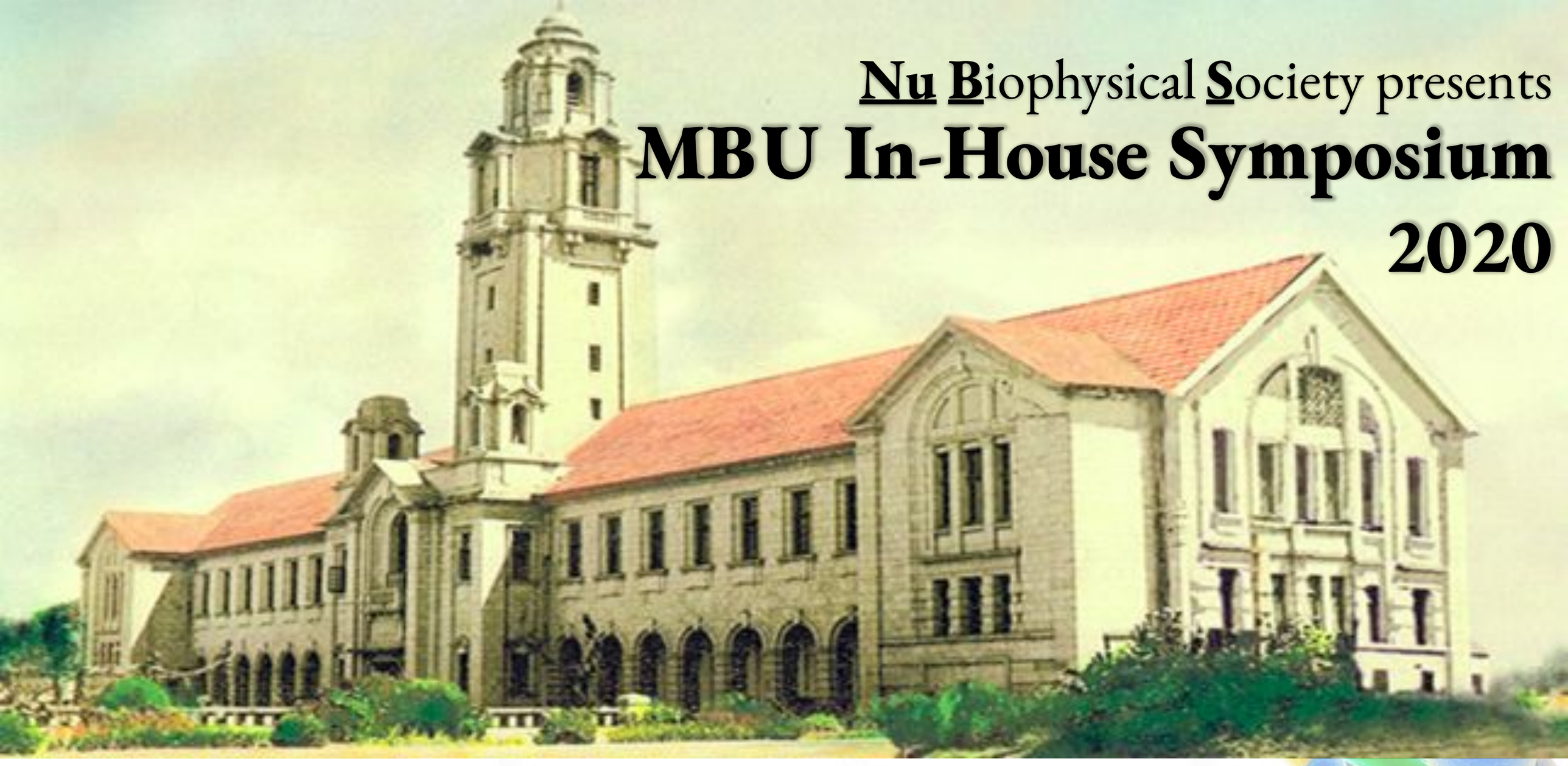
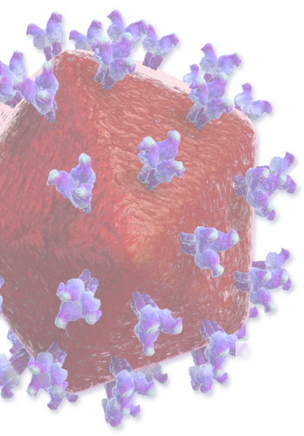


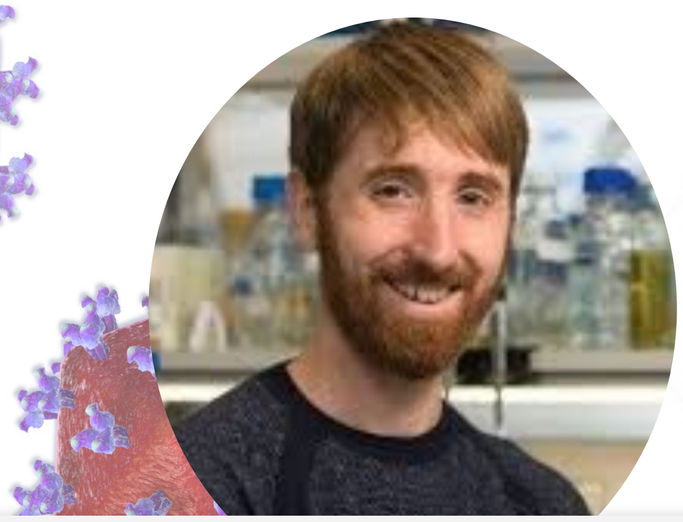
Nu Biophysical Society presents

MBU In-House Symposium 2020

❖ Student talks ❖ Poster sessions

Plenary Talks





Dr. John Briggs

MRC LMB, UK

'Studying proteins within viral particles using cryo-electron tomography'

Dr. Gautam Basu

Bose Institute, India

'Protein functional evolution driven by genome plasticity'

Join us on:

