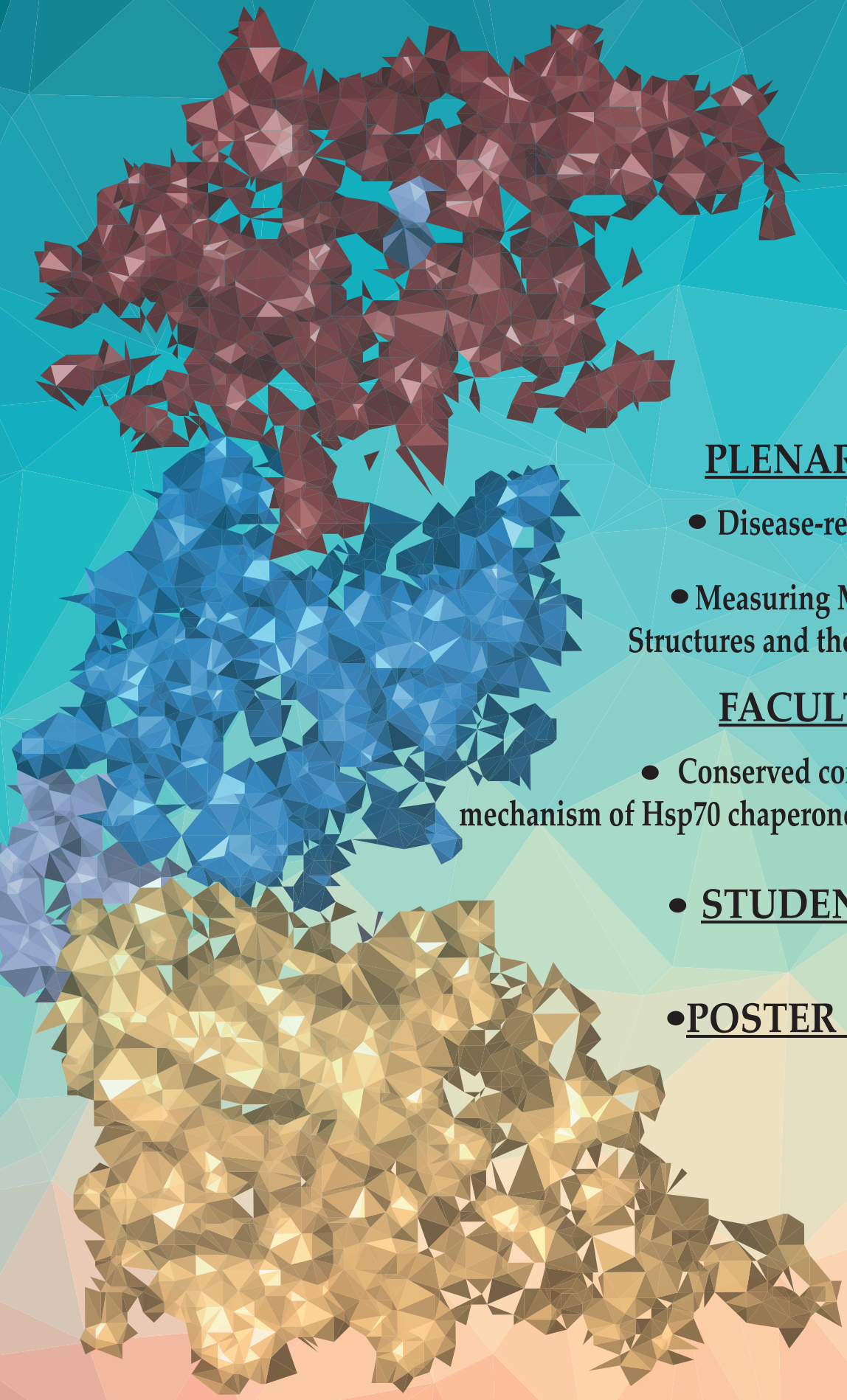
Molecular Biophysics Unit



PLENARY TALKS

- Disease-responsive drug delivery
- Measuring Molecular and Cellular Structures and the Forces That Control it

FACULTY TALK

- Conserved conformational selection mechanism of Hsp70 chaperone-substrate interactions

- STUDENT TALKS

- POSTER SESSIONS

Nu Biophysical Society

INDIAN INSTITUTE OF SCIENCE
भारतीय विज्ञान संस्थान

Table of Content

Molecular Biophysics Unit at Indian Institute of Science

The Molecular Biophysics Unit (MBU) at the Indian Institute of Science was founded in 1971 by G.N.Ramachandran. The Unit is currently engaged in frontline research in contemporary molecular Biophysics and Structural Biology. The research activities in the Molecular Unit focus on the structure, conformation and interactions in biomolecules, with the main objective of explaining biological activity in molecular terms. The unit has grown over a quarter of century, during this period, more than 200 young scientists have obtained their Ph.D. degrees and the number of reserach publicaations exceed 1000.

