Nu Biophysical Society '21

Molecular Biophysics Unit

presents

MBU In-House Symposium

✧ *Student talks* ✧ *Poster sessions*

Session highlights !!

Plenary Talk I

Prof. Ramananda Chakrabarti

'A tale of two craters'

Plenary Talk II

Dr. Amit Baidya

'Bacterial nanotubes: Conduits for intercellular trafficking'

Join us on
December 13, 2021 (Monday)

Time
09:00 hrs - 18:00 hrs IST

Schedule for NuBS In-House Symposium

13th December 2021

TIME	MBU IN-HOUSE SYMPOSIUM	CHAIR
	Oral Session Ⅰ (9.00 AM - 11.00 AM)	
9.00 am to 9.15 am	Chairman's address	Manikandan Parthasarathy
9.15 am to 9.30 am 9.30 am to 9.45 am 9.45 am to 10.00 am	Krishna Kanth B (Dr. Anand Srivastava) Arunabh Athreya (Dr. Aravind Penmatsa) Ahallya Jaladeep (Dr. Ashok Sekhar)	
10.00 am to 11.00 am	Plenary talk Ⅰ: Prof. Ramananda Chakrabarti	
11.00 am to 12.00 pm	Poster Session Ⅰ / Morning High Tea	
	Oral Session Ⅱ (12.00 PM - 1.00 PM)	
12.00 pm to 12.15 pm 12.15 pm to 12.30 pm 12.30 pm to 12.45 pm 12.45 pm to 01.00 pm	Soujanya D Yelamanchi (Prof. Avadhesha Surolia) Sushma Devendra (Prof. Balasubramanian Gopal) Vikas Chaudhary (Prof. Dipankar Chatterji) Bhavesh Khatri (Prof. Jayanta Chatterjee)	Pritha Ghosh
1.00 pm to 2.15 pm	Lunch	
	Oral Session Ⅲ (2.30 PM - 4.00 PM)	
2.30 pm to 3.00 pm	Plenary talk Ⅱ: Dr. Amit Baidya	Debashree Behera
3.00 pm to 3.15 pm 3.15 pm to 3.30 pm 3.30 pm to 3.45 pm 3.45 pm to 4.00 pm	Debostuti Ghoshdastidar (Prof. Manju Bansal) Kavyashree Nadig (Prof. Mahavir Singh) Munmun Bhasin (Prof. Raghavan Varadarajan) Rituparna Roy (Prof. Rishikesh Narayanan)	
4:00 pm to 5.00 pm	Poster Session Ⅱ / Evening High Tea	
	Oral Session Ⅳ (5.00 PM - 6.00 PM)	
5.00 pm to 5.15 pm 5.15 pm to 5.30 pm 5.30 pm to 5.45 pm	Iladeiti Kurbah (Prof. Siddhartha Sarma) Surekha P (Dr. Somnath Dutta) Ashish Apotikar (Prof. Sujit Kumar Sikdar)	Nazia Hussain
5.45 pm to 6:00 pm	Valedictory Session	

PLENARY TALKS

A tale of two craters

Prof. Ramananda Chakrabarti

Center for Earth Sciences, Indian Institute of Science

Impact craters, formed by collisions between planetary objects, are ubiquitous in the Solar System. Such collisions played a critical role in the accretion of planetary bodies and their evolution. On Earth, impact events have also affected the evolution of life. In my talk, I'll provide a brief overview of impact cratering and focus on geochemical and isotopic studies of the Lonar and Dhala impact craters. The 1.8 Km diameter Lonar impact crater, hosted on the Deccan basalts, formed approximately 0.5 million years ago and is one of the best-preserved terrestrial impact craters. In contrast, the 11 km diameter Dhala crater is approximately 2 billion years old and is poorly preserved. Geochemical studies of impactites from these two craters reveal the type of impactor and insights into the evaporation-condensations processes and hydrothermal alteration that takes place due to an impact event.

Bacterial nanotubes: Conduits for intercellular trafficking

Dr. Amit Baidya

Molecular Biophysics Unit, Indian Institute of Science

Nanotubes are intercellular membranous bridges that provide a path for intercellular trafficking of nutrients, antibiotic resistance and toxic proteins and even plasmids, in an intra- and inter-species manner. However, the identity of the machinery producing nanotubes remained elusive. We found that conserved components of the flagellar type 3 secretion system (T3SS) export apparatus (FliO,P,Q,R, FlhB,A), herein termed CORE, dually serve for flagellar and nanotube assembly. Deleting the CORE genes in the Gram positive model organism Bacillus subtilis, but not other flagellum components, eliminated nanotube formation along with intercellular molecular trade. Furthermore, pathogenic bacterium Enteropathogenic E. coli can acquire nutrients from mammalian cells utilizing injectisome T3SS CORE encoded nanotubes. CORE components localize to sites of nanotubes, enabling their emergence, indicating a previously unreported bacterial CORE-nanotube organelle. Deleting COREs of distinct bacterial species established that CORE-mediated nanotube formation is widespread. Furthermore, exogenous COREs from diverse species could restore nanotube generation and functionality in B. subtilis lacking endogenous CORE. Intriguingly FlhA, the major component of CORE interacts with the cAMP phosphodiesterase to act as a switch dictating nanotube or flagella formation. Taken together, CORE functions as a common platform for nanotube biogenesis by a large number of bacterial species belonging to diverse phyla.

ORAL PRESENTATIONS

Integrating molecular simulation with small angle scattering data to refine the structure of multidomain proteins with flexible linkers

Krishnakanth Baratam, Kirtika Jha, and Anand Srivastava

Dr. Anand Srivastava lab, Molecular Biophysics Unit, IISc

Elastic network models (ENMs) are often used to investigate the dynamics and normal modes of well folded proteins. However, ENMs are not flexible enough to reliably reproduce the configurational landscape of large multidomain proteins with long flexible linker regions. Moreover, due to their high flexibility at the linker regions, the structure-function relationship of these proteins are better represented with an ensemble of structures rather than a single structure. In this work, we have developed a genetic-algorithm based "integrative modeling" optimization approach where the low-resolution structural information from small angle scattering data is integrated with molecular simulations to produce experimentally realistic ensemble of structures of multi-domain proteins at all-atom resolution. We use ENM to model the well-folded domains in the conventional manner. However, to match data from SAXS/SANS experiments, we train the force-field parameters of the linker regions to minimize the error against the experimentally observed pair distance distribution function. We demonstrate that conformational profiles across diverse multidomain proteins, such as HIV-1 Gag, RNA-binding TIA-1 and human growth hormone receptor (hGHR-ECD), match well with the experimental SAXS/SANS data. Given the low-resolution scattering data, the method has also opened doors for extracting high-resolution conformations of IDPs/IDRs.