18th December 4 pm - 6.30 pm IST

19th December 9 am - 5.45 pm IST

SYMPOSIUM SCHEDULE

DATE	SESSION CHAIR	FROM	TO	AGENDA	STUDENTS	
DAY-1						
18/12/2020	Ishika Pramanick	1600	1605	Chairman Inauguration		
		1605	1720	John Briggs		
		1720	1735	Anand Srivastava	Gaurav	ORAL
		1735	1750	Aravind Penmatsa	Archishman	
		1750	1757	Mahavir Singh	Kavyashree	POSTER
		1757	1804	A. Surolia	Soujanya	
		1804	1811	K Suguna	Sreeparna	
		1811	1818	N Srinivasan	Sohini	
		1818	1825	S K Sikdar	Ashish	
DAY-2						
19/12/2020	Arunabh Athreya	9000	1015	Gautam Basu		
		1015	1030	Ashok Sekhar	Vaishali	ORAL
		1030	1045	B Gopal	Amit	
		1045	1100	Dipankar Chatterji	Sudhanshu	
		1100	1107	Siddhartha P Sarma	Mihir	POSTER
		1107	1114	Saraswathi Vishweshwara	Arinnia	
		1114	1121	Rishikesh Narayanan	Rituparna	
		1121	1128	Rahul Roy	Rohit	
		1128	1135	Raghavan Varadarajan	Priyanka	
		1135	1142	N Srinivasan	Adithyan	
		1145	1200	BREAK		
	Malyasree Giri	1200	1215	Jayanta Chatterjee	Venkateshwar Rao	ORAL
		1215	1230	Mahavir Singh	Niranjan	
		1230	1245	M Vijayan	Sivaji	
		1245	1400	LUNCH		
	Priyanka Bajaj	1400	1415	N Srinivasan	Sandhya	ORAL
		1415	1430	Raghavan Varadarajan	Gopinath	
		1430	1445	Rishikesh Narayanan	Sameera	
		1445	1500	Siddhartha P Sarma	Mihir	
		1500	1507	N Srinivasan	Ashraya	POSTER
		1507	1514	Somnath Dutta	Alakta	
		1514	1521	N Srinivasan	Yazhini	
		1521	1528	M Vijayan	Prateek	
		1528	1535	Jayanta Chatterjee	Swati	
		1535	1542	Dipankar Chatterji	Anirban	
		1545	1600	BREAK		
	Twinkle Patel	1600	1615	S K Sikdar	Monica	ORAL
		1615	1630	Somnath Dutta	Suman	
		1630	1645	A. Surolia	Nisha	
		1645	1652	B Gopal	Ankur	POSTER
		1652	1659	Ashok Sekhar	Claris	
		1659	1706	Aravind Penmatsa	Deepthi	
		1706	1713	Anand Srivastava	Kirtika	
		1713	1720	Anand Srivastava	Rajlaxmi	
		1730	1745	VALEDICTORY TALK		

STUDENT TALKS

DAY 1 (18th december, 2020) - SESSION I (5:20 PM-5:50 PM)

Integrative biology of membrane remodeling : Using continuum mechanics and molecular simulations to bridge microscopy data with structural and biochemical data

Gaurav Kumar, Kirtika Jha, Anand Srivastava

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Biological membranes undergo dramatic changes in curvature and morphology during processes such as fusion and fission in endocytic recycling, morphogenesis, immune response and metastasis to name a few. During these tightly-regulated membrane remodeling processes, membrane lipids and accompanying proteins pass through several intermediates. Experimental difficulties associated with capturing the evolving membrane curvatures and the proteins assembly intermediates point to the requirement for high-fidelity multiscale simulations. In an ongoing work, we are developing an integrative biology framework with the aim of constructing particle based coarse-grained molecular simulation models of membrane-protein system (BAR-domains family) using a combination of imaging data and structural/biochemical data from experiments. The project intends to develop a framework to explore how the chemical specificity and molecular interactions at residues level propagate to macroscopic scales. Towards that, we arrive at deformation profiles observed in microscopy images by optimizing Hamiltonian parameters of a high throughput continuum mechanics model. Next, we reconstruct the corresponding coarse-grained particle based models from the continuum representation using an algorithm developed in the lab. Finally, we intend to carry out large scale molecular simulations on the reconstructed membrane-protein system for molecular-level insights into these emergent behavior. I shall present a few aspects of this ongoing work.

Surface engineering facilitates epitope transfer between neurotransmitter transporters

Archishman Dakua, Smruti Ranjan Nayak, Deepthi Joseph, Aravind Penmatsa

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γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter found in mammalian CNS, with altered GABAergic neurotransmission being implicated in several neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, depression and epilepsy. After activation of the GABA receptors in the post synaptic neuron, GABA is cleared from the synaptic cleft by reuptake. Reuptake of GABA is facilitated by GABA transporters of which GAT-1 is predominantly expressed in the presynaptic neuron whereas GAT-3 in astrocytes. GAT-1 is a Na^+-Cl^- coupled neurotransmitter transporter, belonging to the SLC6 family of transporters. Despite the structural similarities, GAT has a very distinct pharmacology in comparison to monoamine transporters. Inhibitors of GAT are prescribed in the treatment of epilepsy. Elucidation of GAT structure would provide insights into the unique pharmacology of this transporter. In order to effectively study its structure, we have attempted to engineer the intracellular face of the transporter with substitutions that mimic the epitope of the *Drosophila* dopamine transporter. The crystal structure of dDAT was solved in complex with an antibody Fab fragment called 9D5. We grafted the 9D5 epitope of dDAT onto the corresponding region of GAT-1 from *Rattus norvegicus* (rGAT1), that has enabled 9D5 binding to rGAT. This would facilitate future investigations into the structure and pharmacology of GAT-1.

DAY 2 (19th december, 2020) - SESSION I (10:15 AM-11:00 AM)

Understanding the structural basis for N-glycan recognition by Horcolin, a plant lectin

Vaishali Narayanan, Nisha GJ, A Surolia, Ashok Sekhar

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N-linked high-mannose glycans on the surface glycoprotein gp120 of HIV1 are crucial for viral replication and transmission, and is the target for most broad neutralizing antibodies. Horcolin derived from *Hordeum vulgare* can reversibly bind to mannose sugars and has been reported to be a non-mitogenic, anti-HIV1 agent which makes it a promising candidate for therapy. Here, we investigate the conformational perturbations resulting from glycan binding and characterize the three-dimensional structure of Horcolin using NMR spectroscopy. We employed standard 3D NMR experiments coupled with various isotope labelling schemes to assign all backbone chemical shifts. Using chemical shifts and three-bond HN-Hα couplings, we found that Horcolin is composed of β-sheets interspersed with loops. Furthermore, the antiparallel β-sheet geometry is evident from long-range inter-proton NOE contacts between strands. We monitored the binding of Horcolin to methyl-α-mannose and observed that chemical shift perturbations during binding are localized to the conserved GXXXD motif and to neighboring loops. The movement of backbone amide peaks during the binding event is manifest in the fast-intermediate timescale of exchange indicative of weak binding in the mM range.

Design and characterization of modulators of the *Mycobacterium tuberculosis* transcription machinery

Amit Kumar Dinda, **Twinkal Patel, K. Saravanan, Sandeep Sundriyal and B. Gopal**

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Mycobacterium tuberculosis RNAP exhibits an inherently unstable RP_O complex compared to *E. coli* RNAP on the same promoter. It is hypothesized that the inherent instability of RNAP-promoter complexes would allow the mycobacterial RNAP to be poised to respond to changes in the environment by being easily modified in activity by additional factors. Here we describe the design and characterization of chemical modulators of mycobacterial transcription. The modulators were designed to interact with the most conserved region on the RNAP-σ-factor interface. The feasibility of this approach was first revealed in *Bacillus subtilis* wherein a conserved glutamine 322 was mutated and the results show that the ability of σ^A to bind to β′ was reduced. In this case, the major interactions were observed between coiled region of β′ and domain 2 of a σ factor. In *M. tuberculosis* $\sigma^A{}_2$, a cluster of four residues (D319, Q322, N323 and L326) interact with β′ residues (R350, R353, L357, L360, Q362, I366, N369 and E370). Alignment of four σ factors ($\sigma^A{}_2$, $\sigma^C{}_2$, $\sigma^J{}_2$, $\sigma^K{}_2$) revealed that these interface residues are highly conserved across σ factors. There are 21 σ^A structures (both X-ray and Cryo EM) in the PDB. These provided a basis to design ligands that could be specific for this surface of a σ factor thereby inhibiting its binding to the RNAP. The binding of these ligands was evaluated by diverse biophysical techniques. Evaluation of these compounds using *in vitro* transcription assays and in *Mycobacterium tuberculosis* cultures is currently being pursued. As this approach is thus based on inhibiting protein/protein interaction as opposed to inhibition of catalytic activity, it is likely that these would be less prone to resistant mutations in the target protein complex.

