensembles. While pico-second to one digit nano-seconds are well covered by relaxation measurements and several 10 micro-seconds to millisecond by relaxation dispersion, relying on the variation of isotropic chemical shifts, the region between one digit nano-seconds and several 10 mikro-seconds is difficult to access. High power relaxation dispersion can assess the amount and kinetics of motion in this region. This will be discussed in the context of protein motion and protein/protein recognition[1] with approaches to get information about the region between ns and µs. The importance of optimal control pulses for high field NMR[2] of proteins will be emphasized. In the third project, we have studied the process of aggregation of α-synuclein on membranes in vitro and identified key time points in the aggregation process, that enable targeted isolation of a so called intermediate 1[3] and the fibrillar endpoint. Intermediate 1 has the functional characteristics of a toxic oligomer and the structure will be presented. In addition, we determined the structure of anle138b, a clinical drug candidate, bound to lipidic fibrils that are doped with anle138b as compared to a PET candidate. Comparison with binding of this molecule to lipidic Aβ fibrils will be discussed searching for commonalities.

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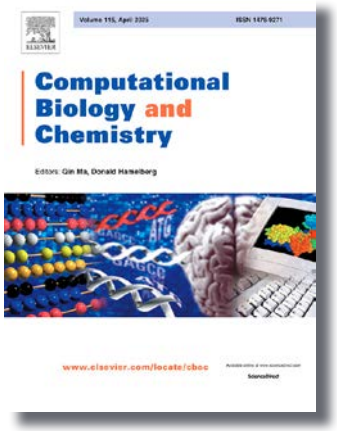

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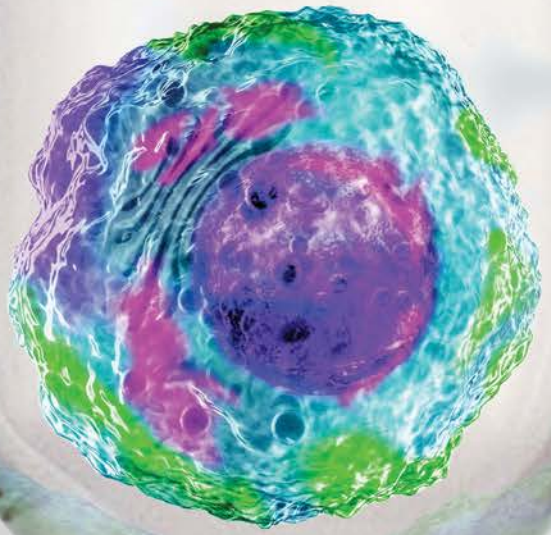

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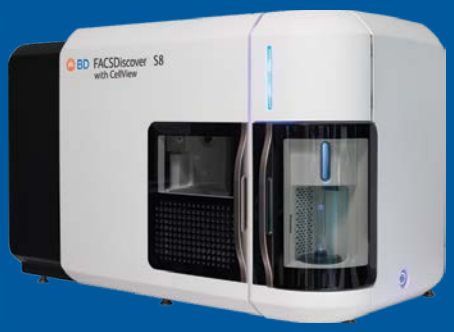

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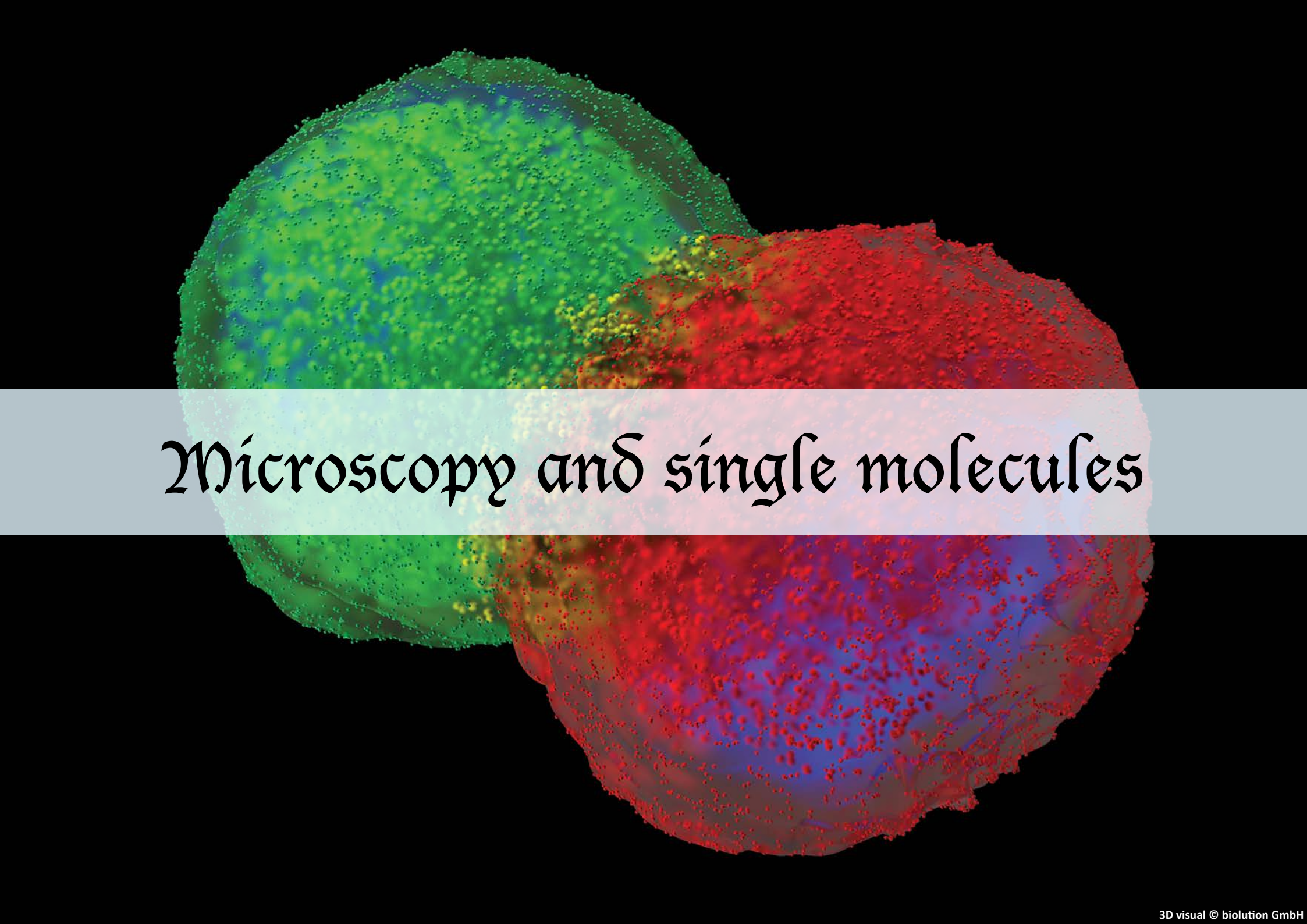
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Changes in Global RNA-Protein Metabolism During Ageing Cause Alteration in Stress Granule Properties

MSM

Compartmentalization plays a crucial role in orchestrating complex biochemical reactions within cells through the presence of both large membrane-bound organelles and smaller, dynamic nucleic acid-protein condensates. In higher eukaryotic cells, numerous RNA-binding proteins possess extensive disordered domains, rendering them susceptible to phase separation on interaction with RNA. RNA and RBPs make nuclear PML bodies, Cajal bodies, nuclear speckles and nucleolus; similarly, in the cytoplasm, P-bodies and Stress Granules (SG) are two important condensates. We hypothesised that altered physiological states like senescence and ageing would have implications on cellular organization and function of the condensates. We developed multiple cellular senescence and organismal ageing models to map the dynamics of translation inhibition-induced stress granules. Stress granules sequester RNA released from ribosomes during transient translation inhibition, which quickly dissolves once translation re-initiates. We found that stress granules formed faster and increased in number and enrichment but decreased in size during ageing and cellular senescence. Stress granules also persisted for longer after withdrawal of stress conditions in the aged condition the SGs persisted. I will present our recent results obtained using quantitative, high-resolution fluorescence microscopy, showing that the condensates are enriched for prion-like RBPs and are depleted in RNA, which may result in their increased viscosity and persistence. I will also present evidence about altered RNA and RBP metabolism, which leads to changes in the proportions of RNA and RBPs in condensates. Our findings will help delineate the contribution of RNA and RBPs towards the physical properties of condensates during altered metabolic states of senescence. This will also help in finding the implication of existing antiaging and novel therapeutics on intracellular condensates organization which can be used as biomarkers of cellular health in near future.

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Structural and Functional Insights into Cren7: A DNA-Bending Protein from Extremophiles

MSM

Crenarchaeal organisms inhabit extreme environments, which are characterized by high temperatures, hypersalinity, and hyperacidity. The proteins found in these organisms exhibit exceptional structural stability, and this can be attributed to their unique amino acid compositions. Cren7, a DNA-bending protein found in these organisms, possesses a β-pleated structure with extended loops. UV melting assays demonstrated that when Cren7 is bound to DNA, it enhances its thermal stability, preventing denaturation. The bound complex is stabilised through both, electrostatic and non-electrostatic interactions. Fluorescence-based kinetic studies show that a solvent-exposed Trp26 (W26) plays an important role in stabilizing the above complex under hypersaline conditions. Cren7 preferentially binds AT-rich DNA with micromolar affinity. Circular dichroism (CD) spectroscopy demonstrates that DNA experiences a cooperative structural alteration upon binding to Cren7. But the Cren7 itself remains structurally intact. In the complex, the electrostatic interactions are primarily mediated by Lys31 (K31) and Lys27 (K27). They are essential for DNA binding and the stability of complexes, as demonstrated by Biolayer interferometry and fluorescence anisotropy analyses. The hydrophobic core of Cren7 reinforces its structure through π-stacking interactions, with Tyr58 (Y58) serving as a gatekeeper. Alanine mutagenesis confirms that core residues are essential for orienting Trp26 (W26). These findings underscore the essential role of Cren7's core in DNA binding and bending, which enhances its stability and functionality in extremophilic adaptation.

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Insights into the Malarial Parasite Cytoskeleton using Cryo-Electron Microscopy

MSM

Plasmodium falciparum, the causative agent of malaria, undergoes a series of transformations as part of its life cycle in two hosts, humans and mosquitos. The cellular integrity of the parasite during these changes is largely maintained by the underlying cytoskeleton. The cytoskeleton, which is made up of actin, microtubules, and intermediate filaments controls the size and shape of all eukaryotic cells. The filaments assembled from building blocks of protein are bound by multiple factors which regulate their dynamics. Studying the cytoskeletal system in living cells is challenging due to its indispensable and complex nature. In our study, we took a bottom-up approach involving in vitro reconstitution and ultrastructural studies to investigate various aspects of filament organisation. Using cryo-electron microscopy, we determined the three-dimensional structures of *P. falciparum* microtubules polymerised in different states from parasite-isolated tubulin building blocks. We observed parasite-specific variations in the tubulin structure that explain its susceptibility to particular drugs. Examination of the *P. falciparum* microtubule lattice contacts revealed smaller but energetically more favourable lateral interfaces. Further, molecular dynamics simulations and cryo-electron tomography showed that the protofilaments in *P. falciparum* microtubules are stiffer than their mammalian counterparts. Our findings thus demonstrate that, despite the high degree of tubulin conservation, distinct parasite cytoskeletal architectures emerge from small changes in tubulin dimer structure and lattice contacts because of the polymeric nature of microtubules. These microtubules form an integral part of the adaptable cytoskeleton critical for parasite survival.

Shubhasis Haldar

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

MSM

Chaperones are vital custodians of cellular proteostasis, traditionally celebrated for their holdase and foldase roles in protein folding. Our research unveils a groundbreaking transformation in chaperone functionality under mechanical force, challenging and expanding our current understanding of protein folding within the cell. Leveraging the unprecedented resolution of covalent magnetic tweezers (CMT), we examined the mechanical behavior of tunnel-associated chaperones such as Trigger Factor (TF) and DsbA, revealing an unexpected phenomenon: these chaperones, long associated with passive substrate stabilization, actively accelerate protein folding under applied mechanical force. This represents a fundamental departure from their canonical roles in force-free conditions. Even more striking are the force-responsive dynamics of BiP and ERdj3, two endoplasmic reticulum (ER) chaperones. In quiescent states, these chaperones exhibit classic holdase activity. However, under mechanical force, they transition into potent foldases, a transformation that starkly contrasts with their cytoplasmic counterparts, DnaK and DnaJ, which remain inert to force. Remarkably, BiP and ERdj3 channel mechanical energy to deliver up to 54 zJ, enhancing the force transmission along the Sec61 translocon tunnel and stabilizing folding intermediates. Our investigations reveal that this force-driven functional switch is governed by strain energy dynamics. Unlike conventional unfoldases, which accumulate strain energy with unfolded substrates, BiP and ERdj3 exhibit a unique affinity for folded substrates, leveraging heightened strain energy to stabilize them in a force-dependent manner. This strain-energy-based feedback mechanism establishes a new paradigm in protein folding: one where mechanical cues, rather than simply challenging proteostasis, actively enhance it. These findings, achieved through the advanced capabilities of CMT, highlight an unexplored layer of cellular mechanics. By exposing the dynamic interplay between chaperones and mechanical forces, our study redefines the functional landscape of protein folding under physiological constraints. This paradigm shift not only deepens our understanding of intracellular proteostasis but also unlocks new possibilities for biotechnological innovation and therapeutic intervention. Covalent magnetic tweezers thus emerge as a transformative tool for probing biological mechanics at an unprecedented resolution, offering an unparalleled opportunity to elucidate the molecular dance of life under force.

Replicating Active Transport of Micro-Organisms in Synthetic Systems

MSM

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The utilization of the self-propelling capabilities of microorganisms as "smart" drug delivery systems represents a promising frontier in targeted therapeutics, with potential applications in tumour-specific drug delivery, traversal of complex biological barriers, and precision medicine. Despite these advantages, the approach is accompanied by inherent biological risks and translational challenges. To address these limitations, we explore the development of artificial, biocompatible, cell-like microorganisms with active transport mechanisms designed to mimic natural motility while minimizing immunogenic risks. This work outlines the de novo design principles underlying the creation of these autonomous systems, capable of both rotational and translational movement. Furthermore, we evaluate their efficacy as drug delivery agents, emphasizing their potential to enhance targeting precision and therapeutic outcomes in clinical settings.

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Sequence-Dependent Phase Separation by Mycobacterium Nucleoid-Associated Protein Lsr2 Mediates DNA Compaction

MSM

The xenogeneic silencer protein Lsr2 from *Mycobacterium tuberculosis* (Mtb) plays a critical role in its survival and pathogenesis. Lsr2 is a nucleoid-associated protein that interacts with DNA in vivo and regulates many genes. However, the physical mechanism underlying Lsr2-mediated DNA compaction, resulting in gene regulation, remains elusive. We employed a combination of biochemical assay, single-molecule imaging, in situ Lsr2 imaging inside Mtb, and molecular dynamics simulations to investigate the governing principles of Lsr2-mediated DNA compaction. We show that Lsr2 undergoes phase separation and addition of DNA substantially lowers the required concentration for its phase separation. Strikingly, our single-molecule and simulation data establish that Lsr2 forms condensates with long stretches of AT-rich DNA, providing strong evidence for sequence-dependent co-condensation. Up on tagging Lsr2 with green fluorescent protein, we observe punctate structures inside Mtb cells confirming the phase-separation of Lsr2 in vivo. This observation is contrary to the classical view of sequence-dependent binding of individual protein molecules to DNA, our findings rather suggest that protein-DNA co-condensates 'sense' the average binding energy landscape. We present a physical model for Lsr2-mediated DNA compaction and gene regulation, describing a novel mechanism for NAP-mediated genome organization in bacteria.

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Determination of Thermally Induced Cell Membrane Slope Fluctuations using Rotational Optical Tweezers

MSM

A rigid body can have 3 degrees of rotational freedom. Among these, in optical tweezers community, the yaw or in-plane degree of rotational freedom has been explored extensively. However, the out of plane or the pitch or roll degrees of freedom have not been explored much. It is here that we show a technique to measure the out of plane or pitch rotational motion to high resolution in optical tweezers. We use this to measure the cell membrane slope fluctuations to find properties like bending rigidity to higher accuracy than conventional techniques. We shall also show how this bending rigidity changes while the we induce hypertension to the cell, and also what kind of drugs recover from hypertension. Thus this approach can also be used for drug discovery.

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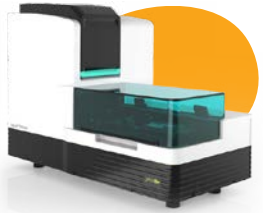

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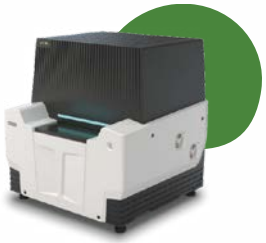

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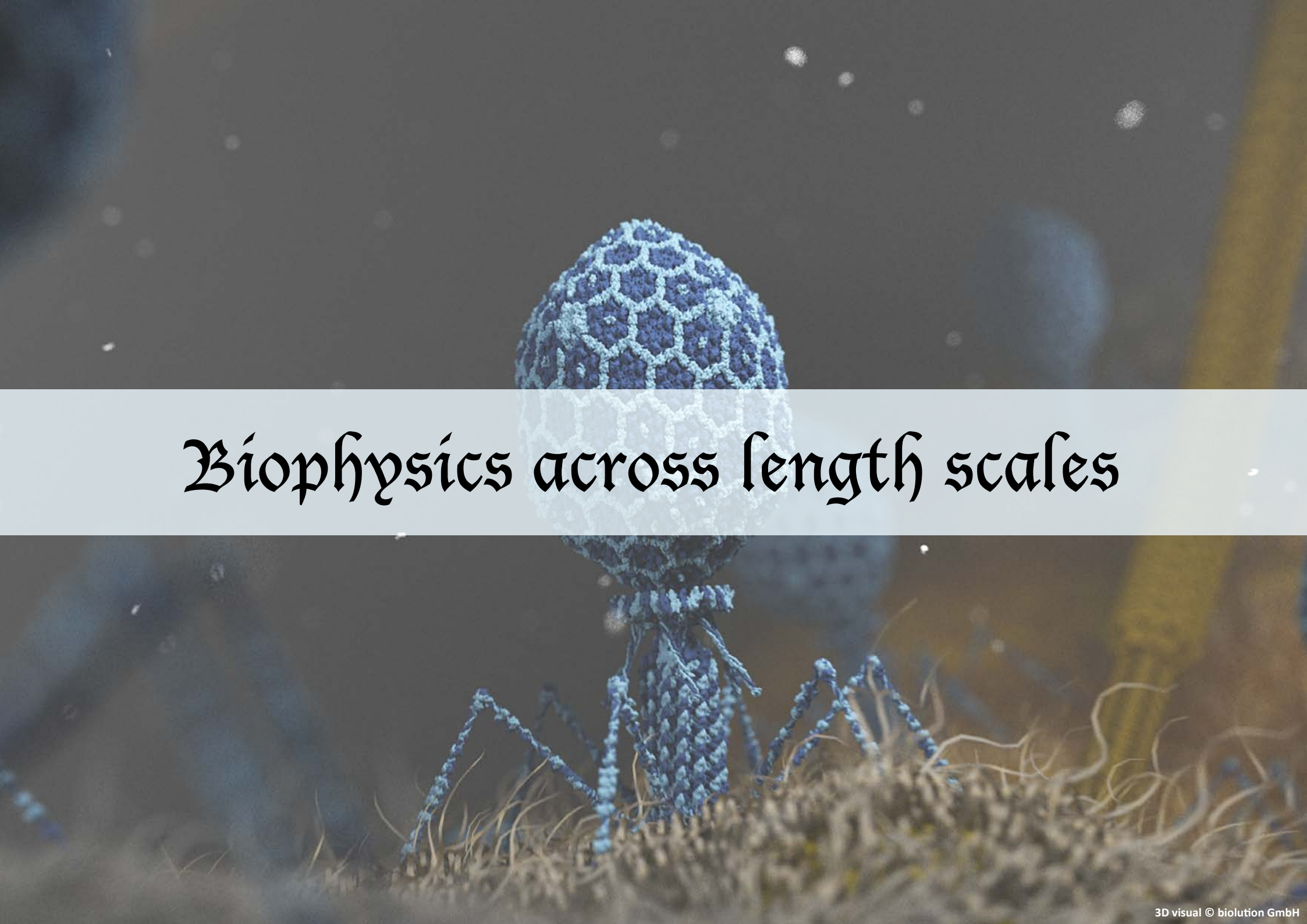
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Aravind Penmatsa

Molecular Biophysics Unit,
Indian Institute of Science

Insights into the architecture and channel properties of Pannexins

BLS

ATP generally considered the energy currency of the cell has the ability to act as an extracellular signal through purinergic receptors. ATP is released from cells through the action of large pore ion channels like pannexins that exist in three isoforms. The talk would focus on the organization of the pannexins isoforms that resemble gap junction channels but remain as hemi channels. I will focus on our work on pannexin 1 and pannexin 3 isoforms which have broad similarities but distinct structural and functional di erences. A particular difference is a second constriction site in pannexin 3 and the di erences in its pore lining residues. A germ line mutant of pannexin 1 was also explored that suggested an allosteric e ect of the mutant in closing the pore for ATP release through a long range conformational shift. The talk would delve into the structural, functional and pharmacological di erences in pannexin isoforms particularly in their architecture and pore-lining residues.

Role of Metastable Conformational States in Proteins: From Function to Drug Discovery

BLS

Suman Chakrabarty

Department of Chemical and Biological Sciences
S. N. Bose National Centre for Basic Sciences, Kolkata

Structural biology of proteins is dominated by a native structure-centric view. However, biomolecular functions are intimately connected to protein motion/dynamics and often low-lying metastable or "excited" conformational states play an important role.[1,2] In this talk, we shall discuss several such examples based on large-scale classical molecular dynamics (MD) simulations. We shall demonstrate how conformational plasticity and dynamics can be crucial to the function. Finally, we shall discuss our ideas on identifying allosteric hotspots on the protein surface to modulate therapeutically important protein-protein interactions (PPI) as a promising alternative strategy in computer-aided discovery of allosteric inhibitors.[3]

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Tarak Karmakar

Department of Chemistry,
Indian Institute of Technology,
Delhi, New Delhi

Thermodynamics and Kinetics of Biomolecular Recognition

BLS

Biomolecular recognition processes, like the binding of small molecules to proteins and nu cleic acids, protein-protein and protein-nucleic acid associations, occur over milliseconds to seconds timescales, posing a challenge for brute-force molecular dynamics simulations in capturing their thermodynamics and kinetics. Enhanced sampling (ES) simulations, particularly collective-variable (CV)-based methods such as umbrella sampling and metadynamics, alleviate this limitation by enabling efficient sampling. A crucial aspect of CV-based ES methods is selecting appropriate CVs that distinguish between the system's metastable states, such as bound and unbound states in biomolecular recognition. A bias, based on these CVs, is introduced into the system's Hamiltonian to enhance CV fluctuations, improving sampling efficiency. However, identifying CVs for biomolecular systems is complex, often requiring a large number of variables to describe the system's states, making the use of such large dimensional CVs in ES methods impractical. To tackle this, deep learning methods are employed to non-linearly combine a large number of descriptors, typically pairwise contacts between host and guest atoms, to create effective CVs. These CVs are then used for ES simulations to study peptide-RNA binding, small RNA-protein binding, and small molecule DNA binding and calculate the free energy landscapes for the host-guest binding process. Moreover, infrequent metadynamics simulations enable the calculation of binding kinetics, with rate constants showing excellent agreement with spectroscopic measurements.

M Hamsa Priya

Indian Institute of Technology, Madras

Self-Assembly Pathway, Energetics and Morphology of Peptide Nanostructures

BLS

Amphiphilic peptides self-assemble to form ordered nanostructures that can be potentially employed as drug delivery systems. The kinetics of self-assembly, morphology of the nanostructure and their stability depends on the peptide length and sequence. Using an amphiphilic tetrameric peptide comprising 3 contiguous stretch of hydrophobic residues and 1 hydrophilic residue at an end as the model system, we have identified that the hexagonal packing of peptides in anti-parallel orientation stabilizes peptide assembly in membrane like lamellar structure. Our results agree well with the signature features of the reported XRD pattern. Peptide lamellar structures, unlike lipid membranes, are stabilized by hydrogen bonds. Hydrophobic residues that form the core of the membrane, therefore, assume extended β-conformation to maximize the inter-peptide hydrogen bonding, while hydrophilic residues at the membrane surface exhibit high conformational flexibility to enrich peptide-solvent interactions. The potential of mean force for bringing two peptides in β-conformation, however, is highly unfavorable owing to the entropic restriction on the interstitial water. The translational entropy of interstitial water molecules is highly compromised as they need to act like a hydrogen bond bridge between the peptides. Any two peptides, therefore, tend to self-assemble as polyproline helix, a dominant conformation in dilute solution, and slowly extend to beta form only in their close proximity. The transition network of peptide conformations in dilute solutions and the peptide clustering during their self-assembly in concentrated solutions have been analyzed using graph network and machine learning techniques.

Anup Padmanabhan

Trivedi School of Biosciences,
Ashoka University

F-Actin Architecture in the Cytokinetic Ring Restricts E-Cadherin Mobility at Furrow Zone During Cell Division

BLS

To maintain tissue integrity, cell division in many tissue types must ensure that the inter cellular adhesion is not compromised. This results in an interesting cellular conundrum. While the division apparatus is trying to pull the furrow inwards, the cell adhesion proteins on the surface are engaged with receptors presented on neighboring cells, e ectively resisting the inward pull of cytokinetic furrow. A 'tug of war' model is an attractive idea where the cytokinesis proceeds when the contractile force of furrow exceeds that of the inter cellular adhesion. We think there is more to it. Our work on cytokinesis in the *C. elegans* zygote uncovered an additional mechanism, that the cells might be employing in resolving this conundrum. Specifically, we wanted to know -

(1) how cell adhesion receptors modulate cytokinesis dynamics, and,

(2) how cells manage their adhesion receptors during cell division.

We observed that, cell adhesion receptors slowed down the rate of cytokinesis, by associating with actin cytoskeleton and reducing the motor activity of myosin at the cell surface. This mode of regulation is independent of its cell-adhesion function. We also discovered that the actin architecture at the division site restricts mobility of cell adhesion receptors, thereby excluding cell adhesion activity at the site of division. Taken together, we believe, we have discovered a rather fascinating phenomenon of reciprocal antagonism between the cell division and the cell adhesion processes.

Tapomoy Bhattacharjee

National Centre for Biological Sciences
Tata Institute of Fundamental Research, Bangalore, India

Physical Confinement Selectively Favours Bacterial Growth Based on Cell Shape

BLS

How are bacterial communities altered by changes in their microenvironment? Evidence from homogeneous liquid or flat plate cultures implicates biochemical cues — such as variation in nutrient composition, response to chemoattractants and toxins, and inter-species signaling — as the primary modes of bacterial interaction with their microenvironment. However, these systems fail to capture the effect of physical confinement on bacteria in their natural habitats. Bacterial niches like the pores of soil, mucus, and infected tissues are disordered microenvironments with material properties defined by their internal pore sizes and shear moduli. Here, using three-dimensional matrices that match the viscoelastic properties of gut mucus, we test how altering the physical properties of their microenvironment influences bacterial growth under confinement. We find that low aspect-ratio bacteria form compact, spherical colonies under confinement while high aspect-ratio bacteria push their progenies further outwards to create elongated colonies with a higher surface area, enabling increased access to nutrients. As a result, the population level growth of high aspect-ratio bacteria is more robust to increased physical confinement compared to that of low aspect-ratio bacteria. Thus, our results capture the first experimental evidence that physical constraints play a selective role in bacterial growth based on cell shape

Ranjith Padinhateeri

Indian Institute of Technology Bombay

Polymer Simulation Study of the Spatiotemporal Organization of Chromatin at the Gene-Length Scale

BLS

We present polymer models to understand the spatiotemporal organization of chromatin. We derive chromatin polymer properties at different scales by simulating chromatin at nucleosome resolution. We also examine how loop extrusion parameters influence time-dependent changes in 3D organization.