Plugging a transporter with a nanobody: structure of NorC-VHH complex and it's implications on the transporter's dynamics

Arunabh Athreya, Sushant Kumar, Ashutosh Gulati, Rahul M Nair, M Ithayaraja, Rakesh Ranjan, Aravind Penmatsa

Dr. Aravind Penmatsa lab, Molecular Biophysics Unit, IISc

Transporters play a major role in acquiring drug resistance in pathogenic bacteria. NorC is a transporter belonging to the Major Facilitator Superfamily and is implicated in providing resistance against fluoroquinolones in Staphylococcus aureus. To elucidate it at an atomic resolution, we crystallized and solved its structure in complex with a nanobody that we generated through camel immunization against NorC. This nanobody, called ICab, showed peculiar characteristics that were not observed in previous studies, from an abnormally long CDR1 region to a natural presence of Zinc coordination in place of a general disulfide involving CDR3. While analyzing the structures of ICab in its Apo and NorC bound forms, we were also able to explain how the ICab, jutting into the vestibule of NorC to almost half the length, can block a substrate's accessibility to NorC's vestibule. The ICab thereby locks NorC in one state, resembling a bottle cork. Screening for substrates revealed that a compound with a micromolar dissociation constant for NorC loses its affinity in the presence of ICab. Together with the information on the altered dynamics of NorC conformations in the presence of ICab, this study provides a steady example of how single-domain antibody fragments can be repurposed to act as diagnostic probes and antibiotic adjuvants in clinical practices.

Measuring radiofrequency fields in NMR spectroscopy using offset-dependent nutation profiles

Ahallya Jaladeep, Claris Niya Varghese, Ashok Sekhar

Dr. Ashok Sekhar lab, Molecular Biophysics Unit, IISc

The application of NMR spectroscopy for studying molecular and reaction dynamics relies crucially on the accurate measurement of the magnitude of radiofrequency (RF) fields that are used to lock or nutate the nuclear magnetization. We report a method for measuring RF field amplitudes that leverages the intrinsic modulations observed in offset-dependent NMR nutation profiles of small molecules. RF fields ranging from 1-2000 Hz, as well the inhomogeneity in their distributions, can be determined very accurately and precisely using this approach. In order to measure RF fields associated with NMR experiments carried out on protein or nucleic acids, where these modulations are damped by the large transverse relaxation rate constants of the analyte, our approach can be used in combination with a suitable external small molecule standard, expanding the scope of the method to large biomolecules.

Pranlukast mediated metabolic dysregulation in *Mycobacterium tuberculosis*

Soujanya D. Yelamanchi, S. T. Arun Kumar, Archita Mishra, T. S. Keshava Prasad, Avadhesha Surolia

Prof. Avadhesha Surolia lab, Molecular Biophysics Unit, IISc

Mycobacterium tuberculosis has been infecting millions of people worldwide over the years causing tuberculosis. Drugs targeting distinct cellular mechanisms including synthesis of the cell wall, lipids, proteins and nucleic acids in *Mtb* are currently being used for the treatment of TB. Although extensive research is being carried out at the molecular level in the infected host and pathogen, the identification of suitable drug targets and drugs remains under explored. Pranlukast, an allosteric inhibitor of *Mt*ArgJ (*Mtb* ornithine acetyltransferase) has previously been shown to inhibit the survival and virulence of *Mtb*. Here in this study, metabolomics was carried out using LC-MS/MS-based approach to identify the differentially expressed metabolites of *Mtb* by pranlukast. Collectively, 50 metabolites were identified to be differentially expressed with a significant p-value through a global metabolomic approach using a high-resolution mass spectrometer. Metabolites downstream of argJ were downregulated in the arginine biosynthetic pathway following pranlukast treatment. Predicted human protein interactors of pranlukast treated *Mtb* metabolome were identified in association with autophagy, inflammation, DNA repair and other immune-related processes. Further metabolites including N-acetylglutamate, argininosuccinate, L-arginine, succinate, ergothioneine and L-phenylalanine were validated by multiple reaction monitoring, a targeted mass spectrometry-based metabolomic approach. This study facilitates the understanding of pranlukast-mediated metabolic changes in *Mtb* and holds the potential to identify novel therapeutic approaches using metabolic pathways in *Mtb*.

Structural studies of R-specific ω-transaminase crucial for the bioconversion of chiral amines

Sushma D Jagatap, Ashish A Deshmukh and B. Gopal

Prof. Balasubramanian Gopal lab, Molecular Biophysics Unit, IISc

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Pyridoxal-5'-phosphate (PLP) dependent enzymes play a crucial role in the asymmetric synthesis of chiral amines. While S- selective transaminases belong to fold type I, R-selective transaminases adopt a different conformation and are referred to as fold type IV PLP dependent enzymes. *In silico* studies led to the identification of only 140 bonafide R-selective transaminases. These enzymes form homo-dimers and the active site is located at the dimeric interface. Blind docking of putative substrates with R-specific transaminases suggested the possibility of annotating these enzymes with specific substrates. Here we describe structural features of selected phylogenetically diverse R-selective transaminases. These experiments are being performed to validate the molecular models that formed the basis for enzyme substrate correlations. We anticipate that this study would lead to an experimentally validated curated library of substrates and R-specific transaminases for diverse applications.

Exploring c-di-AMP driven key phenotypes in *Mycobacterium smegmatis*

Vikas Chaudhary, Aditya Kumar Pal & Anirban Ghosh

Prof. Dipankar Chatterji lab, Molecular Biophysics Unit, IISc

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Cyclic-di-AMP (c-di-AMP) is a newly discovered secondary messenger molecule that plays a critical role in monitoring several important cellular processes, especially during stress adaptation. In *Mycobacterium smegmatis*, c-di-AMP is synthesized from condensation of two ATP molecules by enzyme DNA integrity scanning protein A (DisA) and degraded by enzyme Phosphodiesterase (Pde) into pApA. In this study, we seek to unravel the physiological significance of c-di-AMP in *M. smegmatis* under different conditions, using a c-di-AMP null mutant (*ΔdisA*) and a c-di-AMP over-expression mutant (*Δpde*). We found that, modulating intracellular concentrations of c-di-AMP could alter a few basic phenotypes such as cell size, cell shape, colony morphology, as well as, some surface-related properties including cellular aggregation, sliding motility, and Biofilm/Pellicle formation. The complementation of these knockout mutants with the same gene cloned in an integrative vector reverted the phenotype, which was necessary to conclude the direct gene-phenotype correlation. Our Phenotypic Microarray (PM) data indicated differential drug susceptibility/resistance profile of the mutant strains compared to *wt,* which involves antibiotics from all major classes; these observations were subsequently confirmed by Growth Kinetics and Minimum Inhibitory Concentration (MIC) assay. Further, a genome-wide transcriptome analysis (by RNA-seq) of the mutants indicated few possible cellular mechanisms behind distinctive antibiotic responses and it also highlighted critical metabolic functions and cellular pathways regulated by c-di-AMP *in vivo*.