Yet another second messenger (c-di-AMP) from *Mycobacterium smegmatis*

Sudhanshu Gautam, **Avisek Mahapa, Dipankar Chatterji**

(sudhansu@iisc.ac.in)

The role of cyclic-di-nucleotides (CDN) in bacterial stress signalling and their interaction with the human innate immune system has attracted the pharmaceutical industry's interest to develop novel antibacterial compounds. In 2008, a new messenger molecule 3',5'-cyclic di-AMP (c-di-AMP) came into the fray and was also known to regulate various cellular pathways. Intercellular homeostasis of this molecule was maintained by a synthetase (which carries a **d**i-**a**denylate-**c**yclase domain or DAC domain) and a **p**hospho-**d**i-**e**sterase (PDE). In this investigation, an approach was made to understand the in-vitro and in-vivo regulation of a c-di-AMP synthetase DisA (which is also a DNA **I**ntegrity **S**canning **P**rotein) from *M. smegmatis.* Different biochemical, biophysical and *in vivo* assays were used to reveal the regulatory network by c-di-AMP. *In vitro* functional analysis of MsDisA demonstrated a diverse array of protein regulation under lower osmolyte and alkaline conditions. Structural studies also illustrated some exciting phenomena about this protein such as substrate induced structural alteration by transmission electron microscopy. *In vivo* promoter activity assays suggested the involvement of c-di-AMP in bacterial osmoregulation and pH adaptation. In this work we propose a novel c-di-AMP regulatory network in mycobacterium, which improves our understanding of bacterial second messenger signalling.

DAY 2 (19-12-2020) - SESSION 2 (12:00 PM-12:45 PM)

Impact of *cis* peptide bond on cyclic peptide conformation: Implications in membrane permeability

Venkateswara Rao Nuthakki, Jayanta Chatterjee

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Cyclic peptides are important therapeutic modalities that have gained significant attention in drug discovery owing to their high selectivity, affinity, and low toxicity. While cyclic peptides are considered as sweet spot between antibodies and small molecules, they pose serious challenges which are hindering their transition to clinics. Some of the disadvantages of cyclic peptides include low metabolic stability, poor membrane permeability which in turn affects bioavailability. Physicochemical principles that have been derived based on small molecules and natural products are often used to improve membrane permeability. Cyclosporine is a natural product-based drug which is known for its high oral bioavailability. Its highly N-Methylated backbone and *cis* peptide bond is often attributed to high bioavailability. Although, the role of N-methylation on membrane permeability has been thoroughly investigated, the impact of *cis* peptide bonds per se is not yet clear. In this study, by using cyclic peptide model system, we deconvoluted its impact on transcellular membrane permeability. We analyzed the solubility, lipophilicity and membrane permeability of cyclic *cis* peptide variants and our results suggest that cis peptide bond containing peptides have high permeability than its *trans* counterparts. We rationalized membrane permeability of these peptides using solution conformation studies and HD exchange using NMR spectroscopy.

Interplay among human regulator of telomere elongation helicase 1, replication protein A and telomere DNA

Niranjan Kumar and Mahavir Singh

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Regulator of telomere elongation helicase 1 (RTEL1) is a Fe-S cluster containing DNA helicase that plays roles in telomere DNA maintenance. RTEL1 is a modular protein with an N-terminal helicase domain, two tandem harmonin-N-like (HNL) domains - HNL1 and HNL2, and a C-terminal C4C4 type RING domain. The C4C4 RING domain interaction with TRF2 facilitates recruitment of RTEL1 to telomere whereas the N-terminal helicase domain disassembles telomere t/D-loop and unwinds G-quadruplex via its helicase activity. HNL domains are predicted to be putative protein-protein interaction domain, however not much is known about the protein partners of these domains in RTEL1. Here, we show that RTEL1 interact with replication protein A (RPA) via the HNL domains. Initially, we identified RPA as a protein binding partner of RTEL1 using a mass-spectrometry based approach. This interaction was further validated using co-immunoprecipitation and NMR titration experiments. Our results have also revealed that the HNL domain of RTEL1 binds to single-stranded DNA. The interplay among DNA, RTEL1 and RPA provide novel insights in understanding telomere DNA remodelling processes.

Mevo lectin specificity towards high-mannose structures with terminal αMan(1,2)αMan residues and its implication in inhibiting the entry of _Mycobacterium tuberculosis_ into mouse macrophages

Nukathoti Sivaji, Nikitha Harish, Samsher Singh, Amit Singh, Mamannamana Vijayan and Avadhesha Surolia

(sivaji@iisc.ac.in)

Mannose-binding lectins can specifically recognize and bind complex carbohydrates/glycan structures on pathogens and have potential applications as anti-viral and anti-bacterial agents. We previously reported an unusual quaternary structure of a lectin from an archaeal species, *Mevo* lectin, which has specificity towards terminal α1,2 linked manno-oligosaccharides. *Mycobacterium tuberculosis* (*M. tuberculosis*) expresses mannosylated structures including, lipoarabinomannan (ManLAM) on its surface and exploits C-type lectins to gain entry into the host cells. ManLAM structure has mannose capping with terminal αMan(1,2)αMan residues and is important for recognition by innate immune cells. In this study, we aim to address the specificity of *Mevo* lectin towards high-mannose type glycans with terminal αMan(1,2)αMan residues and its effect on *M. tuberculosis* internalization by macrophages. ITC studies demonstrated that *Mevo* lectin shows preferential binding towards a mannoheptose (Man7) and high mannose glycan (yeast mannan). *Mevo* lectin showed a strong affinity for ManLAM, whereas it binds weakly to *Mycobacterium smegmatis (M. smegmatis*) lipoarabinomannan (MsmLAM), which displays relatively fewer and shorter mannosyl caps. Crystal structure of *Mevo* lectin complex with a Man7 revealed the multivalent cross-linking interaction, which may explain avidity-based high affinity for these ligands when compared to previously studied manno-oligosaccharides lacking the specific termini. Results from functional studies suggest that *M. tuberculosis* internalization by the macrophage was impaired by binding of *Mevo* lectin to ManLAM present on the surface of *M. tuberculosis*. Selectivity shown by *Mevo* lectin towards glycans with terminal αMan(1,2)αMan structures, and its ability to compromise the internalization of *M. tuberculosis in vitro*, underscore the potential utility of *Mevo* lectin as a research tool to study host-pathogen interactions.