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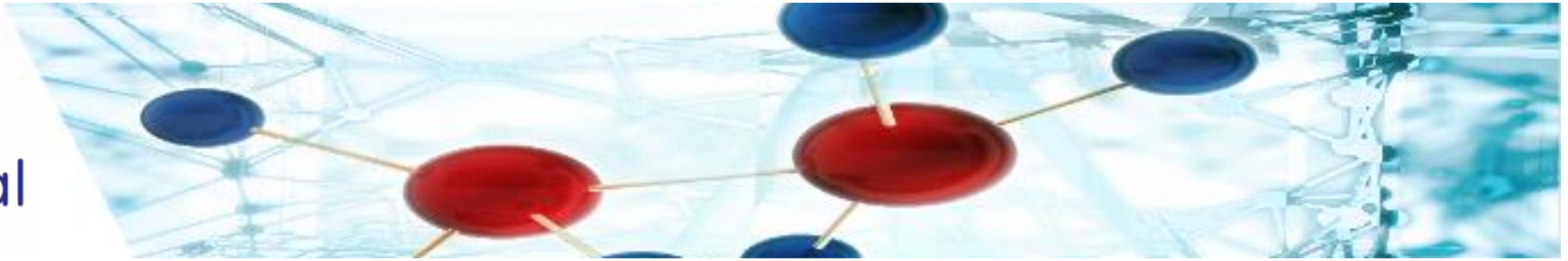

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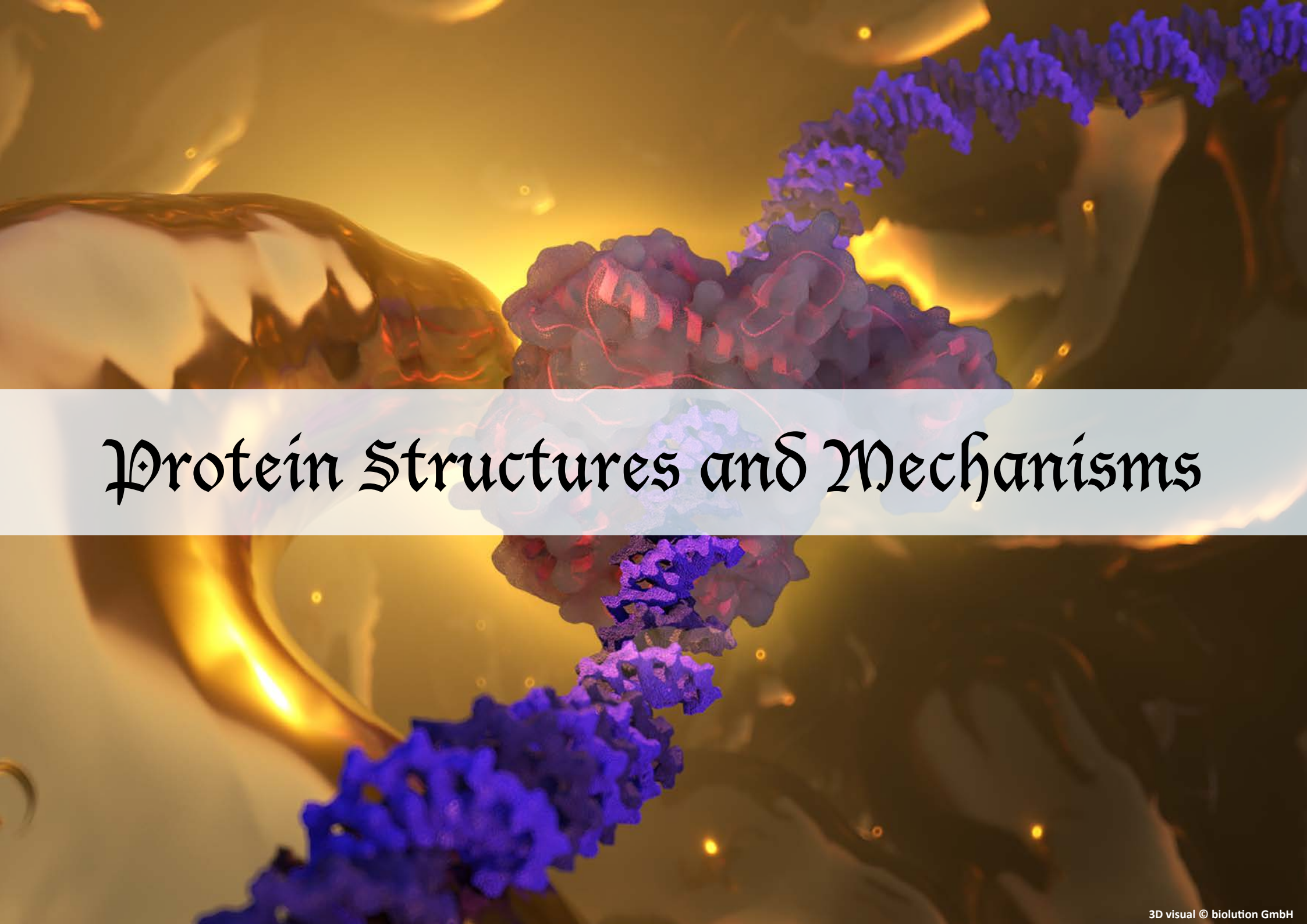
Protein Structures and Mechanisms
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Swagata Ghatak

School of Biological Sciences, National Institute of Science Education and Research (NISER), Bhubaneswar

Ion Channel–Mediated Regulation of Excitatory/inhibitory (E/I) Imbalance in Alzheimer's Disease

PSM

Excitatory/inhibitory (E/I) balance, defined as the balance between excitation and inhibiton in a neuronal network, is required for normal functioning of the brain. In early stages of Alzheimer's Disease (AD), this fine balance is perturbed leading to excessive excitation relative to inhibition reflected in network hyperexcitability. This hyperexcitability precedes neurodegeneration and further cognitive decline. Using electrophysiological and cell biological techniques, we discovered that both active and passive mechanisms acting at the synaptic and neural network level cause the aberrant hypersynchronous activity in hiPSC-derived neuronal models of AD. Among the various ion channels involved in the disease, we found that extrasynaptic NMDA receptors contribute to neuronal hyperexcitability such that their inhibition leads to a decrease in the aberrant neuronal network activity. On the other hand, two pore domain leak potassium channels (K2P) like TREK1 and TASK3 which are known to set the resting membrane potential of neurons, renormalize the aberrant neuronal activity. We observed that the expression of both TREK1 and TASK3 are upregulated by amyloid-β mediated hyperexcitability via independent mechanisms. This upregulation of K2P is also observed in various trangenic AD murine models and the increased expression is important for decreasing the excess neuronal calcium transients. This study emphasizes the role of ion channels in regulating aberrant activity in AD and suggests a possible neuronal survival mechanism in the early stages of the disease.

Hema Chandra Kotamarthi

Department of Chemistry, IIT Madras

Fate of Knotted Proteins During Directed Degradation and Constrained Folding Conditions

PSM

Knotted proteins are a special class that challenges 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 poorly 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 and degradation of two knotted proteins with 41 and 52 topologies. Degradation of these knotted proteins by ATP dependent proteases ClpXP and ClpAP show that the knots do not provide any considerable mechanical stability against mechanical degradation. The ability to degrade the knotted proteins varies based on the translocating mechanisms of the protease, as was observed in the difference in degradation by ClpXP and ClpAP. Further, we have probed the folding/unfolding of the knotted proteins under constrained conditions where their termini are hindered. Our SM-AFM-based studies revealed that many of these molecules fold into their knotted conformation despite constrained ends. These results support a hybrid model for knot formation in which loop twisting occurs during co-translational folding.

Sulakshana P. Mukherjee

Department of Chemical Sciences, Indian Institute of Science Education and Research Berhampur

A Plausible Mechanism of Transcription activation Of a subset of NF-kappaB Repressed IRF3 Target Genes

PSM

The Nuclear Factor-kappaB (NF-κB) and the Interferon (IFN) signaling pathways involve the NF-κB and the Interferon Regulatory Factor (IRF) families of transcription factor (TF) proteins, respectively. These TFs are critical for immune response in the vertebrates. The members of both the TF families function as dimers and binds to their respective cognate DNA sequence/s on the promotor/enhancer region of their target genes to regulate their transcription. While NF-κB members recognize the 9-11 bp κB DNA sequence the IRF proteins have A-rich Interferon Regulatory Element (IRE) as their cognate sequence. p50 and RelA are two of the five NF-κB family members that form p50-p50, RelA-RelA and RelA-p50 dimers of which RelA-p50 and RelA-RelA can activate transcription through the acidic transcription activation domain (TAD) on RelA. Due to absence of the TAD, p50-p50 homodimer can bind to the κB DNA sites but cannot activate gene transcription. Thus, p50 p50 homodimer can function as transcription repressor as physiologically observed in the case of a sub-set of IRF3 target genes[1]. The sub-set of p50 repressed NF-κB target genes share a special DNA sequence at their promoter site called the G-rich IRElement (G-IRE) which is a combination of a half κB and an IRE sequence recognized by the NF-κB subunits and the IRF3 protein, respectively. In my talk, I will discuss a plausible mechanism for activation of IRF3 target genes that are maintained in a repressed state by the NF-κB p50 subunit.

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Subhabrata Majumder

Saha Institute of Nuclear Physics, Kolkata

Profiling Functional Dynamics of High Molecular Weight Proteins using Solution NMR based Methods at Natural Abundance

PSM

Nuclear Magnetic Resonance (NMR) spectroscopy is an important tool to probe structure and dynamics of proteins, in solution, at an atomistic level. However increase in molecular weight leads to adverse transverse relaxation leading to low sensitivity in NMR spectra, thereby limiting the scope of NMR for high molecular weight proteins. In our work, we have applied solution NMR based methodology in case of L-asparaginase (MW ~ 135KDa), at natural abundance. Despite the spectral sparseness, our methodology reveals the dynamic basis of the enzyme function. The methodology has been extended for intact monoclonal antibody (MW ~ 150kDa). The talk will demonstrate the utility of the methodology for probing dynamic basis of protein-function using specific case studies.

Utilising Simple Building Blocks for Complex Catalytic Functions

PSM

Dibyendu Das

Dept of Chemical Sciences and CAFM,
IISER Kolkata, India

Significant gaps remain in our understanding of how functional biopolymers and the complex reaction networks underlying metabolic activities emerged during the origins of Earth's biosphere. In this context, extant proteins, evolved over millions of years, carry out an impressive array of responsibilities, from catalysis and molecular recognition to the out-of-equilibrium work such as motility and load bearing. One of the goals of our lab is to investigate the possible origins of such advanced enzymatic functions from simple building blocks and short peptides.[1-3] Via my talk, I will show our attempts to develop simple chemical-based systems that can demonstrate advance enzyme like traits. Further, I will show how simple chemicals can be driven to access higher energy self-assembled states, just as seen in natural microtubules.

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Shachi Gosavi

National Centre for Biological Sciences, TIFR

Are the Transmembrane Domains of Class I Viral Fusion Proteins Passive Anchors or do they Enable Viral Fusion?

PSM

The homotrimeric class I fusion proteins (CI-FPs) enable the fusion of viral envelopes with host cell membranes. The large ectomembrane domains of CI-FPs are anchored to the viral envelope through the transmembrane domain (TMD) which is a single-pass transmembrane helix homotrimer. I will talk about some recent and ongoing work where we investigate the dynamics and self-assembly of the TMD of the SARS-CoV-2 CI-FP, spike, using multiscale MD simulations in order to understand the possible functional role of these dynamics.

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Glycans as Attachment Factor of SARS-CoV-2

PSM

Nagma Parveen

Department of Chemistry, Indian Institute of Technology Kanpur

Viruses utilize cell surface glycans and plasma membrane receptors to attain adequate attachment strength for initiating cellular entry. Virus particles engage in multivalent binding with receptors and attachment factors on the cell surface. Such multivalent binding leads to an increased residence time and reduced detachment of the virus particles bound to lipid membranes. I will discuss the membrane attachment of intact particles of SARS- CoV-2. The majority of literature reports demonstrate the binding of the S protein of the virus to ACE2 receptor which is weakly expressed in alveolar cells of the lower lungs. Also, there is a decreased gradient of ACE2 from nasal epithelial cells to bronchial airways epithelial and alveolar cells. This led to the speculation that the viral infection cannot be directly correlated to the ACE2 expression level and that other cellular attachment factors are required for efficient infection of the lower lungs. We have reported that SARS-CoV-2 particles bind to gangliosides embedded in the membrane and even the gangliosides lead to a synergistic binding of the virus to endogenous ACE2 receptor. The data confirm that the terminal sialic acid group (s) of the gangliosides interacts with the spike protein of the virus. Even different variants of the virus (variants of concern, VOC), such as Delta, Omicron etc. show binding specificity to the gangliosides. Based on these binding data, we are aiming to design glycan-rich inhibitors against the VOC.

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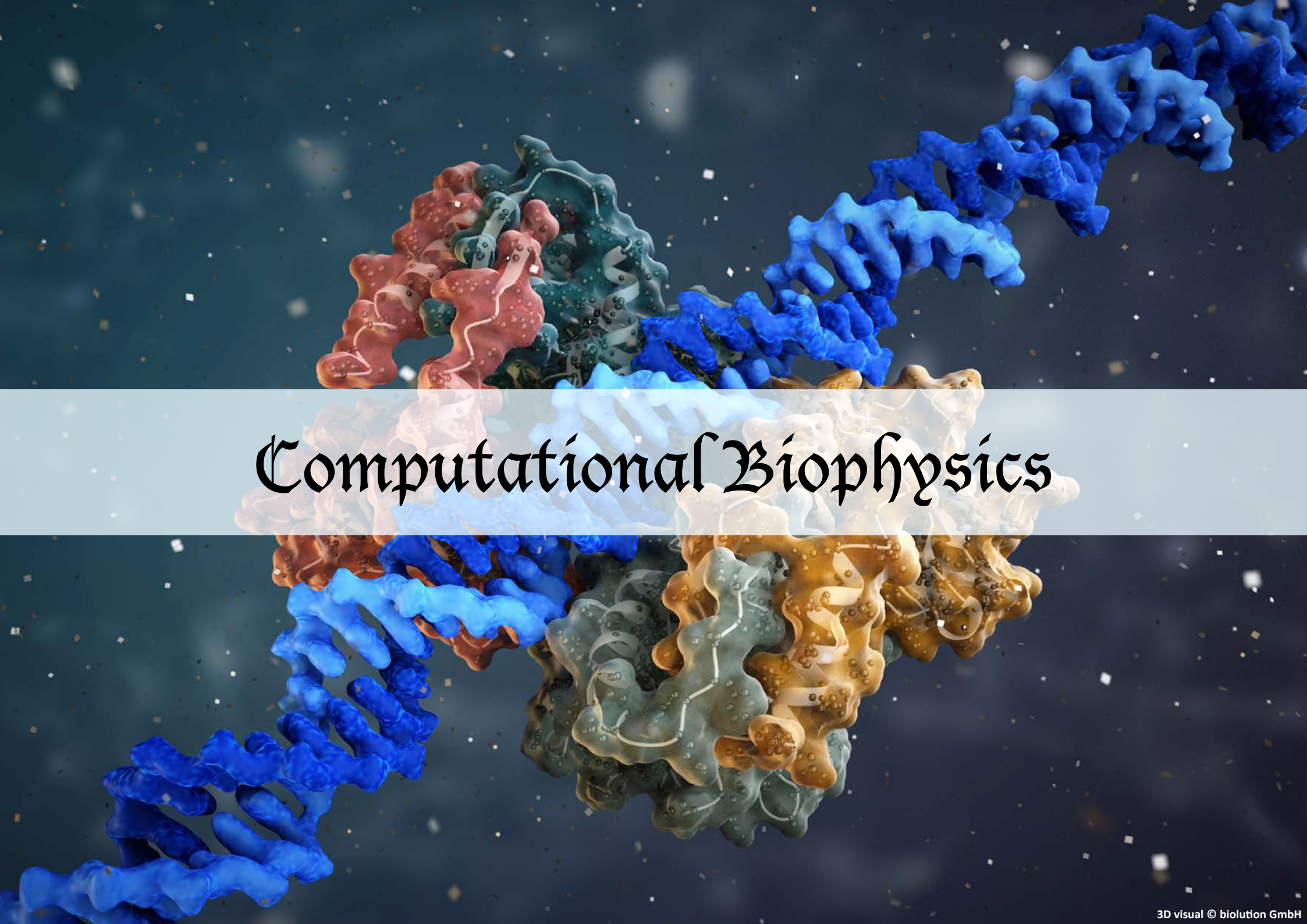
Computational Biophysics
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Arnab Bhattacherjee

School of Computational and Integrative Sciences
Jawaharlal Nehru University, New Delhi

Uncovering High-Resolution Organization of Genomic Loci via Multiscale Polymer Simulations

CB

Higher eukaryote genomes are organized in a multi-scale hierarchy, with nucleosomes forming the fundamental units of chromatin. Nucleosome positioning plays a crucial role in regulating gene accessibility and expression. To better understand this relationship, we developed a simple polymer model capable of reconstructing chromatin structural ensembles from low-resolution contact data and MNase-derived nucleosome positioning information. We applied this model to three human genomic loci: the active Nanog locus and the inactive HoxB4 and HoxA1 loci. Our analysis revealed distinct structural features associated with active and inactive chromatin states. These findings provide crucial insights into the link between genomic organization and transcriptional activity, offering a promising approach to understanding the structure-function relationship of genomes and their implications for developmental disorders and gene misregulation-related diseases.

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Debayan Chakraborty

The Institute of Mathematical Sciences

Exploring the Energy Landscapes of Intrinsically Disordered Proteins using Coarse-Grained Simulations

CB

Unlike folded proteins, intrinsically disordered proteins (IDPs) do not have a well-defined three-dimensional structure, challenging the conventional structure-function paradigm. Recent studies reveal that IDPs constitute about 40% of the human proteome and carry out a diverse range of functions, ranging from participation in cell signaling pathways to transcription regulation. However, computer simulations of IDPs remains challenging. Despite some successes, existing all-atom force fields often fail to accurately reproduce experimental observations and provide a balanced description of IDP conformational ensembles. We illustrate how coarse-graining based on polymer-physics models provides a viable route for exploring the energy landscapes of IDPs. Our work also sets the stage for probing interactions of IDPs with one another, or with binding partners, such as RNA, which are critical for understanding the assembly mechanisms of biomolecular condensates.

Rati Sharma

Dept. of Chemistry, IISER Bhopal, India

Decoding Thermosensation and Associated Response Mechanisms in *Caenorhabditis elegans*

CB

All living organisms need to respond to changing environmental signals for their survival. Therefore, organisms have developed elaborate intracellular and intercellular signaling mechanisms to respond appropriately to environmental changes.[1,2] In this talk, I will focus on our study of signal-response mechanisms associated with sensing temperature changes using the roundworm, *C. elegans*, as a model system. In the first part of the talk, I will discuss a theoretical model that helps in understanding how temperature fluctuations are relayed across the thermosensory AFD neuron through the cGMP pathway leading to changes in Calcium response dynamics.[3] In the second part of my talk, I will discuss our experimental study where we look into how the heat shock response dynamics is affected by the AFD neuron specific receptor guanylate cyclases (rGCs). Through behavioral assays and molecular biology studies at the mRNA and protein levels, we find that the rGCs in the neuron play a very important role in upregulating the small heat shock proteins present in the other cells during high temperature (>30 deg celcius) conditions.[4]

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Shruthi Viswanath

NCBS, TIFR, India

Recent Developments in Integrative Structural Modeling of Macromolecular Assemblies

CB

Integrative structure determination allows us to determine the structures of large macromolecular assemblies by combining data from complementary experimental methods with physical principles, statistical inference, and prior models. The advantage of the approach is that the accuracy, precision, completeness, and efficiency of structure determination are maximized by using all available information. In our group, we applied these methods to characterize chromatin remodeling assemblies, assemblies at cell-cell junctions, and mitochondrial complexes. Usually, these exercises involve integrating data from X-ray crystallography, NMR, cryo-electron microscopy, cross-linking mass spectrometry (XLMS), biochemical binding assays, AlphaFold, and physical principles. Two common themes emerged across multiple studies, motivating us to pursue method development in these areas. One, often, disordered regions (IDRs) of proteins in such assemblies are challenging to localize, since they are usually associated with sparse structural as well as other interaction data. We developed Disobind, a deep-learning method to predict binding regions for IDRs in large assemblies. Two, often these systems are associated with tomograms or low-resolution EM maps. Methods to better localize and identify macromolecules in tomograms, and combine them with other information for integrative modeling, are needed.

Deciphering Thermostability and Structural Dynamics in Carbohydrate Active Enzymes

CB

Ragothaman M. Yennamali

Department of Bioinformatics, School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur

Understanding the structural and dynamic determinants of enzyme function is fundamental for enzymes that function under extreme conditions. Using an integrative approach combining sequence-structure analysis, molecular dynamics simulations, and machine learning, we explore the biophysical principles governing thermostability and substrate specificity in two carbohydrate-active enzyme families: Endoglucanases and Lytic Polysaccharide Monooxygenases (LPMOs). Our comparative analysis of thermophilic and mesophilic endoglucanases reveals that thermostability arises from fold-dependent amino acid compositions, altered intramolecular interactions, and global dynamic behavior. Also, a single mutation in Trichoderma reesei endoglucanase induced thermostability through alosteric rigidity, demonstrating that long-range interactions can significantly impact enzyme stability. Structural dynamics studies on endoglucanases using elastic network models further highlight distinct slow-mode motions that influence catalytic residues. In LPMOs, coarse-grained and all-atom molecular simulations reveal family-specific flexibility in substrate-binding regions, with dynamic loop regions playing a critical role in function. Frustration analysis indicates that local energetic variations modulate enzymatic activity, particularly in substrate recognition and binding. Additionally, machine learning-based classification of LPMOs using sequence and structural features improved sequence-based functional annotation, outperforming traditional methods. By looking at sequence and structural dynamics, we highlight basic biophysical understanding of enzyme stability, flexibility, and function. We demonstrate how computational methods can uncover fundamental biophysical principles governing protein behavior of endoglucanases and LPMOs.

Rajarshi Chakrabarti

Department of Chemistry,
Indian Institute of Technology
Bombay, Powai

Dynamics of Active Tracer Particles in Complex Environments: Insights from Computer simulations

CB

The dynamics of tracer particles in crowded and complex environments, such as biological hydrogels, polymer matrices, and porous media, is a subject of fundamental interest in many fields including biophysics, materials science, and medical engineering. These tracers can be passive, such as colloids, or active (self-propelled), for example, synthetic nanomotors or bacteria. Biological gels serve as a barrier against pathogenic microorganisms and invaders, and their transport properties are determined by the shape and size of the microorganism as well as the pore size of the biological gels. The mechanical and chemical properties of the gels, as well as the interactions of the microbial with the mucin membrane (biopolymer matrix), influence the transport phenomena. Therefore, it is important to develop a nanocarrier with high permeability in cross-linked networks. We use computer simulations to elucidate the effect of active forces, mesh-to-tracer size ratio, tracer–polymer interaction, and network stiffness. Our results show that the activity helps the tracer to escape from polymer-network meshes resulting in a transition from short-time subdiffusion to intermediate-time superdiffusion. There is a competition between the suppressed motion of the tracer inside a mesh and its tendency to escape from that mesh due to self-propulsion. Interestingly, when the activity is high, the particle always undergoes superdiffusive dynamics at an intermediate time, while for a weakly active tracer, a short-time subdiffusion emerges before it becomes superdiffusive. Our analyses of the self-part of the van-Hove correlation functions of the tracers show that the functions become broader and non-Gaussian with activity.

Allosteric Regulation of β-Arrestin 1 and 2 Effector Specificity by GPCR Phosphorylations

CB

Rajesh K Murarka

Department of Chemistry, Indian Institute of Science Education and Research Bhopal

β-arrestin (βarr) isoforms βarr1 and βarr2 are key transducers of signals from G protein-coupled receptors (GPCRs) at the plasma membrane, orchestrating both overlapping and distinct intracellular signaling pathways. The functions of βarr1 and βarr2 are determined by phosphorylation patterns on the receptor's C-terminal tail (Rp-tail), introduced by GPCR kinases (GRKs), which regulate their interactions with downstream effectors, though the underlying molecular mechanisms remain unclear. In this study, we employed an integrated approach combining all-atom molecular dynamics (MD) simulations, machine learning (ML), and graph neural networks (GNNs) to investigate the structural dynamics of βarrs bound to the Rp-tail of vasopressin receptor 2 (V2Rpp) with distinct phosphorylation patterns. Our results indicate that the binding stability of V2Rpp is higher with βarr1 than with βarr2, exhibiting distinct interresidue interactions that generate specific allosteric triggers influencing downstream signaling. We identified unique effector-binding regions significantly impacted by each phosphorylation pattern on V2Rpp, providing a framework for future experimental studies targeting specific signaling pathways through βarrs. Phosphorylation-dependent allosteric pathways from V2Rpp to distal functional regions are highlighted, revealing key structural elements that mediate isoform-specific differences in effector interactions. These residue-level insights enhance our understanding of how βarrs decode diverse signals from GPCRs, which are encoded as specific phosphorylation patterns on the Rp-tail, and thereby activate distinct downstream signaling processes.

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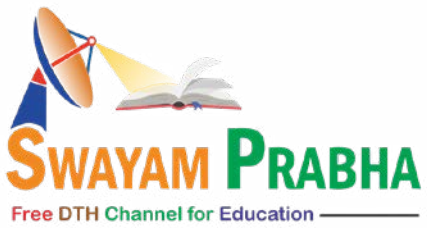
Technical Talks

Biacore™ Surface Plasmon Resonance (SPR) Systems: Addressing Analytical Complexity During Cell-line Development (CLD) for Bi-Specific Antibodies

Shubhendu Seal

High-throughput titer measurement is important for an efficient cell line development (CLD) process. For titer selection early in the workflow, the chosen technology must allow low sample consumption and high sensitivity. Generation of bispecific antibody (bsAb) producing cell lines can be challenging, since large numbers of clones might have to be screened to find high-producing clones with the correct bsAb composition. Biacore™ surface plasmon resonance (SPR) system can be a valuable tool in the CLD processes. The Biacore instrument together with Sensor Chip Protein A offers a ready-to-use solution for CLD of standard mAbs for unattended titer assessment of up to eleven 96-well plates in 8 to 10 h with a dynamic range of ~ 0.05 mg/L to 10 mg/L. For bsAbs, assessing both the level and purity of the correct bispecific form is critical to identify high-performing producer clones. The protein A (ProtA) titer screening assay can be complemented with molecule specific sandwich assay to quantitate the correct bsAb in the presence of product-related impurities. By combining a bispecific sandwich assay with a Protein-A assay, bsAb purity can be obtained and used for clone selection. Biacore SPR data agreed with LC-MS and purification data and could be used as a single method to choose clones for further development.



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TT

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Advancing Scientific Innovation: Twist Bioscience & Zelle Biotechnology

TT

Twist Bioscience is a leading and rapidly growing synthetic biology and genomics company that has developed a disruptive DNA synthesis platform to industrialize the engineering of biology. The core of the platform is a proprietary technology that pioneers a new method of manufacturing synthetic DNA by “writing” DNA on a silicon chip. Twist manufactures synthetic DNA-based products, including synthetic genes, tools for NGS preparation, and antibody libraries for drug discovery and development. Building on its foundation since 2004, Zelle Biotechnology Pvt. Ltd. has been a trusted partner many global players in India for life sciences and biopharma that includes Twist Bioscience. Over the years, we have expanded beyond biopharma to serve industries in nutrition, agriculture, and diagnostics. Our customer-centric approach and scientific expertise allow us to deliver innovative research solutions, fostering long-term collaborations that go beyond conventional partnerships. Together, Twist Bioscience & Zelle Biotechnology are committed to pushing the boundaries of innovation, accelerating research, and delivering tailored scientific solutions for a better future.

Imaging a New Dimension in Confocal Microscopy

TT

J. Sebastian Raja

Confocal microscopy has long been a cornerstone technique in biological imaging, offering high resolution and optical sectioning capabilities. However, recent advancements have pushed the boundaries of traditional confocal microscopy by introducing novel approaches that unlock new dimensions of information. This talk explores the latest innovations in confocal microscopy, including developments in Highly sensitive detectors, super-resolution techniques, multi-modal imaging modalities, new photon counting ability and lifetime-based separation for biological image analysis. By integrating these advancements, researchers are now able to visualize cellular structures with unprecedented clarity and depth, revealing intricate details and metabolic physiology of intra cellular behaviour for various biological applications. This talk provides an overview of these emerging techniques and their potential applications in various fields, from fundamental biology to biomedical research and beyond.

Advancing Research and Education Through Enhanced Visual Learning

Abigail De Souza

TT

Visual learning is revolutionizing the way we teach and conduct research in STEM fields. JoVE (Journal of Visualized Experiments), a peer-reviewed video scientific journal is the leading producer and publisher of high-quality and scientifically accurate video resources with a vast repository of 25000+ videos. JoVE bridges the gap between complex scientific concepts and practical applications to increase the productivity and reproducibility of research as well as enhance student learning and comprehension. This talk will explore how JoVE resources can be integrated into classroom teaching, research labs, training programs, workshops, and much more with dedicated customer support allowing users to create personalized playlists, facilitating efficient learning and research workflows. In addition, it will also cover how scientists and research scholars can publish their impactful work in a dynamic, accessible format.

BD FACSDiscover S8 : Image enabled spectral cell sorter

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Revealing Structural Changes: A Case Study Using MMS Technology

TT

Fatima D'Souza

A Case Study Using MMS Technology: The investigation of structural changes in biomolecules is critical for understanding their functional properties and stability. In this study, we explore the application of Microfluidic Modulation Spectroscopy (MMS) technology to detect structural differences in samples that exhibit minimal aggregation in High-Performance Liquid Chromatography (HPLC) analysis. Our findings underscore the sensitivity and precision of MMS technology contributing to the advancement of analytical methodologies in identifying and characterizing structural variations, providing valuable insights for the development and quality control of biopharmaceuticals.

Toshniwal
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Innovative Analytical Solutions to Characterize Molecular Interactions, Stability and Higher-Order Structure of Biomolecules – A Biophysical Approach

TT

T. Muraleedhara Reddy

Significant advancements in analytical techniques over the past few decades have enhanced our understanding of biological molecules. These tools are essential for characterizing complex macromolecules such as proteins, DNA, RNA, carbohydrates, and lipids, allowing researchers to identify, quantify, and analyze their structures and properties. Beyond fundamental characterization, analytical techniques play a crucial role in modern life sciences by providing deep insights into biomolecular structures, functions, and interactions. These insights drive progress in fields such as drug discovery, diagnostics, and cellular biology. Notably, recent developments in calorimetry have yielded exceptional sensitivity, leveraging heat exchange to create a universal and highly responsive sensor capable of detecting and monitoring nearly all chemical and biological processes. Understanding biomolecular interactions and structural stability is critical for applications ranging from drug discovery to protein engineering. Key biophysical characterization techniques—including **Isothermal Titration Calorimetry (ITC), Differential Scanning Calorimetry (DSC), Grating Coupled Interferometry (GCI) via the Creoptix WAVEsystem, Circular Dichroism (CD), Differential Scanning Fluorimetry (DSF), and Stopped-Flow Spectroscopy**—offer quantitative insights into molecular binding, conformational changes, and thermodynamic properties.

Unraveling Molecular Interactions with Gator Bio's Next Generation Biolayer Interferometry (BLI)

TT

Hendrick Loei

Bio-Layer Interferometry (BLI) is a powerful, label-free technology that enables real-time monitoring of biomolecular interactions. Unlike conventional methods such as ELISA, ITC or SPR, BLI's microfluidics-free nature, high throughput, and ease of use has made it the technology of choice in both the academic research and biopharmaceutical development. In this presentation, we will describe the key innovations in Gator Bio's next generation BLI technology that enabled users to accelerate biological drug discovery, elucidate protein structure-function relationship, and interrogate biologics product biophysical characteristics.

Biophysical Tools for Understanding Challenging Molecules

TT

Saji Menon

Knowing the strength of an interaction between any biomolecule or the stability of these biological entities is crucial in understanding the details behind how biological systems work. When studying about different diseases and the therapeutic or recombinant molecules, we encounter technical challenges that come from working with proteins that aggregate or are intrinsically disordered. Many target proteins that lack enzymatic activity, unstable in given conditions making screening for potential drug candidates very challenging. NanoTemper provides a new approach that brings a fast and reliable solution for the screening of small molecule-protein and protein-protein interactions. The benefits are multiple: reduced costs due to low protein consumption, time saving for screening campaigns due to fast run times, and easy setup so everyone in the lab can use it. Easy detection of disordered proteins and the various process by which they aggregate or stabilize themselves. With shorter time and smaller volumes it becomes easy to characterize complex systems and get lots of data from your precious sample to get insights about molecular interactions or thermal unfolding and sizing.

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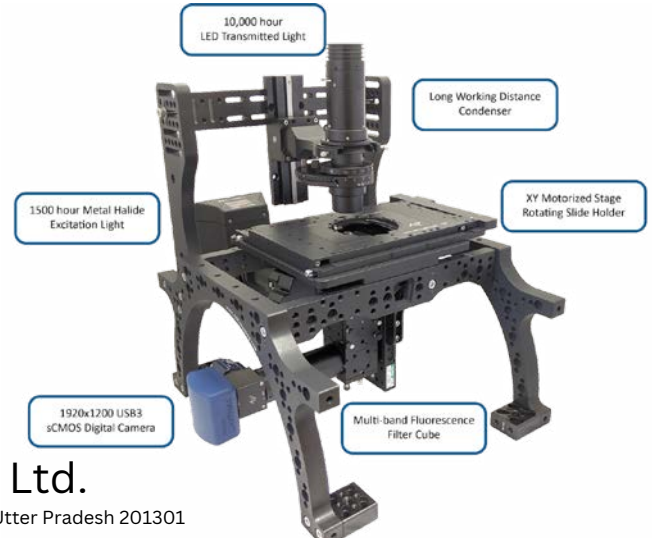

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Poster Presentations

Session 1: March 7

Reliability of Calcium-Binding Site Prediction with AlphaFold 3: Insights from Analysis of Crystal Structure

1

Abdul Basit

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Accurate prediction of calcium-binding sites in proteins is essential for understanding their structural and functional dynamics, presenting a significant challenge in the field of biophysics. In this study, we evaluated the performance of AlphaFold 3 (AF3) in predicting calcium-binding sites by comparing its predictions with experimental structures. The results showed that AF3 performs well in structured protein environments, achieving minimal deviations. However, AF3 overestimated many calcium-binding sites, predicting them in locations where no electron density was observed or where the site was meant for other metal ions. Several common issues were identified, including difficulties with low-coordination sites, closely spaced binding sites, overestimating polar residues, misidentifying metal-binding sites, and low-confidence scores, often associated with failed predictions. Additionally, artefacts commonly found in Protein Data Bank entries, particularly in metal-binding sites, further complicate prediction accuracy and highlight the importance of using validated and high-quality structures for reliable analysis. These findings underscore the robust capabilities of AF3 while emphasizing the need for continued improvements.