A proteomimetic prevents SARS-CoV-2 infection by dimerizing the spike protein

Bhavesh Khatri, Ishika Pramanick[⊥], Sameer Malladi[⊥], Suhail, Raju Rajmani, Pritha Ghosh, Nayanika Sengupta, Rahisuddin, S. Kumaran, Rajesh P Ringe, Raghavan Varadarajan, Somnath Dutta[*], Jayanta Chatterjee[*]

Prof. Jayanta Chatterjee lab, Molecular Biophysics Unit, IISc

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Protein tertiary structure mimetics with well-defined conformation are valuable tools to target large and flat protein-protein interaction interfaces. Here, we demonstrate a strategy for the design of dimeric helix-hairpin motifs from a three-helix bundle miniprotein that targets the receptor binding domain (RBD) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Through truncation of the third helix and optimization of the interhelical loop residues connecting the helix 1 and helix 2 of the miniprotein, we developed thermostable dimeric helix-hairpin that shows reversibility upon removal of the thermal stress. The dimeric four-helix bundle efficiently compete out the human angiotensin-converting enzyme 2 (ACE2) in binding to RBD in a 2:2 stoichiometry. The high-affinity between the helix-hairpin protomers and the helix-hairpin with RBD lead to the formation of stable dimeric spike protein, where all the three RBDs from either spike protein are firmly attached head-to-head in an open conformation, revealing a novel mechanism for virus neutralization. The in vivo efficacy of the dimeric helix-hairpin as SARS-CoV-2 entry inhibitor demonstrates the promise of this class of proteomimetics that act by inhibiting protein-protein interaction through target dimerization.

Dissecting the specificity landscape of Transcription Factor-DNA interactions

Debostuti Ghoshdastidar[1], Shravan Sukumar[2], Devesh Bhimsaria[2], Aseem Ansari[2] and Manju Bansal[1]

[1]*Prof. Manju Bansal lab, Molecular Biophysics Unit, IISc and* [2]*Aseem Ansari Lab, St Jude Children's Hospital*

Therapeutic interventions in gene regulatory disorders require a mechanistic understanding of how transcription factors (TFs) scan through millions of putative binding sites (BS) across the genome to specifically recognize the motif of interest. Elements of this recognition code include base-specific h-bonding, DNA-TF shape complementarity and DNA flexibility. Teasing out the role of shape and flexibility from sequence is challenging. Hence, TFBSs are popularly represented as a uniform string of features, e.g., narrow groove width of the homeodomain binding site or large bendability of the catabolite activator protein binding site, etc.

Here we propose an alternative approach to dissect a TFBS, by scoring its nucleotides, not based on property (sequence/shape/flexibility) but based on indispensability in TF binding. When tested on high-throughput *in vitro* binding affinity data for ~1174 human TFs, our scoring function dissected each TFBS into a heterogeneous mix of "shape-clamp" nucleotides and "specificity-lock" nucleotides. "Shape-clamps" possess an ideal shape/flexibility feature that weakly clamps the TF at an ideal BS following which "specificity-locks" lock the interaction by direct h-bonding with TFs. Site-directed mutagenesis and molecular simulations further revealed that shape-clamp mutants alter TF binding specificity, and could potentially serve as a powerful tool to orchestrate DNA:TF binding modalities in DNA-based therapeutics.

Purification of type III toxin-antitoxin systems for biophysical studies

Kavyashree Nadig M, Manikandan P, and Mahavir Singh

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Bacterial toxin-antitoxin (TA) systems are genetic modules that play essential roles in bacterial persistence phenotype, antibiotic resistance, and are speculated to regulate the existential processes of the cell. The type III TA system is an interesting system consisting of a protein toxin and a non-coding RNA antitoxin. The binding of antitoxin RNA neutralizes the activity of the protein toxin under homeostatic conditions. Specific bacteriophages can trigger the system upon infection, causing the toxin to be released, resulting in an altruistic behaviour of the bacterium, such as retardation in cell growth or cell death. The type III systems are classified into three families: tenpIN, cptIN, and toxIN, based on toxin antitoxin sequence homology. We have identified the toxIN and tenpIN systems in *E. coli*. Further, we have standardized the expression and protein purification strategy for three toxIN TA systems that enabled us to obtain free toxin protein, protein-RNA TA complex, and free antitoxin RNA from a single purification experiment. This protocol helps us obtain milligram quantities of toxin, antitoxin, and TA complex required for the biophysical characterization (using methods that include CD, NMR, X-ray, cryoEM, and ITC) of these systems to understand their assembly, structure, and activation mechanisms.

Structure and stability predictions from saturation mutagenesis

Munmun Bhasin, Shahbaz Ahmed, Kavyashree Manjunath, Raghavan Varadarajan

Prof. Raghavan Varadarajan lab, Molecular Biophysics Unit, IISc

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Mutational scanning can be used to probe effects of large numbers of point mutations on protein function. Positions affected by mutation are primarily at either buried or at exposed residues directly involved in function, the latter are referred to as active-site residues. In the absence of prior structural information, it has not been easy to distinguish between these two categories of residues. We curated and analysed published deep mutational scanning datasets. The analysis revealed differential patterns of mutational sensitivity and substitution preferences at buried and exposed positions. Prediction of buried-sites solely from the mutational sensitivity data was facilitated by incorporating predicted sequence-based accessibility values. In addition to the computational predictions, using CcdB as a model system, we used FACS and deep sequencing to reconstruct the mean fluorescence intensity of each mutant's binding and expression. The reconstructed mean fluorescence intensity was utilised to distinguish between buried sites, exposed non-active-site positions, and exposed active-site positions. The method was extended to the receptor binding region of the spike protein of SARS-CoV-2, indicating its general applicability. This highlights the ability of deep mutational scans to provide important structural and functional insights, even in the absence of three-dimensional structures or limited numbers of orthologous sequences.

Dominant role of calcium and calcium-dependent potassium channels in regulating complex spike bursting in a heterogeneous population of CA3 pyramidal neuron models

Rituparna Roy and Rishikesh Narayanan

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Complex spike bursting (CSB) is a characteristic neuronal signature of hippocampal CA3 pyramidal neurons. Despite the roles of CSB in synaptic plasticity and place-cell formation, the role of neural heterogeneities on CSB output has not been assessed. Here, we generated a heterogenous population of morphologically realistic CA3 pyramidal neuron models that accounted for their ion-channel biophysics and associated heterogeneities. Validation against electrophysiological properties yielded a population of 236 valid models endowed with weak correlations amongst the underlying parametric space. We identified two distinct functional subclasses of intrinsic bursting (IB) and regular spiking (RS) neurons employing dimensionality reduction techniques, and found differential expression of calcium and calcium-activated potassium conductances across these subclasses. We triggered CSB in all 236 models by subjecting them to 5 different kinds of inputs and observed considerable heterogeneity in their propensity for exhibiting CSBs. Finally, we employed virtual knockout analyses across 8 ion channels and receptors and showed that synergistic interactions among several ion channels regulated CSB, with dominant roles for NMDA receptors, *N*-type calcium and SK channels. Together, our analyses unveiled the expression of ion-channel degeneracy in CA3 pyramidal neurons and emphasized the dominance of calcium and calcium activated potassium channels in the emergence of CSB.