DAY 2 (19th december, 2020) - SESSION III (2:00 PM-3:00 PM)

The Obvious and the Hidden: Useful outcomes of computational predictions

Sankaran Sandhya, Gayatri Kumar, Richa Mudgal and Narayanaswamy Srinivasan

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While the number of sequenced genomes grows, experimentally verified functional annotation of predicted proteomes remains patchy resulting in large numbers of uncharacterized proteins. Although annotation efforts are straightforward for proteins showing high sequence similarity to characterized homologues, challenges remain in highly divergent sequences. Annotation for such proteins are immensely benefited by using sequence profiles or methods that employ 'linker' sequences. Homologues of known structure are an advantage and intuitively facilitate recognition of functional residues. Our own methods, specifically developed for difficult protein targets in the last decade, have demonstrated the power of 'multiplicity' in computational function prediction. We highlight scenarios and challenges to address different levels of annotation like a) use of natural and 'artificial linkers' to annotate uncharacterized protein families such as DUF3050, DUF4918 and DUF5131 b) predict function for proteins expressed under oxidative stress in *M.tuberculosis* and c) discovery of novel toxin-antitoxin proteins in genomes.

Protein function prediction does not offer "one-size-fits-all" solutions. Linking evidence through multiple tools and consideration of different aspects of function are key to infer usefulness and effectiveness of annotation. *Ab-initio* protein function prediction can be improved by (1) being conservative about evolutionary distances (2) considering the ambiguous meaning of "functional similarity," and (3) taking cognizance of limitations of functional databases.

Mechanistic insights into global suppressors of protein folding defects

Gopinath Chattopadhyay, Jayantika Bhowmick, Kavyashree Manjunath, Raghavan Varadarajan

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Amino acid substitutions in protein are mostly destabilising and difficult to comprehend mechanistically. Several studies have shown the effect of second-site mutations that can rescue the defect caused by the initial mutation. However, the mechanism of such suppression is poorly understood. In a previous study from the laboratory, a second site-saturation mutagenesis (SSSM) approach coupled to FACS has been utilised to exhaustively screen for putative suppressors, identifying both proximal and distal suppressors. Based on their improved binding to Gyrase, two distal suppressors, E11R and S12G had been identified in the CcdB protein. In the present work, the effect of E11R, S12G and other known global suppressors: M182T of TEM-1 β-lactamase and N239Y of p53 DBD are characterised in isolation, and in combination with inactive mutants to give mechanistic insights into global suppression of multiple defects. The suppressors alone, and in conjunction with inactive mutants, stabilise the protein both thermodynamically and kinetically *in-vitro*, and result in increased *in-vivo* solubilities as well as regain-of-function phenotypes when coupled to their inactive mutants, reiterating the fact that the principles governing protein stability *in-vivo* and *in-vitro* are similar. The study demonstrates that the global suppressor approach can be used to stabilise wild-type protein.

Heterogeneities *vs.* degeneracy in plasticity rules: Impact of structural, synaptic and intrinsic heterogeneities in dentate gyrus granule cells

Sameera Shridhar, **Poonam Mishra and Rishikesh Narayanan**

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Granule cells in the dentate gyrus exhibit pronounced heterogeneities in their morphology, intrinsic response properties and synaptic connectivity, which are amplified by the expression of adult neurogenesis. Here, we assessed the ramifications of the expression of these heterogeneities on synaptic plasticity profiles. Employing a heterogeneous population of 126 valid GCs, we found that heterogeneities in intrinsic properties resulted in heterogeneities in the modification threshold of the BCM-like protocol and the amount of plasticity obtained with theta-burst stimulation protocol. However, similar plasticity profiles were obtained across all models by adjusting synaptic strengths, thereby demonstrating plasticity degeneracy whereby the impact of heterogeneities in intrinsic properties was counterbalanced by heterogeneities in synaptic strength. When synaptic strengths were fixed, we found a progressive rightward shift in the plasticity profile with increase in neuronal surface area, reflecting neuronal maturation phase. Furthermore, no long-term depression was observed in plasticity profiles of neurons with extremely small diameters. Finally, when the plasticity profiles were tuned to be similar by altering synaptic strengths, we found the emergence of plasticity degeneracy involving intrinsic, structural and synaptic heterogeneities. Our analyses provide an overarching framework for understanding the role of different forms of heterogeneities in engram cell formation and in plasticity degeneracy.

Solution NMR studies of the frog skin peptide LL-TIL

Mihir J. Rami, Dr. Mohd Shafique, Sri Teja Adhada

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The high resolution three-dimensional solution structure of LL-TIL, a trypsin inhibitor from the skin secretions of *Lepidobatrachus laevis* has been solved using multidimensional NMR spectroscopy. LL-TIL belongs to the family of trypsin inhibitor-like (TIL)-type protease inhibitors incorporating five disulfide bonds. Disulfide connectivity was determined using long range NOEs between paired cysteines. 829 NOE distances, 125 dihedral angles and 34 hydrogen bond restraints were used for the structure calculations. The ensemble of 20 structures has a backbone and heavy atom root mean square deviation of 0.30 ± 0.8 and 0.93 ± 0.11 respectively, with all the residues occupying allowed and additionally allowed regions of Ramachandran map. LL-TIL acquires a flat disc shape scaffold and lacks the hydrophobic core. In the absence of the hydrophobic core, five disulfide bonds and multiple hydrogen bonds stabilizes the peptide. The structure consists of two antiparallel β-sheets flanked by tightly packed loops. The reactive site responsible for binding to the protease is situated within a long loop region between second and third β-strands.

DAY 2 (19th december, 2020) - SESSION IV (4:00 PM-4:45 PM)

Role of I_h Currents in Epileptiform Activity in Subicular Neurons

Monica Alfred and Sujit Kumar Sikdar

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Subicular neurons play a critical role in Temporal Lobe Epilepsy. While studies have shed light on how synaptic conductances contribute to epileptiform activity in these neurons, it is still unclear how intrinsic conductances influence epileptic discharges. HCN channels play crucial role in pathophysiology by modulating the electrophysiological properties of neurons. To investigate the ability of intrinsic properties in a subicular neuron to generate epileptiform activity, we studied the effects of I_h (HCN) currents in 4-Aminopyridine magnesium-free epileptic model in various classes of subicular neurons. To study the modulation of epileptiform activity due to I_h currents, we modeled subicular HCN currents in dynamic clamp that mimicked the effects of the blockers. We observed similar changes in input resistances with the addition and subtraction of currents in burst firing, regular firing and interneurons. The firing rate characteristics during epileptiform activity with the addition and subtraction of HCN channels in burst firing and regular firing differed from those observed in interneurons. We also studied the electrophysiological profiles of subicular neurons with mutated HCN current kinetics introduced through dynamic clamp in the presence of ZD7288. Further studies are being carried out to understand the mechanism of epileptogenesis by I_h currents in subicular neurons.