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Unlocking the Puzzles of Self-Assembly and Disassembly of Viral Capsid

2

Aditi G Muddebihalkar[1], Aishwarya. P[2], Yugandhar Reddy BS[2], M. Hamsa Priya[1*]

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Viruses are intracellular parasites comprising of nucleic acid (either DNA or RNA) surrounded by a protective protein layer called capsid. It also plays a significant role in replicative machinery of viruses. Depending on the presence or absence of an additional lipid layer, viruses are further categorized into enveloped and non-enveloped viruses respectively. Capsid structures are highly stable and are comprised of multiple copies of single or multiple proteins, predominantly arranged in helical or icosahedral symmetry. Spontaneous formation of these supramolecular complexes is an interesting example of self-assembly and remains less understood. Deciphering the assembly process would further help in the vaccine and antiviral development. Our study aims at understanding the mechanism of assembly and disassembly of these viral capsids through molecular dynamics simulation. Atomistic simulations would not be feasible owing to their large size and longer time scales involved in the assembly process. Hence coarse-grained simulations of the capsids will be performed. This study would provide insights on the key aspects governing the process of capsid assembly and disassembly.

Benchmarking Computational Models for Relaxation Dispersion NMR: Insights into Sub-Millisecond Protein Dynamics

3

Apurva Phale[1], Aishani Tewari[2], Gayatri Tendulkar[2], Ranabir Das[1], Sivakumar Srinivasan[2], Kalyan S Chakrabarti[2*]

[1]National Centre for Biological Sciences, Bengaluru, Karnataka, India, [2]School of Interwoven Arts and Sciences (SIAS), Krea University, Andhra Pradesh, India

Understanding protein function at a mechanistic level requires insights into the kinetics, thermodynamics, and structural transitions among conformational sub-states. High-power Relaxation Dispersion (RD) NMR is a key tool for probing microsecond to millisecond dynamics, but the accuracy of extracted parameters depends heavilyW on the fitting model and experimental noise. In our paper, we benchmark theoretical models, including Bloch-McConnell, Carver-Richards, and Luz-Meiboom, for analyzing sub-millisecond exchange dynamics. Using ubiquitin as a model system, synthetic data with noise was generated and fitted to these models. Our results reveal that while exchange kinetics are reliably extracted across regimes, structural features such as chemical shift differences and minor state populations exhibit significant uncertainty, particularly in the fast-exchange limit. These findings provide critical guidelines for designing RD NMR experiments and interpreting data, ensuring robust insights into biomolecular dynamics.

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What Drives Vesicle Formation in Peptide Permeation Through the Cancerous Membrane: Insights from Umbrella Sampling Simulations

4

Anjana V. Mathath[1] and Debashree Chakraborty[1]

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Membrane active peptides facilitate the permeation process by disrupting the structural integrity and altering the physical properties of the membrane. We examine the various permeation pathways of novel anti-microbial peptide melittin and its second-generation pH-dependent, pHD108, through the modeled cancerous membrane using multi-dimensional reaction coordinate[1] in umbrella sampling. The number and placement of hydrophilic residues in the peptide were found to be the key to different permeation pathways, from a barrel-stave-like mechanism to toroidal pore and vesicle formation. Vesicle formation is favored by the presence of hydrophilic residues at the middle and terminal ends of the peptide, while the presence of hydrophobic residues leads more toward the barrel-stave-like mechanism. The peptide traverses along a smooth, homogenous pathway during the vesicle formation, whereas the barrel-stave-like mechanism follows a rugged and steep pathway. Because of the favorable mutation in the middle residues and optimum distribution of charge near two terminals, vesicle formation is observed along the permeation of pHD108, N-terminal with a maximum membrane thinning of 54.4%, insertion barrier of 8.20±0.10 kcal mol−1, and a pore radius of 2.33±0.07 nm. A mechanism similar to toroidal pore consists of multiple minima, mainly observed for the permeation through the C-terminal of peptides. Higher free energy was found for the permeating terminal containing charged amino acid residues. The position of charged groups was found to be the determining factor for forming positive or negative curvature on the membrane.[2] The insights gained from this study can help to develop new synthetic strategies for efficient cargo delivery.

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Alteration at a Protein-Water Interface Due to Changes in pH

5

Anusree Sen[1], Jaydeb Chakrabarti[2] , and Rajib Kumar Mitra[2]

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The interaction between proteins and water is vital for biological processes, influencing protein structure and function. Experimental techniques, such as X-ray scattering and neutron diffraction, reveal that the solvent structure near biomolecules differs significantly from bulk water, a finding supported by theoretical studies. Recent investigations using two-dimensional infrared spectroscopy (2D IR) and Molecular Dynamics (MD) simulations[1] show that protonation and deprotonation of acidic residues impact protein structure but provide limited insight into hydration dynamics. In this study, we explore the pH-dependent hydration properties of Hen egg-white Lysozyme (PDB ID 1AKI) using constant pH MD simulations[2] across extreme acidic to basic pH levels. We examine the structural arrangement of water molecules and their hydrogen-bonding dynamics with the protein. Results indicate that at low pH, protein structures become more extended with exposed hydrophobic cores, leading to reduced water density profile and lower tetrahedral order near acidic residues. At higher pH, hydration water forms more stable hydrogen bonds, reflecting pHinduced changes in hydration structure. Additionally, we performed zeta potential experiments to determine the total charge of the protein at various pH values and mid-infrared (Mid-IR) spectroscopy to analyze changes in secondary structure. Using dihedral principal component analysis, density-based clustering, and machine learning[3], we identified metastable states at low pH, dominated by acidic residues. At basic pH, basic and hydrophobic residues govern hydration site exposure due to charge inversion post-isoelectric point. Conformational thermodynamics reveals residue-specific free energy and entropy changes. These findings, corroborated by experimental zeta potential and Mid-IR data, provide critical insights into pH-dependent hydration water properties and their influence on protein structure and function.

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Plasticity of the Proteasome-Targeting Signal Fat10 Enhances Substrate Degradation

6

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Proteasomal degradation, a crucial mechanism for regulating cellular protein levels, relies on posttranslational modifications such as ubiquitination and FAT10ylation. Fat10, a ubiquitin-like protein upregulated during the immune response, marks substrates for rapid degradation. FAT10ylation exhibits distinct characteristics compared to the ubiquitin-mediated pathway. Unlike ubiquitin, FAT10 is degraded alongside its substrate, necessitating the unfolding of both the tag and substrate, making their unfolding kinetics pivotal. Despite its rapid degradation, the factors influencing proteasomal degradation rates, the role of the tag and its structural plasticity remain poorly understood. Our study investigates the biophysical consequences of FAT10ylation on substrate proteins, seeking to elucidate the impact of FAT10's structural plasticity on the proteasomal degradation process. Using MD simulations our studies[1] reveal that certain salt bridges are absent in the Fat10 structure compared to ubiquitin, creating a plastic protein with partially unstructured regions suitable for proteasome engagement. This structural malleability reduces resistance to mechanical unfolding, expediting proteasomal degradation. While the proteasome targets substrates tagged with proteins, the impact of the structural dynamics of these tags on degradation remains poorly understood. Therefore, we investigated the thermodynamics of three FAT10-conjugated substrate proteins[2]. Our findings address fundamental questions regarding FAT10ylation: Is FAT10 a better degradation tag than ubiquitin? Does FAT10 induce thermodynamic changes in substrates? Are they primarily enthalpic or entropic These findings highlight a mechanistic model where Fat10's structural plasticity destabilizes substrates, enabling their efficient unfolding and degradation. This study advances our understanding of Fat10-mediated proteasomal pathways and underscores the strong dependence of degradation efficiency on the structural properties of proteasome-targeting tags.

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Computational Modelling of Levan Biosynthesis Pathway in Bacillus subtilis: Implications in Strain Optimization and Engineering

7

Aruldoss Immanuel[1], Ragothaman M. Yennamalli[2] and Venkatasubramanian Ulaganathan[1,2]

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Levan is a fructan polymer with many industrial applications such as the formulation of hydrogels, drug delivery, and wound healing, among others. To this end, metabolic systems engineering is a valuable method to improve the yield of a specific metabolite in a wide range of bacterial and eukaryotic organisms. In this study, we report a systems biology approach integrating genomics data for the Bacillus subtilis model, wherein the metabolic pathway for levan biosynthesis is unpacked. We analyzed a revised genome-scale enzyme constrained metabolic model (ecGEM) and performed simulations to increase levan biopolymer production capacity in B. subtilis. We used the model ec_iYO844_lvn to (1) identify the essential genes and bottlenecks in levan production, and (2) specifically design an engineered B. subtilis strain capable of producing higher levan yields. The FBA and FVA analysis showed the maximal growth rate of the organism up to 0.624hr^{-1} at 20mmol gDw^{-1} hr^{-1} of sucrose intake. Gene knockout analyses were performed to identify gene knockout targets to increase the levan flux in B. subtilis. Importantly, we found that the pgk and ctaD genes are the two target genes for the knockout. The perturbation of these two genes has flux gains for levan production reactions with 1.3- and 1.4-fold the relative flux span in the mutant strains, respectively, compared to the wild type. In all, this work identifies the bottlenecks in the production of levan and possible ways to overcome them. Our results provide deeper insights on the bacterium's physiology and new avenues for strain engineering.

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**Selected for Flash Talk*

Investigating The Influence of Solvent on Self-Assembly Mechanism of Peptides

8

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Surfactants like Peptides (SLPs) are known to self-assemble into a variety of nanostructures having wide applications in tissue engineering, antivirals, as drug-delivery vehicles and biosensors. Identifying the factors involved in self-assembly at atomic level of these nanostructures experimentally is a challenging task. Molecular Dynamics (MD) Simulations can provide better insights of the self-assembly mechanism which can help in tailoring the self-assembly of these sequences to design desired nanostructures. Our study focuses on understanding the interaction of specific peptide sequences with different hydrophilic head such as Alanine, Leusine and Valine and hydophobic tail - Lysine in solvents like water, ethanol, methanol and their mixtures. Single and multiple peptide (8) simulations were performed in different solvent conditions to assess their intra and inter-peptide interactions through helicity and cluster analysis which depicts the tendency of peptides to self-aggregate. Stability of preassembled nanotubes of different sizes and different solvent environment were also analyzed.

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Molecular Insights into CETP-Mediated Lipid Transfer and Inhibition Mechanisms

9

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Cholesteryl Ester Transfer Protein (CETP) plays a pivotal role in lipid metabolism and has been identified as a key factor in cardiovascular disease (CVD) risk[1]. This study employs advanced computational methods to elucidate CETP's lipid transfer mechanism and inhibition dynamics, with the goal of guiding the development of more effective CVD therapies. Initially, we utilized Molecular Dynamics (MD) simulations to analyze CETP-lipid interactions and dynamic behaviours within the protein's tunnel, focusing on its binding to various neutral lipids and also with the known inhibitors. To further investigate the lipid transfer process, we applied Steered Molecular Dynamics (SMD) and Metadynamics simulations, which uncovered the energy landscape and key intermediates involved in lipid passage through the CETP tunnel. Additionally, we examined the inhibition mechanism of torcetrapib and also the known CETP inhibitors, to understand how it impedes the protein's transfer function. Our multi-faceted computational approach provided atomic-level insights into CETP's functional mechanism, including the identification of crucial protein-lipid interactions, energy barriers associated with lipid transfer, and the molecular basis of inhibitor action. Beyond structural dynamics, we sought to identify critical molecular fingerprints and functional moieties that contribute to CETP inhibition using machine learning (ML) and Quantitative Structure-Activity Relationship (QSAR) modelling. By integrating MD-based mechanistic insights with ML-driven inhibitor profiling, this study advances our understanding of CETP's role in lipid metabolism and its inhibition. These findings offer a valuable foundation for structure-guided drug design, paving the way for next-generation CETP inhibitors aimed at effective CVD prevention and treatment.

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Influence of Cardiolipin on Bacterial Membrane Dynamics Under Osmotic Stress

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Bacterial membranes primarily consist of phospholipids such as Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), and Cardiolipin (CL). In response to increasing salinity, bacteria adjust their membrane composition by increasing the proportion of anionic phospholipids like PG and reducing zwitterionic phospholipids like PE. CL, a negatively charged phospholipid, forms distinct lipid domains within the membrane. Changes in the phospholipid composition, particularly in CL levels, can impact membrane properties. Higher cardiolipin concentrations have been linked to increased drug resistance and reduced pore formation in bacterial membranes. This resistance to pore formation is likely associated with osmotic adaptation, as elevated CL levels in the membrane enhance the ability to withstand osmotic stress. The variation in phospholipid composition under osmotic stress alters the physical properties of the membrane. In this study, we constructed multiple bacterial membrane models with different CL concentrations and PG-to-PE ratios to examine the effects of CL during various bacterial growth phases. We focused on lipid domain formation through lipid interactions and the energetics of these model membranes. To explore membrane physical properties, we assessed membrane elasticity by evaluating curvature and pressure profiles under varying CL concentrations. This research enhances our understanding of bacterial membrane behavior under osmotic stress, offering insights into the mechanisms of action of peptides, drugs, and other biomolecules targeting bacterial membranes.

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The Thermodynamic Architecture of Eukaryotic Protein Kinase

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Sequence variation across members of a protein family can contribute to diverse structural, functional, and ensemble behaviors. A classic example is the family of eukaryotic protein kinases (EPKs), responsible for phosphorylating varied protein substrates, which exhibit high structural but low sequence similarity. In this work, we dissect the consequences of sequence variation on the folding-conformational landscape of 274 EPKs through a structure-based statistical mechanical framework that considers millions of conformational states and the associated non-covalent interaction diversity. We find that most of the EPKs undergo multi-state folding, populating several partially structured states in their native ensemble, some of which are kinetically trapped due to large thermodynamic barriers. Multi-state folding is conserved across the 9 sub-families of EPKs determined primarily by the differential stabilities of the N-terminal and C-terminal lobes. Detailed thermodynamic coupling analysis and in silico alanine scanning mutagenesis reveal how residues far from the catalytic site are differentially coupled across families, highlighting the consequence of sequence variation, including substrate selectivity. Finally, employing Abl kinase as a model system, we showcase how activation drives the ensemble towards a more folded and more thermodynamically coupled system in a graded manner, and in very good agreement with experiments. The ensemble framework presented here provides a wholesome view of the conformational determinants in eukaryotic protein kinases, which can be leveraged to design allosteric inhibitors of activity.

SuBMIT: A Toolkit for Facilitating Simulations of Coarse-Grained Structure-Based Models of Biomolecules

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Biopolymers such as proteins and RNA have multi-dimensional folding energy landscapes, where the number of dimensions (degrees of freedom) increase with the length of the polymer. This makes it difficult to extensively sample the landscape to study large length and time scale processes. To tackle this, computational models are designed to (1) reduce the degrees of freedom by using a coarse-grained representation of the molecule, and (2) simplifying the number of interactions that contribute to the model energy function. Coarse-grained structure-based models (CG-SBMs) are simplified potential energy functions of biomolecules or biomolecular complexes that encode their structure. Molecular dynamics simulations of such SBMs have been successfully used to study long time scale dynamics such as protein and RNA folding, and large conformational transitions of their complexes. Moreover, SBMs are easy to modify and can be adapted for the specific biomolecular problem that needs to be investigated. However, the force-fields of SBMs are not usually included in commonly used simulation packages resulting in a barrier to their use. Here, we present SuBMIT (Structure Based Models Input Toolkit; https://github.com/sglabncbs/submit), a toolkit for generating coarse-grained SBM input files for performing MD simulations with GROMACS and OpenSMOG. The different flavors of SBMs included in the software can be used to simulate systems such as folding and conformational ensembles of proteins with intrinsically disordered regions, 3D-domain swapping of proteins, and dynamics of RNA and protein multimer assembly such as those present in RNA viruses.

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Predictive Modeling to Forecast SARS-COV-2 Variants and Understanding its Virulence by Integrating Machine Learning and Biophysical Studies

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This work report on forecasting mutations on the spike protein of SARS-COV-2 using prompts focusing on receptor binding domain (RBD) and receptor binding motif (RBM), by developing a fine-tuned transformer model (FT-ProtGPT2) trained on spike-protein sequences of SARS-COV-2. The predictive ability of the model was found to be robust and statistically significant and we have predicted novel mutations in the RBM. Among the predicted single mutations, the spike-ACE2 complex of mutated C480H, E484I and T500R exhibit binding free energies on par and lower than that of the Omicron variant. Of the variants with multiple mutations, the combination of A475M, C480H, E484I, G476T, L492I, P491R and T500R ($\Delta G = -93.51$ kcal/mol) and A475M, C480H, E484I, G476T, L492I, P491R, T500R and Y495V ($\Delta G = -92.81$ kcal/mol) exhibit binding free energies. These combinatorial effects of single mutations lead to increased binding energy and affinity better than Omicron. A prominent mutation in which cysteine is replaced by histidine at position 480 (C480H) of RBD, leading to breakage of the disulfide bond making the RBM more flexible. However, the disulfide bridge breakage doesn't affect the overall compactness of the structure due to the presence of multiple disulfide bonds. Moreover, Histidine differs from cysteine in side chain structure, which can modify the electrostatic environment at the binding site as it influences the protein's redox state vital for protein structure and function, and altering redox activities can substantially change how proteins behave and interact and might contribute to the emergence of more virulent variants in the future.

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Emergence of Soft Dynamic Channels in Highly Ordered Lipid Bilayers

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Over the last few decades, extensive investigations on spatial and dynamic heterogeneity in plasma membrane (PM) have been performed on carefully reconstituted membranes. Characterizing the molecular features in heterogeneous membranes is extremely challenging due to experimentally inaccessible time- and length-scales of these emergent systems. In this context, simulations can provide important insights into molecular-level interactions leading to PM heterogeneity and associated functions. To that end, we use the non-affine displacement (χ^2) framework[1] to faithfully capture molecular-scale local membrane order in simulated heterogeneous bilayers. In our latest application of χ^2, we investigate the temperature-dependent spatial and temporal organization on microsecond trajectories of liquid-ordered bilayer systems at all-atom resolution (DPPC/DOPC/CHOL: 0.55:0.15:0.30; 40 nm x 40 nm with a total of 5600 lipids and 2 million atoms) [2]. Lateral organization in these large bilayer patches shows noticeable heterogeneity despite its liquid-ordered nature. Our χ^2 analyses reveal soft dynamic channels within the tightly packed membrane reminiscent of the classical two-component Kob-Andersen glass-forming binary mixture. Hence, we quantified the multiple time scales underlying the lipid dynamics using classical glass physics markers such as self intermediate scattering function, dynamic susceptibility, van Hove etc., Our analyses reveal that highly ordered membrane systems inherently possess glass-like dynamics with distinct soft fluidic channels inside them. Biologically, these soft fluidic channels could act as conduits for facilitating molecular encounters for biological functions even in highly ordered phases.

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**Selected for Flash Talk*

An Integrative Computational Approach to Predict Viral Epitopes by Targeting the MHC-TCR Complexation

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T-cell immunity acts as a major defense system against controlling viral infections in vertebrates. During viral entry, innate immune cells degrade the viral proteins (antigens) and present them on their surface via Major Histocompatibility (MHC) proteins. T-cell receptors (TCRs) recognize these antigens/peptides presented by MHC (pMHC), initiating a T-cell-mediated immune response. Despite its significance, the mechanism by which pMHC-TCR binding triggers T-cell activation remains unclear. In this study, we employed an integrative computational approach combining Bioinformatics, Molecular Dynamics (MD) simulations, and Machine Learning (ML) to identify viral epitopes as potential vaccine candidates. We performed large-scale all-atom and coarse-grained MD simulations on MHC-peptide-TCR complexes embedded into dendritic and T-cells, for which experimental immunogenicity data is available[1]. One hundred fifty such systems are simulated for 1 μs each to capture the conformational and dynamical changes that underlie T-cell activation. Our ML models, trained on simulation-derived structural and dynamical features extracted from 2500 time points, revealed key determinants responsible for T-cell activation with an accuracy of approximately 85%. Notably, we have identified the bending of the TCR alpha membrane region and the buried surface area between pMHC and TCR as critical factors influencing immune response initiation. Our approach unravels the mechanism of T-cell mediated immune response and helps ML-guided screening of viral epitopes for vaccine development.

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A Deep Learning Method for Predicting Interactions for Intrinsically Disordered Proteins

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Intrinsically disordered proteins (IDPs) or regions of (IDRs) are characterized by an ensemble of conformations in contrast to a single well-defined three-dimensional structure. They occupy a significant fraction of the eukaryotic proteome and are involved in several cellular processes including signal transduction, molecular recognition, chaperoning protein folding, and condensate formation. Their intrinsic disorder often makes it challenging to characterize them both computationally and experimentally. Here, we developed Disobind, a deep-learning method that predicts inter-protein contact maps and interface residues for an IDR and a partner protein, leveraging sequence embeddings from a protein language model. Several current methods, in contrast, provide partner-independent predictions, require the structure of either protein, and/or are limited by the MSA quality. Disobind performs better than AlphaFold-multimer and AlphaFold3. Combining the Disobind and AlphaFold-multimer predictions further improves the performance. However, Disobind is limited to binary IDP-partner complexes, where the two proteins are known to bind, and the input sequence fragments are less than one hundred residues long. The predictions can be used to localize IDRs in integrative structures of large assemblies, characterize protein-protein interactions involving IDRs, and modulate IDR-mediated interactions.

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From Contacts to Patterns: Quantifying Structural Changes in HIC data

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Hi-C and other 3C techniques are advanced sequencing techniques that probe the three-dimensional (3D) genome organization within the nucleus. By generating contact frequency matrices, these methods reveal spatial interactions between genomic loci, shedding light on DNA folding patterns that influence gene regulation and cellular function. However, these matrices are typically sparse due to the genome size and sequencing limitations and are affected by technical and sequence-based biases. Such challenges make comparing contact matrices computationally demanding, and traditional metrics like Pearson correlation are inadequate for capturing their complex relationships. To overcome this, we introduce an entropy-based method to quantify similarity by analyzing pattern complexity along matrix diagonals. This approach measures von Neumann entropy, reflecting the unpredictability in contact patterns, and offers a robust metric for comparing 3D genome architectures. In addition to assessing similarity, the method enables detailed exploration of structural changes in genome organization. Comparing entropy profiles across cell types or conditions that are crucial for understanding the biological processes driven by structural changes, including gene regulation, cellular differentiation, and disease mechanisms.

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Synergizing Physics-based Modeling with AI/ML for BioPharma R&D

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AI/ML methods are increasingly complementing physics-based modeling in R&D within the Biopharma industry by enabling faster and accurate predictions. While advances in deep learning architectures in conjunction with the traditional MD simulations have a tremendous impact in protein structure modeling and enzyme engineering, Generative AI is transforming efficient discovery of vast libraries of potential drug candidates with optimal properties. Models trained on the data from High-Throughput Experiments (HTEs), first-principles methods, and empirical models enable rapid molecule design (including covalent binders and PROTACs), property prediction, molecule screening, and hit-to-lead optimization. In this poster, we show how Aganitha leverages the aforementioned synergies at the intersection of Deep Science and Deep Tech. to solve real-world problems[1,2] with the help of the following case studies

- MD-based investigation of drug selectivity[1]
- De novo molecule design to target complex oligomer in tumor cells
- In silico PROTAC design using AI-driven computational chemistry
- LLM-based chatbot to analyze single cell RNA sequence data

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Non-enzymatic Kinetic Error Correction in Primordial Nucleotide Replication through Asymmetric Cooperativity

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Accurate nucleotide sequence replication is essential for self-replicators in primordial environments, where each nucleotide incorporation presents a potential for error. Modern cells have attained remarkably low error rates, as minimal as 10^(-9), through the use of sophisticated enzymes that utilize kinetic proofreading mechanisms[1]. However, the emergence of life must have occurred in the absence of such complex enzymes. We propose a theoretical model of a non-enzymatic kinetic error correction mechanism utilizing asymmetric cooperativity[2], a novel kinetic property that actively rectifies errors immediately after their occurrence. Our analysis employed a time-continuous Markov chain[3] to assess the fidelity of progeny strands by incorporating kinetic discrimination. Our findings indicate that kinetic asymmetry enhances the accuracy of progeny strands when the system operates beyond thermodynamic equilibrium, driven by an influx of energy.

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**Selected for Flash Talk*

An Insight into the Leucine Zippers Present in the Unique Blue Light Photoreceptor of Marine Alga Ectocarpus siliculosus

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Ectocarpus siliculosus, a multicellular brown alga inhabiting the rocky coastlines of temperate regions, possesses a unique blue light receptor, named Aureochrome[1], that also exhibits transcription factor-like activity. While the basic leucine zipper (bZIP) family of transcription factors is prevalent across the three kingdoms of life, an association of this ancient DNA binding module with a light-oxygen-voltage (LOV) sensor has never been observed before. A comprehensive structural characterization, assessment of DNA binding ability, and analysis of dimerization specificity of the bZIP domain of aureochrome have been carried out using in-silico sequence analysis, modelling, docking, molecular dynamics simulations, and in-vitro electrophoretic mobility shift assays. The characterization revealed the presence of a histidine residue [2] exclusive to the signature motif of aureochrome-bZIP, which favored its DNA binding through semi-random interaction with the flanking sequence of the ACGT core. The properties of the aureochrome-bZIP were compared to those of other plant and animal bZIPs in order to understand its uniqueness and evolutionary significance. It has been observed that even a single residue change in bZIP's DNA recognition motif can affect its hydrogen bonding interaction with the DNA. Understanding the substrate preferences of aureochrome-bZIP and its coupling preferences for dimerization[3] with other bZIPs will pave the way for its use as an optogenetic tool in the light mediated gene regulation of plants/animals.

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The Dual Influence of Transcriptional and Translational Kinetics on Gene Expression Noise

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Gene expression noise, the inherent randomness in protein levels, plays a crucial role in cellular heterogeneity and diverse responses to environmental conditions. While transcriptional bursts and their impact on noise have been extensively studied over the last two decades, recent experiments suggest that mRNA folding-unfolding kinetics can also create bursts in gene translation[1,2]. However, the combined influence of transcriptional and translational bursts on gene expression noise remain largely unexplored. To address this, we present a gene expression model that integrates both transcriptional and translational bursting phenomena to provide a detailed understanding of factors governing protein noise. Using the generating function technique, we derived analytical expressions for mean protein levels and noise in protein copy numbers. Our findings show that translational bursting significantly contributes to overall noise, and the noise from transcriptional bursts further amplifies the relative contribution of translational noise. For the first time, we show that translation efficiency—defined as the rate of protein synthesis per mRNA transcript—increases gene expression noise, a finding consistent with experimental studies. This positive correlation becomes evident when mean protein levels are held constan, highlighting the complex interplay between transcriptional and translational regulation. Furthermore, we explore impact of mRNA folding kinetics near the start codon on noise, finding that not only the folding stability but also the kinetics of mRNA folding-unfolding significantly influence gene expression noise. In conclusion, this study enhances our understanding of the factors regulating gene expression noise, providing insights into how transcriptional and translational bursting shape this noise.

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**Selected for Flash Talk*

Computation Analysis of Pathogenic Variants in the POLG2 and TWNK Mitochondrial Proteins

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Mitochondrial disorders encompass a diverse range of conditions caused by mutations in mitochondrial DNA (mtDNA) or in nuclear genes that encode mitochondrial proteins and their subunits. Numerous single nucleotide polymorphisms (SNPs) have been identified in the nuclear genes responsible for these mitochondrial components. Computational frameworks and simulation studies are valuable tools for identifying significant mutations and assessing the pathogenicity of specific variants. In this study, we focused on two key proteins: PolG2, which is crucial for the maintenance and replication of mitochondrial DNA, and Twinkle (TWNK), a mammalian helicase essential for mitochondrial DNA replication and integrity. We selected various variants deposited in these proteins for analysis. Specifically, we examined three African variants of PolG2—L374F, A169T, and H133Y—and three mutations in TWNK involved in the mtDNA replisome—Y508C, R302W, and R543Q. Using molecular dynamics simulations, we analyzed the structural and dynamic properties of the wild-type and mutant proteins. The analysis of protein conformation revealed changes in global properties such as RMSD and RMSF, indicating local fluctuations affecting protein structure. Additionally, the solvent-accessible surface area increased significantly in the L374F mutant and decreased in the A169T mutant of the POLG2 protein. Contact Order analysis showed that main chain–side chain interactions were destabilized in both the A169T and H133Y mutants, with an aromatic-sulfur interaction observed in the H133Y variant of POLG2. Our analyses demonstrate that these mutations lead to destabilizing effects, in POLG2 and TWNK. Our findings shed light on mitochondrial disorder mechanisms and support targeted therapeutic development.

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Family-level Characterization of Lytic Polysaccharide Monooxygenases using Multiscale Modeling

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The lytic polysaccharide monooxygenases (LPMOs) are essential for the oxidative degradation of crystalline polysaccharides like chitin and cellulose. LPMOs use copper to aid in the oxidative breakage of glycosidic bonds. Structural dynamics can be studied in depth using computational methods such as all-atom and coarse-grained (CG) methods[1]. There are currently eight families (AA9-AA11, AA13-AA17) of LPMO in the CAZy database with around 165 solved 3D structures. These structures were analyzed using SignDy (Signature Dynamics) to analyze structural dynamic signatures within a single family of LPMOs. For all the available structures, CG Gō models using SMOG were employed to study the structural mobility and conformational changes of the protein. Structures with missing residues were edited using predictions from AlphaFold2 to build the CG Gō models. Additionally, FrustratometeR was utilized to evaluate both configurational and mutational frustration in the protein simultaneously. Gō model simulations were run for 200 million steps with a time step of 0.0005. All-atom MD simulations were run for 100 ns for all the structures. The AA10 family was observed to have relatively more stable structure profile than AA9. However, AA9 showed higher residue-level fluctuations (between 10 to 75 residues) than AA10. Frustration analysis showed that AA9 family had high local frustration in loop regions spanning residues 10 to 75 and the AA10 family showed reduced local frustration, indicating a more energetically stable configuration. We show that combining structural dynamics analysis from SignDy, Gō models and frustration evaluation provides a detailed understanding of the dynamics and stability of LPMOs.

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Efficient Replication and Information Storage Capacity sets the Genome Length

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Genome size varies widely among organisms, from compact genomes of prokaryotes to the vast and complex genomes of eukaryotes. In this study, we investigate the evolutionary pressures that may have driven this divergence in genome sizes, with eukaryotes having larger genomes and prokaryotes maintaining smaller ones. We use a parameter-free model to study the evolution of genome size under selection pressure to minimize replication time and maximize information storage capacity. Our results show that prokaryotes tend to reduce genome size, constrained by a single replication origin, while eukaryotes expand their genomes by incorporating multiple replication origins. We propose a link between genome size and cellular energetics, suggesting that mitochondria regulate the number of replication origins and thus modulate genome size. These findings offer new insights into the interaction between genome size, replication efficiency, and cellular energy dynamics in the evolutionary divergence of prokaryotic and eukaryotic genomes.