Structural studies on the novel conotoxin Im23.5 from *Conus imperialis*

Iladeiti Kurbah, Siddhartha P. Sarma

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A single marine cone snail species can produce around 50-200 unique peptides in its venom. This sequence diversity allows them to have significant functional diversity, targeting ion channels, G protein-coupled receptors (GPCRs), transporters, or enzymes. The small size, structural stability, potency, and specificity of conopeptides have made them a valuable source of molecular probes for therapeutic studies and lead compounds for drug design and clinical use. Disulphide-rich conopeptides or conotoxins are classified under 1 of 32 Cysteine frameworks.

Im23.5 is a 25 amino acid conotoxin produced by *Conus imperalis*. There are currently eight conotoxins with Cysteine Framework XXIII. Excluding Im23.5, the remaining seven conotoxins not only have a sequence similarity of more than 70%, but they are also much longer (40 or more amino acids) as compared to Im23.5. We are interested in looking at how Im23.5 compares to other members of Cysteine Framework XXIII in terms of structure and function.

Since conotoxins have multiple disulphide bonds, they can adopt conformations with different disulphide bond connectivities. High Performance Liquid Chromatography analyses indicate that Im23.5 has one major conformation with other minor conformations. Preliminary NMR Data also supports the presence of multiple conformations.

Structural insights into the outer membrane protein TolC in native lipid environment

Rupam Biswas, **Surekha P**, Somnath Dutta

Dr. Somnath Dutta lab, Molecular Biophysics Unit, IISc

TolC is a unique bacterial outer membrane channel protein. It facilitates the direct passage of molecules from the inner membrane to outside the cell, bypassing the periplasmic space. It transports a wide range of molecules, from small molecules to large proteins (e.g., hemolysin, colicin). It is a universal outer membrane transporter that works with multiple inner membrane transporters to directly export/import molecules. Tripartite (three-component) RND (resistance nodulation division) pumps also require TolC for exporting molecules outside the cell. These efflux pumps are the primary reason for multidrug resistance in bacteria.

The structures of TolC that have been reported so far do not provide information on the effect of native lipid surrounded environment on TolC structure. In our study, we are comparing the structures of TolC in a non-native environment (amphipol exchanged) and native environment (liposome exchanged) using cryo-EM technique. We have optimized the TolC preparation in amphipol and liposome. Later, negative staining and cryo-EM studies are carried out on the samples. Knowledge of the structure of TolC in a native lipid environment will helps us understand the interaction of TolC with the inner membrane transporters and the substrates.

Studies on modulation of HCN channels by Nicotine

Ashish Apotikar, SK Sikdar

Prof. Sujit K. Sikdar lab, Molecular Biophysics Unit, IISc

Hyperpolarization-activated cyclic nucleotide-gated(HCN) channels are voltage-gated, inward rectifying cation channels that mediate cardiac rhythmicity and perform major functions like setting the resting membrane potential, dendritic integration of synaptic inputs, pacemaking of neurons etc. Pharmacological modulation of HCN channel kinetics can treat disorders like neuropathic pain, heart failure, arrhythmias and epilepsy. Various molecules that can block HCN have been identified but their low affinity and lack of specificity prevents their wide-spread use. Nicotine was found to partially block HCN channels with an affinity more than 240 times (IC50 = 62 nM) that of the known blocker of HCN channels, ZD7288 (IC50 = 15 μM) but the details regarding its HCN isoform specificity and binding remain unknown. In our study, we aim to study modulation of HCN channel isoforms hHCN1 and hHCN4 by nicotine and identify the binding site of nicotine with hHCN1 isoform. Our experiments using patch clamp electrophysiology on HEK293 cells expressing hHCN1 show that, nicotine causes decrease in hHCN1 current. Preliminary molecular docking studies using Autodock vina on closed hHCN1 and modelled open conformation structure using the templete of eukaryotic CNG channel shows that nicotine possibly binds to Tyr361 and Gly362 residues in the pore region which needs to be validated experimentally.

POSTER PRESENTATIONS

Driving forces for interleaflet coupling in lipid nanodomains

Akshara Sharma, Aniruddha Seal, Sahithya S Iyer, Anand Srivastava

Dr. Anand Srivastava lab, Molecular Biophysics Unit, IISc

A biological membrane is a complex self-assembly of lipids, sterols, and proteins organized as a fluid bilayer. Heterogeneities can arise in the leaflets of a lipid bilayer due to differential molecular interactions, leading to formation of phase-separated domains. A fascinating phenomenon from a cell-signaling perspective is the possibility of spatial correlation of domains between leaflets known as domain registration. Several contrasting theories behind mechanisms inducing registration of nanoscale domains have been suggested.[1-3] Following a 2019 study[4] showing how position of unsaturation of lipid tails affects domain registration behavior, we developed an analytical theory to elucidate the driving forces that create and maintain registered nanodomains. We formulated a Hamiltonian for a stacked lattice system with site variables capturing position of unsaturation and other interactions known to affect interleaflet coupling. We solve the Hamiltonian using Monte-Carlo simulations and create a phase diagram showing a gradation of registration/anti-registration behaviour as a function of the Hamiltonian parameters. We find that both lipid composition and entropy between the leaflet act as major design variables for creation and maintenance of non-coalescing registered nanodomains.

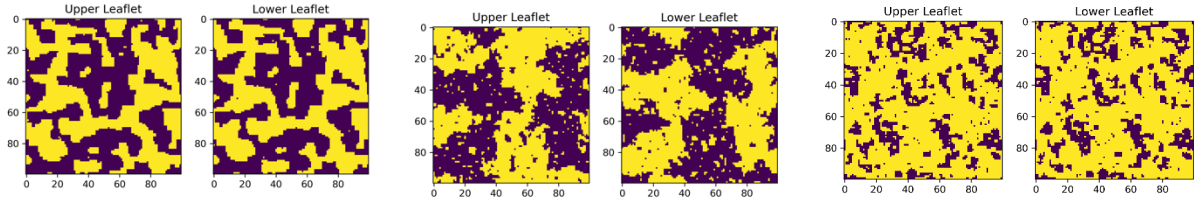

Figure 1: Some points from our parameter space **Left)** A fully registered system. **Middle)** A fully anti-registered system. **Right)** Non-coalescing persistent registered nanodomains.