Areas of Research at the Unit

- Protein folding and dynamics
- Computer modeling and dynamics of biological molecules
- Unusual DNA structures and control of transcription
- Genome organization
- Ion channels and electrophysiology
- Lectins and lectin-carbohydrate interactions
- X-ray crystallography of proteins and viruses
- Theoretical studies on peptide and protein conformation
- DNA-protein interactions
- Ionophores, drugs and their interaction with membranes
- Synthetic protein design and protein engineering
- Membrane channel forming peptides
- NMR studies of proteins and peptides

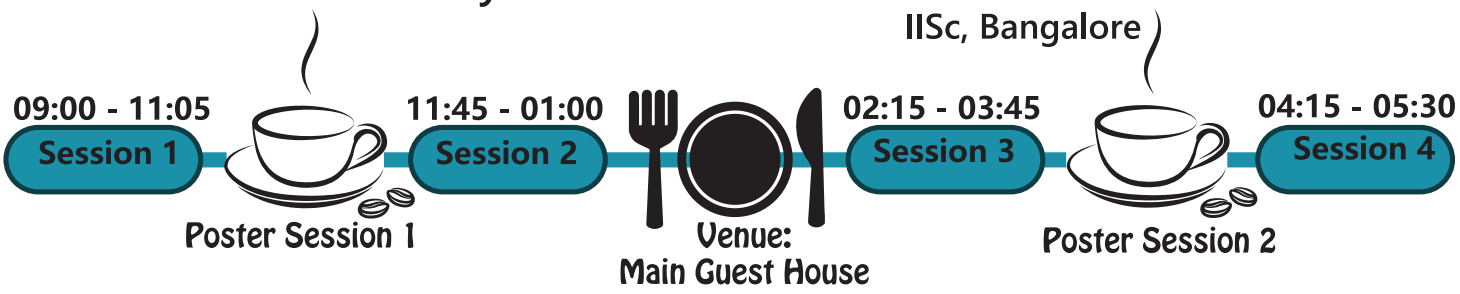
IN-HOUSE SYMPOSIUM 2018

MBU

SCHEDULE (8th Sep 2018)

Plenary lecture
- Dr. Praveen Kumar Vemula
  InStem, NCBS, Bangalore
- Dr. Gautam Soni
  RRI, Bangalore

Faculty lecture
- Dr. Ashok Sekhar
  IISc, Bangalore



Session 1

09:00 – 09:15 AM	CHAIRMAN'S ADDRESS
09:15 – 10:00 AM	PLENARY LECTURE 1
10:00 – 10:20 AM	FACULTY LECTURE
10:20 – 10:35 AM	Dr. Sushant Kumar Dr. A. Penmatsa's group
10:35 – 10:50 AM	Dr. Anushya Petchiappan Prof. D. Chatterji's group
10:50 – 11:05 AM	Dr. Amit Kumar Dinda Prof. B. Gopal's group

Session 2

11:45 – 12:00 PM	Bhavesh Khatri Dr. J. Chatterjee's group
12:00 – 12:15 PM	Dr. Debostuti Ghoshdastidar Prof. M. Bansal's group
12:15 – 12:30 PM	Devanshu Kurre Prof. K. Suguna's group
12:30 – 12:45 PM	Ishika Pramanick Dr. S. Dutta's group
12:45 – 01:00 PM	Kirtika Jha Dr. A. Srivastava's group

Session 3

02:15 – 02:45 PM	PLENARY LECTURE 2
02:45 – 03:00 PM	N. Sivaji Prof. M. Vijayan's group
03:00 – 03:15 PM	Pavithraa Seenivasan Dr. R. Narayanan's group
03:15 – 03:30PM	Pankaj Jadhav Dr. M. Singh's group
03:30 – 03:45 PM	Pooja Jorwal Prof. S.K. Sikdar's group

Session 4

04:15 – 04:30 PM	Dr. Saurabh Yadav Prof. A. Surolia's group
04:30 – 04:45 PM	Sneha Vishwanath Prof. N. Srinivasan's group
04:45 – 05:00 PM	Sunaina Banerjee Dr. R. Roy's group
05:00 – 05:15 PM	Tanaya Basu Roy Prof. S.P. Sarma's group
05:15 – 05:30 PM	Uddipan Kar Prof. R. Varadarajan's group

5:30– 6:00 PM: Awards and Vote of Thanks, followed by High Tea

Designed by: Nazia Hussain,2018

Plenary Talks

MBU In-house symposium 2018

Disease-responsive drug delivery: An emerging concept for the treatment of autoimmune and inflammatory diseases.