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Graphene Hybrid Scaffolds for Laser Stimulated Neuro-regeneration

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Brain, the enigmatic machinery network has a poor regenerative capacity and complex neuronal circuit which confers serious medical challenges and complications to the human race. Interfacing graphene scaffolds driven by laser stimulation has been proven to be effective in developing neuro-regenerative medicine. Graphene hybrid scaffolds promotes the rate of proliferation of neural stem cells into neurons by rational modification of the carbon backbone and through functionalization of materials. Also, the inclusion of laser stimulation can enable the precise control of selective spatial induction in the neurons which increases differentiation rate. Hybrids of MnO_2 and reduced graphene oxide were prepared by reduction method and XPS studies confirmed the surface functionalization of MnO_2 on rGO. FESEM and HRTEM exposes the integration of rGO with tangled 1D MnO_2 nanowires of length ranging from several micrometers. Contact angle measurement emphasizes the superior hydrophilicity and wettability of the prepared scaffold surface. Cytotoxicity of the scaffolds and optimal laser conditions were estimated by MTT assay and with that dosage, the proliferation and differentiation of 3T3 neuronal cell culture were performed. From immunocytochemistry measurements, the frequency of number of Nestin and DAPI positive neural cells were more pronounced on laser stimulated rGO/MnO_2 scaffolds than the control. Insights from Z-scan data exposes the prepared nanomaterials involves reverse saturable absorption which mitigates the risk of localized energy spikes and pave the way for designing scaffolds that can dynamically contribute to neuron proliferation and differentiation rather than being a passive substrate. These findings indicate that laser driven graphene scaffolds could be implicated in the imperative development of noninvasive regenerative medicine and brain repairing functions.

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Bio-Nanoreactor Formation using BMC Shell Protein by Probing the Role of Disordered Regions of Enzyme Cargoes

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Protein cages are widely used for building nanoreactors as they offer many merits. They confine enzymes and enhance their catalytic efficiency by maintaining a concentrated co-factor pool. Bacterial microcompartments (BMCs) are natural examples of protein-based nanoreactors found in bacteria for energy production. These compartments are composed of a protein shell and an enzyme-rich interior for vectoral catalysis, while preventing toxic intermediate leakage. The shell proteins of BMC are often used for artificial nanoreactor design, a key challenge is the successful encapsulation of non-native enzymes, which requires specific enzyme modifications. In our study, we demonstrate that the shell protein PduA, in the presence of the native enzyme PduCDE, self-assembles into a functional compartment. This structure transports 1,2-propanediol (1,2-PD) while preventing aldehyde leakage. Interestingly, we found that the intrinsically disordered regions (IDRs) of enzymes influence their encapsulation. To further explore this phenomenon, we co-expressed shell proteins and enzymes (native and non-native) with varying IDR percentage. Our results show that enzymes with disordered regions are more likely to be encapsulated, even without native BMC elements. Both native and non-native enzymes lacking IDRs do not fold the shell protein. Further the native enzymes without IDRs remain outside the whole BMC protein shell, highlighting the critical role of disorder in enzyme-shell interactions and encapsulation. These findings provide the first evidence that heterologous enzymes can be successfully encapsulated within protein cages without relying on native BMC components. Moreover, this study paves the way for designing customizable protein cages for diverse biotechnological applications through IDR manipulation.

**Selected for Flash Talk*

Ionic Liquid-Induced Modulation of Ubiquitin Stability: The Dominant Role of Hydrophobic Interactions

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Despite the widespread use of imidazolium-based ionic liquids (ILs) in biotechnology, pharmaceuticals, and green chemistry, their detailed interactions with proteins, particularly affecting structural stability, remain poorly understood[1]. In this study, we systematically examine the effects of various imidazolium-based ILs on ubiquitin, a thermodynamically robust protein with a β-grasp structure composed of an α-helix and β-sheets. We found that IL-induced destabilisation follows a consistent order with previous findings: [BF4]- > [MeSO4]- > [Cl]- for anions and $[BMIM]^+$ > $[BMPyr]^+$ > $[EMIM]^+$ for cations. Through pH and ionic strength-dependent studies, we observed that hydrophobic interactions predominantly influence the stability of positively charged ubiquitin, with electrostatic interactions playing a secondary role. Nuclear magnetic resonance (NMR) spectroscopy identified specific residues, such as E16, G47, and L71, that showed significant chemical shift perturbations in the presence of $[BMIM][BF_4]$. However, site-directed mutagenesis of these residues resulted in minimal changes in IL-induced destabilization, suggesting a global rather than a local effect. We conducted a broader empirical analysis incorporating solvent-accessible surface area evaluations, confirming that hydrophobic residues are the primary drivers of stability alterations in ubiquitin, while charged residues play a minimal role. Additionally, single-molecule force spectroscopy, combined with Monte Carlo simulations, indicates that imidazolium ILs decrease the unfolding barrier without altering transition state structures, offering insights into protein folding dynamics. ILs appear to modulate the stability landscape of proteins by energetically and kinetically favouring the unfolded state over the folded state. These insights offer potential strategies for the selective tuning of protein stability in ILs.

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**Selected for Flash Talk*

Exploring Liquid-Liquid Phase Separation in ALS-Associated SOD1 Mutants

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Human Cu, Zn superoxide dismutase 1 (SOD1), a key enzyme in the cellular antioxidant defence system, converts fatal superoxide radicals into hydrogen peroxide and molecular oxygen[1]. More than 175 mutations in the SOD1 gene are linked to Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease characterized by SOD1 misfolding and aggregation[2]. Mutant SOD1 proteins have been shown to undergo liquid-liquid phase separation (LLPS), forming dense protein-rich droplets that mature into insoluble fibrillar aggregates. Although wild-type (WT) SOD1 is structurally ordered, ALS mutations or loss of metal cofactors critically influence its phase separation. Previous studies have explored the evolutionary traits of SOD1, highlighting their association with ALS mutation sites and their effect on the internal dynamics and stability of the protein[3]. However, the links between ALS-associated SOD1 mutations, phase separation behaviour, and disease progression remain underexplored. Here, we investigate LLPS behaviour of two ALS-associated SOD1 mutants, H46R and H80R, chosen for their structural and pathological differences. Histidine 46 resides in an ordered beta-sheet and is associated with slower disease progression, whereas Histidine 80, located in a disordered loop critical for metal binding, is linked to rapid aggregation and severe ALS outcomes. Using computational analyses, chemical denaturation, and in vitro assays, we compared the phase separation behaviours of H46R, H80R, WT, and Apo-SOD1. Our findings reveal distinct LLPS propensities and aggregation behaviours, highlighting the relationship between SOD1 structure, mutation, and phase separation. These insights enhance our understanding of ALS pathogenesis and may facilitate therapeutic strategies targeting phase separation in neurodegenerative diseases.

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Characterization of SARS-CoV-2 Pseudoviruses: Investigating Spike Protein Interactions with Mammalian Cells at Membrane and Global Levels

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SARS-CoV-2 has sparked an urgent demand for safe and effective research methods to examine its interactions with host mammalian cells. Because SARS-CoV-2 is a biosafety-level-3 virus, one approach to simplifying such research is to spike biosafety-level-2 viral particles. In response to this requirement, we have developed pseudotyped viral particles with the SARS-CoV-2 spike protein on their envelope. This novel approach bridges the gap between biosafety concerns and the need to investigate the virus's behaviour within host cells, allowing for a better knowledge of SARS-CoV-2 pathogenesis and prospective intervention techniques. This research aims to thoroughly examine SARS-CoV-2 pseudovirus-host cell interactions, taking into account both membrane-level and global perspectives. We seek to elucidate the dynamic mechanisms behind the initial attachment, entry, and subsequent cellular responses elicited by the SARS-CoV-2 spike protein. The investigation at the membrane level focuses on the molecular interactions between host cell membranes and the spike protein, providing insight into the critical phases of viral attachment and fusion. Furthermore, we study the downstream signalling pathways that are activated by spike protein interaction with host cell receptors to comprehend host cell response to SARS-CoV-2 pseudovirus invasion. In addition to adding to our basic understanding of SARS-CoV-2 infection, our research efforts provide insights that could guide the development of vaccines and therapeutic approaches. We hope to uncover the dynamic processes that govern the initial attachment, entrance, and subsequent cellular responses triggered by the SARS-CoV-2 spike protein.

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Hydrophobicity Dependent Deformation of Bacterial Cell Wall Induced by Imidazolium-Based Ionic Liquids

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Ionic liquids (ILs) are a diverse class of material composed of complex ions, which are liquid at room temperature with a low vapour pressure and excellent thermochemical stability. These characteristics allow for the synthesis of ILs with tunable properties to target specific applications[1]. Recently, researchers have been exploring multiple ways to enhance the potential bactericidal properties of ILs[2]. Generally, these studies constitute single-component mimetic cellular membranes composed of phospholipid molecules[3]. In the present study, mixed lipid monolayers of lipopolysaccharide (LPS) molecules and anionic phospholipids are considered. This system mimic one of the leaflets of a bilayer, which is the structural matrix of a bacteria's cellular membrane. Furthermore, this study explores the hydrophobicity-dependent interaction of ILs with model membranes by comparing the results on 1-decyl-3-methylimidazolium chloride (DMIM) and 1,3-didecyl-2-methylimidazolium chloride (DDMIM). Additionally, by varying the temperature, the measurements have been done on ordered gel and fluid phases of the lipid. In this regard, a Langmuir trough setup has been used where both these ILs are exclusively added to the mixed phospholipid monolayers and characterized by surface pressure-area isotherms. The results so obtained are further quantified through synchrotron X-ray reflectivity (XRR) measurements performed on different monolayers deposited on a silicon wafer substrate utilizing the Langmuir-Blodgett method. The obtained XRR curves and the corresponding electron density profile further depict the monolayer's more significant fragmentation induced by highly lipophilic dialkylated ionic liquid DDMIM compared to DMIM.

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Outer Membrane Vesicle-Derived Membrane Model for Probing the Peptide Interactions

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Rising antimicrobial resistance and the lack of new antibiotics present a critical global challenge. Targeting the Gram-negative bacterial membrane represents a promising yet underexplored approach to overcoming multidrug resistance. Current membrane models fail to fully replicate the composition and architecture of bacterial membrane, highlighting the need for advanced platforms to study drug-membrane interactions. This study employs outer membrane vesicles (OMVs), proteoliposomes derived from the Gram-negative bacterial outer membrane, as an in vitro model for assessing antibiotic effects on membrane components. In this work, OMVs are isolated from *Escherichia coli* via ultracentrifugation and characterized using dynamic light scattering, transmission electron microscopy, and mass spectrometry. Biophysical characterization of OMV converted GUVs is performed including structure, lamellarity and lipid protein content. We employ the biomimetic platform for comprehending antimicrobial peptide interaction with membranes. Such in vitro platform will help understand molecular mechanism of antibiotic interaction with membranes and aid better design of antimicrobial peptides.

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Dominance of Hydrogen Bonding over Aromatic π-π Interactions in the Efficiency of Peptide Gelators in Supramolecular Hydrogelation: Asparagine as a Key Factor

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Short peptide-based supramolecular hydrogels have drawn immense attention due to their inherent biocompatibility, biodegradability and design flexibilities. These self-assembled structures are primarily stabilized by intermolecular hydrogen bonding of the peptide backbone, while the nature of the self-assembled architectures is determined by interactions between amino acid side chains. As previously reported, hydrophobic and aromatic π-π interactions arising from amino acid side chains play a prominent role as driving and stabilizing forces in self-assembled structures, while the influence of side-chain hydrogen bonding remains less explored. Here, we study the role of side-chain hydrogen bonding compared to aromatic π-π interactions in the hydrogelation efficiency. To elucidate this, we have selected two amyloidogenic peptide fragments, Fmoc-NVGGAVVT and (Syn-N) Fmoc-NFGAIL (IAP-N), derived from α-Synuclein and hIAPP, respectively. In the mutated sequences, asparagine was substituted with phenylalanine (Syn-F/IAP-F), alanine (Syn-A/IAP-A), and glutamine (Syn-Q/IAP-Q), to demonstrate the roles of aromatic π-π interactions, hydrophobic effects, and hydrogen bonding effects with varying chain lengths on hydrogelation efficiency. Our results show the highest hydrogelation efficiency/lowest minimum gelation concentration (MGC) for Syn-N/IAP-N and Syn-Q/IAP-Q compared to Syn-F/IAP-F and Syn-A/IAP-A. Interestingly, Syn-N, Syn-Q, and IAP-N exhibit MGC less than 0.1 wt%, classifying them as super hydrogelators. The MGC values are 4-6.6 folders higher for Syn-F and Syn-A compared to that of Syn-N. Likewise, IAP-N demonstrated a notable increase in hydrogelation efficiency, being 8.75, 3.75, and 2.5 times higher than that of IAP-A, IAP-F, and IAP-Q, respectively. The experimental observations are in well agreement with the molecular dynamic simulation results, where -CO-NH_2 group of asparagine side chains plays a key role in hydrogen bonding, effectively trapping water molecules at low gelator concentrations. We propose that point mutation of asparagine/glutamine in short peptide gelators presents an opportunity to tailor their properties for targeted applications.

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Confirmatory Detection and Evaluation of Acid Rain Stress in Neem (Azadirachta indica) using Spectroscopic Techniques

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Acid rain, caused by air pollution, forms when sulphur and nitrogen oxides react with atmospheric water vapor, producing acidic precipitation. Simulated acid rain (SAR) is used in laboratory environment to study its effects on vegetation, which experience visible (foliage damage, biomass loss) and physiological damage (reduced photosynthesis and chlorophyll content). Traditional assessment methods, such as enzyme extraction and colorimetry, are destructive and time-intensive. Spectroscopic techniques offer an efficient alternative for detecting plant stress. Neem (Azadirachta indica) saplings were collected from the NIT Tiruchirappalli campus and acclimated for 30 days before SAR treatment. The SAR solution, prepared using 1N H_2SO_4 and 1N HNO_3 in a 4:3 ratio, was adjusted to pH 3.5. To monitor the physiological and biochemical changes Raman, FT-IR and UV-Vis spectroscopy was employed. Comparing the spectra of the healthy plant leaves with SAR treated one we can do a confirmatory detection of acid rain stress. Raman spectra showed changes in peak intensities corresponding to xylan, carbohydrates, carotenoids and carboxylic acids[1]. Similarly, FT-IR spectra showed changes in peak transmittance corresponding to xyloglucan, carboxylates, amides, cellulose and lipids[2]. The UV-Vis spectra also exhibited variation corresponding to chlorophylls and carotenoids[3]. These key findings showed that as a result of acid rain stress, the photosynthetic ability of the plants have reduced resulting in its dependence on reserved food resources and strengthening of cell walls. This study showed the adverse effects of acid rain on plants and how it can be successfully evaluated using spectroscopic techniques.

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Functional Nucleic-Acid Binding Domain Of TAR-DNA Binding Protein-43 Forms Pathogenic Beta-Aggregates Via Monomeric Early Precursor Intermediate

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Loss of neuronal cells because of protein aggregation is the major reason behind neurodegenerative diseases. Aggregated assemblies of TAR-DNA Binding Protein of a molecular weight 43kDa are observed in the brains of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD) diseased patients.[1] Polypeptide chain attains its functional native from through protein-folding process via different partially unfolded intermediates. Throughout the lifetime of protein in the cell, it undergoes different conformational changes because of environmental changes such as acidification, oxidative stress, ageing etc. These environmental changes are able to destabilize the functional native form of protein followed by formation of misfolded and aggregated protein assemblies. Partially unfolded intermediate forms are considered to be major culprit for misfolding of these aggregation-prone proteins. Due to the characteristics of lower stability and higher energy state in equilibrium, they are highly prone to have unfavorable conformational changes to undergo misfolding and forms toxic aggregates. Therefore, studying these partially unfolded intermediates would be crucial to know their structural information for building therapeutic drugs to inhibit aggregation at early precursor stage of disease progression. In this study, by using principles of thermodynamics and kinetics we have identified an early partially unfolded intermediate of functional domain of TDP-43, which has higher propensity to misfold which forms toxic β-Amyloid aggregates. This information on early precursor stage for pathogenic aggregation of TDP-43 would be helpful for advancement of therapies for TDP-43 Proteinopathies.

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Decoding the Role of Mycobacterial Lipid Remodelling and Membrane Dynamics in Antibiotic Tolerance

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Tuberculosis (TB) treatment strategies predominantly target active *Mycobacterium tuberculosis* (Mtb) infections, often neglecting the complexities of latent tuberculosis infection (LTBI), which demonstrates reduced susceptibility to antibiotics[1]. During dormancy within host granulomas, Mtb undergoes profound physiological adaptations, fostering the development of antibiotic-tolerant strains[2]. Among these adaptations, the mycobacterial membrane plays a pivotal role, significantly influencing drug permeability[3]. Through the integration of membrane lipidomics and biophysical analyses of extracted membrane lipids during active growth and dormancy, this study identifies the differential distribution of specific lipids across the membrane envelope. These lipid redistributions equip the bacterium to adapt to the challenging granuloma microenvironment[4]. Furthermore, these modifications are associated with alterations in the biophysical properties of the mycomembrane, potentially impacting its interactions with antibiotics. By elucidating these lipid-mediated adaptations, the findings of this study provide critical insights into the mechanisms by which Mtb modifies its membrane dynamics to enhance drug tolerance during dormancy.

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**Selected for Flash Talk*

Structural Analysis Of Broadly Neutralizing Antibody Interactions with Dengue Virus

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Dengue virus (DENV) is a mosquito-borne virus that belongs to the Flaviviridae family and has a positive-sense, single-stranded RNA genome. DENV has four distinct serotypes circulating within the human population. Every year there, around 400 million DENV infection cases occur, out of which DENV2 infections are most prevalent and associated with a higher number of cases reaching the severe infection stage. Primary infection of DENV causes mild flu-like symptoms, however, secondary infections can become severe with haemorrhagic fever and shock syndrome. In some patients, secondary infection with a different serotype of DENV can lead to a severe dengue stage and can be fatal. DENV is an enveloped, spherical virion with a diameter of 50nm. There are two glycoproteins, E (envelope) and M (membrane), which decorate the surface of the DENV virus. Out of which, E glycoprotein is involved in host receptor interaction, due to which it receives major antibody-mediated responses. Broadly neutralizing monoclonal antibodies (bnAbs) have shown promise in effectively neutralizing multiple DENV serotypes. In this work, we have used a bnAb that demonstrates broad neutralizing activity across all DENV serotypes without cross-reactivity to other flaviviruses. Using a 3.9Å resolution cryo-EM structure of infectious dengue virus bound to Fab fragments, we have mapped the binding epitope of the bnAb, which has provided insights into the possible neutralization mechanism of the same. These structural studies can contribute to a broader understanding of the 3D architecture of broadly neutralizing epitopes on the DENV surface, potentially aiding towards better design of immunogens for effective vaccine development.

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Oxygen Partitioning into Biomolecular Condensates is Governed by Protein Density

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Biomolecular condensates form through the self-assembly of proteins and nucleic acids to create dynamic compartments in cells. By concentrating specific molecules, condensates establish distinct microenvironments that regulate biochemical reactions in time and space. Macromolecules and metabolites partition into condensates depending on their interactions with the macromolecular constituents, however, the partitioning of gases has not been explored. We investigated oxygen partitioning into condensates formed by intrinsically disordered repeat proteins with systematic sequence variations using microelectrodes and phosphorescence lifetime imaging microscopy (PLIM). Unlike other hydrophobic metabolites, oxygen is partially excluded from the condensate with partitioning constants more strongly modulated by changes in protein length than hydrophobicity. For repeat proteins, the dense phase protein concentration drops with chain length resulting in a looser condensate. We found that oxygen partitioning is anti-correlated with dense phase protein concentration. Several mechanisms could explain such an anti-correlation including excluded volume or salting out effects. Molecular dynamics simulations suggest that oxygen does not form strong and specific interactions with the scaffold and is dynamic on the nanosecond timescale. Biomolecular condensates thus result in variation of oxygen concentrations on nanometer length-scales, which may tune the oxygen concentration available for biochemical reactions within the cell.

**Selected for Flash Talk*

Measuring Chirality of Single Protein Molecule in a Cellular Environment Using Localization Microscopy

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Chirality is an inherent property of large types of biomolecules, and it influences functions such as cell fate, tissue repair, immune response, and receptor-ligand interactions. Conventional techniques measure chirality in the ensemble average but measuring chirality at a single-molecule level remains challenging. We present a novel technique, chiral Single Molecule Localization Microscopy (chiralSMLM), which integrates Fluorescence Detected Circular Dichroism (FDCD) with Single Molecule Localization Microscopy (SMLM) to measure chirality at single molecule level. This technique measures the chiral dissymmetry factor of targeted protein molecules, and it precisely localizes the molecules in the cellular environment with high localization precision(~30nm). Using chiralSMLM, we examined three proteins that are expressed in NIH3T3 cells after transiently transfected with the fusion plasmids (Dendra2-Actin, Dendra2-HA, and Dendra2-NS3 where HA is Hemagglutinin protein of Influenza A virus and NS3 is the Non-Structural protein of Dengue virus type -2). The results show that Dendra2-HA and Dendra2-NS3 proteins aggregate and form clusters. Also, the DBSCAN based cluster analysis shows left-handed molecules are more involved in cluster formation than right-handed molecules. Our findings reveal the role of chirality in protein aggregation and molecular interactions. The chiralSMLM opens new avenues for studying molecular chirality in biological systems with high resolution and holds potential applications in disease biology, drug discovery, and pharmacology.

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Understanding of Mechanical Heterogeneity in Cells by Resistive Pulse Sensing using Glass Microchannel

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Mechanical heterogeneity within biological cells plays a crucial role in various cellular processes and tissue dynamics.[1] In cancer cells, this heterogeneity serves as a key factor influencing metastasis by affecting interactions with the extracellular environment and the ability to traverse tissue matrices.[2] This study examines cellular heterogeneity by analysing their passage behaviour through glass micro electro fluidics devices. Cellular size is determined by its magnitude, while deformability is evaluated through transit time measurements using resistive pulse sensing. Additionally, the results are compared between cancer cells and blood cells to investigate their distinct mechanical properties. These findings contribute to a deeper understanding of cellular biomechanics and hold significant implications for biophysics, particularly in advancing disease diagnostics.

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Probing the Structural Changes in Therapeutic IgG1 mAbs by Solution NMR based Fingerprinting at Natural Abundance

40

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Monoclonal antibodies (mAbs) have become an important class of biotherapeutics for variety of diseases due to their excellent specificity and efficacy. As compared to other small molecule therapeutics, mAbs face unique challenges during development and storage given their structural complexity. An alteration in higher order structure (HOS) of mAbs by denaturation, aggregation, chemical modification etc. can potentially reduce their efficacy. This can in turn affect their quality and compromise the drug-safety by causing unwanted immune response. Thus, high resolution biophysical methods are needed to monitor the structural integrity of these molecules during the research and developmental phase. However, due to large molecular weight and inherent flexibility of mAbs, they have been inaccessible to X-ray crystallography and conventional solution NMR spectroscopy based methods. Although isotope labelling has been applied to mAb fragements produced from Chinese hamster ovaries (CHO) and *E. coli* cells, they do not provide complete insight about the structural and conformational integrity of the intact therapeutic mAbs. Here we have done the structural characterization of three intact therapeutic IgG1 mAbs using the natural abundance-based NMR fingerprinting methods. Although, the structural fingerprints of the mAbs are sparse due to their large molecular weight, they are robust and gives adequate information about their structural aspects. Our approach has provided insight about the aggregation, biophysical stability, peroxide induced instability and antigen binding property of the mAbs from their NMR fingerprint.

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Structural and Pharmacological Insights into Noradrenaline Reuptake Inhibition

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Neurons communicate via synapses, with neurotransmitter reuptake primarily mediated by neurotransmitter sodium symporters (NSS), such as the norepinephrine transporter (NET), which regulates levels of norepinephrine and dopamine. Targeting NET is critical for treating neuropsychiatric disorders like depression, schizophrenia, and chronic pain. Chi(χ)-conotoxin, derived from cone snail venom, selectively inhibits NET and presents significant therapeutic potential. This study aims to investigate the structure of human NET (hNET) to better understand neurotransmitter regulation and advance drug development. Employing innovative strategies like epitope transfer and toxin-mediated purification, we aim to develop novel, highly specific reuptake inhibitors for hNET by modifying Chi(χ)-conotoxin and Tramadol to enhance their potency for NET inhibition. Through Cryo-EM, we will explore the structure of hNET in complex with these toxins, revealing critical insights into their binding sites. This structural information will provide a deeper understanding of hNET's function and enable the binding pattern of more effective NET inhibitors. Ultimately, this research aims to enhance therapeutic strategies for neuropsychiatric conditions by identifying novel inhibitors and refining drug development approaches targeting NET.

Conformational Dynamics of Membrane Porins Control Cyclic Sugar Transport

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Gram-negative bacterial pathogens utilize hydrophilic porin channels to regulate nutrient uptake and restrict harmful molecules. CymA, a passive outer membrane porin from Klebsiella oxytoca, is specialized for the uptake of large cyclic sugars, with its constricted N-terminal region playing a key role in substrate translocation. However, the dynamic changes in the N-terminus that control sugar transport are not well understood. Here, we investigate the conformational dynamics of the CymA using protein engineering, single-channel electrical recordings, and molecular dynamics simulations. We engineered a mutant variant, "stapled CymA," in which the N-terminus is confined within the pore lumen by a disulfide bond tethering the loop and barrel structures. This confinement restricted the translocation of both cationic and neutral cyclic sugars. However, chemical reduction of the disulfide bond enabled a transition to "unstapled CymA," restoring dynamic flexibility to the N-terminus and facilitating sugar translocation. Molecular dynamics simulations revealed that in the stapled form, cyclic sugar translocation is impeded by a significant energy barrier, while the unstapled form exhibits more favourable thermodynamics for substrate movement. We further demonstrate that the N-terminus shifts between internal and external pore positions, dynamically controlling substrate movement across the membrane. Our findings provide new insights into the molecular mechanisms of substrate translocation by CymA, offering valuable implications for the design of antimicrobial systems.

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2D-IR Spectroscopy Shows the Influence of Sequence Specificity on the Macrostructure of Single-Repeat GLFG Hydrogels

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Nuclear Pore Complex (NPC) is a complex molecular machinery which serves as a macromolecular sieve for regulating exchange of materials to and from the nucleus [1,2]. This is due to the presence of GLFG/FG/GxFG (G:Glycine, L:Leucine, F:Phenylalanine, x:other amino acid residue) repeats, which forms mesh-like network imparting NPC with its functionality. However, the secondary structure(s) of this non-globular protein is yet to be fully understood. In this regard, infrared spectroscopy serves as a viable spectroscopic tool, since the amide-I vibration is sensitive to the secondary structure adopted by proteins [3]. Handier than linear Fourier-transform one-dimensional infrared spectroscopy is femtosecond (fs) two-dimensional infrared (2D-IR) spectroscopy, an ultrafast technique which reports not only on the secondary structure of proteins by providing a high-resolution FT-IR spectrum, but also informs on the relative orientation of the oscillators and ensemble heterogeneity through cross-peaks formation and inhomogeneous broadening, respectively. Dynamic information is imparted by using sub-100 fs pulses which allows observation of ultrafast processes which govern local fluctuations in proteins. Therefore, this contribution explores the role of a single GLFG repeat in three short chain amino-acid sequences differing in N- and C- terminal sequences. 2D-IR spectra reveal fibers composed of β-sheets in one of the sequences whereas non-fibrous β-sheets constitute the other two sequences with unstructured regions composed of random coils and turns present in all three sequences. Our findings show that amino-acid sequences which are not integral to GLFG sequence may play a role in determining the macrostructure of the NPC.

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Translational Regulation by *Huntingtin* and its Rhes-Mediated Pathogenesis in Huntington's Disease

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Huntington's Disease (HD) is a neurodegenerative genetic disorder characterized by the expression of a mutant Huntingtin (mHtt) protein with an expanded poly-glutamine tract leading to the progressive death of neurons in the striatum and the cortex. It was identified that a striatal-specific small GTPase called Rhes specifically SUMOylates mHtt and reduces its tendency to aggregate[1]. This presents Rhes-mHtt as a key complex orchestrating the progressive neurodegeneration in the striatum and cortex in HD. No structural information is available on the selective SUMOylation properties of Rhes and its effect on the structural properties of Htt. We are investigating this less-studied complex using structural biology and biochemical methods. Additionally, both wtHtt and mHtt have been shown to interact with ribosomes directly and inhibit protein synthesis with the mutant protein causing increased translational defects in comparison to the wild-type counterpart[2]. We are exploring the structural basis of interaction using cryo-electron microscopy to better understand the mechanism of translational regulation.

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Investigation into a Multi-Domain Transporter Reveals New Modes of Allosteric Regulation in Cation: H^+ Antiporters

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Cation: H^+ antiporters constitute one of the largest superfamilies of secondary active transporters, the majority of which are represented by Na^+/K^+: H^+ antiporters. These transporters are present in every domain of life and are primarily responsible for metal cation homeostasis in the cells. The transporters are assembled as dimers and consist of a highly conserved transmembrane region, responsible for substrate recognition and proton coupling. In some instances, a small cytoplasmic region is also present which may play regulatory roles, and until now, little was known about the modes and extents of such regulations in these transporters.

We present the Cryo-EM structure of one such K^+ transporter from *Escherichia coli,* called YcgO at an approximate resolution of 3.7 Å in complex with another protein called PtsN that is part of a phospho-relay system. YcgO forms a homodimer, with its C-terminus extending into two distinct domains: Regulator of Conductance of K^+(RCK) and CorC-Homology Domain (CorC). We investigated the structural and biophysical foundations of its inhibition. Using Cryo-EM Single Particle Analysis, we gained insights into its complex formation. Additionally, biophysical assays combined with previous genetic studies involving multiple small molecules allowed us to propose a model for the multimodal regulation of this transporter.

The N-Terminal Domain of *Mycobacterium leprae* HSP18: Key to its Structural Integrity and Chaperone Activity

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HSP18, a major immunodominant antigen of *Mycobacterium leprae,* is a small heat shock protein (sHSP) and exhibits molecular chaperone function. This particular function of HSP18 plays a pivotal role in regulating the growth and survival of *Mycobacterium leprae* in leprotic patients. Like other sHSPs, HSP18 comprises three distinct domains: the N-terminal domain (residues 1-38), the alpha-crystallin domain (residues 39-121) and the C-terminal domain (residues 122-148)[1]. Evidences of the presence of bis-ANS binding sites, zinc-binding sites and T-cell epitopes within the N-terminal domain[1, 2] of HSP18 motivates us to hypothesize that this domain may be crucial for its structure, stability and chaperone function. To test this hypothesis, we constructed and purified several N-terminal deletion mutants of HSP18 alongside the wild-type/full-length variant. Chaperone activity of HSP18, as determined by aggregation and thermal inactivation assays, decreased progressively with increasing lengths of N-terminal deletions and minimal activity was observed for the mutant protein devoid of entire N-terminal domain. Far-and near-UV circular dichroism studies revealed significant secondary and tertiary structural perturbations in the mutant proteins compared to wild-type/full-length protein. Additionally, surface hydrophobicity and stability of the mutant proteins decreased with increase in the length of deletion. These findings highlight the critical role of N-terminal domain of HSP18 towards its chaperone activity, substrate specificity and structural stability. Altogether, this study provides a possible foundation for rational designing and development of suitable HSP18 inhibitors in the context of effective treatment for leprosy.