References

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2. Putzel, G. G., M. J. Uline, I. Szleifer, and M. Schick, *Biophysical Journal*, 2011, 100 (4), 996
3. Galimzyanov, T. R., P. I. Kuzmin, P. Pohl, and S. A. Akimov, *Biophysical Journal*, 2017, 112 (2), 339
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Role of glycans in recruitment of lipid molecule in Lili-Mip protein

Harini SureshKumar and Anand Srivastava

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In the aspect of nutrient storage, in-cellulo crystals have been largely unexplored due to their size constraints, multimeric nature, heterogeneity and poor diffraction capacity. Lili-Mip (PDB ID: 4NYQ) is one such case of lipid-binding glycoprotein [1] with high dietary prospects [2], found in the developing embryo of a viviparous cockroach. To understand the molecular mechanism of the nutrient storage in Lili-Mip, we have employed all-atom molecular dynamics simulations on various glycosylated stages of a bound and free Lili-Mip protein and characterized the impact of glycans and lipid ligand on the dynamics of this glycoconjugate. Our analyses show that the glycans locally stabilize spatially proximal residues and regulate the low amplitude opening motions of the residues at the entrance of the binding pocket. A simple but effective distance-based network model of the protein reveals that the residues of the distal-end cavity are highly interconnected and the presence of glycans prevent the collapse of the binding pocket upon lipid recruitment. Our work reveals that the glycosylation causes local stabilization that possibly aids the protein in lipid-recruitment process.

References:

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Ingenious clustering algorithm for representing IDP ensembles

Rajeswari Appadurai, Irawati Roy, Anand Srivastava

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Conformational landscapes of intrinsically disordered proteins (IDPs) represent heterogeneous ensemble of structures with large dimensionality that can prove difficult to interpret effectively. The heterogeneous conformations often need to be grouped into "structurally similar" clusters for molecular recognition studies, functional screening and also for constructing kinetic models. This is a difficult task for IDPs since the conformational clusters are defined by the choice of low dimension feature space or collective variables and therefore result in ambiguous classification. The problem is further compounded for IDPs that are very long (example: 100+ residue-long hnRNPA-1 low complexity domain and K2P channel TREK-1 C-terminal domain), and dynamically shift populations within an ensemble under different context (example: Amyloid beta protein in presence and absence of ligand). Here, we employed tdistributed stochastic neighbour embedding, a non-linear projection technique for analysing the disordered ensembles of the three long and dynamic IDPs. The projection aids in faithful clustering of IDP ensemble and identifies distinct metastable conformations with unique topological features. The method also allows for the quantification of conformational population and its relative shift due to ligand binding.

Insights into RNA interaction mechanism of FUsed in Sarcoma protein

Sangeetha Balasubramanian[1], Shovamayee Maharana[2] and Anand Srivastava[1]

Dr. Anand Srivastava lab, Molecular Biophysics Unit[1], IISc, [2]Department of Microbiology and Cell Biology, IISc

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Fused in Sarcoma (FUS), is an abundant RNA binding protein which drives phase separation of cellular condensates and plays multiple roles in RNA regulation. Mutations and abnormalities in FUS are directly linked to neurodegenerative diseases like ALS and FTLD. FUS recognizes a large variety of RNA sequence/structure motifs in biomolecular condensates and expresses RNA dependent phase behavior. Identifying the atomic level interactions in FUS-RNA complexes forms the basis for understanding the RNA dependent liquid-liquid phase separation (LLPS) of FUS. Our on-going study explores the structure, stability and interaction of RRM and ZnF domains with different RNA sequences. Interestingly, our simulations highlight the sequence specificity of ZnF and shape as well as sequence specificity of RRM. The RRM domain recognizes a stem-loop junction with YNY motif and the inclusion of RGG improves the interaction with RNA. Similarly, the recognition of GGU motif by the ZnF domain was reinforced by simulating a ZnF-polyA RNA complex. The uniqueness of FUS-RNA binding lies in the stabilizing nature of both hydrophobic as well as electrostatic interactions. As a next step, the bipartite binding of RRM-RGG2-ZnF domains with ssRNA-stem-loop-GGU motif will be modeled. These results would facilitate further modeling of a full-length FUS in complex with RNA to study RNA dependent LLPS.

Plugging a transporter with a nanobody: structure of NorC-VHH complex and its implications on the transporter's dynamics

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Transporters play a major role in acquiring drug resistance in pathogenic bacteria. NorC is a transporter belonging to the Major Facilitator Superfamily and is implicated in providing resistance against fluoroquinolones in *Staphylococcus aureus*. To elucidate it at an atomic resolution, we crystallized and solved its structure in complex with a nanobody that we generated through camel immunization against NorC. This nanobody, called ICab, showed peculiar characteristics that were not observed in previous studies, from an abnormally long CDR1 region to a natural presence of Zinc coordination in place of a general disulfide involving CDR3. While analyzing the structures of ICab in its Apo and NorC bound forms, we were also able to explain how the ICab, jutting into the vestibule of NorC to almost half the length, can block a substrate's accessibility to NorC's vestibule. The ICab thereby locks NorC in one state, resembling a bottle cork. Screening for substrates revealed that a compound with a micromolar dissociation constant for NorC loses its affinity in the presence of ICab. Together with the information on the altered dynamics of NorC conformations in the presence of ICab, this study provides a steady example of how single-domain antibody fragments can be repurposed to act as diagnostic probes and antibiotic adjuvants in clinical practices.

Structural, mechanistic and pharmacological studies of inhibitory neurotransmitter transporters

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γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). The synaptic levels of GABA are controlled by the activity of GABA transporters (GATs) present in the presynaptic and glial membranes. Impaired GABAergic signaling leads to hypersynchronous excitatory discharges that can result in epileptic conditions. The absence of a high-resolution structure of GAT1 limits the understanding of its pharmacology and the basis of GAT1 specific inhibition. The *Drosophila melanogaster* dopamine transporter (dDAT) can be used as a suitable model to study inhibitor interactions among the NSS members due to the availability of thermostabilizing mutants and an antibody chaperone for rapid crystallization. We have therefore used the dDAT, as a template to engineer the binding pocket of GAT1 (DAT_{GAT}) and determined X-ray structures of the construct in complex with GAT1 inhibitors. The high-resolution crystal structures of DAT_{GAT} in complex with NO-711 and SKF89976a displayed an altered subsite architecture in the primary binding pocket revealing discrepancies in inhibitor interactions among GATs and biogenic amine transporters. Interestingly, we observed an additional density for SKF89976a in the vestibule alongside that of the SKF89976a bound in the primary binding site. We also deciphered a crucial role of the extracellular loop 4 in influencing substrate gating in NSSs. The structural findings obtained from DAT_{GAT} structures were further validated using thorough biochemical analysis performed in a mammalian orthologue of GAT1.