Praveen Kumar Vemula

Institute for Stem Cell Biology and Regenerative Medicine (inStem)
Bangalore
www.praveenlab.net

Abstract:

In this talk, we will discuss about one of the emerging concept in the field of biomaterials and drug delivery, i.e., disease-responsive biomaterials.
A significant leap in drug delivery is an autonomous system that titrates the amount of drug released in response to a disease, for instance, inflammation, ensuring the drug is released only when needed at therapeutically relevant concentration. Diseases have inherently fluctuated in nature such as inflammatory and autoimmune diseases, in particular, pose an enormous challenge to deliver drugs in safe, efficient and compliant manner. In what follows we will take a brief look at current approaches about biomaterials-based therapeutics and with examples taken from our work to examine how disease-responsive biomaterials have developed to i) improve the lifetime of the transplanted organs, ii) locally injectable hydrogels for the treatment of inflammatory arthritis, and iii) inflammation-targeted drug delivery to alleviate inflammatory bowel diseases.

Measuring Molecular and Cellular Structures and The Forces That Control it

Gautam V. Soni
Raman Research Institute

Structure and Function are deeply connected concepts in self-assembled biological systems. Interactions forces, crowding, shape and deformability, charge and active/passive motion are some of the biophysical parameters that shape and modulate biological structures and their function. This happens at both molecular scale, Chromatin is one excellent example of this, as well as the cellular scale (cell shape and stiffness). Failure of any of these structural elements lead to various diseases and disorders. To be able to precisely measure and understand the biophysical principles underlying these structures and changes their-in is the main aim of my lab. In this talk, I will introduce you to the various experimental approaches we build and employ to understand molecular and cellular structure. I will talk about high-resolution single molecule measurements on chromatin system using the nanopore and AFM platforms and a novel in-house developed electrofluidic method to measure single-cell volume and stiffness.

Faculty Talk

MBU In-house Symposium 2018

Conserved conformational selection mechanism of Hsp70 chaperone-substrate interactions

Ashok Sekhar, Algirdas Velyvis, Guy Zoltsman, Rina Rosenzweig, Guillaume Bouvignies and Lewis E. Kay

Abstract:

Molecular recognition is integral to biological function and frequently involves preferred binding of a molecule to one of several exchanging ligand conformations in solution. In such a process the bound structure can be selected from the ensemble of interconverting ligands *a priori* (conformational selection, CS) or may form once the ligand is bound (induced fit, IF). Here we focus on the ubiquitous and conserved Hsp70 chaperone which oversees the integrity of the cellular proteome through its ATP-dependent interaction with client proteins. We directly quantify the flux along CS and IF pathways using solution NMR spectroscopy that exploits a methyl TROSY effect and selective isotope-labeling methodologies. Our measurements establish that both bacterial and human Hsp70 chaperones interact with clients by selecting the unfolded state from a pre-existing array of interconverting structures, suggesting a conserved mode of client recognition among Hsp70s and highlighting the importance of molecular dynamics in this recognition event.

Student Talks

Structural studies of antibiotic efflux transporter NorC from *Staphylococcus aureus*

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NorC of *Staphylococcus aureus* is a 14-transmembrane containing transporter protein belonging to major facilitator superfamily. Transporter proteins are integral membrane proteins that regulate the movement of biomolecules across the plasma membrane and play a key role in driving and regulating physiological processes that are essential for maintaining cellular homeostasis.

NorC was reported to be involved in providing multi-drug resistance to methicillin resistant *S. aureus* (MRSA) strains against quinolone class of drugs. Our work aims to elucidate the structure of this transporter and undertake its functional characterization. Integral membrane transporters are challenging molecules to crystallize given the high levels of conformational heterogeneity and minimal solvent-exposed surface area that impedes formation of lattice contacts, a prerequisite for crystal formation.

To overcome this hurdle, we have used single chain Indian camelid antibody (ICab) as crystallization chaperone.