Cryo-EM structural characterization of SARS-CoV-2 S-protein

Ishika Pramanick, Nayanika Sengupta, Suman Mishra, Suman Pandey, Nidhi Girish, Alakta Das, Somnath Dutta

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SARS-CoV-2, the causative agent of COVID-19 pandemic, employs Spike (S) glycoprotein to interact with the human ACE2 receptor (hACE2) and subsequent pathogenesis. Three major conformations of S glycoprotein have been observed- 1-RBD up "open", 2-RBD up "open" and 3-RBD down "closed" conformations. Open conformation of S protein is responsible for binding with hACE2. However, the study of intermediate conformations of open & closed S protein still remains an enigma. It is proposed that S-protein undergoes significant conformational changes as a function of pH. Hence, in our current study we implemented cryo-EM based structural studies to visualize the conformational heterogeneity and intermediate conformational states of S protein at physiological pH 7.4 and near-physiological pH's 6.5 and 8.0. Our study shows that 68% of S- protein exists in open conformation at physiological pH. It is also interesting to note that we observed diverse movements in the NTD and S2 subunit along with the high flexibility showed by RBD region. Several important residues involving RBD-neutralizing epitopes have been found to have differences in solvent-accessible surface area in each conformation. This study will assist in developing novel therapeutics and vaccines against SARS-CoV-2.

Exploring the mechanism and interaction dynamics of high mannose glycans and Horcolin, a potential antiviral microbicide

Nisha GJ and Avadhesha Surolia

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N-Linked glycans are critical to the infection cycle of HIV, and most neutralizing antibodies target the high-mannose glycans found on the surface envelope glycoprotein-120 (gp120). Carbohydrate-binding proteins, particularly mannose-binding lectins, have also been shown to bind these glycans. Despite their therapeutic potency, their ability to cause lymphocyte proliferation limits their application. In this study, we report one such lectin named horcolin, seen to lack mitogenicity owing to the divergence in the residues at its carbohydrate-binding sites, which makes it a promising candidate for exploration as an anti-HIV agent. Extensive isothermal titration calorimetry experiments reveal that the lectin was sensitive to the length and branching of mannooligosaccharides and thereby the total valency. Modeling and simulation studies demonstrate two distinct modes of binding, a monovalent binding to shorter saccharides and a bivalent mode for higher glycans, involving simultaneous interactions of multiple glycan arms with the primary carbohydrate-binding sites. This multivalent mode of binding was further strengthened by interactions of core mannosyl residues with a secondary conserved site on the protein, leading to an exponential increase in affinity. Finally, we confirmed the interaction of horcolin with recombinant gp120 and gp140 with high affinity and inhibition of HIV infection at nanomolar concentrations without mitogenicity.

POSTER ABSTRACTS

Day 1 (18th december, 2020) – SESSION I (5:50 PM – 6:25 PM)

Strategy for large scale expression and purification of type III toxin-antitoxin complexes

Kavyashree Nadig M, Manikandan P, Sandhya Sankaran, N. Srinivasan and Mahavir Singh

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Bacterial toxin-antitoxins are genetic modules that play important roles in bacterial persistence phenotype, antibiotic resistance, and speculated to be involved in regulation of existential processes of the cell. The type III system is a unique system consisting of a protein toxin and a non-coding RNA antitoxin. Binding of antitoxin RNA neutralizes the activity of protein toxin under homeostatic conditions. Certain phages are able to trigger the system upon infection causing the toxin to be released which results in an altruistic behavior of the bacterium such as retardation in cell growth or cell death. The type III systems are classified as; tenpIN, cptIN, and toxIN, based on the sequence homology of the toxin and antitoxin. We have identified the toxIN and tenpIN systems in *E. coli*. We have standardized the expression and protein purification strategy 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 to obtain milligram quantities of toxin, antitoxin, and TA complex that is required for the biophysical characterization of these system to understand their assembly, structure and activation mechanisms

Proteomic characterization of pranlukast mode of action on altered host processes upon *Mtb* infection

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Arginine biosynthetic enzyme, *ArgJ* is essential for survival of *Mtb*. Pranlukast (PRK) an anti-asthamatic drug is recently shown to inhibit the activity of ornithine acetyltransferase *(ArgJ)* in a non-competitive manner killing *Mtb*. In this study, proteomic analysis was carried out in PRK treated mouse macrophages in order to understand the altered host response processes and pathways in response to the drug. Raw264.7 mouse macrophage cell line infected with *Mycobacterium tuberculosis* H37Rv was subjected to PRK treatment. Subsequently, the samples were processed for downstream proteomic analysis using Orbitrap Fusion Tribrid mass spectrometer in duplicates. LC-MS/MS analysis of infected, PRK treated infected and uninfected samples led to the identification of 4,524 proteins of which, 1,006 proteins in infected and 131 proteins in PRK infected groups (1.5-fold) were identified to be differentially expressed in both the replicate data sets. A number of differentially expressed proteins were identified that are known to augment autophagy while decrease ROS and inflammation processes. In addition, a total of 21 proteins were enriched in oxidative phosphorylation of which Ndufs1, Ndufa10, Ndufv2, Ndufa2, Ndufc2 and Ndufa5 were upregulated in pranlukast (10 µg/ml) group while downregulated in untreated infected group. This study serves as a foundation for understanding the effect of Pranlukast on altered host processes and signaling due to *Mtb* infection.

Structural Diversity of small Heat Shock Protein Assemblies

Sreeparna Biswas, Somnath Dutta and K. Suguna

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Small heat shock proteins (sHSPs) take up several types of oligomeric forms in the process of performing their chaperone activity on protein substrates. They interact with unfolding cellular proteins during heat and other types of stress to prevent their aggregation. In our laboratory, we have characterized sHSPs from different organisms. Structures of several sHSPs were determined by X-ray crystallography and Cryo Electron Microscopy. Their oligomers range from dimers to 60-mers. AgsA, an sHSP from *Salmonella typhimurium*, forms dimers, 18-mers and 24-mers. *Mycobacterium marinum* sHSP has a dodecameric structure. One of the sHSPs of *Entamoeba histolytica* forms a 3-dimensional network with unique dimer-dimer interactions. A cyanophage sHSP showed multiple oligomeric structures: a 24-mer, a 48-mer and a 60-mer. An analysis of the sHSP oligomeric structures revealed certain common features and provided structural insights into the diversity observed in these large assemblies

NOD: A webserver to predict New use of Old Drugs to facilitate drug repurposing

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Computational methods accelerate the drug repurposing pipelines which are a quicker and cost-effective alternative to discovering new molecules. However, there is a paucity of webservers to conduct fast, focused, and customized investigations for computational drug repurposing. We present the NOD webserver which has the mentioned characteristics. It is primarily a sequence-based *in silico* resource that aids repurposing of drugs against any disease of interest. Exploiting the benefits of polypharmacology mediated by similar binding sites shared between related proteins, NOD suggests potential repurpose-able candidates from the DrugBank database. Furthermore, it facilitates the expansion of chemical space through similarity searches. Performance of NOD has been tested extensively with ~10000 query sequences from pathogens and human implicated in various diseases. These results have been validated against available experimental and/ clinical reports. In 65.6% of the investigated cases in a control study, NOD out-performs DrugBank. We believe, NOD could be an essential component for preparing against any future unpredictable outbreak of novel pathogenic diseases, when the structures of the drug targets in the pathogen may be unavailable, but the sequences of the proteins could be used for identifying potential repurpose-able drugs. The development of NOD is currently underway and it is freely-available at http://pauling.mbu.iisc.ac.in/NOD/NOD/ .