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Structural and Biochemical Characterization of Cauliflower Mosaic Virus Reverse Transcriptase

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Reverse transcriptases (RTs) are multifunctional enzymes that play a key role in the replication of retroviruses and retroelements, facilitating the conversion of single-stranded RNA into double-stranded DNA through the process of reverse transcription. These enzymes possess two distinct catalytic activities: DNA polymerase, which utilizes either DNA or RNA as a template to synthesize DNA, and RNase H, which specifically targets and cleaves the RNA strand within RNA-DNA hybrids formed during replication. The Caulimoviridae family, a major group of plant viruses, lacks integrase activity, unlike retroviruses, but uses reverse transcription to replicate its circular dsDNA genomes. These viruses are closely related to Ty3 retroelements, which are important models for understanding RT structure and function. However, the detailed structural and biochemical characterization of caulimoviral RTs remains unknown. Here, we report the first crystal structure of Cauliflower Mosaic Virus (CaMV) RT complexed with an RNA-DNA hybrid, revealing a unique monomeric configuration, unlike the dimeric structure observed in Ty3 RT. Functional studies of the polymerase activity assay results have confirmed that a single molecule of CaMV RT is able to carry out the efficient polymerase function. Interestingly, our studies revealed that a second CaMV RT molecule is required to interact transiently with the polymerase-competent RT to enable RNase H cleavage of the RNA strand. Our results provide the first detailed understanding of the structure and function of CaMV RT and reveal a novel mechanism of RNase H activity that distinguishes it from other well-characterized RTs. This unique mechanism may facilitate the development of novel virucidal agents against caulimoviruses.

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Structure-Function Relationship Studies of a Mitoribosomal Protein MRPL50

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Mitochondrial ribosome (mito-ribosome) consists of two subunits, the 39S large subunit and the 28S small subunit. These subunits are composed of rRNA and several mitoribosomal proteins of unknown function. One such protein is MRPL50, a nuclear encoded protein, which is a part of the large subunit of the mito-ribosome assembly. A recent clinical study has shown premature ovarian insufficiency (POI) in women due to a single mutation in this protein, inducing multiple metabolic disorders. In order to understand the molecular basis for the observed effects, we have structurally characterized the protein using circular dichroism, fluorescence studies, and NMR. Our studies disclose that the MRPL50 mutation causes partial loss of structure, exposing hydrophobic residues.

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pH and Chemical Induced Denaturation of Esterase from *Clostridium acetobutylicum*

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The activity and stability of enzymes depend on the protonation and deprotonation of specific amino acid residues at different pH, highlighting the critical role of pH in the functionality of enzymes. This study investigates the impact of pH on the functional and structural stability of Ca-Est, an esterase from *Clostridium acetobutylicum*, purified and expressed in *E. coli* BL21 (DE3) cells. Deactivation kinetics at different pHs ranging from 3.0 to 10.0 has been studied and the functional stability has been assessed in terms of half-life and deactivation rate constant. The highest stability was observed at pH 7 with a half-life of 462 min. Interestingly, Ca-Est exhibits a wide range of pH stability with a half-life of more than 3 h at pH 4 to pH 8. The conformational changes at different pH, monitored using far UV-Circular Dichroism spectroscopy has shown that the secondary structural signatures are retained at a pH range of 4 to 10 and lost at pH 3, subsequently, the structural reversibility of Ca-Est has been explored. The structural stability was monitored by measuring the melting temperature (Tm). The highest Tm of 75.12 °C was observed at pH 7. Additionally, chemical denaturation of Ca-Est using Guanidine hydrochloride and urea has been studied. A two-state transition from native to denatured state was observed and the values of midpoint of transition (cm) for Guanidine hydrochloride induced and Urea induced unfolding were 1.67 M and 3.32 M respectively. Understanding the mechanism of pH and chemical induced denaturation of Ca-Est is vital for developing strategies to improve the industrial application of Ca-Est and other similar enzymes.

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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). 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.

Dissecting the Pathophysiology of Full-Length Human Tau as an IDP and the Spread Function of Neuro-Degeneracy through Protein-Membrane Interaction

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Tau, a microtubule-associated protein under normal physiological conditions, is a natively unfolded soluble protein with a very limited tendency for aggregation in the solution state. However, Tau aggregates are the characteristic feature of several neurodegenerative conditions like Alzheimer's Disease and Pick Disease. The pathological aggregations are possibly driven by the interaction of Tau proteins with biological membranes[1]. Our work focuses on understanding the membrane-Tau interactions leading to these aggregate-driven Tauopathies. Experimentally, we use in vitro reconstitution-based techniques, confocal imaging and micromanipulation to assess the binding kinetics of Tau and quantify subsequent membrane deformation by employing micropipette aspiration with rigidity modulus of the membrane (Kb) as the dependency factor. We observe that the aggregate state of Tau, artificially induced in the solution state by Heparin, has a higher binding affinity towards the membrane as compared to oligomeric and monomeric states. To unravel the molecular driving forces behind this observation, we use Martini 3.0-based coarse-grained molecular dynamics simulation with Tau proteins in its solution state and while it interact with the membrane. We use the available solution state SAXS data for Tau to reparameterize the Martini force-field that faithfully captures the available experimental observables[2,3]. We apply the improved force-field parameters to study the protein-protein and protein-membrane interactions. The summation of our experimental and computational findings indicates membrane deformation mediated through Tau aggregation and the subsequent propagation of neurodegeneration mediated through Tau-membrane interaction.

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Linker Mediated Phase Separation of Rubisco in Algal Pyrenoids: A Tale of Stickers and Spacers

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Algal pyrenoids are a multiprotein complex organelle that contribute to one-third of global carbon fixation. They are formed by phase separation of enzyme Rubisco in the presence of a disordered linker protein. The linker protein features repetitive helical Rubisco binding motifs that function as stickers and are separated by flexible disordered spacer regions. The current work investigates how crucial it is to fine-tune sticker numbers and spacer length in linker proteins in order to facilitate Rubisco phase separation. Our results indicate a correlation between the sticker numbers and critical linker concentration needed to recruit Rubisco into the condensed phase. A comparative study between linkers from two algal species indicate that in addition to the binding affinity of stickers towards Rubisco, the spacer properties like amino acid composition and spacers length also play a role in partitioning Rubisco between dilute and dense phase. The *in vivo* expression of the linkers with varying sticker numbers confirms that a suitable number of stickers in linker protein is necessary for localization and partitioning of Rubisco within the matrix of pyrenoids. The Rubisco partitioning governs the shape and size of the pyrenoid matrix, which in turn helps survival of algae under lower CO_2 availability. Altogether, this study highlights the physiological significance of linker in facilitating Rubisco phase separation and pyrenoid function. We believe that the information generated through our work will enable engineering of minimalistic pyrenoids in crops to improve their yields, thereby meeting the rising need for food in the near future.

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Role of RNA Pseudoknot Antitoxin in Type III Toxin-Antitoxin Complex Assembly and Toxin Inhibition

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Toxin-antitoxin (TA) systems are crucial bacterial defense systems present in either chromosomes or plasmids. Type III TA systems feature a protein toxin acting as a sequence-specific endoribonuclease and an RNA antitoxin that neutralizes the toxin by forming a protein-RNA TA complex(Blower et al., 2011). Type III TA complexes form closed-cyclic assemblies of toxins and their cognate antitoxins. In TA assemblies, antitoxin RNA adopts a complex tertiary structure comprising a central pseudoknot flanked by toxin-binding 5' and 3' single-stranded regions. The pseudoknot fold within the antitoxin is a conserved feature of all known type III TA systems(Manikandan et al., 2022; Rao et al., 2015). We have shown that closed, cyclic assembly of ToxIN complex is required for the complete inhibition of ToxN in *E. coli*. In this study, on the *E. coli* Type III TA system, we have identified critical tertiary contacts within the pseudoknot that are essential for toxin inhibition. Furthermore, we investigated the impact of mutations on antitoxin RNA stability, structure, and TA complex assembly using UV thermal melting, NMR spectroscopy, analytical SEC and Isothermal Titration Calorimetry (ITC) studies. We have shown that ToxI mutants form structures different from ToxI wild-type repeat. In altered conformations, mutants were unable to assemble with toxin into closed assemblies but could still form lower oligomeric assemblies with reduced affinity for toxin, resulting in incomplete inhibition of toxin. Our findings emphasize the significance of the pseudoknot structure in complex assembly and reveal that even a single nucleotide alteration within the pseudoknot can induce structural changes in antitoxin that prevent neutralization of toxin.

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Understanding the Mechanism of Human Gamma D-Crystallin Aggregation at Acidic and Physiological pH

54

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Human γD-crystallin is an eye lens protein of crystallin family which helps to maintain the transparency of eye lens. Several factors like aging, mutations and environmental changes causes crystallins to aggregate leading to cataract. Earlier reports have suggested the differential aggregation behaviour of human γD-crystallin at different pH. At pH 4.5 human γD-crystallin forms amyloid like structure while at physiological pH, it forms amorphous aggregates. The detailed mechanism which leads to different final states of protein aggregates at different pH is missing. To understand differential behaviour of human γD-crystallin at different pH, we correlated stability of human γD-crystallin with the aggregation behaviour at different pH. Equilibrium unfolding suggests that at pH 4.5, human γD-crystallin forms intermediate like structure which may leads to the amyloid fibril formation, whereas no such intermediate was observed at pH 7.4. Kinetics of the aggregation at both pH were followed by fluorescent probes like Thioflavin-T (ThT) and Nile red, while surface hydrophobicity was measured by 8-Anilinonaphthalene-1-sulfonic acid (ANS). Morphology of protein aggregates were visualized by transmission electron microscopy (TEM) and atomic force microscopy (AFM). Taken together, our data suggests that partial unfolding at pH 4.5 plays an important role in amyloid fibril formation.

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Unambiguous Assignment of Kinked Beta Sheets Leads to Insights into Molecular Grammar of Reversibility in Biomolecular Condensates

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Kinked-β sheets, found in fibrillar amyloid structures, are short peptide motifs that appear as distortions in the β-strands. Recent studies have postulated that these esoteric motifs are critical for the formation of reversible amyloid fibrils under physiological conditions. The kinked site prevents the interdigitation of the β-sheet interface, resulting in reduced buried surface area compared to the disease-associated irreversible amyloid fibrils. However, the detailed structural characterization of these motifs is lacking due to their transient appearance. In this work, we have provided a machine learning-based structural quantification technique to unambiguously capture the Kinked-β sheets. We have used a set of established structural parameters called θ_B and θ_R angles to label kinked and non-kinked β sheets and implemented these as a training dataset on an SVM model to differentiate the kinked peptide segments for different systems. We have shown that these kinked β-strands, although deviating from standard β-strand signature domains of the Ramachandran plot, scatter around the allowed regions. We have demonstrated the applicability of our technique in wresting out LARKS, which are kinked β-strands with designated sequences. Additionally, from our simulation-obtained conformations, we have created a repository of potential kinked peptide-segment through structural comparison against experimentally solved reversible fibrils which can be used as screening-libraries. Our technique has added a step toward precisely classifying another functionally important secondary structure motif.

Systematic Biophysical Approach to Study Plastic Degradation: Polycaprolactone (PCL) Degradation by *Mycobacterium marinum* Cutinase

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The excessive use and improper disposal of synthetic plastics have led to a significant environmental crisis across ecosystems. Biodegradable alternatives like PET, PCL, PBS, PLA, and PES offer potential solutions. Among them, PCL stands out for its efficient biodegradability in diverse environments and structural similarity to the natural polymer cutin[1,2]. This study investigates the systematic approach on biodegradation of PCL through bio-physical techniques by Cutinase from *Mycobacterium marinum* (MmCut3). Recombinant MmCut3 was purified and characterized, followed by PCL biodegradation analysis. Surface morphology changes were examined using SEM, structural alterations were assessed with ATR FT-IR, and nano-emulsion stability was studied using DLS. Additionally, the Langmuir kinetic model was applied to determine biodegradation rates. To further understand the enzymatic mechanism of MmCut3, DSC studies, molecular docking, and dynamics simulations were performed. Our findings show that MmCut3 has strong potential for bioplastic degradation, positioning it a promising enzyme for environmental applications. Future work will focus on protein engineering to improve substrate specificity and extend MmCut3 activity to polymers like PET, PES, and PBS, advancing sustainable plastic waste management strategies.

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Molecular Crosstalk between Wctodomains of Adherens Junction Proteins, E-Cadherin and Nectin-4

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The association of cell adhesion molecules (CAM) facilitates physiological events necessary for growth and development of multicellular organisms. The cooperative role of CAMs like cadherins and nectins drive the formation of adherens junctions necessary for mechanical coupling of interacting cells. The complex intercellular crosstalk among the cytoplasmic domains of nectin and cadherin facilitated through actin binding cytoplasmic adaptors is fundamental to the study of various developmental and morphological processes. Although, the interplay of cytoplasmic adaptor molecules drives recruitment of cadherins and nectins by each other, we have reported a direct interaction between extracellular domains of E-cadherin and nectin-4 as demonstrated by atomic force microscopy (AFM)-based single molecule force spectroscopy (SMFS)[1]. From AFM topographic images, complex formation between the membrane-distal N-terminal extracellular domains of E-cadherin and nectin-4 is clearly observed. AFM-based SMFS experiments indicate direct interaction between E-cadherin EC1-2 and nectin-4 IgV domains with the kinetic off-rate constant (koff) value of 31.48 ± 1.53 s^{-1} and the lifetime of the complex of 0.036 ± 0.0026 s. The dynamic force spectroscopy study indicates weak and transient heterophilic interaction of E-cadherin with nectin-4. This is further investigated by surface plasmon resonance (SPR) spectroscopy study which also supports the SMFS-based findings. We thus propose E-cadherin and nectin-4-mediated novel cell adhesion mechanism which can have functional significance in early embryogenesis as evident from the expression pattern of both the proteins during early development.

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Investigation of Amlodipine Besylate and Ranitidine Hydrochloride as Bacterial RNA Polymerase inhibitors

58

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The multi-subunit RNA polymerase (RNAP) is a validated bacterial therapeutic target, involved in transcription and cell growth[1]. In this study, we attempt to repurpose non-antimicrobial FDA approved drugs to act as RpoC inhibitors against *Streptococcus pyogenes* using comprehensive computational methods and biophysical techniques. *S. pyogenes* causes wide range of infections and toxin-mediated diseases in human beings of all age groups. With a fatality of 10-30%, limited antibiotic efficacy and the non-availability of vaccines, *S. pyogenes* is a global burden[2]. High throughput screening followed by Free Energy Perturbation (FEP) based binding free energy calculations and classical MD simulations identified Amlodipine Besylate (Amd) and Ranitidine hydrochloride (Rnt) as promising candidates with high binding affinity to functionally significant pockets of RpoC. The thermodynamic characterization done using Isothermal Titration Calorimetry provided insight into the stoichiometry, enthalpy and entropy of the interactions. The structural implications of the interaction were assessed using Infrared spectroscopy and Fluorescence spectroscopy. The major shift in the Amide-I peaks in FT-IR along with the non-linear dynamic quenching observed in Fluorescence spectroscopy confirmed that Amd causes significant structural alterations in RpoC on interaction. Conversely, RpoC-Rnt complex was highly stable with low binding energy but did not significantly alter the secondary structure of RpoC. Further investigation through Invitro Transcription (IVT) assays provided insights into the enzyme inhibition mechanism of the drugs. This study highlights Amd and Rnt as potential therapeutic candidates against *S. pyogenes*.

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A study of aggregation kinetics of Poly-ubiquitin fibrils

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Ubiquitin, a 76-residue polypeptide, acts as a post-translational modifier and forms poly-ubiquitin chains for diverse cellular functions. Ubiquitin is often found co-localized with β sheet-rich fibrillar aggregates – popularly known as amyloids - in the form of lesions associated with neurodegenerative disorders like Alzheimer's, Huntington's disease, etc. In contrast to its highly stable structure in the monomeric form, ubiquitin becomes thermodynamically unstable when it is conjugated with another ubiquitin molecule. Recent studies have shown that poly-ubiquitin chains form amyloid-like fibrils under in vitro conditions; however, the exact mechanism of this conversion and the role of ubiquitin chains in amyloidosis remain areas of active research. This study aims to characterise these poly-ubiquitin fibrils to understand under what conditions do these thermodynamically destabilised poly-ubiquitin chains undergo fibrillation, thereby elucidating its significance in the case of neuropathological conditions. Using various spectroscopic approaches like CD and Thioflavin-T assay, it was observed that the rate of fibrillation is regulated by pH and salt concentration. Additionally, it has been observed that neutral polymers like polyethylene glycols regulate the rate of fibrillation, which depends on both the concentration as well as the length of the polymer. Further investigation about how fibrils respond to specific conditions would allow a detailed perspective of disease progression and thus, guide the development of innovative therapies and diagnostic tools.

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. Proteins, subjected to biologically relevant mechanical forces 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. Traditionally, the mechanical response recorded from AFM is ascribed to the feature of individual protein domains. However, many proteins exist in parallel/antiparallel fashion to execute their biological functions.[1] For example, in striated muscle sarcomere, the giant muscle protein titin exists as hexameric bundles to mediate the passive elasticity of muscles. A special molecular biology design is necessary to probe the mechanical features of parallelly arranged protein domains. A previous study[2] on the mechanical response of parallely arranged protein domains, using alpha-helical coiled coils, showed that the unfolding force of parallel dimers was double that of individual domains. Another study[3] using SpyCatcher-Spytag chemistry and covalent immobilization of proteins on coverslip suggested that the ratio of unfolding force in parallel arrangement versus monomer was always less than 2. Both the previous studies had drawbacks related to the design of the system. Our study provides a molecular biology design more suitable to study the mechanical features of proteins arranged in parallel. We use incorporation of unnatural amino acids to create a junction in the polyprotein construct and keep the alpha-helical coiled-coil domain at the terminus of the construct for heterodimerization. This forms a conserved parallel construct whose mechanical features may directly be probed through AFM. Additionally, our design may potentially be extended to parallel tetramers which should mimic a more natural biological condition. Our study aims to provide a suitable molecular biology design to probe and draw a clear conclusion regarding the mechanical properties of parallelly arranged proteins.

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Bridging the Knowledge Gap: Investigating K18 and K19 Aggregation through Protein Charge Transfer Spectra (ProCharTS)

61

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Tau K18 and K19 are two truncated forms of the microtubule-associated protein Tau, implicated in neurodegenerative diseases[1] like Alzheimer's disease. K18 contains four microtubule-binding repeats (4R) while K19 lacks the second repeat (3R).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.

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Understanding Self-Peptide Insertion During Protein Folding

62

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Self-peptides are peptides derived from the same protein. Previous studies have shown that self-peptides from specific segments can affect the folding of their parent protein by competing with the corresponding native segments during folding, causing the protein to fold onto the peptide instead — which effectively inserts the peptide into the protein. However, why specific self-peptides insert and how, is not yet understood. Here, we used MD simulations of Cα structure-based models to examine the same. In SARS-CoV-2 MproC (discontinuous folding nucleus), only self-peptides derived from the early folding segments could insert. Moreover, the order of contact formation by the self-peptide during insertion was similar to that of the corresponding native segment during folding. However, in ubiquitin (localized folding nucleus), a self-peptide derived from outside the folding nucleus could also insert. Moreover, for the self-peptide derived from the folding nucleus, the peptide-insertion route did not follow the native folding route. This is probably due to the lack of connection between the self-peptide and the protein, which allows the peptide to form non-local native contacts more easily than the corresponding native segment and thus, insert via an alternative non-native route. Previous studies on folding modifiers had identified small molecules and peptides which correspond to the protein's early folding regions or bind to its folding intermediates. Our results indicate that self-peptides derived from segments which do not fold early in the native folding route, also have the potential to act as folding modifiers.

Silver Incorporated Reduced Graphene Oxide-Molybdenum Disulfide (Ag-rGO-MoS2) Hybrid Material for Bio-photonic Application

63

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Cancer is one of the pernicious diseases in the human life due to its high death rate and incurability. Though there are several advances in treatment modalities, several factors like rapid metabolism, non-specific cytotoxicity and multi-drug resistance limits its effectiveness in therapy. In this regard, architecting novel advanced hybrid materials are the ultimatum entail for multifunctional traits and enhanced responses to the desired inputs. The advent of exceptionally interesting class of 2D materials like graphene and graphene like materials has triggered a wide range of applications including bio-photonics owing to their unique properties. Further, the incorporation of metals to these organic-inorganic complex system enhances the effectiveness of anti-cancer activity due to their improved biocompatibility, pharmacokinetics and excellent target specificity. Thus, the present work is on preparation of multifunctional metal incorporated organic-inorganic hybrid nanocomposite (Ag-rGO-MoS2) suitable for laser based targeted cancer therapy. The limiting action of the Ag-rGO-MoS2 hybrid under 532 nm nanosecond green laser excitation was studied using Z-scan technique. MTT assay was carried out to study the antiproliferative action on MCF cancer cell line. The cytotoxicity studies revealed that the percentage of live cells decreased in the presence of hybrid showcasing higher percentage of inhibition. The morphological evidence for apoptosis and the detection of nuclei alterations activated by the prepared hybrid were analyzed using AO/EB staining and Hoechst staining respectively. The elemental, morphological, vibrational and ground state absorption studies were also analyzed. Thus, pleiotropic actions of Ag-rGO-MoS2 hybrid were demonstrated for the possible use in laser bio-photonics applications.

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Phase Transitions in Intrinsically Disordered RNA Binding Proteins and Crucial Role of RGG Motifs

64

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Recently, it has been appreciated that intrinsically disordered proteins (IDP) form condensates through liquid-liquid phase separation and exhibit reversible assembly/disassembly in response to thermodynamic conditions, physiological conditions, and post-translational modifications. These condensates play a crucial role in many neurodegenerative disease, such as, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Our current work consists of understanding how post-translational modifications, sequence modifications of protein fused in sarcoma (FUS) alter the transport and thermodynamic properties of the IDP in presence or absence of RNA. We consider the case where IDP is characterized into multiple domains based on their amino acid composition and sequence pattern. By means of coarse-grained and all-atom simulations we characterize the role of arginine-glycine (RG) rich regions containing multiple RGG and RG motifs, termed as RGGRG regions, on the propensity of phase separation. First, we systematically show the effect of changes in RNA binding regions on the phase behavior of FUS variant sequence. Using the phase diagram as benchmark, we further explore the role of electrostatics and cation-π interactions in the stability or destabilization of the condensate. We further characterize the effect of point mutations on the material properties of the condensate, which are important for engineering purposes.

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Exploring the Anti-Fibrillating Effect of the Anthocyanidin Petunidin on the Aggregation and Amyloid Fibril Formation of α-synuclein Involved in Parkinson's Disease

65

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Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, is characterized by the pathological aggregation and amyloid fibril formation of α-synuclein (α-syn). Reducing or inhibiting the aggregation of α-synuclein can be a promising therapeutic approach for Parkinson's disease. One potential approach to inhibit α-syn aggregation is the use of naturally occurring polyphenols. In this study, we investigated the anti-fibrillating properties of Petunidin, an anthocyanidin known for its antioxidant, anti-inflammatory, anti-obesity, and neuroprotective effects, on α-syn aggregation and amyloid fibril formation. Fluorescence titration-based studies revealed that Petunidin weakly interacts with α-syn, with an association constant of 10^4 M^{-1}, and modulates the fibrillar process towards the formation of amorphous aggregates, as observed through the imaging techniques, AFM. ThT assay, ANS assay, and static light scattering data have shown concentration-dependent suppression, formation of less hydrophobic species and reduction in the size of aggregates of α-syn in the presence of Petunidin. Fourier-transform infrared (FTIR) spectroscopy also demonstrated that the resulting aggregates are rich in α-helical content. The results suggest that Petunidin is an effective inhibitor towards the aggregation and fibrillation of α-syn and could be further explored as a therapeutic candidate for Parkinson's disease.

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ARP-2/3 regulates cell surface dynamics of CeTOCA-1 in the *C. elegans* zygote

66

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ARP-2/3 mediated polymerization of branched actin filaments plays critical roles in diverse cellular processes. This polymerization is tightly controlled through precise regulation of the upstream CDC-42 GTPase, by effectors such as members of WASP/N-WASP and TOCA protein families. Although TOCA-1 has been implicated in the ARP-2/3-dependent polymerization of branched actin filaments, its interaction with cortical F-actin structures- both branched and linear- remains poorly understood. By analyzing iPTM values associated with the interaction between different domains of CeTOCA-1 and CDC-42, we found that while the HR-1 domain is essential, SH3 domain is dispensable for the complex formation between the two proteins. Furthermore, we disrupted ARP-2/3 and CYK-1/formin polymerized F-actin structures in *Caenorhabditis elegans* zygotes, to examine the role of cortical F-actin on CeTOCA-1 assembly dynamics, and biophysical properties. Co-localization studies revealed a preferential association between CeTOCA-1 and the pool of F-actin structures polymerized by ARP-2/3. Disruption of ARP-2/3 led to formation of larger CeTOCA-1 clusters, prolonged cluster lifetime on the cell surface, and reduced cluster mobility. These findings suggest that distinct F-actin structures play specific roles in mediating plasma membrane interactions and surface dynamics of CeTOCA-1 clusters.

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Phase Separation Dynamics of FLTDP-43: A Protective Mechanism or Pathological Driver in Neurodegeneration?

67

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The accumulation of TDP-43 (Tar DNA-Binding Protein-43 kDa) aggregates in the central nervous system is a common feature of many neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTLD). When the cells sense some stress they undergo phase separation for their survival but when stress is prolonged they undergo aggregation. TDP-43 is a nucleic acid-binding protein that performs physiologically essential functions and is known to undergo phase separation and aggregation during stress. Initial observations have shown that TDP-43 forms heterogeneous assemblies, including monomers, dimers, oligomers, aggregates, phase-separated assemblies, etc. However, the significance of each assembly of TDP-43 concerning its function, phase separation, and aggregation is poorly known. We employ biophysical techniques to conquer the phase separation and aggregation mechanism of FLTDP-43. We also checked the dynamicity of the phase-separated assemblies using FRAP (Fluorescence Recovery After Photobleaching) to understand their rigidity. This study enlightens on the early steps involved in phase separation to aggregation which gives us a path to understand the toxicity of the assemblies, which could help to design the drug to cure protein misfolding diseases.

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Integrative Structure Determination of Sub-Complexes of the Mitochondrial Contact Site and Cristae Organizing System (MICOS)

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The mitochondrial contact site and cristae organizing system (MICOS) complex is an inner mitochondrial membrane (IMM) assembly, present at the cristae junction (CJ). It regulates cristae formation and remodeling[1]. The structure of the MICOS complex is not well-characterized and functions of its subunits are not known. We applied Bayesian integrative structure determination to characterize structures of sub-complexes of the MICOS complex, containing Mic60, Mic19, Mic10, and Mic13, combining information from crosslinking mass spectrometry (XLMS), biochemical assays, electron tomography (ET), homology modeling, AlphaFold predictions, and sequence alignments[2]. Our models suggest that Mic10 and Mic13 together mediates the interaction between the MICOS sub-complexes. Further, we were able to localize the Mic19 coiled-coil domain and its interaction with Mic60 coiled-coil tetramer. Overall, this approach provides insights into the structure and function of MICOS subunits that can be used as hypotheses for future experiments.

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A First Passage Time Study of Bacterial Eradication Under the Influence of Antibacterial Agents

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Studies concerning bacterial eradication have become important in the light of growing antibiotic resistance observed around the world. Hence, it is important to develop and optimize new strategies and discover alternatives for treatment against drug resistant bacteria. Considering this, we apply the method of first passage time (FPT) to theoretically scrutinize the bacterial population dynamics in the presence of antibacterial agents. First, we demonstrate that the extinction time for the bacterial population in the presence of antibiotics can be lowered by reducing the growth rate of bacteria. Next, we examine the antibacterial role of silver nano-particles (AgNPs) against the pathogenic bacteria E*scherichia coli.* We find that in comparison to antibiotics, the same concentration of AgNPs require more time for complete clearance of the bacterial population. Therefore, in order to reduce this extinction time,we investigate the combined effect of AgNP and Ampicillin. Our results suggest that AgNP combined with Ampicillin can be a substitute in tackling resistance against *E. coli.*

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Two-Dimensional Light-Sheet Optical Tweezer (2D-LOT) Facilitates the Trapping and Imaging of Live Cells in a Selective Plane

70

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We report a Two-Dimensional Lightsheet Optical tweezer for trapping and interrogating live biological specimens within a selective, well-defined 2D plane. It utilizes an IR laser directed through a cylindrical lens and a high NA objective, generating a tightly focused, diffraction-limited thin light sheet. When subjected to the Lightsheet, the target specimens (live cells) get trapped in the plane. The illumination is along the Z-direction, with sample holder aligned along the XZ plane. However, the detection subsystem is placed orthogonal to the XZ plane, i.e., along the Y-axis, to visualize the specimen getting trapped in the selective light sheet plane. A switchable illumination subsystem is integrated with the 2D-LOT system to illuminate the specimen in widefield and fluorescent mode. The trap region is measured to be 2073.84 μm^2, with a cylindrical lens of focal length 75 mm and objective of 0.5 NA. With a trap stiffness of $k_z = 1.17 \pm 0.034$ pN/μm and $k_x = 0.78 \pm 0.021$ pN/μm, along Z and X- axes respectively, the 2D-LOT system can trap 5-6 cells in a plane in one go. The system provides a powerful platform to trap and image simultaneously in fluorescent mode. Results show successful trapping and imaging of live cells, including HEK-293T human cells, NIH3T3 mouse fibroblasts, and HeLa cells, within the trap region. The technique offers efficient, stable trapping of live specimens with sub-cellular resolution, demonstrating its potential for selective 2D confinement for biophysics and cell biology studies.

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Poster Presentations

Session 2: March 8

Unravelling the Influence of Cholesterol and Membrane Composition on Drug Transport Dynamics

1

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Phospholipids are the basic building blocks of biomembranes that act as semipermeable barriers and play crucial roles in cell signaling, protein binding, fusion, transport, etc. Recent advancements in computational studies, specifically molecular dynamics (MD), have been quite promising in describing the dynamic behaviour of membranes and their structural features. Even though there are approaches to building realistic biomimetic membrane models, they lack experimental validations and no consensus owing to their structural diversity; thus, model membranes are primarily used for MD studies. Here, the effect of membrane composition on the passive diffusion of small molecules is studied using MD. The primary focus is on elucidating the influence of cholesterol on membrane packing and its subsequent effect on drug transport. Through umbrella sampling simulations, the potential of the mean force of drug transport across the membrane has been determined[1], and drug diffusivity is estimated through positional autocorrelation functions[2]. The study provides crucial insights into the mechanistic role of membrane composition and cholesterol on drug-membrane interaction studies and its potential implications for designing drug delivery systems and developing pharmaceuticals targeting membrane-associated processes.