Surface engineering facilitates epitope transfer between neurotransmitter transporters

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Antibodies are popular tools in structure determination of integral membrane proteins. These antibodies can reduce conformational heterogeneity by stabilizing flexible regions, provide surfaces for crystal contacts and increase size of the molecule and serve as fiducial markers in Cryo-EM studies. But generating such antibodies and characterizing their interaction with antigens is time consuming and challenging. To circumvent this, we used a strategy to generate antibody interactions in a GABA transporter 1 (GAT1) homologue, by grafting the epitope from *Drosophila melanogaster* Dopamine transporter (dDAT) onto GAT1 to facilitate interactions with a Fab fragment called 9D5 that is used to crystallize dDAT. After visual inspection of 9D5 binding site in dDAT as well as SASA calculation we identified the residues where were part of the epitope and generated a set of mutations in GAT1 called epitope mutants (epi mutants). We screened all the mutant constructs for their ability to interact with the Fab 9D5, by checking their FSEC shifts. GABA uptake experiments performed with epi mutants displayed transport activity and a mutant combination called epi4 had optimal shifts with 9D5 binding. 2D classification and a low-resolution 3D model showed GAT1-Fab complex surrounded by a micelle and bound to antibody fragment.

Characterizing sparsely populated intermediates in the free energy landscape of a metamorphic protein

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The field of structural biology has advanced to an extent that currently there are more than 1,50,000 structures in the PDB. However, these colorful structures that are used to represent the field of structural biology can be deceptive as they portray proteins as rigid molecules which are functional only in their lowest energy conformations. With the development of advanced biophysical tools it is now established that understanding protein structure-function relationship in its entirety requires an additional dimension, time. One classic example which signifies the importance of studying dynamics in protein structure is the class of metamorphic proteins. Metamorphic proteins exist in more than one native state in biological systems while undergoing reversible exchange amongst each other. In this study, we are investigating the mechanism of interconversion between the two native forms of a metamorphic protein, lymphotactin. Using sophisticated NMR experiments which can detect sparsely populated high energy transient states, we are trying to characterize an intermediate conformation that facilitates the interconversion between the two metamorphic conformations of lymphotactin (Ltn10 and Ltn40). We have been able to detect a low populated minor state in slow exchange with Ltn10 with a $k_{ex} \sim 500\ s^{-1}$ and having a population of ~1.8%.

Understanding the dynamics of a metamorphic protein, MAD2 using NMR spectroscopy

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‘One sequence-one structure’ paradigm has been a long-standing notion in the world of protein folding. With advancements in biophysical techniques, various protein classes like intrinsically disordered proteins and metamorphic proteins have emerged that challenge this paradigm. Metamorphic proteins have more than one global minima in the free energy landscape and therefore can adopt at least two native structures that are in equilibrium with each other. MAD 2 (Mitotic arrest deficiency 2) is one such metamorphic protein that has a role in spindle assembly checkpoint. The protein along with other binding partners is recruited during chromosome misalignment in the mitosis phase of cell cycle and halt its progression by inhibiting the anaphase promoting complex. It has been reported that MAD 2 exists in two native conformations, Open O (N1) and Closed C (N2) wherein C form is the active form and the interconversion between the two forms takes at a time scale of ~9 hours in-vitro. Using NMR spectroscopy, we aim to understand the interconversion between these two forms and probe the binding mechanisms of other protein ligands with the active form of MAD 2 (C form).

Deciphering the role of N-terminal region of *Mycobacterium tuberculosis* σ^A

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Gene expression in bacteria is regulated at the transcription initiation step. This step involves recruitment of the RNA polymerase (RNAP) holoenzyme (core enzyme with its small dissociable subunit σ factor) to DNA promoter element. Mycobacterial σ^A which belongs to Group 1 of classical sigma 70 family, is essential and responsible for basal gene expression for bacterial survival. The mycobacterial σ^A has a 210 aa disordered polypeptide N-terminal region and its unique to Actinobacterial class. Recent structural findings on the mycobacterial RNAP holoenzyme do not provide any structural cues to the role of this N-terminal polypeptide. In this study, we explored the role of N-terminal region of σ^A with different N-terminal deletion constructs in DNA binding, transcriptional efficiency and growth assays in conditional knockout background of σ^A. We inferred from our study, mycobacterial SigA neither contribute directly to promoter recognition nor it adopts an autoinhibition regulatory mechanism of Group1 sigma factors. This study revealed the importance of the unique N-terminal region of σ^A and discovered the physiological relevance of N-terminal phosphorylation.

Elucidating the Role of C-di-AMP in Antibiotic Tolerance in *Mycobacterium smegmatis*

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Nucleotide-derived second messengers play critical roles in bacterial physiology including Mycobacteria species. Here, we probe the role of cyclic di-AMP (c-di-AMP) in *in vitro* drug tolerance, which includes both persister and resistant mutant characterization. In *Mycobacterium smegmatis*, c-di-AMP is constitutively synthesized from condensation of two ATP molecules by enzyme DNA integrity scanning protein A (DisA) and hydrolyzed by enzyme phosphodiesterase (PDE) into phosphadenylyl-adenosine (pApA); often this steady-state homeostasis gets unbalanced as a part of stress adaptation. In this study, we used c-di-AMP overexpressing mutant (*Δpde*) and c-di-AMP null mutant (*ΔdisA*) to check the frequency and rate of antibiotic resistance against different drugs and genotoxic agents. Our data suggests a direct correlation between the elevated intracellular c-di-AMP level and high mutant generation, which might be possibly linked to compromised DNA repair mechanism. We further characterized the resistant mutants into the target and non-target mutation categories, highlighting the importance of homology-based repair. c-di-AMP was also found to play a role in putting unique amino acid substitutions impacting the overall fitness cost of the strains, and then modulating specific epistatic interactions between resistance genes, resulting in the evolution of multi-drug tolerance. Finally, we identified the role of c-di-AMP in persister cells regrowth and mutant enrichment upon cessation of antibiotic treatment. The implications of these findings will be helpful in the context of understanding the link between second messenger signaling and antimicrobial resistance.

A proteomimetic prevents SARS-CoV-2 infection by dimerizing the spike protein

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Protein tertiary structure mimetics with well-defined conformation are valuable tools to target large and flat protein-protein interaction interfaces. Here, we demonstrate a strategy for the design of dimeric helix-hairpin motifs from a three-helix bundle miniprotein that targets the receptor binding domain (RBD) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Through truncation of the third helix and optimization of the interhelical loop residues connecting the helix 1 and helix 2 of the miniprotein, we developed thermostable dimeric helix-hairpin that shows reversibility upon removal of the thermal stress. The dimeric four-helix bundle efficiently compete out the human angiotensin-converting enzyme 2 (ACE2) in binding to RBD in a 2:2 stoichiometry. The high-affinity between the helix-hairpin protomers and the helix-hairpin with RBD lead to the formation of stable dimeric spike protein, where all the three RBDs from either spike protein are firmly attached head-to-head in an open conformation, revealing a novel mechanism for virus neutralization. The in vivo efficacy of the dimeric helix-hairpin as SARS-CoV-2 entry inhibitor demonstrates the promise of this class of proteomimetics that act by inhibiting protein-protein interaction through target dimerization.