Studies on modulation of hHCN channels by nicotine

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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 and pacemaking of neurons. Various molecules that can block HCN have been pharmacologically identified but their low affinity and lack of specificity prevents their wide-spread use to treat cardiac and neurological problems. 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 (1 mM) causes ~45% decrease in hHCN1 current at -120 mV. Preliminary molecular docking studies using Autodock vina on closed hHCN1and modelled open conformation structure using the template of eukaryotic CNG channel shows that nicotine possibly binds to Tyr361 and Gly362 residues in the pore region which needs to be validated experimentally.

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Day 2 (19th december, 2020) – SESSION II (11:00 AM – 11:42 AM)

Solution NMR studies of the frog skin peptide LL-TIL

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The high-resolution three-dimensional solution structure of LL-TIL, a trypsin inhibitor from the skin secretions of *Lepidobatrachus laevis* has been solved using multidimensional NMR spectroscopy. LL-TIL belongs to the family of trypsin inhibitor-like (TIL)-type protease inhibitors incorporating five disulfide bonds. Disulfide connectivity was determined using long range NOEs between paired cysteines. 829 NOE distances, 125 dihedral angles and 34 hydrogen bond restraints were used for the structure calculations. The ensemble of 20 structures has a backbone and heavy atom root mean square deviation of 0.30 ± 0.8 and 0.93 ± 0.11 respectively, with all the residues occupying allowed and additionally allowed regions of Ramachandran map. LL-TIL acquires a flat disc shape scaffold and lacks the hydrophobic core. In the absence of the hydrophobic core, five disulfide bonds and multiple hydrogen bonds stabilizes the peptide. The structure consists of two antiparallel β-sheets flanked by tightly packed loops. The reactive site responsible for binding to the protease is situated within a long loop region between second and third β-strands.

The SARS-CoV-2 spike protein structure from the side-chain network perspective

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We have examined the closed and the partially open states structures of the SARS-CoV-2 spike protein from the perspective of side chain connectivity network. We highlight the advantages of using the side-chain based network metrics in discriminating conformational changes, which are elusive to the measurements at the backbone level. Such a conformational change in the spike protein is crucial for coronavirus entry and fusion into human cells. Our analysis of network parameters reveals global structural reorientations between the two states of the spike protein, despite small changes between them at the backbone level. We also observe some differences at strategic locations in the structures, correlating with their functions, asserting the advantages of the side-chain network analysis. The detailed analysis is presented in [aRXiv, and Frontiers(MS-accepted: in https://www.frontiersin.org/articles/10.3389/fmolb.2020.596945/abstract]. Further, the cluster analysis of the receptor binding domain (RBD) of the spike protein through graph spectral method has provided insights into the intra-protein connectivity of the residues binding to the ACE receptor. A detailed study of ACE2 receptor binding to the spike proteins of SARS-CoV-2 and SARS-CoV-1 are in progress.

Dominant roles of calcium and calcium-activated potassium channels in regulating complex spike bursting of CA3 pyramidal neurons

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Complex spike bursting (CSB) is a burst-firing pattern displayed by hippocampal pyramidal neurons and has been implicated in place cell formation. Here, we employed a stochastic search algorithm to generate a heterogenous population of neuronal models that accounted for ion-channel heterogeneities, towards assessing the biophysical basis of CSB in CA3 pyramidal neurons. Validation against signature electrophysiological properties yielded 236 neuronal models, which were endowed with broad distributions of underlying parameters showing weak pair-wise correlations. We found two subclasses of valid models: intrinsically bursting (IB) and regular spiking (RS). We employed principal component (PC) analysis on the underlying parameters and found clustering of IB *vs.* RS parameters along the first PC. We analyzed the parameters contributing to the first PC and found that an interplay between calcium and calcium-activated conductances distinguished IB from RS neurons. We triggered CSB employing 5 different kinds of inputs and observed diversity in CSB generation across models. Finally, we employed virtual knockout of individual ion channels and showed that synergistic interactions among several ion channels yielded CSB. Together, our analyses unveil the expression of ion-channel degeneracy in CA3 pyramidal neurons and emphasize the dominance of calcium and calcium-activated potassium channels in the emergence of CSB.

Heterogeneity in arabinose inducible gene expression in bacteria

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The arabinose inducible pBAD series of bacterial vectors is considered a tuneable and tightly regulated system with protein expression increasing monotonically with arabinose concentration for a bacterial population. However, single cell studies have shown that the arabinose expression vectors have an 'all-or-none' response. Initially attributed to the heterogeneity in the expression of the transporter AraE, this heterogeneity persists in non-arabinose metabolizing cells with a constitutively expressed transporter. To characterize this heterogeneity and understand the underlying cause, a fast degrading(fd) version of GFP expression under the P_{BAD} promoter was monitored by flow cytometry and fluorescence microscopy. Upon arabinose induction at intermediate levels, only a fraction of cells initiate gene expression resulting in a bimodal distribution of proteins per cell. Even with increasing amounts of arabinose, a large delay in initiation of gene expression leads to an 'all-or-none' behaviour over long timescales (~1.5 hrs). Further, we employed a single molecule RNA quantification technique, smRNA-FISH, to determine whether the observed protein heterogeneity stems from transcriptional heterogeneity at the single cell level. Our results suggest that a 'inducer-threshold' model explains the observed heterogeneity in the bacterial arabinose inducible system.

Mutational sensitivity of CcdB mutants in its native operonic context

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Autoregulation of transcription of Toxin Antitoxin (TA) operons through a mechanism known as conditional cooperativity is a hallmark of TA operons. The genomic architecture of the operon substantially influences toxin activation, by altering T:A ratio and affecting its oligomeric state. It is therefore important to study autoregulation of toxin-antitoxin systems in its native context. CcdA-CcdB is a convenient system to study mutational effects in an operonic context. Here, a comprehensive site-saturation mutagenesis library of CcdB (~1664 single-site mutants) was generated in its native operon and phenotypes were examined by transforming in two strains, one resistant and the other sensitive to the toxic activity of CcdB. Deep sequencing results revealed the mutational sensitivity of each mutant based on the relative population of the mutant in sensitive versus resistant strain. Most of the mutants grew similarly in both strains while several mutations at the CcdA binding site caused cell death more efficiently than the WT toxin, hence displaying a hyperactive phenotype. One such hyperactive mutant was used to understand the molecular mechanism behind this effect. This strategy can be deployed to identify prominent antitoxin binding residues within a toxin in the absence of the structure. The majority of the buried mutants showed an active phenotype in its operonic context, suggesting *invivo* cotranslational folding of the proteins in the operon allows CcdA to rescue the folding defect of CcdB mutants, thereby forming functional complexes.