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All-Atom Molecular Dynamics Simulations of Glioblastoma Patient-Derived Calcitonin Receptor Mutants Elucidate Mechanisms Behind their Loss-of-Function

2

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Calcitonin receptor (CALCR), a member of the class B family of GPCRs, binds calcitonin (CT), a neuro-active peptide, to maintain calcium homeostasis in bone and kidney. Interestingly, in a recent clinical cancer study[1], several mutants of CALCR were identified in Glioblastoma patients, which were inadequate in tumour suppression. Using all-atom MD simulations as the primary tool, our work here tries to understand the effect of mutants on the CALCR signalling pathway from a structural and molecular dynamics perspective. Towards that, we constructed distinct CALCR-bilayer systems with calcitonin and the G-protein complex. We analyse our trajectories using contact-map evolution, residue-wise interaction energy and cross-interaction with the lipids and CT peptide. Our simulations data show that the position of the mutation affects the protein conformations and dynamics that could explain the phenotypic behaviour observed in experiments. For example, our data shows that mutation near intracellular loop 2 (V250M) significantly enhances the ICL2 flexibility and in turn affects the G-protein binding. We also find that R404C and R420C mutations on the intracellular region remarkably reduces interaction with membrane and could potentially impact the protein's activity. Our preliminary analyses on mutants located in the extracellular domain (R45Q, A51T and P100L) indicate that these mutants affect the conformational landscape of the ECD of CALCR. Together, in conjunction with the available experimental data, our in silico conformational landscape investigations of the WT and mutant CALCR systems can be used to establish the molecular mechanisms of CALCR activation in health and disease states.

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Mechanistic of repeat RNA folding and its interaction with drug molecule using molecular dynamics simulations

3

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Underearning the folding of repeat expansion in RNA sequence to non-canonical secondary structures is important due to their established implications in neurodegenerative diseases and dementia.[1,2] Distilling several microseconds long equilibrium molecular dynamics (MD) simulation trajectories, we have explored the role of sequence length and salt type in the structure and folding dynamics of GGGGCC (G4C2) RNA repeat sequence. Taken together with the limitation of equilibrium MD simulations, we found that six and 4-repeat units of G4C2 leads to ensemble of structures, sometimes resembling to G-quadruplex like structures, other time hairpin like structures. While increasing the temperature, we find that the self-assembled G-quadruplex remain stable across 27–61°C in explicit solutions. Next, we have identified a natural compound as a prospective drug target and shown its binding to the terminal cytosine residues of RNA G-quadruplexes in MD simulations. Our findings, for the first time, shed light on folding dynamics of G4C2 RNA repeat sequence. Results from the drug and RNA G-quadruplex simulations present the natural compound as a potential target for therapeutic by destabilizing the RNA secondary structure which will be tested further using circular dichroism.

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Biologically Meaningful Regulatory Logic Enhances the Convergence Rate in Boolean Networks and Bushiness of their State Transition Graph

4

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Boolean models of gene regulatory networks (GRNs) have gained widespread traction as they can easily recapitulate cellular phenotypes via their attractor states. Their overall dynamics are embodied in a state transition graph (STG). Indeed, two Boolean networks (BNs) with the same network structure and attractors can have drastically different STGs depending on the type of Boolean functions (BFs) employed. Our objective here is to systematically delineate the effects of different classes of BFs on the structural features of the STG of reconstructed Boolean GRNs while keeping network structure and biological attractors fixed, and explore the characteristics of BFs that drive those features. Using 10 reconstructed Boolean GRNs, we generate ensembles that differ in BFs and compute from their STGs the dynamics' rate of contraction or 'bushiness' and rate of 'convergence', quantified with measures inspired from cellular automata (CA) that are based on the garden-of-Eden (GoE) states. We find that biologically meaningful BFs lead to higher STG 'bushiness' and 'convergence' than random ones. Obtaining such 'global' measures gets computationally expensive with larger network sizes, stressing the need for feasible proxies. So we adapt Wuensche's Z-parameter in CA to BFs in BNs and provide four natural variants, which, along with the average sensitivity of BFs computed at the network level, comprise our descriptors of local dynamics and we find some of them to be good proxies for bushiness. Finally, we provide an excellent proxy for the 'convergence' based on computing transient lengths originating at random states rather than GoE states.

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Effect of Hydropathy Scale on Intrinsically Disordered Protein Properties Predicted using Implicit Solvent Simulations

5

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Intermolecular interactions in proteins and their interaction with solvents, driven by their electrostatic and hydrophobic nature, determine the conformation of proteins. In particular, hydrophobic interactions play a crucial role in determining the conformations of intrinsically disordered proteins and form the driving force for liquid-liquid phase separation. Molecular dynamics simulations with explicit water molecules, which recapitulate hydrophobicity interactions naturally, have a high computational cost and are not feasible for large systems. Reducing the computational cost by coarse-graining the amino acid representations is a commonly used approximation to study biomolecular properties. Unlike electrostatic and bonded interactions, coarse-grained modelling of hydrophobic interactions by theoretical methods – especially in a solvent-free representation – is a formidable task and often replete with errors. To accurately capture the hydrophobicity of each amino acid, researchers have relied on experimental data to develop hydropathy scales that assign a number called the hydropathy parameter, which is derived from various biophysical properties. Since these scales are derived from experiments designed to capture a particular biophysical property, one scale does not apply to all IDPs. In this study, we tested the accuracy of 24 popular hydropathy scales to capture the behaviour of 16 commonly studied IDPs using coarse-grained simple polymer models. We compare the simulation results with the experimental values and show that different scales demonstrate varying performance, each showing optimal accuracy for specific IDPs.

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Non-Equilibrium Kinetics of Chaperone Molecules in Protein Folding

6

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Proteins must fold into their native structures to perform essential cellular functions. However, they can sometimes misfold into non-native structures, resulting in reduced functionality or malfunction. To counteract this, cells employ chaperones that prevent misfolding and assist proteins in achieving their functionally active state by utilizing the energy released from ATP hydrolysis. While this energy-driven process is essential, its impact on chaperone action, particularly the limits and constraints imposed on their efficiency by energy flow and dissipation, still needs to be fully understood. In this work, we built a detailed kinetic model of the Hsp70 chaperone system in *E. coli* by incorporating all key structural and kinetic details. Using a chemical kinetic framework, we analyze the consequences of energy expenditure on the chaperone's protein folding efficiency. Our results show that ATP consumption significantly enhances the folding of proteins into their native states. Chaperones achieve optimal efficiency when their binding to misfolded proteins is much faster than the misfolding kinetics of those proteins. We further demonstrate the presence of an upper bound on a chaperone's efficiency in protein folding and its overall rescue rate. This upper bound increases with energy dissipation but saturates beyond a certain point. Additionally, we highlight a speed-energy-efficiency trade-off in chaperone action, showing that it is impossible to simultaneously optimize the efficiency of chaperone-assisted protein folding and the energy efficiency of the process. These findings deepen our understanding of chaperones as vital components in maintaining cellular viability and proper functioning.

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Structure and Self-assembly of DNA Nanostructure in Lipid Bilayer Membrane

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Creating synthetic biosystems that reproduce the functionality and outperform naturally evolved biological systems is one of the most challenging and intriguing aims of nanoscience. In past three decades, DNA has established itself as a building block for designing custom shaped nanostructures with sub-nanometer precision. Among myriads of nanostructures, DNA nanostructure tethered to lipid membranes are particularly interesting and have shown some promising applications as tools in synthetic biology. These hydrophobically modified DNA nanostructures can also form transmembrane ions channels, hence can be used in synthetic biology for numerous applications such as mimicking membrane remodelling proteins, working as artificial enzymes etc. The binding and assembly process of hydrophobically modified DNA nanostructures in lipid bilayer membranes is too fast to be probed in molecular detail using conventional biophysics approaches. With dramatic improvement in the architecture of high performance computers (HPC), artificial intelligence-based methods and simulation algorithms, computer simulations have become a powerful tool in predicting the biomolecular behavior and properties at nanoscale. In this work, we propose to deploy high performance computer simulations to reveal the self-assembly of membrane spanning DNA nanopores and decipher the molecular level interactions. The outcome of the simulation is expected to improve the design of DNA based nanopores for next generation technologies.

A Hydrophobic Mismatch in the Transmembrane Helices of Class I Viral Fusion Proteins may Facilitate Viral Fusion

8

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We recently reported that the sequence of the SARS-CoV-2 spike protein transmembrane (TM) helix makes it very dynamic[1]. We found that the composition of the lone TM helix of the spike protein allows it to self-assemble in different conformations and stoichiometries. Moreover, we found that the spike TM helix has a hydrophobic mismatch to the viral envelope it resides in. We argue that both of these properties of the spike TM helix facilitate the dynamics of the spike protein ectomembrane region during viral fusion. New data suggests that this might be a general feature of TM domains of fusion proteins of many unrelated viruses. Specifically, the predicted lengths of a curated dataset of viral fusion protein single pass TM helices are on an average 3-8 residues longer than those involved in human cell fusion processes. Considering that these viral fusion proteins undergo a large conformational change to fuse viral envelope to cellular membranes, perturbation of the viral envelope by the dynamics of a hydrophobically mismatched fusion protein TM helix would assist the process. This could be a conserved feature in fast evolving virus fusion proteins to reduce the kinetic barrier to viral membrane fusion.

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π - π and Cation-π Interactions are Required in Intrinsically Disordered Protein Coarse-Grained Models to Recapitulate Experimental Reality

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Here, we present an improved version of the 2-site per amino-acid resolution Self-Organized polymer (SOP) model for studying Intrinsically Disordered Proteins (IDPs). The originally SOP-IDP model works well for well-solvated chains that adopt expanded conformations[1,2]. However, we found that SOP-IDP force-field parameters need to be recalibrated for IDP chains whose Flory exponents deviate from the random-coil limit. For example, for hnRNPA1-LCD, the SOP-IDP force field produces conformational ensembles (with mean Rg of 3.26 nm) that are much more expanded as compared to the experiments (with mean Rg of 2.70 nm) with an error of ~20%. Other examples also suggest that there is scope for refining the SOP-IDP model[3]. Most condensate forming IDPs are rich in residues that can form π - π and cation-π interactions, which are weak but important interactions for biomolecular recognition and also for driving and stabilizing biomolecular condensates. In our improved model, we reparametrized the force field to account for π - π and cation -π interaction at a very coarse-grained level such that the simulations provide experimentally-consistent behaviours for IDPs both in terms of single-chain and bulk properties – especially those rich in cation and aromatic residues. Our method addresses an immediate need in the IDP-biophysics and biomolecular condensate simulations community by providing a well-grounded prescription to simulate and generate faithful conformations of IDPs that are better suited to recapitulate experimental realities.

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Evolutionary and Functional Insights Among CRISPR-Cas Systems Through Direct Repeat Analysis

10

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The CRISPR-Cas system is a vital component of prokaryotic genomes, functioning not only as a defense mechanism through its three key stages—adaptation, expression, and interference—but also contributing to gene regulation, genome evolution, and DNA repair[1]. Central to these functions is the CRISPR repeat, which plays a critical role in regulating the various aspects of CRISPR-Cas activity[2]. Despite its importance, the comparative genomic analysis of CRISPR-Cas systems based on direct repeats remains underexplored, leaving key evolutionary and functional insights unaddressed. This study aims to fill this gap by analyzing the sequences of direct repeats across various classes, types, and subtypes of CRISPR-Cas systems to identify mechanistic similarities. Our findings reveal conserved nucleotide arrangements and consistent length distributions in direct repeats. We also uncover a significant relationship between specific nucleotide motifs and length distributions, providing a foundational understanding of this correlation utilizing a kinetic model based the DNA unzipping kinetics[3]. By clustering different types and subtypes of CRISPR-Cas systems based on their nucleotide motifs, we establish evolutionary and functional parallels within each cluster.

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NBAR-Mediated Membrane Deformations at Multiples Scales Using Integrated Continuum Mechanics and Molecular Simulations

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Membrane remodeling is an essential process in cellular functions that occurs by changing the curvature of the membrane through the actions of various proteins and lipid molecules. This process takes place at different timescales, which makes it difficult to study using a single computational technique. To understand this process in a more sophisticated way, we present an integrative biology framework that models membrane deformation using a continuum mechanics model and then maps the continuum results into a coarse-grained molecular model to study the process further. In this study, we choose the N-BAR mediated membrane deformation process as our model system[1]. We model the membrane as a triangulated surface and represent proteins as nematics. We then use a Helfrich-type free energy function to describe the system and perform Monte Carlo simulations to investigate the protein rearrangements that drive membrane remodeling. Once the model has converged, we use the continuum output and convert the system from continuum representation to a coarse-grained membrane-protein system with higher molecular detail by representing a protein with 26-bead hENM model and membrane with 3-bead highly coarse-grained lipids. The higher resolution model, built from the continuum modeling, is used for coarse-grained molecular simulations to get deeper molecular-level insights into processes leading to membrane remodeling due to N-BAR proteins[2].

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Understanding RNA Chaperon Activity of ProQ Protein using Molecular Dynamics and Enhanced Sampling Method

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ProQ, recently identified bacterial RNA chaperone, plays a pivotal role in post-transcriptional gene regulation by promoting sRNA-mRNA interactions. While its RNA binding activity and the importance of its N-terminal domain (NTD) have been established, a comprehensive understanding of the molecular mechanism underlying its RNA binding remains elusive. To address this gap, we employed extensive molecular dynamics simulations, including spontaneous binding simulations and on-the-fly probability-based enhanced sampling (OPES) simulations, to investigate the binding mechanism of raiZ sRNA to ProQ and its free energy calculation. Our simulations revealed that raiZ binds to the concave surface of ProQ in a semi-folded state, stabilized by strong electrostatic interactions. The binding process involves a concerted effort from the NTD, CTD, and linker domain of ProQ, highlighting the importance of a coordinated interaction for efficient RNA binding. Furthermore, in-silico mutagenesis studies identified some key residues as critical for RNA binding. Disrupting these interactions led to the unbinding of raiZ from the ProQ binding pocket. To enhance the efficiency of our simulations and overcome time scale limitations in classical MD simulation, we employed advanced sampling techniques such as OPES to get a complete free energy landscape . To design effective collective variables (CVs) for OPES, we utilized Deep-Targeted Discriminant Analysis (Deep-TDA) to identify key features and patterns in the conformational space. Our findings provide valuable insights into the molecular mechanisms underlying ProQ-mediated RNA interactions. These insights can be leveraged to design novel strategies for modulating gene expression and developing therapeutic interventions targeting RNA-based regulatory pathways against Bacterial Disease.

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Beyond Annotation: Identifying Potential Proteins in *D. melanogaster* and *C. elegans*

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We systematically translated the genomic sequences of *Drosophila melanogaster* (DM) and *Caenorhabditis elegans* (CE) in all six reading frames, terminating translation at the first stop codon encountered. We found approximately 8 million translations for both organisms. We restricted our analysis to 813,000 DM and 478,000 CE translations of lengths greater than 60 amino acids. The known protein sequences (exons) in DM, CE, and their respective close species were identified using BLAST searches against the DM and CE protein databases. This way, approximately 40,000 and 24,000 sequences were excluded from further analysis. The remaining sequences, 772,000 in DM and 454,000 in CE were further annotated through BLAST, but this time, against the entire Uniref90 database. Out of which only 18,000 sequences in DM and 7,000 sequences in CE matched to any protein against the database. After excluding the low-complexity sequences, the final dataset contained 16,000 DM and 6,100 CE sequences, matching organisms ranging from bacteria to fish. We also obtained the structural predictions for these sequences using OmegaFold. These sequences could represent sequencing artefacts or potential new/dormant functional proteins that are a few mutations away from being translatable. We believe such mutations might already exist in small subpopulations. Preliminary experimental validation via mass spectrometry identified one sequence in the hemolymph proteome of DM, corresponding to a PH domain-containing protein from Anopheles christyi with 295 amino acids. Future work will characterize these "new" proteins and explore the pathways and interactions they are involved in.

Metal Ion Driven Folding of GAAA Tetraloop Receptor Complexes

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GAAA-tetraloop receptor complexes are frequently found in self-splicing group I and II introns and help to stabilize the folded structure of introns during the self-splicing activity for the formation of mature messenger-RNA. To investigate how the GAAA-tetraloop receptor complexes help to stabilize the folded structure of introns, we have studied the Mg^{2+} induced folding of P4-P6 domain of Tetrahymena thermophila ribozyme[1] using coarse-grained (CG) and all-atom (AA) computer simulations. Coarse-grained simulations[2] using the TIS RNA model revealed that the P4-P6 domain of Tetrahymena thermophila ribozyme folds to its native structure via the formation of an intermediate. Metadynamics simulations[3] using all-atom models show that water-mediated outer-shell (OS) interactions between Mg^{2+} ions and the phosphate backbone of RNA, along with the condensation of Mg^{2+} ions on the major groove, are crucial for maintaining the stability of the native folded state. The non-native intermediates populated during folding contributed to increasing the folding energy barriers of this complex. This study provides critical insights into the folding and function of more complicated intron structures.

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Unveiling a Novel Inhibitor of EGFR Tyrosine Kinase: An In-Silico study

15

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The epidermal growth factor receptor (EGFR) is a transmembrane protein essential for controlling cell migration, differentiation, proliferation, and survival in a variety of cellular activities. EGFR overexpression is linked to numerous cancers, making it a promising target for the development of anticancer drugs. Given this, the primary goal of the current investigation is to identify the best phytoconstituent of well-known medicinal plants that has the ability to inhibit EGFR tyrosine kinase (EGFR TK). We have considered 2000 such molecules and the standard drug Gefitinib as a control of our study. Though many compounds displayed binding affinities that were better than the cut-off -7.4 kcal/mol (docking score of Gefitinib, the standard drug), Withacoagulin is the most promising candidate by the in-silico ADMET analysis. Further, from molecular dynamics (MD) simulation and binding free energy calculations, we found that Withacoagulin interacts with EGFR TK more strongly than Gefitinib and induces conformational changes that move the protein closer to its inactive state. Thus, after validation from the wet lab experiments and clinical trials, Withacoagulin can be considered a potential anticancer drug linked to EGFR overexpression.

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Computational Approach for Decoding Malaria Drug Targets from Single-Cell Transcriptomics and Finding Potential Drug Molecule

16

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Malaria is a deadly disease caused by Plasmodium parasites. While potent drugs are available in the market for malaria treatment, over the years, Plasmodium parasites have successfully developed resistance against many, if not all, front-line drugs. This poses a serious threat to global malaria eradication efforts, and the continued discovery of new drugs is necessary to tackle this debilitating disease. With recent unprecedented progress in machine learning techniques, single-cell transcriptomic in Plasmodium offers a powerful tool for identifying crucial proteins as a drug target and subsequent computational prediction of potential drugs. In this study, We have implemented a mutual-information-based feature reduction algorithm with a classification algorithm to select important proteins from transcriptomic datasets (sexual and asexual stages) for Plasmodium falciparum and then constructed the protein-protein interaction (PPI) networks of the proteins. The analysis of this PPI network revealed key proteins vital for the survival of Plasmodium falciparum. Based on the function and identification of a few strong binding sites on a couple of these key proteins, we computationally predicted a set of potential drug molecules using a deep learning-based technique. Lead drug molecules that satisfy ADMET and drug-likeliness properties are finally reported out of the generated drugs. The study offers a general computational pipeline to identify crucial proteins using scRNA-seq data sets and further development of potential new drugs.

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Structure, Dynamics Small Molecule Modified DNA Hydrogel

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The same molecular properties which make DNA a suitable choice for building nanostructures also makes it attractive target for engineering hydrogel. It has been shown that the mechanical properties of DNA hydrogels can be enhanced by modifying them using small molecules like cyanuric acid. However, their viscoelastic response and understanding molecular contributors to their stiffness is still not well-understood. Here we have investigated the nanoscale structure and dynamics of cyanuric modified DNA hydrogels. Through equilibrium all-atom molecular dynamics simulation trajectories, we delineate the molecular level interactions governing the interplay of stiffness and elasticity of modified DNA hydrogels. We also study the effect of salt concentration and the strand length on the hydrogel structures and mechanical properties. The study encompasses all-atom simulations of DNA-cyanuric acid hybrids, revealing how adenine lengths impact hydrogen bonding and network stability. The findings from our study are expected to improve the rheological understanding of DNA hydrogel and pave the way for crafting next-generation biomaterials with tailored functionalities, drug delivery, and gene silencing applications.

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Dynamics of Broadly Neutralizing Antibody Interactions and Glycan Modulation in Glycosylated HIV-1 Env: A Clade-Specific Perspective from Molecular Dynamics Simulations and Machine Learning Approaches

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HIV-1 broadly neutralizing antibodies (bNAbs) interact with the highly glycosylated envelope spike protein (Env), which comprises a trimeric assembly of gp120 and gp41. This study investigates the molecular mechanisms underlying bNAb binding to fully glycosylated Env, focusing on the key epitope, utilizing atomistic molecular dynamics (MD) simulations. We examine clade-specific interactions (clades A, B, and C) of the Env protein with a clinically relevant bNAb to uncover binding-induced conformational changes, glycan dynamics, and the impact of glycan removal on Env stability and dynamics. Accelerated MD simulations further enable the identification of rare glycan-mediated events, providing a detailed view of glycan behavior and its impact on modulating bNAb interactions. To enhance our insights, we integrate machine learning models to predict binding affinities for bNAb interactions across various HIV-1 clades. This combined approach helps identify key glycan features influencing antibody binding and offers a comprehensive analysis of the relationship between glycan structure and bNAb efficacy. These findings could improve the rational design of effective therapeutic antibodies and vaccines against diverse HIV-1 clades.

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Mathematical Modelling of Cellulose and Chitin Degradation in the Presence and Absence of Lytic Polysaccharide Monooxygenases

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Chitin is the most abundant natural polysaccharide which is commonly found in crustacean shells and fungal cell wall. On the other hand, Cellulose is the most abundant biopolymer on earth, which is an important structural component in the plant cell wall. These polysaccharides make rigid and compact networks that provide durability and recalcitrance to the cell wall. CAZymes such as cellulases, chitinases, and lytic polysaccharide monooxygenases (LPMOs) plays a major role in biotechnological transformation of chitin into chitosan and chitooligosaccharides, cellulose into glucose and other smaller oligosaccharides. LPMOs are monocopper enzymes that catalyze oxidative cleavage of glycosidic bonds in these crystalline polysaccharides. It assists in the degradation process of chitin and cellulose and boost the yield of oligosaccharides. The current gap is a kinetic and mechanistic model of LPMOs working in synergy with other CAZymes. Here, in this study functionally-based mathematical models have been developed that captures the enzymatic cocktail (consisting of endoglucanase, cellobiohydrolase, and β-glucosidase) with LPMO in the presence of the crystalline substrates, chitin and cellulose. We have incorporated Michaelis-Menten kinetics, various environmental conditions such as pH and temperature both in the presence and absence of LPMOs. Our results indicate that the presence of LPMOs indeed boosts the glucose yield and there is a significant difference when substrate (i.e., cellulose and chitin) concentration increases. Specifically, the yield of glucose for the 6500 Mol L^{-1} amount of solid cellulose, the amount of K-mer released in the absence of LPMOs is 9000 Mol L^{-1} whereas in the presence of LPMOs it is 22000 Mol L^{-1}.

A Comparative Study of Electrical Activity in Human Cardiac Cell Models: The Effects of Pacing and Ionic Conductance on EADs

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Early afterdepolarizations (EADs), defined as a reversal of the action potential (AP) before the completion of its repolarization phase, are a leading cause of irregular APs in cardiac cells, which can trigger fibrillation in the heart. However, the occurrence of EADs depends on how frequently a cardiac cell is paced by external stimuli. In this study, we carry out a comprehensive numerical simulation to investigate and compare the effects of pacing and ionic conductance variations on the induction of EADs using two human ventricular cell models: the TP04 and TP06 models[1,2]. Our systematic numerical studies reveal the existence of three distinct AP states in the TP04 model: (1) normal AP (nAP), (2) EADs AP (eAP), and (3) repolarization failure AP (rAP). In contrast, the TP06 model, an enhanced version of the TP04 that incorporates more detailed calcium dynamics, exhibits an additional AP state, namely, oscillatory AP (oAP), along with the above-mentioned AP states. We also observe that both models are capable of initiating backward waves through the EAD mechanism, a key factor in spiral instability within cardiac tissue, which can lead to fibrillation.

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Morpheus: An Fragment-Based Algorithm to Predict Metamorphic Behaviour in Proteins

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While the majority of proteins adhere to the traditional one-structure/one-function model, an increasing number of folded proteins have been found to possess dual structures and functions. These proteins can undergo significant structural changes under native conditions or in response to specific cellular stimuli and are classified as metamorphic proteins . This ability to "switch folds" allows proteins to perform multiple functions and enables the cellular machinery to tightly regulate various biochemical processes. Despite their importance in health and disease, not much work has happened that could assign a fold-switching protein from sequence information[2]. Here, we present a fragment-based approach to build a classifier that can predict metamorphic behavior in protein sequences with an average accuracy of 85% and a Matthew's correlation coefficient (MCC) of 0.7. The classifier works by making use of existing structural databases, both experimental (PDB) and machine learned (AlphaFold DB), to analyze the diversity of structures the fragments within a query protein sequence occupy. We employed our algorithm on 57 different proteomes consisting of a total of 618,458 protein sequences. We identified about 12% of these proteins to have the ability to fold-switch, which significantly expands the known metamorphic reservoir of proteins. Our work helps in the screening of a proteome for metamorphic proteins and in the selection of a potential candidate protein to evaluate its metamorphic behavior through further experimentation.

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Molecular Design Principles Underlying the Conformational Dynamics Diversity in EHD Paralogs

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Several reports have now established the important role of EHD, a class of ~60kDa ATPase that contains a C-terminal EH domain, towards partitioning and trafficking of proteins through endocytic recycling pathways. Mammals possess four paralogs of EHD; EHD1-4, which share ~70% or more sequence identity but exhibit high diversity in enzymatic activity, membrane-binding affinity, and membrane-remodeling functions. We use molecular dynamics simulations to address the molecular level differences among EHD paralogs by studying their ability to change conformation in solution state and membrane bound state and how it affects oligomerization, which inturn stimulates ATPase activity of EHD proteins. We build upon the modeling work using the limited structural information from the experimental studies reporting structures of EHD2 and EHD4 paralogs. Our large-scale simulation studies show that the Switch-1 loop acts as flap in the catalytic site and likely plays a substantial role in ATPase activity mediated by a $Mg2^{+}$ cation. In our simulations, we were able to observe differences between EHD2 and EHD4 paralogs in the protein residues and number of water molecules that are coordinating $Mg2^{+}$ ion in the first and second coordination sphere. The Switch-1 flap acts as a gateway for water entry to the catalytic site and is present in the interface oligomers. What triggers Switch-1 flapping motion, how does it affect oligomerization and what relation does it have with the non-conserved residues found among the EHD paralogs are few of the many questions that are being currently addressed.

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siRNA Complexation with Galactose-Functionalized Dendrimer: PAMAM vs PETIM

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siRNA-based therapies hold promise for treating various diseases, but efficient and targeted delivery remains challenging. This study explored the potential of galactose-functionalized poly(amidoamine) (Gal-PAMAM) and poly(propyl ether amine) (Gal-PETIM) dendrimers as siRNA carriers using all-atom molecular dynamics simulations. This study revealed distinct complexation behavior for both the system in physiological (pH ~ 7) and high (pH ~ 10) environments. Electrostatic interactions dominated complexation, with PMF profiles revealing stronger binding affinity for GalPAMAM/siRNA at pH 7. siRNA displayed lower flexibility at pH 7 for both complexes, indicating enhanced stability. These findings suggest that Gal-PAMAM dendrimers at physiological pH hold promise for efficient siRNA delivery due to their superior complex stability and interaction strength. However, in polar solvents, Gal-PETIM exhibits hydrophobic behavior, enabling rapid delivery.

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Validation of Various Urea Force Fields through Vibrational Spectroscopy and Interface Surface Area

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Self-aggregation of external solutes in an aqueous solution is an important factor in understanding its stabilizing/deaturing effect. Urea is a well-known denaturant and highly soluble in water in this work, we aim to validate various urea forcefields by leveraging theoretical isotope dilute IR spectroscopy and correlating it with Interface Surface Area. We compare different types of urea force fields (Smith Kirkwood Buff [1], GAFF-D3, Duffy, Duffy (OPLS-AA-D)). To interconnect the vibrational spectra with self-aggregation we choose Interface Surface Area as a parameter by following the methodology of Grubmüller et al[2]. Reduction of interface surface area suggests self-aggregation of urea. Smith Kirkwood Buff derived model of urea produces the most accurate IR spectra in comparison with experiments without any concentration dependence, and GAFF-D3[3] produces a peak at a higher frequency referring to weakening of W-W hydrogen bonding upon increasing urea concentrations. Both versions of the Duffy[3] model show strong concentration dependence in the IR spectra. Upon investigating the self-aggregation of urea in these four models we find that Smith (KWB) is slightly repulsive in nature on the other hand both versions of Duffy are attractive in nature whereas GAFF-D3 is neutral with a large standard deviation from the average interface surface area.

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Characterization of Aureochromes: A Unique Photoreceptor cum Transcription Factor

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Photoreceptors absorb light across a wide spectrum (ultraviolet-B to far-red) of electromagnetic radiation from regulating visual transduction, circadian rhythm in animals to photoperiodism, photosynthesis in plants, and photo-activated movements in bacteria. Herein, we primarily discuss the characterization of blue-light responsive photoreceptor aureochromes, which are exclusively present in photosynthetic marine stramenopiles. Aureochromes are unique Light-Oxygen-Voltage (LOV) sensors containing basic leucine zipper (bZIP) transcription factors (TFs). Upon sensing light, these sensory transcription factors execute gene expression and can potently modulate behavioral responses. We conduct the structural and functional characterization using biochemical, biophysical and in silico methods. A synthetic photoreceptor-TF construct is further generated. We use UV-Vis spectroscopy studies to study dark recovery kinetics for natural aureochrome, its mutants as well as the synthetic one. Temporally diverse dark recovery kinetics in these constructs establishes the modular nature of both the sensor and effector domains of aureochromes. In silico modeling, docking as well as mutational analyses of C-terminal LOV and N-terminal bZIP domain of aureochromes reveal conserved 3D structures as well as interaction with 'Aureo-box' containing substrate DNA. Upon blue-light illumination, the designed synthetic construct show about a ten-fold increase in DNA binding activity as verified by Electrophoretic Mobility Shift Assay. Both the native and the synthetic constructs facilitated the formation of higher order structures, which could be significant for transcriptional activation. At the end, we discuss the possibility of using ours mutant/ synthetic constructs as scaffolds for designing optogenetic tools.