Structure and DNA binding analysis of AT-rich interaction domain present in human BAF-B specific subunit BAF250b

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BAF250b is the DNA-binding protein present in BAF-B class of SWI/SNF chromatin-remodelling complexes. It contains an AT-rich interaction domain (ARID) and C-terminal BAF250_C domain. ARID is a helix-turn-helix motif-containing DNA-binding domain present in several eukaryotic proteins. The BAF250b ARID likely recruits SWI/SNF to the target gene promoters for their activation. BAF250b ARID structures had been deposited in the protein data bank by a structural genomics consortium. However, it is not studied for its DNA-binding properties. Here, we report complete backbone NMR resonance assignments of human BAF250b ARID. The structure and chemical shift indexing results revealed the presence of a short β-sheet in the DNA-binding region of BAF250b ARID that was absent in the structure of its paralog BAF250a ARID. NMR chemical shift perturbations identified DNA-binding residues and revealed the DNA-binding interface on BAF250b ARID. NMR data-driven HADDOCK models of BAF250b ARID – DNA complexes revealed its plausible mode of DNA-binding. Isothermal titration calorimetry experiments showed that BAF250b ARID interacts with DNA sequences with moderate affinities like BAF250a ARID. However, distinct thermodynamic signatures were observed for binding of BAF250a ARID and BAF250b ARID to AT-rich DNA sequence, suggesting that subtle sequence and structural differences in these two proteins influence their DNA-binding.

TFDB: A comparative structural analysis platform for DNA-transcription factor complexes

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Understanding the mechanistic basis of high-specificity recognition of DNA binding site by transcription factors (TFs), among millions of putative binding sites in the genome, is a first step to alleviating gene regulation disorders. DNA:TF recognition is highly paradoxical - the same TF binds identical sites with varying affinities, while unrelated TFs bind identical consensus sites with identical affinities. Hence, DNA:TF recognition principles cannot be deciphered by investigating individual complexes. As part of the PDB-India initiative, we present the construction and application of an exclusive TF-DNA database (TFDB) embedded with a platform for comparative analysis of TF-DNA complexes. From a manually curated collection of DNA:TF PDB structures deposited in the database, the user can simultaneously compare sequence, structure and interactions in multiple TF:DNA complexes and correlate the results with observed functional differences. Every TF:DNA complex is linked with a manually curated list of keywords that makes user-based query of the database highly efficient in comparison with other databases. Addtionally using our database, the structures/models in PDB format generated from molecular dynamics simulations can be analysed for changes in interactions and structures in comparison with crystal structures.

Pseudokinases repurpose flexibility signatures associated with the protein kinase fold for noncatalytic roles

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The bilobal protein kinase-like fold in pseudokinases lack one or more catalytic residues, conserved in canonical protein kinases, and are considered enzymatically deficient. Tertiary structures of pseudokinases reveal that their loops topologically equivalent to activation segments of kinases adopt contracted configurations, which is typically extended in active conformation of kinases. Herein, anisotropic network model based normal mode analysis (NMA) was conducted on 51 active conformation structures of protein kinases and 26 crystal structures of pseudokinases. Our observations indicate that although backbone fluctuation profiles are similar for individual kinase-pseudokinase families, low intensity mean square fluctuations in pseudo-activation segment and other sub-structures impart rigidity to pseudokinases. Analyses of collective motions from functional modes reveal that pseudokinases, compared to active kinases, undergo distinct conformational transitions using the same structural fold. All-atom NMA of protein kinase-pseudokinase pairs from each family, sharing high amino acid sequence identities, yielded distinct community clusters, partitioned by residues exhibiting highly correlated fluctuations. It appears that atomic fluctuations from equivalent activation segments guide community membership and network topologies for respective kinase and pseudokinase. Our findings indicate that such adaptations in backbone and side-chain fluctuations render pseudokinases competent for catalysis-independent roles.

Highly similar conformational states of a protein could have markedly different fluctuation profiles

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The dynamic nature of protein structures arises from the several degrees of freedom available to the protein for motion. The structures of some globular proteins available in the protein data bank capture a diversity of conformations exhibited by different forms of the protein (free / bound to different ligands). In this work, we use flexibility information derived from multiple conformers of a given protein to understand variability in the intrinsic fluctuations of specific sites / regions of a protein. We employed a network-based approach and observe that changes in the intra-residue connectivity within the protein leads to variation in fluctuations between the conformers. Certain sites within the structure of a protein are found to have higher variance in fluctuations that may sometimes be associated with its biological function. Results presented here show that the conformation in which the protein is captured play an important role in determining its inherent flexibility. Taken together, we show that multiple conformations of a protein, that are expected to have similar fluctuation profiles, can have different global motions.

Mechanistic insights into viral evasion of B.1.351 SARS-CoV-2 variant

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Coronavirus infectious disease (COVID-19) is caused by SARS-CoV-2. The receptor binding domain (RBD) on the Spike glycoprotein presented on SARS-CoV-2 is the major target of neutralizing antibodies. Host immune selection pressure and disease burden drive the selection of neutralization escape variants that lead to evolution of major variants of concern (VOC). The B.1.351 VOC contains three mutations in the receptor binding domain, namely K417N, E484K and N501Y mutations that contribute to immune evasion. We hypothesized that VOC mutations that may enhance the proteolytic cleavage susceptibility, will lead to destabilization of RBD and disrupt major neutralizing epitopes. We set out to explore if such a mechanism of immune escape and enhancement of the viral entry exists for the B.1.351 variant. B.1.351 variant RBD mutations were investigated for proteolytic cleavage, thermal stability, and binding affinities. B.1.351 RBD showed lower thermal stability than wild type (WT) RBD and got digested faster than the WT RBD with respect to trypsin digestion with an approximate half-life of 20 minutes at 37^0C. B.1.351 as well as the E484K single mutant of RBD were expressed at considerably lower yield than WT RBD in mammalian cell culture, and also degraded upon storage at 4^0C after a few days whereas the WT RBD protein could be stored for months under these conditions. We are currently probing the effects of these mutations in the context of pseudoviral stability and infectivity to validate whether the increased proteolytic sensitivity is indeed associated with immune evasion.

A stabilized receptor binding domain elicits high-titer neutralizing antibodies against all SARS-CoV-2 variants of concern

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Saturation suppressor mutagenesis was used to generate thermostable mutants of the SARS-CoV-2 Spike Receptor Binding Domain (RBD) that were expressed in high yield in both mammalian cells and the microbial host, Pichia pastoris. Lyophilized proteins were resistant to high temperature exposure and could be stored for over a month at 37 ℃. In mice and hamsters, SWE adjuvanted formulations elicited antibodies that neutralized all four current variants of concern. A cocktail comprising of B.1 and B.1.351 RBDs elicited antibodies with improved breadth of protection after a single immunization in mice. Immunized hamsters were protected from high dose viral challenge. Such vaccine formulations can be rapidly and cheaply produced, lack extraneous tags or additional components, and can be stored at room temperature. They are a useful modality to combat COVID-19, especially in remote and low resource settings.