CatBoost-PPI: Partner-specific protein-protein interfacial residue prediction

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The functions performed by proteins in living organisms are carefully regulated through interactions with other proteins and consequently form intricate complex networks of protein-protein interactions. Identifying the residues involved in these interactions may better our understanding of why certain interactions occur and pave the way for engineering interactions. Computational protein-protein interaction prediction methods are now valued in the wake of the fact that experimental methods to determine protein-protein interactions are time-consuming and expensive rendering fast high throughput protein-protein interface characterisation prohibitive.
We have developed CatBoost-PPI, a protein-protein interfacial residue predictor that employs CatBoost - a type of gradient-boosted-decision tree-based classifier, capable of predicting residue-residue contacts in protein complexes. CatBoost-PPI is a 'partner-specific' interaction predictor, and uses information derived from the sequence and structure of interacting proteins. The model has been trained and tested on Docking Benchmark v5.0 with leave-one-complex-out cross-validation and achieves a mean ROC-AUC performance of 0.8314 across 230 complexes. Our model is computationally inexpensive to employ in prediction and a compact easy-to-use Python package encapsulating the model is presently being developed and tested.
(Adithyan Unni was a summer intern in Prof. N. Srinivasan's lab at MBU IISc)

Day 2 (19thdecember, 2020) – SESSION III (3:00 PM – 3:42 PM)

Improvement in stereochemical quality of cryo-EM structures

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Over the past few years, several macromolecular structures solved using cryo-Electron Microscopy (cryo-EM) are determined at atomic level. We analyzed the stereochemical quality of more than 300 atomic cryo-EM structures using metrics such as sidechain rotamer outliers, Ramachandran angles, steric clashes and ω angle planarity. We identified certain examples among these structures which had unacceptable geometry in local regions. Using real-space refinement in these regions, we were able to re-fit models with better stereochemistry (correcting unreliable *cis* prolines, relieving steric clash, improving sidechain torsion angles and correcting disallowed Ramachandran angles), with improved fit to the potential map, thereby enhancing the reliability of these structures. Through this work, we demonstrate ways by which structures with improved stereochemistry can be obtained, without compromising fit to the experimental data.

Analysis of clinically approved therapeutics as potent SARS-CoV-2 entry inhibitor : An EM based study on Spike homotrimers

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SARS-CoV-2 is the pathogenic human coronavirus that caused the outbreak of the global pandemic COVID 19. Currently, efforts are being made worldwide for development of vaccine which can help to contain the disease in the long run; other therapeutic drugs are also necessary to treat the infected patients at present. Most of the recent cryo-EM studies indicate that the spike protein of SARS-CoV-2 adopts two major conformations, 1-RBD up open and all three RBD down closed conformation. Open state of RBD of the S protein is essential for interaction with hACE2 receptor facilitating subsequent viral entry into the host cell. Thus, SARS-CoV-2 S protein represents one of the prime targets for COVID-19 vaccine and therapeutic research. Our study aims at structural characterization of the S protein in the presence of clinically approved drugs including viral entry inhibitors like hydroxychloroquine, cepharanthine, emetine and analogs of linoleic acid. We used cryo electron microscopy (Cryo EM) and negative staining TEM analysis to elucidate the conformational changes in the S protein structure in the presence of these drugs. We have characterized the high-resolution structures of S protein under various drug conditions. The study also reveals significant decrease in spike-receptor binding with hACE2 receptor, with two different drug conditions. We further intend to screen various essential free fatty acids which may bind to the previously reported fatty acid binding pocket and stabilize an inaccessible closed form of homotrimeric S protein, thereby reducing viral entry.

D614G substitution at the hinge region enhances the stability of trimeric SARS-CoV-2 spike protein

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SARS-CoV-2 spike protein with D614G substitution has become the dominant variant in the ongoing COVID-19 pandemic and linked to higher infectivity than the ancestral D614 variant. Here, we demonstrate that the D614 is located at the hinge region that mediates domain-domain motions and hence glycine substitution potentially influence the flexibility of the spike protein. Further, *in-silico* mutagenesis and energy calculations reveal that the local interactions mediated by D614 are energetically frustrated and create unfavourable environment. Whereas G614 confers energetically favourable environment and strengthens intra- as well as inter-protomer association. Consequently, the thermodynamic stability of the spike protein trimer is enhanced as the free energy difference ($\Delta\Delta G$) upon glycine substitution is -2.6 kcal/mol and -2.0 kcal/mol for closed and open conformations, respectively. Our results on the structural and energetic basis of enhanced stability hint that G614 may confer increased availability of functional form of spike protein trimer thereby contributes to the higher infectivity.

Structural and related studies on *Mycobacterium smegmatis* MutT1

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M. smegmatis MutT1 (*Ms*MutT1) is a sanitation enzyme made up of a N-terminal Nudix hydrolase domain and a C-terminal domain resembling a histidine phosphatase. Action of MutT1 on 8-oxo-dGTP, 8-oxo-GTP and diadenosine polyphosphates has been established to be modulated by molecular aggregation. In order to further explore this aspect and to elucidate the structural basis of its differential action on 8-oxo-NTPs and unsubstituted NTPs, the crystal structures of the complexes of the enzyme with 8-oxo-dGTP, GMPPNP and GMPPCP have been determined. It was ensured through replacement soaking that the complexes are isomorphous to one another. The analysis of structural data led to the elucidation of a relation among aggregation, molecular plasticity and enzyme action on nucleotides. The dominant mode of aggregation involving a head-to-tail arrangement, leads predominantly to the generation of NDPs. The other mode of aggregation appears to prefer the generation of NMPs. The work also provides interesting insights into the dependence of enzyme action on the conformation of the ligand. The possibility of modulation of enzyme action through differences in molecular aggregation and ligand conformation, makes *Ms*MutT1 a versatile enzyme.

Increasing the therapeutic index of β-hairpin antimicrobial peptides to tackle multidrug-resistant bacteria

Priyanka Lahiri, Swati Priyardarshini, Sanmoy Pathak, Chitralekha Sen Roy, Nayanika Sengupta, Aparna Asok, Sunita Prakash, Somnath Dutta, Dipankar Nandi and Jayanta Chatterjee

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Despite being the most potent among different classes of antimicrobial peptides (AMPs), the high cytotoxicity, low serum stability, short half-life and rapid clearance precludes the development of β-hairpin AMPs as therapeutics. So, we reconfigured the naturally occurring β-hairpin AMPs (Arenicin1, Protegrin1, Tachyplesin1, and Polyphemusin1) by using a minimalistic design – i) substituting the Cys residues with Thr, and ii) introducing a stable engineered β-turn motif. This led to reduction in their affinity towards eukaryotic membranes while improving their bactericidal activity against ESKAPE pathogens and thereby enhancing their therapeutic indices. A mirror-image strategy was adapted to circumvent their proteolytic cleavage in biological fluids, resulting in increased stability of 72 h. Thus, among our four designed peptides, TP-D was found to be the most potent with rapid killing behavior against both gram-positive and gram-negative bacteria. TP-D exhibited low resistance generation against *A. baumanii*, *P.aeruginosa* and *S.aureus* and had high efficacy against MDR strains (MIC: 1-8 µg/mL). Encouraged with the results, an ex vivo bacteraemia model was established employing *A. baumanii* and *S.aureus*, where TP-D caused 3-log fold reduction in viable bacteria within 1 h. Finally, an intraperitoneal injection of a single dose of 5 mg/kg of TP-D rescued mice from succumbing to sepsis caused by *A. baumanii*.