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Exploring the Interplay Between Bacterial Microcompartments and Outer Membrane Vesicles in Salmonella Pathogenesis

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Bacterial microcompartments (MCPs) are proteinaceous organelles that optimize metabolic pathways in bacteria, offering a growth advantage and facilitating catalytic efficiency by sequestering toxic or volatile intermediates. Emerging evidence suggests a critical role of MCPs in the pathogenesis of *Salmonella enterica* serovar Typhimurium, an intracellular pathogen and a leading cause of gastrointestinal illnesses. Pathogenesis is influenced by virulence factors translocated into host cells, with outer membrane vesicles (OMVs) serving as one of the delivery vehicles. These nanosized particles, originating from the outer membrane of Gram-negative bacteria, play a crucial role in various biological processes, including the transport of pathogenic factors to host cells. Despite individual roles of MCPs and OMVs in bacteria, their interconnected contribution to Salmonella pathogenesis remains unexplored. In our study, we aimed to investigate OMV production under MCP-induced conditions in *Salmonella enterica* serovar Typhimurium. Our findings indicate that OMVs are actively produced in MCP-inducing conditions, independent of nutrient availability. Additionally, to provide an insight into how MCPs influence the pathogenic potential of Salmonella within host environments, we checked for it's survival in MCP induced conditions using HCT116 epithelial cell lines and RAW 264.7 murine macrophages cell lines as targets. Our results demonstrate a significant multi-fold increase in the survival of bacteria expressing MCPs. Overall, our study aims to find the mechanistic link between MCPs and OMVs, providing a deeper understanding of Salmonella pathogenesis and opening avenues for therapeutic strategies to counteract bacterial infections by targeting these interconnected systems.

Extent of Stochasticity in Folding Dynamics Determines the Force-tolerance and Longevity of Mechanosensing Proteins

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Anti-cross-correlated motions among β-strands are crucial in determining β-rich protein mechanics. Although in-silico approaches estimate these motions, their direct link to protein molecular mechanics remains elusive. Here, we identified three genotypic variants of cadherin-23 (Cdh23)—a mechanosensory protein essential for force transmission in hearing mechanotransduction. These variants, encompassing two wildtype and one mutant, differ by a single amino acid yet display significant mechanical phenotype variations. Using high-precision magnetic tweezers, we investigated their stochastic kinetics and folding patterns to model aging-related pathologies in biological mechanosensors. All three variants exhibit consistent microstates in their folding-energy landscape. However, their response to external forces varies significantly. The variant with pronounced correlated motions among β-strands demonstrates superior force tolerance, enduring tensile forces for extended durations. Conversely, the variant with weaker inter-strand correlations shows enhanced folding cooperativity and faster intrinsic folding but is more susceptible to force-induced landscape distortion. This study elucidates parameters enabling proteins to adapt to diverse mechanical environments, highlighting crankshaft-type motions among β-strands as a key biophysical factor in force adaptation. This study unveils a distinctive relationship between the cooperativity of transitions among sparsely populated conformational states and the adaptability to external forces in β-rich proteins. Furthermore, the correlation between protein aging and age-related diseases underscores the potential for therapeutic interventions to mitigate disease phenotypes. By leveraging the mechanistic insights from this research, we can understand aging in mechanosensory proteins and their broader implications in biophysics.

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Detection and Characterization of DNA-Cas9 Interactions Using Glass Nanopores

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In recent years the CRISPR-Cas9 technology has revolutionized genome editing due to its efficient targeting of specific sequences in the genome. The CRISPR-Cas9 system involves binding and cleavage of target DNA at specified locations with the help of Cas9 protein and guide RNA. Quantifying the spatial location of Cas9 protein on the DNA and cleaved DNA fragments is important to understand the effectiveness of CRISPR-Cas9 technology. In this study, we used a resistive pulse technique to characterize DNA-Cas9 interaction using a glass nanopore-based assay. Under the applied voltage DNA-Cas9 complex were translocated through the nanopore resulting in instantaneous drop in current, which produces distinct current signature for Cas9-bound and free DNA molecule. We find our ECD-based calculation of these events was reasonably well in predicting the actual location of protein on DNA, volume, and size of cleaved fragments. Next, we quantified the rate of release of the cleaved DNA fragments in 2M salt and found a rate constant of 0.02 1/min. Additionally, we demonstrate detection of multiple Cas9 bound on DNA with 12 equally spaced targets. The translocation events produce distinct signatures for each cas9 molecule bound on the DNA, which allows us to measure the number and location of bound proteins. Our experimental results open future possibilities in assessing the effectiveness of CRISPR-Cas9 technology.

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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. 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. 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+ superfamily. It recognises, mechanically unfolds, and translocates the substrate protein having a degron tag called Pup using ATP as the energy source[2]. 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 between its OB domain[3]. 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 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.

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Sequestration Mechanisms in MerR Family Antibiotic Binding Proteins

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TipAS and AlbAS (structural homolog of TipAS) are naturally synthesized short isoforms of the MerR family antibiotic binding proteins. These proteins protect host organisms by sequestering peptide antibiotics. A disordered N terminal motif in TipAS accommodates promiscuous binding to several thiopeptide ligands while domain duplicated structure of AlbAS enables sequestration of its ligand albicidin in a solvent-inaccessible binding tunnel. The underlying molecular heterogeneity that shapes the folding–function landscapes in TipAS and AlbAS is studied by combining equilibrium and time-resolved experiments, statistical modeling, and simulations. The native ensemble of TipAS exhibits a pre-equilibrium between binding-incompetent and binding-competent substates. The binding-competent state characterized by a partially structured N-terminal sub-domain displays slow conformational exchange across multiple conformations. Binding to the antibiotic thiostrepton follows a combination of induced-fit and conformational-selection-like mechanisms, via partial binding and stabilization of the binding-competent substate. These ensemble features are evolutionarily conserved across orthologs from select pathogenic bacteria, underscoring the functional role of partial disorder in the native ensemble of TipA. AlbAS displays large differences in local and global stability and dynamics across the entire structure. The conformational landscape is quite rugged encompassing numerous partially structured states in equilibrium, including partial unlocking of the N-terminal sub-domain at a time-constant of 6 milliseconds that exposes the binding sites to aid in albicidin binding. In addition, there is a correspondence between the structural motifs that aid both TipAS and AlbAS to bind their cognate ligands, highlighting the role of domain duplication in enabling acquisition of new function.

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Decoding the Structural Insights of Prothymosin-α by Novel Label-Free Approach: Protein Charge Transfer Spectra

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Studying biomolecules in their native state is the first step towards revealing their structure and function. Nonetheless, the sequences of a number of Intrinsically Disordered Proteins (IDPs), are rich in charged and lacking in aromatic amino acid residues. These pose a number of limitations on tracking the structural alterations in IDPs. Our lab has discovered a new intrinsic chromophore in proteins in the past few years originating from charge transfer transitions that results in a wide UV-Visible absorption band across 250 to 800 nm[1]. In this study, we tracked the structural changes in an IDP protein using this recently developed Protein Charge Transfer Spectra (ProCharTS) tool. We recorded the UV visible absorption of prothymosin-α and Histone H1, whose sequences are rich in negatively and positively charged amino acids, respectively. These proteins have a significant ProCharTS profile with a characteristic tail that extends up to 800 nm. Our findings demonstrate considerable variations in the ProCharTS spectrum of prothymosin-α when pH is varied between 2.5 and 10.5. Furthermore, the presence of salt in the media also influences the intensity of ProCharTS spectrum. ProCharTS absorbance and luminescence are dictated by the number of charged amino acid sidechains in close proximity. The interaction of prothymosin-α and H1 is important for chromatin dynamics and can be observed using ProCharTS to explore their structural complex. In conclusion, we present ProCharTS as a novel label-free intrinsic probe for monitoring structural changes and interactions in IDPs like prothymosin-α and H1.

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A MarR/DUF24 Family Transcriptional Regulator directs Bacterial Nanotube Formation

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Bacterial nanotubes are intercellular membranous connections that facilitate the transfer of antibiotic resistance, toxins, metabolites, cytoplasmic proteins, and genetic material. These conduits mediate both antagonistic and cooperative intra/inter-species interactions, as well as host-pathogen interactions. Previously, a calcineurin-like metallophosphodiesterase, YmdB, a global regulator, was found to influence nanotube biogenesis among various other cellular processes. However, the molecular pathway through which YmdB regulates nanotube biogenesis remains elusive. Using Bacillus subtilis as a model, we explored the effectors interacting with YmdB to enable nanotube production. Proteomic analyses revealed a previously uncharacterized transcriptional regulator, YdzF, significantly engaged in nanotube production and antibiotic resistance spread. A phylogenetic analysis of YdzF across the bacterial kingdom showed its presence primarily in Gram-positive classes, including Bacilli and Clostridia. Remarkably, homologs of YdzF from major human pathogens such as Bacillus anthracis, Bacillus cereus, Listeria monocytogenes, and Clostridium botulinum restored nanotube formation in B. subtilis lacking YdzF indicating its implication on bacterial interactions. YdzF belongs to the MarR/DUF24 family of transcriptional regulators, traditionally associated with redox sensing. However, our biochemical, biophysical, and structural studies suggested that despite its classification in the DUF24 family, YdzF does not function as a redox sensor. Further experiments demonstrated that YmdB utilizes its unfolded C-terminal MFFE tail to interact with YdzF, an interaction critical for nanotube production. Altogether, we propose a mechanistic model highlighting a novel function of a DUF24 family protein in regulating nanotube biogenesis within the bacterial kingdom.

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Impact of Nucleosome Dynamics on Condensed Chromatin Globules

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Chromatin is a polymer made of double-stranded DNA wrapped around histone proteins, forming nucleosomes, the fundamental units that regulate chromatin's organization and function. These nucleosomes are dynamic entities and are influenced by ATP-dependent remodelers that induce sliding, dissociation, and rebinding. These processes play a crucial role in the formation of euchromatin and heterochromatin regions. Within the nucleus, the extent of chromatin condensation is closely linked to transcriptional activity, with heterochromatin being more condensed and transcriptionally repressed, while less condensed euchromatin is transcriptionally active. Recent in-vitro experiments with short chromatin filaments show that chromatin forms phase-separated chromatin condensates with nucleosome concentrations comparable to those found in the nucleus[1,2]. Building on these findings, we present a study, using hybrid Molecular Dynamics-Monte Carlo simulation, on the impact of nucleosome dynamics on chromatin organization in these globules. We modeled an active multipolymer system that accounts for nucleosome sliding, dissociation, and rebinding. Our results indicate that condensates with passive filaments, consisting of chromatin with regularly spaced nucleosomes, forms a more liquid-like structure compared to chromatin condensate with randomly placed nucleosomes, consistent with experimental observations[3]. We observed that increased activity accelerates chromatin fluidity, rendering it more liquid-like than passive chromatin, which matches with the experimental data on Fluorescence Recovery After Photobleaching (FRAP)[3] under varying activity levels.

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Impact of Multidrug Therapy Drugs on the Structure and Chaperone Function of *Mycobacterium tuberculosis* Hsp16.3

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Mycobacterium tuberculosis is a human pathogen that secretes Hsp16.3, a major immunodominant antigen, throughout the course of infection[1]. Hsp16.3, a member of the small heat shock protein family, plays critical role as a molecular chaperone, aiding in the growth and survival of *M. tuberculosis* within host macrophage[2]. During tuberculosis treatment, Hsp16.3 is frequently exposed to multidrug therapy (MDT) drugs, including rifampicin, isoniazid, pyrazinamide and ethambutol. However, whether MDT drugs do interact with Hsp16.3 and how these interactions affect its structure and chaperone function is still unclear. In this study, we have reported Hsp16.3-rifampicin/pyrazinamide/isoniazid/ethambutol interaction using fluorometric experiments. All these drugs interact efficiently with Hsp16.3, exhibiting sub-micromolar binding affinity with a 1:1 stoichiometry. These interactions significantly alter the secondary structure of Hsp16.3 and perturb the microenvironment around its tyrosine residue. Furthermore, the binding of these four drugs brought alteration in the surface hydrophobicity, oligomeric size and structural stability of Hsp16.3. These structural changes collectively lead to variation in the chaperone function of Hsp16.3. Hsp16.3-rifampicin/pyrazinamide interaction reduced the chaperone function of Hsp16.3, which may additionally impair *M. tuberculosis* survivability during MDT treatment. Whereas Hsp16.3-isoniazid/ethambutol interaction enhanced the chaperone function of Hsp16.3, which might aid in the cause of prolonged treatment and relapse of the disease. Altogether, this study highlights the potential of targeting Hsp16.3 for therapeutic intervention and lays a foundation for the rational design and development of specific Hsp16.3 inhibitors to enhance the effectiveness of tuberculosis treatment.

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Polyphosphate Discriminates Protein Conformational Ensembles More Efficiently than DNA Promoting Diverse Assembly and Maturation Behaviors

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Disordered proteins and domains often assemble into condensates with polyanionic nucleic acids, primarily via charge complementarity, regulating numerous cellular functions. However, the assembly mechanisms associated with the other abundant and ubiquitous, anionic, stress-response regulating polymer, polyphosphate (polyP),[1] is less understood. Here, we employ the intrinsically disordered DNA binding domain (DBD) of cytidine repressor (CytR) from *E.coli* to study the nature of assembly processes with polyP and DNA. CytR forms metastable liquid-like condensates with polyP and DNA, while undergoing liquid-to-solid transition in the former and solubilizing in the latter. On mutationally engineering the ensemble to exhibit more or less structure and dimensions than the WT,[2] the assembly process with polyP is directed to either condensates with partial time-dependent solubilization or spontaneous aggregation, respectively. On the other hand, the CytR variants form only liquid-like but metastable droplets with DNA which solubilize within a few hours. Polyphosphate induces large secondary-structure changes, with two of the mutants adopting polyproline II-like structures within droplets, while DNA has only minimal structural effects. Our findings reveal how polyphosphate can more efficiently discern conformational heterogeneity in the starting protein ensemble, its structure, and compactness, with broad implications in assembly mechanisms involving polyP and stress response in bacterial systems.

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**Selected for Flash Talk*

Constraints of Liquid-Liquid Phase Separated Droplets

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Liquid-liquid phase separation (LLPS) is a notable feature of biological systems and plays a crucial role in spatiotemporal regulation[1]. Furthermore, by acting as reaction crucibles, phase-separated droplets might have played a major role in the emergence of life on early Earth[2]. LLPS involves various non-covalent interactions, such as π-π, dipole-dipole, and hydrogen bonding, between biomolecules, resulting in a condensed microenvironment inside these phase-separated droplets. In vitro, such a complex environment can be mimicked by the phase separation of different polymers, including RNA and peptides, resulting in 'liquid-like' droplets[3]. Even though these droplets support various biomolecular functions, we lack a comprehensive and quantitative understanding of how the internal heterogeneous and crowded environment influences the dynamics of biomolecules.
Employing various biochemical and biophysical approaches, my lab is interested in a quantitative understanding of parameters governing the dynamics of structured and functional RNAs in the complex environment of LLPS. Furthermore, we also exploit these phase-separated droplets as a protocell model to address some pertaining questions regarding the Origins of Life. In our recent work, we have analyzed oligonucleotide transport dynamics[3] and RNA-ligand binding function in the complex environment of LLPS droplets. The high spatiotemporal measurements show that the microenvironment of these phase-separated droplets is very constrained and impacts the dynamics[3]. Furthermore, in the case of RNA-ligand binding, a mere excess of RNA-ligand complexes inside these droplets does not dictate the binding function of the aptamer, but rather, polymer packing and transport dynamics could be the major drivers.

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Unravelling the Origin of Polymorphic Behavior in G-Quadruplexes using Integrated NMR Spectroscopy and All-Atom Molecular Dynamics Simulations

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G-quadruplexes (GQs) are higher order structures of nucleic acids formed by guanine-rich sequences. They are of significant interest due to their role in a wide range of regulatory functions in cells like telomere maintenance, gene regulation and genomic stability. Synthetic G-quadruplexes are also designed to behave as aptamers, biomarkers, and nanomaterials in a structure specific manner. Structural polymorphism is a common trait of GQs resulting in structural diversity due to varying conditions like sequence, ionic conditions and cation coordination. The structural features responsible for defining the ion specificity with respect to GQ topology and for regulating the ion exchange with bulk ions have been overlooked till date. Understanding the structural diversity of GQs is crucial for unraveling their biological functions and for developing therapeutic strategies targeting the GQ-forming sequences. Our NMR studies have shown the polymorphism of d[A2(G3T2)3G3A2], where a parallel GQ was found in K^+ solution and a 3+1 hybrid GQ was found in Na^+ solution. In this study, we integrate the NMR data with all-atom molecular dynamics (AAMD) simulations to obtain high-resolution insights into the dynamic behavior and structural polymorphism of this three quartet GQ. AAMD simulations provide atomic-level molecular insights into the conformational landscape of GQs under different ionic conditions and help us explore the properties of these GQs that are responsible for their polymorphism.

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A Futuristic Microfluidic Platform for SiHa Cancer Cell Sorting and Stiffness Profiling

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The development of advanced technologies for detecting and analyzing circulating tumor cells (CTCs) promises to revolutionize cancer diagnostics and personalized therapy[1-2]. This proposed work envisions the creation of a multifunctional microfluidic platform to address the challenges associated with isolating and characterizing rare CTCs, using SiHa cervical cancer cells as a model system. The platform will integrate a hybrid sorting mechanism combining lateral dielectrophoresis (DEP) and magnetophoresis sorting unit to isolate epithelial and mesenchymal subpopulations of SiHa cells with high efficiency, specificity, and viability. Following sorting, a resistive pulse technique (RPT) unit[3] will measure the stiffness of isolated cells, providing insights into their mechanical properties and metastatic potential. This device will be fabricated using photolithography and soft lithography technique to create PDMS-based microchannels with optimized geometries, interdigitated electrodes, and microelectromagnets for combined DEP-magnetophoresis sorting. Multistage micropores will facilitate RPT measurements of the sorted SiHa cells, enabling precise analysis of their size and stiffness. This integrated system aims to simultaneously perform sorting and mechanical characterization, addressing key limitations in existing methods such as low throughput and separation efficiency, reduced cell viability, and limited mechanical characterization capabilities. The anticipated outcomes include precise sorting of rare SiHa cancer cells from whole blood and detailed biomechanical profiling, enabling a deeper understanding of metastatic progression and tumor heterogeneity. This futuristic platform has the potential to advance liquid biopsy technologies, offering a scalable and efficient tool for early cancer detection, disease monitoring, and personalized treatment planning, bridging the gap between cell isolation and mechanical analysis.

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Unravelling Anti-Amyloidogenic and Fibril-Disrupting Effects of a Gut Microbiota Metabolite on Insulin Fibrillation through Multispectroscopic, Machine Learning and Hybrid QM/MM Approaches

39

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The misfolding of insulin into cross-β aggregates complicates both its manufacturing process and its use in diabetes treatment[1]. The present study explores the potential of indole-3-acetic acid (IAA), a gut-derived metabolite, in inhibiting amyloidogenesis and disaggregating insulin fibrils. Based on Thioflavin T (ThT) fluorescence assays and transmission electron microscopy (TEM), IAA showed a strong inhibitory effect on both primary and seed-induced insulin fibrillation. IAA was observed to reduce the size of insulin aggregates as indicated from the scattering profiles while circular dichroism studies revealed that IAA helps maintain the native α-helical structure, likely minimizing exposed surface hydrophobicity of insulin. IAA also demonstrated effectiveness in disassembling preformed fibrils, as evidenced by a time-dependent reduction in ThT fluorescence and further corroborated by TEM analysis. Additionally, biolayer interferometry interaction studies revealed a moderate 2:1 binding affinity between IAA and insulin. Two key binding sites on insulin were identified via machine-learning-based-docking and hybrid QM/MM studies where IAA interacts. The interaction energetics of Site-I (Leu13A, Tyr14A, Glu17A, Phe1B) were more favorable than those of Site-II (Tyr19A, Phe25B, Thr27B), as shown by SAPT0 residue-wise interaction energy analysis. IAA also effectively protected cells from fibril-induced cytotoxicity and hemolysis making it a potential therapeutic option for amyloid-related disorders, with dual action in preventing fibril formation and promoting fibril disaggregation.

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Role of Attractive Crowder Interactions in the Hydration Shell Driving Biomolecular Aggregation, Collapse, and Folding

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Water and crowders occupy a notable volume within cell, influencing protein behavior in these cells[1,2,3]. Prior studies have shown that proteins aggregate entropically due to size effect caused by hard interaction, while soft interactions can either stabilize or destabilize them[4,5]. Therefore, the folding or unfolding of proteins is influenced by the interplay between sizes of crowders and soft interactions, with the chemistry of crowders and test macromolecules also playing a crucial role. Firstly, to understand the role of neutral crowders in the hydration shell of PIC dyes, we studied oligomerization of PIC dyes, known for its propensity to self-assembled into fibrillar structure called J-aggregates, in water and crowded aqueous ethylene glycol solutions, employing soft interaction. The J-oligomerization is entropically opposed, but entropic penalty is not high in crowded solutions as in pure water because water and crowders can occupy interstices of oligomers and the loss of degrees of freedom of water and crowder to form bound state. The experimental measurements qualitatively agree with the observations obtained from simulations[6]. Secondly, to comprehend further the role of charged crowders in hydration shell, we studied folding equilibria of hydrophobic polymer with varying architecture. Moreover, the charged crowders are found to be preferentially adsorb, replacing water, on the polymer surface. The discriminating behavior of anionic crowders, and exhibit surfactants behaviour, particularly in their ability to improve the hydrophobic collapse, is related to how water molecules arrange themselves around the termini or central region of polymer beads in the two polymers. In summary, the neutral crowder (ethylene glycol) disfavor the self-assembly of PIC dye molecules through favorable dye-crowder interaction in first hydration shell. However, charged crowders (CR^-, CR^+) favor the folded over unfolded state. In both scenarios, crowders preferentially adsorb to the surface of solute molecules by replacing water molecules and therefore, promoting the most favorable state.

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Elucidating the Structural and Functional Aspects of the Novel Heterophilic Interaction between E-Cadherin and Nectin-4

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Cell adhesion molecules (CAMs) are a group of cell surface glycoproteins that help in cell-cell or cell-matrix adhesion, regulating various functions including cell migration, differentiation, and proliferation. Cadherins and nectins are the major cell adhesion molecules forming cell-cell adhesion junctions. Cadherins are $Ca2^{+}$-dependent CAMs containing five extracellular domains participating in homotypic cell-cell adhesion using membrane distal ectodomain (EC1), regulating various physiological and pathophysiological events. Nectins, conversely, contain three immunoglobulins (Ig) fold-like extracellular domains, where the membrane distal IgV domain mediates $Ca2^{+}$-independent heterophilic interaction among themselves in cell adhesion junctions[1,2]. Interestingly, the direct heterophilic associations among cadherins and nectins have recently come into the limelight, however, the molecular basis and functional significance of these interactions are yet to be deciphered[2]. We have identified a novel heterophilic interaction between E-cadherin and nectin-4 using biophysical studies[3]. AlphaFold 3 based prediction of E-cadherin: nectin-4 complex, followed by molecular dynamics simulation and residue-wise binding energy decomposition helped us to identify probable binding interface residues of E-cadherin and nectin-4 complex. Site-directed mutagenesis of the identified residues followed by their expression, purification, and surface plasmon resonance-based binding studies confirmed their contribution to E-cadherin: nectin-4 complex formation. Interestingly, the expression levels of nectin-4 and E-cadherin are linked with the progression of aggressive carcinomas. Moreover, nectin-4 has very minimal expression in healthy adults[1]. Thus, understanding the downstream signaling events associated with the heterophilic interaction between E-cadherin and nectin-4 can help us design novel therapeutics to combat aggressive metastatic phenotypes of cancers.

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Elucidating the Molecular Mechanism of Thioesterase 2-Acyl Carrier Protein Interaction

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Fatty acid biosynthesis (FAS) is upregulated in most types of cancers. Thus, several enzymes of the FAS pathway have been regarded as a drug target. One such enzyme is the type II thioesterase (TE2), that regulates the chain length of fatty acids. TE2 generates a broad lipid distribution and has been implicated in breast cancer. The closed conformation of TE2 facilitates its interaction with the ACP domain of FASN, promoting the release of shorter length fatty acids (C8–C12), rather than the longer chain fatty acids produced by FASN. However, the ability of TE2 to compete with the endogenous TE1 domain within the FASN mega-synthase for acyl substrates is a brain-teaser, as the cleavage should ideally be favoured by TE1 based on proximity. Thus, the goal of the present study is to gain structural insights into the ACP-TE1 interaction using various biophysical tools. The study will advance our understanding of the molecular mechanisms underlying the regulation of fatty acid biosynthesis, with implications for both metabolic disorders and cancer therapy.

Probing the Mechanistic Basis of the Redox Regulated Chaperon Hsp33 Activity

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Hsp33 (MW ~ 33kDa) is a bacterial holdase chaperone that protect proteins from oxidative stress. The Hsp33 activity is primarily dependent on the redox environment of the cell, as in oxidative environment structural alterations leading to dimerization or oligomerization has been reported to be the active form of the chaperone[1,2]. Although crystal structures of Hsp33 are available, the substrate bound state of the chaperone is not available, which corresponds to its “active” state. Hence, the mechanistic basis of the Hsp33 activity remains elusive. In this preliminary work, high resolution NMR of Hsp33 has been performed to probe the structural basis of chaperone action, in solution. Use of methyl 2D 1H 13C HSQC NMR experiments, at the natural abundance, exhibits a unique methyl fingerprint, possibly from a flexible segment of the protein. Supporting biophysical experiments were performed to demonstrate the chaperone activity of Hsp33, by use of a substrate protein L-asparaginase. The role of protein dynamics will be probed for the substrate protein, will be investigated in future.

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Phosphorylation Acts as a Switch Inducing Destabilization and Controlling Context-Dependent Phase-Separation in HMG Proteins

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Chromatin remodeling is essential for gene regulation and DNA repair. Apart from histones, High Mobility Group (HMG) proteins are the most abundant genome-associated proteins present in Saccharomyces cerevisiae. They play a vital role in the assembly and disassembly of chromatin, by binding and bending DNA via both sequence-specific and sequence non-specific modes. All HMG proteins are subjected to extensive post-translational modifications (PTMs) like phosphorylation, acetylation, methylation, etc. In this work, we employ NHP6A and NHP6B, a class of paralogous non-specific DNA binding proteins with similar stabilities and folding relaxation rates, to explore the role of crowding and phospho-mimetic mutations on stability and phase separation (PS) tendencies. We find that phospho-mimetic mutations progressively destabilize the native ensemble irrespective of the location of the mutation. On the other hand, a significant context-dependence is observed in PS tendencies in relation to crowder concentration and dsDNA, with the T69D mutant of NHP6B undergoing PS at lower crowder concentrations. Phase separation is enhanced with dsDNA and is also characterized by the protein unfolding within droplets, with the NHP6B nearly fully unfolding compared to its paralog. Taken together, the site of phosphorylation and its extent (one-, two- or three-phosphorylation events) could fine tune the extent of phase-separation, despite not exhibiting dramatic changes in DNA-binding affinities.

Structural Characterization of Mitochondrial Acyl Carrier

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The role of mitochondria as “power-house of the cell” has been grossly over-simplified. Infact, mitochondria are hubs of important biosynthetic reactions such as the mitochondrial fatty acid synthesis (mtFAS). Any disruption in this pathway is lethal to mammalian cells. mtFAS resembles the type II prokaryotic fatty acid synthesis system with each enzyme being a discrete protein. Central to mtFAS is a soluble, small, helical, acidic, the mitochondrial acyl carrier protein (mtACP). Holo-mtACP acts as a scaffold for the growing acyl chain, keeping it sequestered in its hydrophobic cavity. The enzymes of mtFAS need to interact with mtACP via protein:protein interactions to flip the nascent fatty acyl intermediate into their active site. Recent research has revealed diverse roles of mtACP in addition to fatty acid synthesis, viz. as a component of mitoribosomes, as essential assembly factors or subunits of electron transport chain, and Fe-S cluster assembly complexes. Octanoyl-ACP also acts as a donor of acyl chain in the lipoic acid biosynthesis pathway. Thus, mtACP is indispensable to mitochondrial function. However, many crucial questions about the mtACP functioning remain to be answered. This study aims to decipher the mechanism of post-translation modification of mtACP which is essential for activation of ACP and is also the inception of mtFAS pathway.

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BLM Helicase Unwinding Mechanisms: Implications for DNA Repair and Genome Maintenance

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BLM helicase is a molecular motor protein that unwinds DNA by utilizing the energy released by ATP hydrolysis. It plays an important role in maintaining chromosomal stability by resolving aberrant structures that arise during DNA replication and repair. Previously, biochemical studies have attempted to study the kinetic step size, with which BLM unwinds DNA, and describe its mechanism of action. However, it is difficult to identify intermediate steps through such bulk studies and therefore accurately describe its mechanism. Here, we use optical tweezers combined with confocal fluorescence microscopy, to monitor the real time changes of the unwinding of a double stranded DNA. In order to mimic the replication bubbles found in vivo, the DNA is stretched at 35 pN to form transient single stranded bubbles where the protein can bind. It can unwind long stretches of DNA up to 1000 basepairs at the rate of 47 bp/s. Interestingly, we also observe instances of spontaneous strand switching that are largely reduced with the introduction of a single stranded binding protein (RPA) that binds to the growing bubble and prevents backtracking by BLM. Additionally RPA can also recruit more BLM proteins to the junction between the double stranded and single stranded DNA and enhance the unwinding rate of BLM. The results provide an insight into the role played by BLM in propagating replication bubbles and shed light on the mechanism it employs during DNA repair.

Characterization of Fatty Acid Synthase (FasN): A Multimeric Enzyme Complex

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Abnormal lipid metabolism is one of the hallmarks of cancer. Most cancers display upregulation of fatty acid synthesis for the generation of signaling molecules and energy. Thus, fatty acid synthase (FasN), the key enzyme responsible for catalyzing the synthesis of fatty acids has been regarded as a target for drug intervention against cancer. It's a type I enzyme, a dimer, comprising seven different domains, β-ketoacyl synthase, malonyl-acetyl-transferase and dehydratase, enol reductase, keto-acyl reductase, acyl carrier protein and thioesterase, that work in cooperation. To understand the function of individual enzymes at the molecular level, and identify inhibitors specifically against them, we have cloned individual domains and followed their activity and binding studies using biophysical techniques like CD, fluorescence and NMR along with activity measurements.

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Engineering Collagen Mimicry with Short, Hyperstable Collagen-Mimetic Peptides via Terminal π-Capping

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Collagen model peptide (CMP)-based fibrils are promising alternatives to mimic natural collagen for tissue regeneration.[1,2] However, most CMPs composed of GXY/XYG repeats (X=Proline (P) & Y=Hydroxyproline (O)) fail to replicate collagen's fibril networks, and their lengthy sequences (≥9 GXY/XYG tripeptide repeats) limit large-scale applications.[2,3] While aromatic residues in animal collagen's telopeptide regions (N- and C-terminal regions of collagen triple helical domain) enhance triple helix stability and fibril formation, their role in CMPs remains underexplored. To address this, we designed minimalistic CMPs with just 3-6 GPO repeats, capping the N-terminus with a fluorenylmethoxycarbonyl (Fmoc) group where fluorenyl moiety represents extended aromatic π-system and adding a tyrosine residue at the C-terminus. Interestingly, single and diFmoc-capping enabled triple helix formation with just five and four GPO repeats, respectively, at physiological pH. DiFmoc-capped CMPs showed exceptional stability, forming fast-folding triple helices with melting temperatures up to 76°C. At pH 5.5, even three GPO repeats were able to form stable helices, marking the first report, to the best of our knowledge, of such stability without covalent cross-linking between the three strands. This underscores the significant stabilizing influence of the aromatic π-system. Biophysical and computational analyses revealed that stability enhanced through a combination of π-π interactions, hydrogen bonding from Fmoc carbamate groups, and CH-π interactions. Furthermore DiFmoc-capped CMPs self-assembled into fibril networks with mesh like morphology resembling natural collagen, exhibiting excellent biocompatibility. These findings highlight the potential of π-system (Fluorenyl) capping in the design of hyperstable CMPs capable of forming robust fibril networks, advancing biomaterial innovations for tissue engineering and regenerative medicine.