Mutational scans of intrinsically disordered bacterial antitoxins to map functional sites and structure

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In contrast to globular proteins, mutational effects on function of intrinsically disordered proteins (IDP) are not well studied. Yeast Surface Display of a mutant library coupled to next generation sequencing allows facile parallel investigation of protein-protein interactions for large number of variants. Using this methodology, we probed binding of a saturation mutagenesis library of bacterial IDP antitoxin, CcdA to its cognate partner, CcdB. Data was analyzed to infer the apparent binding constants for ~1290 mutants in the library. This provides insights into the sequence-function relationships in CcdA and enables prediction of the interacting interface and the local structure of CcdA in its bound form. We identified the non-interface residue, Gly63 with non-canonical backbone conformations, to be essential for optimal binding to the high affinity site on CcdB. Based on this exhaustive mutational sensitivity data, we also describe an empirical model to predict mutational effects on binding affinity of IDPs and extended alpha-helical proteins. We have also employed Aspartate mutational scan on *Mtb* antitoxin MazE6 and predicted secondary structural footprints and functional sites. This rapid inexpensive method can be readily applied to any IDP to study interactions with protein partners.

Structure, Function and Dynamics of rare cysteine framework *Conus* peptides

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A single marine cone snail species can produce around 50-200 unique peptides in its venom. This sequence diversity allows them to have significant functional diversity, targeting ion channels, G protein-coupled receptors (GPCRs), transporters, or enzymes. The small size, structural stability, potency, and specificity of these conopeptides have made them a valuable source as molecular probes for therapeutic studies and lead compounds/structural scaffolds in drug design and clinical use. Disulphide-rich conopeptides or conotoxins are classified into 33 Cysteine frameworks and 12 families based on their pharmacological target. Cau25 (*Conus austini*), Cbt9 (*Conus betulinus*), Cim23 (*Conus imperialis*), Cstr2 (*Conus striatus*) are some of the conopeptides, each belonging to unique cysteine framework. We are currently interested in determining the disulphide connectivity and structure of these peptides followed by studying their dynamics using NMR spectroscopy. These peptides are currently being recombinantly purified in lab. Preliminary experimental data has shown that each of these peptides exist in multiple conformations.

A Novel Broad-Spectrum Anti-fungal T3SS Effector Bg_9562 Unveils the Helical Structural Polymorphism of Phage Tail Assembly Chaperone Protein

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Bacterial pathogens are always in a constant race to evolve innovative strategies to overcome the host defenses, colonize and cause severe infectious human diseases. Mycophagy- 'feeding on fungus by pathogenic bacteria' had conferred an ability to bacterial pathogens to survive and to colonize in certain ecological races. Mycophagous bacteria will also provide an eco-friendly way to treat fungal biomass as a targeted host instead of using highly hazardous pesticides and fungicides. Apart from Psoriasin (S100A7), a calcium (Ca2+) binding epithelial antifungal protein, a T3SS effector namely Bg_9562 from Burkholderia gladioli NGJ1 stain has recently been identified to be another broad spectrum anti-fungal protein (BSA) so far. Although the mycophagous ability of Bg_9562 has been studied elaborately earlier, knowledge about detailed molecular, structural, and functional study remains completely unknown. Surprisingly, the BSA protein Bg_9562 belongs to a phage_TAC7_superfamily. Tail assembly chaperones (TACs) are likely to stabilize tape-measure protein (TMP) during the tail morphogenesis of long tailed bacteriophages. Herein, we showed that the BSA protein Bg_9562 belonging to phage_TAC7_superfamily, an important component needed for the morphogenesis of long tail of bacteriophages had self-assembled into both low and high molecular weight homo-oligomeric states along with long nano-filamentous structures having a diameter of about 6 nm at physiological buffer condition. Solution state cryo-EM and asymmetric helical reconstruction analysis has suggested that the recombinant Bg_9562 rearranged into a more compact right handed helical array inside the core of nano-filamentous geometry. A certain level of inherent conformational and geometrical heterogeneity was also associated with its filamentous states as demonstrated by cryo-EM 2D class averages and from their respective layer line profile of filamentous TAC protein, Bg_9562. However, herein we have provided the detailed structural

evidence of a TAC protein, namely Bg_9562 forming helical filaments along with closed and open ring-like oligomers in physiological solution, considered to be an important geometrical feature evolved for their biological activity.

Structural and functional analysis of NRPS (MODULE-2) of leinamycin biosynthesis gene cluster of *Streptomyces atroolivaceus*

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Non-ribosomal peptide synthetases (NRPSs) are important multidomain enzymes for the assembly of complex peptide natural products. It includes many products that exhibit broad biological activities and include some of the most critical clinical drugs. An excellent understanding of the structures and activities of isolated domains of NRPS has been known, but much less is known about how multidomain work with respect to each other as a part of the larger NRPS.

To gain the structural insights of multidomain NRPS, we localised module-2 of hybrid NRPS/PKS of leinamycin biosynthetic pathway. We have performed cryo-EM of module 2 (PCP-Cy1-Cy2-A-PCP-Ox) which was found to be too flexible. It is difficult to solve this structure without substrates. However, to elucidate crucial interdomain interfaces and interactions that occur during different steps of the NRPS catalytic cycle, we have considered multidomain fragments (PCP-Cy1-Cy2) and (A-PCP-Ox) of module2.We have determined the structure of multidomain constructs (PCP-Cy1-Cy2) and (A-PCP-Ox) with a resolution of 5.2 angstrom and 7 angstrom respectively. The unravelling of architecture, organization, and mechanism of NRPS module 2 of leinamycin biosynthesis by cryo-EM will help design bioengineering approaches to understand the mechanistic insight into this novel pathway (swapping modules and domains).

Structural insights into the RND efflux pump from multi-drug resistance pathogenic bacteria

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The RNDs efflux pumps are responsible for the extrusion of antibiotics into the external environment in gram-negative bacteria. At the molecular level, the RND efflux systems are three component transporters that include an integral membrane pump protein (IMP), a periplasmic membrane fusion protein (MFP), and an outer membrane pore protein (OMP), and these three proteins work together to form a continuous channel for proton motive force-dependent substrate extrusion. Previous structural studies have highlighted the mechanism of synchronized translation and rotation of each unit in homo-trimeric IMPs ($AcrB_3$, $MexB_3$) during the substrate transport process. Nevertheless, the above study is not valid for hetero-trimeric IMPs such as $MdtB_2C$, since only one unit of hetero-trimeric IMPs (MdtC) has been suggested to be functional in this case. In our current study, we wanted to show how an inactive IMP ($MdtB_3$) can look, which will potentially assist in understanding the structural rearrangement required to generate an active hetero-trimeric IMP ($MdtB_2C$). We also intended to investigate the three protomer conformations (L: loose, T: tight, O: open) of a tightly controlled IMP ($MdtF_3$) using atomic resolution cryo-EM structure.