Elucidating the dual role of c-di-AMP synthesizing enzyme DisA in *M.smegmatis*: differential promoter induction and associated physiological roles

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c-di-AMP is an essential secondary messenger in *Mycobacterium smegmatis*, which is synthesized from condensation of two ATP molecules by enzyme DisA in response to different environmental stimuli such as osmolyte stress. Interestingly, DisA protein is also known to contribute in DNA repair pathway in other bacteria such as *Bacillus sp*. Our main interest is to know how two such completely non-related stresses contributes to *in vivo* promoter induction of the same gene *disA*. Using GFP promoter fusion constructs, we have identified specific stress conditions contributing to differential induction of core promoters of *disA* and upstream gene *radA* in population level, highlighting the complex operon structure. Currently, the auto-regulation and cross-regulation aspects are studied for *PdisA* and *Ppde*. Overexpression constructs of *disA* have shown better stress tolerance to the corresponding stresses, further emphasizing the physiological relevance of c-di-AMP/DisA. By introducing SNPs in catalytic and DNA-binding domains of DisA, we try to uncouple the two activities of DisA, whereas cross-protection assays have demonstrated how two functions of DisA are interconnected. Recently, basic phenotypic characterization of *M. smegmatis disA* deletion mutant has revealed interesting functional insights of c-di-AMP in *M.smegmatis*.

Day 2 (19th December, 2020) – SESSION IV (4:45PM – 5:20PM)

Calcium promotes the activity of a metal dependent ribonuclease, RNase J2 in *Staphylococcus epidermidis*

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RNA degradation and recycling in bacteria are governed by several exo and endoribonucleases. Some of these ribonucleases are associated with the RNA degradation assembly, RNA degradosome. RNase J paralogues, RNase J1 and RNase J2 are the components of degradosome assembly in most of the gram-positive bacteria. RNase J2 is a bifunctional enzyme, exhibiting both endo and 5'-3' exoribonuclease activity. Crystal structure of *S. epidermidis* RNase J2 with manganese ion revealed a β-CASP, β-lactamase and a C-terminal polypeptide segment. Unlike its paralogue RNase J1 and other metallo β-lacatamase enzymes, RNase J2 has one metal ion at its active centre. The difference in the active centre geometry is the reason for adapting one metal ion catalysis by RNase J2. The activity assay on RNase J2 with different metal ions revealed that Ca^{+2} to be the most preferred by the enzyme. This observation is intriguing as Ca^{+2} is known to inhibit the catalytic activity of several metal dependent nucleases as opposed to our experimental observation on RNase J2. We are currently working towards understanding the unique behaviour of *Staphylococcus epidermidis* RNase J2 in the presence of Ca^{+2}.

Determination of hydroxyl proton exchange rates: a key to hydrogen bonds in glycans

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The dark matter of biology - That's what glycans are often called for how ubiquitous they are and yet how little we know about them.1 Structure of glycans like DNA and proteins must be utilizing hydrogen bonds in assembling to 3D scaffolds that would form the basis of biomolecular carbohydrate recognition and function.2 However, the hydrogen bonds (H-bonds) in carbohydrates can be transient and difficult to detect in protonated solvents. Hydrogen exchange rates are key indicators of hydrogen bonding in biomolecules and enable discrimination between persistent and transient H-bonds.3 We developed a new method to measure isothermal hydroxyl proton exchange rate constants in glycans using chemical exchange saturation transfer (CEST) of 13C in two states, 13C-O-H and 13C-O-D existing in a mixture of H2O and D2O. We illustrate the utility of selective excitation of peaks for 13C CEST with a weak B1 field to detect the two states which differ in a chemical shift as small as 0.15 ppm. We have measured the hydroxyl proton exchange rates in sucrose and observed a reduction in exchange rates of an order of magnitude as we change the pH from 6 to 5.8.

Structural and pharmacological studies of GABA transport inhibition using an engineered neurotransmitter transporter

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Chemical neurotransmission occurs through the calcium-induced release of neurotransmitters into the synaptic space that activate receptors in the post synaptic neuron. The uphill transport of neurotransmitters is mediated by the Na^+/Cl^- dependent neurotransmitter sodium symporters (NSSs). Among NSSs, our work is focused on γ-aminobutyric acid (GABA) transporters (GATs), which are responsible for sustaining synaptic GABA concentration in the neural synapse. Dysregulation of GATs can lead to seizures in epilepsy and other neurological disorders. Drugs like tiagabine inhibit GAT and elevate GABA levels in the synapse. Till now, there is no experimental structure of GAT(s). We engineered the primary binding pocket of the *Drosophila* dopamine transporter (dDAT), which shares 60% sequence similarity to resemble the human GAT1, for structural studies. The modified DAT_{GAT} showed binding to GAT1 inhibitors such as Tiagabine, NO-711 and also retained affinity towards the Fab which is used for dDAT crystallization. We could successfully solve the atomic resolution structures of substrate free and NO-711 bound DAT_{GAT}. The structures display a unique reorganization of the binding site in comparison to the monoamine transporters that allows us to rationalize the pharmacology of GAT inhibition.

Membrane dissociation free energy of AKT1 PH domain depends on protonation states of Phosphoinositides lipids: Insights from molecular simulations

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AKT1 proteins target the plasma membrane via specific binding of its pleckstrin homology domain (PHD) to $PI(3,4,5)P_3$ lipids. Interestingly, a single charge reversal mutation (E17K) greatly increases the protein's affinity for $PI(4,5)P_2$ lipids leading to promiscuous membrane binding. We use all-atom molecular dynamics (MD) simulations and umbrella sampling based free-energy calculations to understand the molecular mechanism behind the loss of PIP_3 specificity due to the charge reversal mutation. In particular, we explore the effect of ionization states of the lipids on binding geometries and free energy of dissociation. For a given charge state, we also explore the effect of proton position on lipid headgroups to get insights into the association and dissociation dynamics of AKT1 PH domains. Towards that, we use QM calculations to extract the altered charge distribution in PIP_2 and PIP_3 headgroups for given ionization states and use the information in MD simulations. We find that the binding geometries and dissociation free energies have remarkable variations for different protonation states of lipids. Our work highlights the role of membrane composition in PIP_3 binding as the presence of hydrogen-bond donor lipids (e.g. phosphatidylethanolamine) can have a significant effect on the charge and phase behaviour of individual $PI(3,4,5)P_3$.

Membrane catalyzed formation of nucleotide clusters and its role in the origins of life

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One of the challenges in studying the molecular "Origin of Life" has been about the emergence of RNA and RNA-based life forms where non-enzymatic polymerization of nucleotides is a crucial hypothesis in formation of large RNA chains. Non-enzymatic polymerization can be mediated by various environmental settings such as cycles of hydration and dehydration, temperature variations and proximity to a variety of organizing matrices such as clay, salt, lipid membrane and mineral surface. In this work, we explore the influence of different phases of the membrane towards nucleotide organization and polymerization in a simulated prebiotic setting. We calculate the free energy cost of localizing a mononucleotide, UMP, in distinct membrane settings and we perform all-atom molecular dynamics simulations to estimate the role of the monophasic and biphasic membrane in modifying the behavior of UMPs and their clustering mechanism. Based on the thermodynamics and kinetics data from molecular simulations, we also develop a reaction-diffusion based mathematical model to explain the observations from the molecular simulations. Our work shows that the lipid layers can act as unique substrates for 'catalyzing' polymerization of mononucleotides due to the inherent spatiotemporal heterogeneity and phase change behavior.

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