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**Selected for Flash Talk*

Chaperone-Mediated Phase Separation Modulates the Liquid-to-Solid Transition of an Amber Stop Codon Mutant of the Prion Protein (Y145X)

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Eukaryotic cells perform various cellular functions with the help of conventional membrane-bounded organelles. In addition to these, cells also harbor, non-canonical, membrane-less biomolecular condensate formed via phase separation. These regulatable supramolecular assemblies can orchestrate a diverse array of cellular functions in a spatiotemporal fashion and thereby maintain cellular homeostasis. Different transient multivalent interactions are known to promote the formation of such non-stoichiometric, reversible liquid-like condensates enriched in intrinsically disordered proteins/regions and nucleic acids. However, unregulated phase separation results in the maturation of such dynamic assemblies into gel-like or solid-like irreversible aggregates implicated in various fatal diseases[1]. Elements of protein quality control (PQC) machinery like molecular chaperones are often enriched in these dynamic biomolecular condensates and are known to modulate them[2]. However, the exact molecular mechanisms behind such regulation remain elusive. Here, we show that one such class of heat shock protein, Hsp40 (Ydj1), modulates the phase separation and aggregation of a disease-associated stop codon mutant of the prion protein (Y145Stop or Y145X). Hsp40 shows holdase-like activity within these Y145X-Hsp40 complex condensates. Utilizing a multitude of techniques involving mutagenesis, biophysical tools, and fluorescence imaging, we decipher the complex network of interactions that regulates the heterotypic condensation of Y145X and Hsp40. Further, we show that Hsp40 can precisely regulate condensates formed in the presence of RNA, which results in multiphasic protein-RNA condensate formation. Taken together, our study provides unique mechanistic insights into the condensate-mediated chaperoning of Y145X, thereby preventing its aberrant transition into amyloid aggregates[3].

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Mechanical Degradation and Unfolding Studies of Knotted Proteins using Single Molecule Force Spectroscopy

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Knotted proteins are a particular class of proteins where the polypeptide backbone crosses itself, resulting in a physical entanglement akin to mathematical knots. Based on the number of crossings, these knotted proteins are subdivided into categories such 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[3], we delve deep into understanding the degradation mechanism of model knotted protein substrates (miRFP709 and human UCHL1) by proteases ClpXP and ClpAP using SDS-page densitometry and steady-state fluorescence loss assays. ClpAP is known to be a more efficient protease for degradation owing to its higher processivity. 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 account for 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. In another study, to understand how these knotted proteins fold when the free ends are hindered, we used single-molecule AFM. Pre-existing studies suggest that knots can form when the free ends are hindered for simple 3_1 knotted proteins. In the current study, we have explored such folding for much more complex 4_1-knotted miRFP709 and 5_2-knotted UCHL1. By studying the unfolding-folding intermediates, we have tried to map the folding energy landscape of these proteins and investigate the possibility of knot formation under such hindered conditions.

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Structural and Functional Basis of Erm-Mediated Antibiotic Resistance

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Antimicrobial resistance is a silent pandemic rapidly spreading across the globe, primarily driven by the misuse and overuse of antibiotics[1]. Among the various mechanisms pathogenic organisms employ to evade the effect of antibiotics, drug target modification stands out as both efficient and straightforward. Erythromycin-resistance methyltransferases (Erms) site-specifically mono- or di-methylates A2058 (*E. coli* nomenclature), a nucleotide located deep within the nascent peptide exit tunnel (NPET) of the 50S ribosomal subunit[2]. This modification confers resistance to the macrolide, lincosamide, and streptogramin B classes of antibiotics, which bind within the NPET to inhibit protein translation. Although it is established that Erms act on 50S ribosomal precursors[3], the true in vivo substrate of these enzymes has remained elusive until now. By employing cryogenic electron microscopy (cryo-EM) and single-molecule Förster resonance energy transfer (sm-FRET), we have successfully captured this elusive substrate and uncovered the complex, dynamic nature of the enzyme's interaction with its ribosomal target. Our findings reveal critical structural determinants—located outside the enzyme's catalytic domain—that define substrate specificity. Furthermore, we observed a unique molecular motion exhibited by these enzymes, that aids in facilitating precise methylation of the target base. Collectively, our study provides a comprehensive understanding spanning from substrate recognition to the dynamic behaviour of these enzymes, and helps uncover new avenues for the development of drugs to reverse resistance.

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Establishing the Organization of Cellular Condensates in Aging

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Cellular reactions are compartmentalized in membrane-bound organelles and membrane-less condensates. These membrane-less condensates are mostly composed of RNA and RNA binding proteins (RBPs). They play crucial roles in facilitating biochemical reactions regarding RNA metabolism, stress response, DNA repair and cell cycle regulation[1]. For example, cytoplasmic condensates like P-bodies and stress granules are involved in mRNA quality control and stress responses respectively[2]. Nuclear condensates, like nuclear speckles and nucleolus, are involved in RNA processing (splicing) and ribosome biosynthesis. These nuclear and cytoplasmic condensates dissolve quickly after the release of processed RNA or when translation is resumed. Hence the condensate dynamics is closely coupled to the cellular metabolism. Aging at the cellular level or senescence is associated with altered cellular energy, protein and RNA metabolism. It is very unclear how cellular condensates change with altered cellular state during senescence. RBP condensate formation is often driven by RBPs [eg. FUS, TDP43 and hnRNPs] with large low complexity domains and several studies have shown that these RBPs form aggregates during age-related neurodegeneration[3]. Therefore, we hypothesize that low-complexity RBP containing condensates can change its dynamics during aging, eventually forming irreversible solid-like aggregates. In our study, we observed enriched nucleolus, nuclear speckles and P-bodies in DNA-damage induced senescence. In future we are planning to explore the potential of anti-aging compounds to reverse these age-related condensate alterations. Linking the mechanisms of senescence recovery with phenotypic changes in condensates can effectively reveal the connection between condensates and aging.

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Dynamic Coacervate-lipid membrane Interaction Regulates Membrane Bending

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Compartmentalization is crucial in both prokaryotic and eukaryotic cells, enabling essential processes and biochemical reactions to occur simultaneously. Cellular compartments can be either membrane-bound or membrane-less. Membrane-less organelles (MLO) or condensates arise through liquid-liquid phase separation (LLPS), enabling dynamic and reversible organization of biomolecules within them. Membrane-less organelles (MLOs) are also known to interact with membranes to facilitate critical cellular processes such as endocytosis, exocytosis, invagination, and cell division. Coacervates mimicking the LLPS characteristics of condensates are engineered and encapsulated within GUVs for biophysical studies. Enzymatically formed condensates are dynamically regulated within GUVs designed to be semipermeable, enabling the transport of small molecules across their membranes. We reveal electrostatic interactions between the condensates and the membrane, significantly influence the membrane's morphology. The molecular diffusion property of lipids, quantified using FRAP, is modulated at the coacervate-membrane interaction site restricting the coarsening of coacervates. Our findings provide mechanistic insights of lipid bilayers controlling condensate sizes that plays a prominent role in comprehending nucleation and localization of cellular condensates.

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Immune Recognition of Dengue Virus: A Structural Analysis of Surface Epitopes

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Dengue virus (DENV) is an RNA virus that causes mild to life-threatening hemorrhagic fever in humans. DENV infection can be by any of its four serotypes, DENV1-4, which are genetically related but antigenically distinct. The immune system of humans produces broadly neutralising antibodies (bnAbs) as a response to exposure to any four serotypes of DENV. First exposure to any DENV serotype generates neutralising antibodies, which provide long-term but sometimes incomplete protection against reinfection by the same or other serotypes. Cross-reactive, non-neutralising antibodies and pre-existing antibodies may enhance disease severity upon secondary exposure, attributing to antibody-dependent enhancement (ADE), which can lead to lethality. Thus, the discovery of cross-reactive neutralising antibodies that can target the conserved epitopes of all four serotypes will be a successful vaccine candidate in the future. Among many research groups, Dr Leslie Goo and her team recently reported a few bnAbs isolated from infected individuals that showed a cross-reactive neutralisation effect on all four serotypes of DENV[1]. We considered one of the bnAb, D14.F05.S03, from their reported families of antibodies to identify the epitopes of DENV crucial for antibody interaction structurally. The 4.51 Å cryo-EM structure showed a significant interaction between the E protein and the variable region of the Fab. The Heavy chain of the Fab region predominantly interacts with the fusion loop and N153 residue of E protein, which are crucial for viral replication. Our study reveals the structural insights of antibody interaction with E protein, delineating epitopes that may be crucial for neutralising DENV. Hence, the study will have a promising role in identifying critical epitopes that can be targeted for DENV prevention and treatment in future.

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Structural and Functional Studies of the Enzyme PseF Involved in Pseudaminic Acid Biosynthesis Pathway from Helicobacter pylori

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The enzyme PseF is an essential glycosyltransferase involved in the biosynthesis of pseudaminic acid. The sixth step in the Pse biosynthesis pathway, a metal-dependent enzyme pseudaminic acid biosynthesis protein F (PseF) catalyzes activation of pseudaminic acid (Pse) using cytidine 5'-triphosphate (CTP) as a cofactor by addition of a cytidine 5'-monophosphate (CMP) to generate CMP-Pse. The disruption of PseF function impairs flagellar motility, weakening the bacterium's ability to establish infection. Moreover, glycosylation mediated by PseF contributes to H. pylori's structural integrity, biofilm formation, and immune evasion. In order to unravel the structure function mechanism of PseF and understand it's antimicrobial resistance (AMR), we intend to elucidate the structure of PseF by integrating computational and experimental methods. In this context we have purified the enzyme (PseF) with and without the presence of cofactor (CTP). Crystallization trials were done and we found crystals for the apo form and it was further confirmed by izit dye. We have performed isothermal calorimetry studies using the cofactor (CTP) to understand the thermodynamic behavior of enzyme and the cofactor. We have also modeled the structure of PseF and identified CTP mimics using virtual screening. Currently we are working on crystallization trials for PseF with CTP to get the plausible crystals followed by X-ray crystallography to unravel the structure function mechanism of PseF. Our study would pave the way for the design of mimics and inhibitors targeting PseF of H. pylori in order to combat antimicrobial resistance.

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Miniprotein-Driven IgG Oligomerization for Targeting Protein-Protein Interactions

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Protein-protein interactions (PPIs) play crucial roles in numerous biological processes and represent challenging yet promising therapeutic targets. The large interaction surfaces characteristic of PPIs are difficult to target with traditional small molecules, necessitating alternative approaches. Here, we introduce a strategy employing miniprotein-driven oligomerization of immunoglobulin G (IgG) to enhance its efficacy in targeting PPIs through the avidity effect. This approach utilizes a dimeric variant (Dhh) of a helical hairpin peptide (hh), designed by us to bind to the Fc region of human IgG[1]. Dhh, engineered by substituting hydrophobic residues at the solvent-exposed face of hh, induces IgG oligomerization upon binding to its Fc region resulting in a multivalent IgG. Multivalent interactions resulting from oligomerization would significantly enhance efficacy of binding to the target, thereby, causing the avidity effect[2]. This study underscores the potential of Dhh as a versatile scaffold for antibody engineering and highlights the advantages of miniprotein-mediated IgG oligomerization in tackling large PPI surfaces. By exploiting the avidity effect of multivalent antibodies and the structural benefits of miniproteins, this platform provides a robust and scalable method for expanding the landscape of druggable PPIs.

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Understanding the Design of Ultrastable α-Helical Hairpin Motif

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Miniproteins are a specific class of proteins comprising less than 40 residues and can adopt a stable structure in aqueous conditions[1]. In our pursuit of miniprotein design, we aimed to design a short helical miniprotein from a naturally occurring 63-residue α-helical hairpin protein, Rop[2]. Truncation of Rop resulted in a significant loss of hydrophobic packing within the core, consequently unfolding the molecule. By introducing a conformationally stable loop connecting the two helices, we could impart a stable structure to the miniprotein. Further optimization of the hydrophobic core resulted in a dimeric miniprotein that is thermally more stable than Rop. Crystallographic evidence shows that the nature of hydrophobic residues at the core determines the topology of the dimeric assembly. In the design workflow, we also observe a specific site in the protein's hydrophobic core, where the incorporation of a single substitution (His to Leu) in synergism with the engineered loop causes the transition of the molecule from an unfolded to a folded state. Thus, we show the cooperative effect of loop and hydrophobic residues to govern the structure and stability of the dimeric α-helical hairpin motif, which can be utilized as a scaffold to target protein-protein interactions (PPIs).

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Systematic Mutation and Unnatural Base Pair Incorporation Improves Riboswitch-Based Biosensor Response Time

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RNA-based sensors have a wide range of applications in synthetic biology and biotechnology, including use in regulatory circuits, metabolic engineering, and diagnostics. A key factor in their effectiveness is the activation kinetics, which are critical for monitoring sensitive and dynamic biological processes. Despite significant advancements in developing biosensors for various ligands, optimizing their activation speed remains underexplored. In this work, we designed RNA-based fluorescent (RBF) biosensors for guanidine, a common chaotropic agent that serves as a precursor for fertilizers and explosive compounds.[1] The cell permeability of guanidine, along with its non-toxic nature to *E. coli* at millimolar concentrations, allowed us to directly compare the activation kinetics of a riboswitch-based biosensor and a reporter in cells. Our findings show that the RBF biosensors respond within 4 minutes of guanidine exposure which is 15 times faster than the reporter derived from the same riboswitch and this rapid sensing capability is stable for up to 1.6 weeks. Additionally, we systematically introduced mutations, including transposed or transitioned base pairs, as well as unnatural orthogonal base pairs. Both natural and unnatural orthogonal base pair mutants enhanced the biosensor's response time without compromising ligand affinity or fluorescence fold activation.[2] This study outlines the design of rapid-responding RNA-based biosensors and presents strategies to enhance the performance of RNA-based tools.

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Interaction Dynamics of Mycobacteria and Phage Revealed by In-Situ Cryo-Electron Tomography

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The increasing prevalence of antibiotic-resistant strains of Mycobacteria has revived the interest in phage therapy. Therapeutic application of Mycobacteriophages has already shown clinical potential in Mycobacterium infection. However, the structural and mechanistic details of host-phage interactions remain poorly understood. In this study, we employed cryo-focused ion beam-enabled cryo-electron tomography to visualize different stages of infection by Mycobacteriophage Bxb1, a well-studied phage targeting Mycobacterium smegmatis. Along with the complete Bxb1 structure elucidated by single particle cryo-electron microscopy, the in-situ sub-tomogram averages of phage during infection provide insights into virus architecture and assembly. Ultrastructural analysis of phage-bound mycobacterial membrane reveals the mechanism by which phage particles attach prior to infection. Our findings highlight key structural transitions that facilitate free phage particles to bind to the bacterial surface and navigate through the cell wall to enable DNA injection into the cytoplasm. These insights provide a foundation for optimizing mycobacteriophage-based therapies for tuberculosis and nontuberculous mycobacterial infections.

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Exploring the Interaction between Protein Z Dependent Protease Inhibitor(ZPI) and Plasma Pre-kallikrein

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Blood coagulation is a complex biological process involving extrinsic, intrinsic and common phase. Protein Z Dependent Protease Inhibitor (ZPI), being a member of serpin super family, inhibits FXIa without the presence of procoagulants such as PZ, Calcium and Lipid membrane. Unlike other coagulation protease FXI is a vitamin-k independent coagulation protein. FXI polypeptide is organised into four 90-91 amino acid repeats called apple domains designated as A1 to A4 from the N-terminus and a C-terminal protease domain. The plasma pre-kallikrein (PK), the precursor of protease plasma kallikrein (PKa) is the only other protein known to share this particular configuration.[1] Also, the gene F11 that codes for FXI is duplicated from the gene KLKB1 that codes for PK. We propose that ZPI might interact with PK as it is a FXI homolog and the gene (F11) codes for FXI and PK are orthologs.[2] The current study is therefore aimed to probe the interaction between ZPI and PK. Genome analyses of evolutionarily significant organisms, multiple sequence alignment analyses, phylogenetic tree construction, synteny analyses and molecular docking were used for the current study. It was observed that Zebra fish genome consists of two genes homologous to KLKB1 in chromosome number 12 and 14. The pattern of branching in the phylogenetic tree constructed for Serpina10 orthologs and KLKB1 orthologs showed significant similarity. Docking pattern of the PK proteins from different organisms and the corresponding ZPI of those organisms also showed significant similarities. As the SerpinA10 gene known to exist before the divergence of cartilaginous fish, ZPI should have a significant function other than anticoagulation by interacting with the predecessor protease of FXI and Plasma pre-kallikrein.

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Bi-Directional DNA Translocation in Conical Nanopores

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Studying DNA folding is crucial for understanding gene regulation, chromatin structure, and genomic stability. This study investigates the translocation dynamics of linear DNA molecules through a nanopore device. Nanopores have proven to be excellent label-free, high-throughput tools for studying conformational changes in various biomolecule structures. We employed a nanopore device to understand the polymeric behaviour of different lengths of linear DNA (3, 5 &10 kbp). By utilizing both forward and reverse translocation through voltage polarity modulation, we aimed to comprehend the bidirectional movement of DNA through the nanopore. We conducted a quantitative comparison of various parameters, such as the drop in conductance, translocation time, event charge deficit and percentage of DNA folding, in both directions. Additionally, we focused on estimating the sensing length of the conical nanopore, a challenging parameter to measure using optical instruments. The sensing length is crucial for calculating the size of different biomolecules and it is directly related to the spatial resolution of a nanopore. We estimated this parameter through the electrical signatures of DNA events and verified it using a theoretical model. Our study reveals significant insights into the dependence of DNA length, translocation directionality, and applied voltage on the translocation mechanism, contributing to the optimization of nanopore-based sensing technologies. We also discuss several advantages of reverse translocation compared to the more commonly studied forward direction. Through this study, we provide a comprehensive picture of linear DNA folding with proper quantifications, enhancing our ability to detect and analyse biomolecules with greater accuracy and efficiency.

**Selected for Flash Talk*

Phase Transitions of Mammalian Prion Protein: Molecular Mechanisms, Structural Insights, and Disease Relevance

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Prion protein (PrP) can undergo different types of phase transitions. In one type of phase transition, the protein forms misfolded aggregates, either oligomers or fibrils, which are associated with a group of neurodegenerative diseases, known as 'Prion diseases'. Our results show that the dynamic nature of PrP allows it to unfold and refold continually, cycling through all possible high-energy forms. Using hydrogen-exchange mass spectrometry (HDX-MS) and other techniques, we are able to identify these partially unfolded high-energy intermediates (PUFs) and show that these PUFs act as direct precursors to misfolding. Our results also suggest the existence of strong evolutionary pressure to prevent misfolding by placing conserved gatekeeper residues at specific positions, maintaining a high-energy barrier between the native (N) state and the PUFs to reduce the sampling of PUFs from the N state. In another type of phase transition, PrP undergoes liquid-liquid phase separation under physiological conditions to form liquid protein condensates. Upon aging, the liquid-like condensates become solid-like. Structural changes in the protein during the aging process have been studied by fluorescence methods as well as HDX-MS. In the structured C-terminal domain of the protein, some segments become more stable, some 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 oligomer and fibril formation, attains significant structure during the aging of the liquid condensates. These results suggest that similar to folding, misfolding also can occur through different structural mechanisms.

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**Selected for Flash Talk*

Electrostatics Dominate Au-S Interaction in Governing Protein Orientation on Citrate-Capped Gold Nanoparticles

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Gold nanoparticles (GNPs) show great promise for drug delivery, but ensuring their biocompatibility remains a challenge. Coating GNPs with proteins improves stability and functionality, though optimizing protein orientation on the nanoparticle surface is crucial1. This study investigates the interaction between GNPs and single-cysteine mutants of Protein L, a small bacterial protein, to identify factors influencing coating stability. The interactions of protein mutants, varying in cysteine exposure and local environment, were studied using isothermal titration calorimetry (ITC), UV-Vis spectroscopy, and fluorescence spectroscopy to assess binding strength and interaction dynamics. Our findings reveal that binding occurs in two steps: electrostatic forces initially guide the protein's orientation on the GNP surface, followed by covalent bonding that stabilizes the complex. Mutants with exposed cysteine residues and a predominantly electropositive local environment exhibited stronger and more stable binding, highlighting the significance of cysteine's accessibility and surroundings in determining interaction strength. These results emphasize the complementary roles of electrostatic and covalent interactions in stabilizing protein coatings on GNPs. They provide a foundation for designing biocompatible and robust protein-nanoparticle systems for effective drug delivery applications.

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Biophysical Characterization of RelA-p52 NF-kB Dimer – A Link Between the Canonical and the Non-Canonical NF-kB Pathway

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The NF-κB family consists of the key transcription factors, which are well known for their role in innate immune response, organogenesis and a variety of cellular processes. The five NF-κB subunits, RelA, RelB, c-Rel, p50, and p52, are functional dimers, each of which shares a conserved DNA binding domain, which contains the dimerization domain (DD) as well. These proteins can form 15 possible dimers among themselves, of which RelA-p50 is extensively studied for transcription regulation. There is an importance of NF-κB subunit specificity in the transcription regulation of certain genes, and the dynamic nature of the NF-κB dimers plays a crucial role in increasing the specificity. In this study, we have biophysically characterized six combinatorial dimers formed by three NF-κB subunits, RelA, p50 and p52, using NMR spectroscopy and Differential scanning calorimetry. In this study, we have come to the conclusion that the dimer composition is dynamic and can readily undergo exchange to form other possible homodimers and heterodimers at varied rates. Among the six dimers formed, RelA-p52 is found to be the most stable dimer, with RelA-RelA being the least. These results explain the existence of RelA-p52 heterodimer at the later stages of the NF-κB activation and show a link between the canonical and non-canonical NF-κB pathways.

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Single-Molecule FRET Investigation on the Long-Range Contact Formation in the Folding of a gRNA Granule of SARS-CoV-2

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SARS-CoV-2 is a positive-sense single-stranded RNA virus possessing ~30-kb genomic RNA (gRNA). gRNA in virus binds with the N protein and forms ~40 granular structures having a diameter of ~15 nm, each of which consists of ~12 N proteins and ~800 base regions of gRNA[1,2]. However, it is still unresolved how gRNA is folded into the granules and packaged in the virus particle. Here, we synthesized a possible granular region of gRNA, whose 5' and 3' ends separated by several hundreds of bases were assumed to form long-range base pairing, and conducted the single-molecule fluorescence resonance energy transfer (sm-FRET) efficiency observations to examine the long-range contact formation. After a heat denaturation of the labeled RNA fragment, we incubated the sample in the presence and absence of 2 mM $Mg2^{+}$ and 150 mM of NaCl at 37 °C. We found that only the sample in the presence of the salts showed a population having the high FRET efficiency. Furthermore, the population shifted its efficiency to a lower value in the presence of a short oligonucleotide whose sequence was complementary to the possible long-range pairing sites between 5' and 3' ends. These results strongly suggest that the fragment of gRNA forms a compact globular structure having the long-range contacts even in the absence of the N protein. The data also demonstrated that the sm-FRET spectroscopy can be a powerful method for unveiling the gRNA folding and packaging.

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The Role of Isoaspartic Acid Residues at Different Positions in the Fibrillation of Amyloid Beta Peptides and its Inhibition by Protein-L-Isoaspartyl Methyltransferase (PIMT)

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Formation of isoasparate (isoAsp) residues from isomerization of aspartate/asparagine residues, on ageing, can cause fibril formation in brain. Protein-L-isoaspartyl methyltransferase (PIMT) is a ubiquitous repair enzyme which recognizes abnormal isoAsp residues and converts them back to normal Asp[1]. Our previous study with a small hexapeptide Val-Tyr-Pro-(isoAsp)-His-Ala (designated as VA6) showed fibril formation, while the equivalent peptide with normal Asp did not[2]. The presence of PIMT inhibited the fibrillation of VA6. We have extended the study to show that PIMT is also active against oligomers/fibrils of Aβ42, the neuropathological hallmark of AD[3]. Along this line the present work aims to investigate the fibrillation propensity of mutants of Aβ42 peptides. Aβ42 has three Asp residues at positions 1, 7 and 23, which have been replaced by isoAsp. In addition to Asp, Aβ42 also contains two Phe residues at positions 19 and 20, which are also known to contribute to fibrillation. To confirm the role of isoAsp in fibrillation, a mutant of Aβ42 was synthesized by replacing the two Phe by Ala and all the three Asp residues by isoAsp. The anti-fibrillation role of PIMT on the Aβ42 mutants has been studied using various biophysical methods, such as dynamic light scattering, far-UV CD, Thioflavin T fluorescence etc. The cytotoxicity assay of these mutant peptides on human neuroblastoma SH-SY5Y cell line has also been explored. Overall the present study aims to understand the effect of isoAsp residues, unencumbered by the presence of any aromatic residues, in the fibrillation of Aβ42 peptides.

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Biophysical Characterization and Interaction study of WhiB6 Protein of *Mycobacterium tuberculosis* with espA

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Tuberculosis (TB) remains one of the leading causes of death worldwide, primarily due to the pathogenic capabilities of *Mycobacterium tuberculosis* (Mtb). One of the major challenges in combating this disease is understanding the molecular mechanisms that enable Mtb to regulate gene expression and maintain its virulence. The transcriptional regulator protein WhiB6 has been identified as a critical player in this process, as it regulates genes, like espA, essential for the bacterium's survival and pathogenicity. However, the precise mechanisms of WhiB6-espA interaction and its impact on gene expression are not well understood. In this study, we undertook a detailed biophysical examination of WhiB6 and its interactions with espA. Utilizing techniques such as fluorescence spectroscopy, CD spectroscopy, Surface-enhanced Raman spectroscopy (SERS), and ITC, we unveiled the intricate details of WhiB6-DNA binding. Our findings revealed that WhiB6 binds to the espA gene in an entropically driven manner. We studied the effect of varying NaCl concentrations on binding affinity, finding a shift from endothermic to exothermic enthalpy, underscoring the importance of ionic strength in modulating these interactions. SERS provided us with structural insights into the WhiB6 protein and identified the amino acids involved in its interactions. Further, ITC data for the arginine-to-leucine mutant (G**R**A**R**AF) of WhiB6 suggested that these specific residues are crucial for DNA binding. By preventing WhiB6 from binding to the promoter DNA of virulence genes, we could potentially hinder the functioning of *Mycobacterium tuberculosis,* offering a new avenue for therapeutic intervention against tuberculosis.

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Understanding the Structural and Functional Mechanisms Underlying the Dynamic Regulation of Pyruvate Kinase Muscle Isoform 2 (PKM2) in Cancer

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Cancer cells reprogram their metabolism and rely on aerobic glycolysis (Warburg-Effect) for uncontrolled proliferation. PKM2 (Pyruvate kinase muscle isoform 2), a critical factor in this shift, exists in a dimer-tetramer equilibrium[1], the lesser-active dimeric form of which facilitates tumorigenesis through its metabolic and non-metabolic functions[2].The latter is facilitated by its interaction with JMJD5, a positive regulator of tumorigenesis, which promotes its nuclear translocation and the transactivation of tumor-associated genes[3]. Interestingly, PKM2 interacts with JMJD5 around the same region important for its tetramerization. We aim to design PKM2 variants that will shift the equilibrium from dimer to tetramer (non-tumorigenic form), preventing Warburg Effect as well as negate JMJD5 interaction. Using in silico tools: ClusPro and PDBSum, critical amino acid residues at the dimer-tetramer interface of PKM2 (PDB ID:1T5A), important for its oligomerization and PKM2-JMJD5 (PDB ID:4GAZ) interaction were identified. Based on these insights, several PKM2 variants (double, triple, or patch mutants) were generated to disrupt PKM2-JMJD5 interaction and facilitate tetrameric conformation. Biophysical characterization, including Circular Dichroism (CD) and fluorescence emission, depicted well-folded secondary and tertiary structures with minimal conformational changes suggesting the mutants are not structurally perturbing. Biochemical studies validated significant disruption in PKM2-JMJD5 interaction by introducing PKM2 patch mutations, thus underscoring the importance of the specific residues. Comparative size-exclusion chromatographic analyses of PKM2 variants with PKM1 (its non-tumorigenic isoform) implicated an equilibrium shift toward tetrameric ensemble. These studies provided an important platform toward the design of peptides/mimetics to disrupt PKM2-JMJD5 interaction and shift the equilibrium towards its non-tumorigenic form.

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Tuning Geometry and Functionality of α-Helical Nanopores through Site-Specific Incorporation of Natural and Unnatural Amino Acids

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Synthetic α-helical nanopores offer a powerful platform for tuning pore geometry and functionality through precise molecular chemical modifications for various applications in nanobiotechnology and synthetic chemical biology. Here, we designed a synthetic α-helical peptide pore pPorA, derived from the porin PorACj that autonomously assembles into octameric monodisperse pores. By incorporating either natural or unnatural amino acids at a specific position, we modulate the pore size and conductance, generating two distinct conductance states despite the identical octameric subunit composition. This established the structural flexibility of α-helical pores. Larger pores with higher conductance can sense bulky cyclic sugars and PEGylated molecules, while smaller pores with lower conductance selectively detect linear peptides or small molecules. The site-specific covalent chemical modification with small and large thiol reagents confirmed the two distinct pore geometries. These findings demonstrate the versatility of site-specific modifications in α-helical peptide pores, enabling the design of tunable sensors with broad molecular sensing capabilities. This work lays the groundwork for developing peptide-based sensors with applications in biosensing and complex single-molecule detection.

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Unraveling Transition Path and Membrane Topological Characteristics in Bacteriorhodopsin Fragment Folding Pathway Implying Artificial Intelligence

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Synthetic α-helical nanopores offer a powerful platform for tuning pore geometry and functionality through precise molecular chemical modifications for various applications in nanobiotechnology and synthetic chemical biology. Here, we designed a synthetic α-helical peptide pore pPorA, derived from the porin PorACj that autonomously assembles into octameric monodisperse pores. By incorporating either natural or unnatural amino acids at a specific position, we modulate the pore size and conductance, generating two distinct conductance states despite the identical octameric subunit composition. This established the structural flexibility of α-helical pores. Larger pores with higher conductance can sense bulky cyclic sugars and PEGylated molecules, while smaller pores with lower conductance selectively detect linear peptides or small molecules. The site-specific covalent chemical modification with small and large thiol reagents confirmed the two distinct pore geometries. These findings demonstrate the versatility of site-specific modifications in α-helical peptide pores, enabling the design of tunable sensors with broad molecular sensing capabilities. This work lays the groundwork for developing peptide-based sensors with applications in biosensing and complex single-molecule detection.

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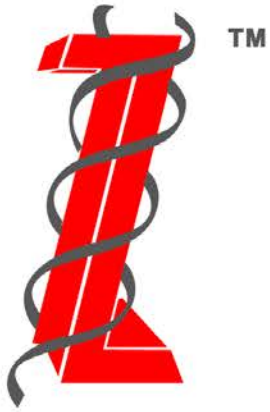

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