In this work, we cloned and expressed NorC in *E.coli* and successfully purified the protein. We immunized an Indian camel with NorC and isolated NorC-specific ICabs from the cloned antibody library using yeast surface display platform, and expressed those in *E.coli*. Further, we purified the ICabs and characterized their binding with NorC using ITC. To gain structural insights on ICab, we crystallized one of them and determined its structure. We then used this ICab as a crystallization chaperone and successfully crystallized NorC-ICab complex. The crystals diffracted to a resolution of 4.25 Å. Our current efforts are focused on improving the resolution of this complex.

Differential role of the (p)ppGpp synthetases Rel and RelZ from *Mycobacterium smegmatis*

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Stringent response is a highly conserved physiological response by bacteria to stress and it is orchestrated by the second messengers guanosine pentaphosphate and guanosine tetraphosphate. In Mycobacteria, (p)ppGpp regulates several phenotypes such as antibiotic tolerance, biofilm formation, cell division, long-term survival etc. Rel is the major (p)ppGpp synthetase in *M. smegmatis.* In addition to Rel, *M. smegmatis* contains a second (p)ppGpp synthetase RelZ. RelZ possesses a unique combination of a RNaseH domain along with a (p)ppGpp synthesis domain. This raises an important question as to the role played by RelZ *in vivo*. We previously showed that RelZ can hydrolyze the triple-stranded DNA/RNA hybrids called R-loops. We now report that RelZ has the additional capability of synthesizing the nucleotide derivative pGpp, whose function in bacteria remains unknown. In contrast to Rel, RelZ preferentially uses GDP and GMP as substrates over GTP giving rise to ppGpp or pGpp as products, respectively. Furthermore, the biochemical activity of RelZ is regulated by single stranded RNA. Phenotype microarray analysis showed that the deletion of Rel from *M. smegmatis* makes the bacteria more resistant to antibiotics with altered biofilm formation ability. However, the removal of the RelZ leads to increased senstivity to antibiotics. Thus, it appears that both the (p)ppGpp synthetases of *M. smegmatis* have a different substrate utilization pattern and mode of regulation, which helps the bacteria efficiently utilize (p)ppGpp signalling under different stress conditions.

Understanding extracytoplasmic function σ factor – Pribnow box interactions: Implications for transcription initiation

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Transcription in prokaryotes is mainly regulated at the initiation step. Sigma (σ) factors, the dissociable subunit of RNA polymerase play a prominent role in transcription initiation. *M. tuberculosis* encodes 13 σ factors (σ^A to σ^M, divided into 4 different groups) out of which 10 belong to the group 4 σ factors. The group 4 σ factors contain only regions 2(σ_2) and 4(σ_4), and accommodate the highly diverged extracytoplasmic function (ECF) subfamily. σ_4 recognizes the −35 promoter/extended −10 element while, the Pribnow box (−10 element) is recognized and melted by σ_2. It is hypothesized that L3 loop of ECF σ_2 determines the specificity for the single flipped out base at the -10^{th} position of Pribnow box. Despite extensive studies on promoter recognition, –10 consensus sequences for *M. tuberculosis* σ factors are not well defined. In addition, genes regulated by particular σ factors remain unannotated. To find out the specificity of *M. tuberculosis* ECF σ factors for the -10^{th} position of the Pribnow box, we constructed 10 chimeric proteins by introducing the heterogeneous L3 loop of the 10 ECF σs separately within the *E. coli* σ^E_2 scaffold. Specificity of these 10 L3 loops towards nucleobase was investigated using Trp fluorescence quenching experiments. These are being validated by X-ray crystallographic studies and *in vitro* transcription assays. We also identify different sets of genes under regulation of only one σ factor as well as multiple σ factors by *in silico* analysis. These studies provide a basis to understand the partitioning of bacterial transcription space in the presence of multiple σ factors.

Rethinking hydrogen bond and the influence of local environment in protein stability.

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Hydrogen bonds play a crucial role in maintaining the protein structure and its importance cannot be overemphasized. The energy of a hydrogen bond contributes around 5-6 kcal/mol for the isolated bond to 0.5-1.5 kcal/mol for proteins in solution. However, this contribution is context dependent and is rather difficult to assess. Although few approaches have been used to study the contribution of hydrogen bonds by replacing amide bond of interest in a protein with an ester or an olefin moiety, but this mutation eliminates a H-bond donor or both H-bond donor and acceptor. Our goal is to gain a better understanding of hydrogen bond strength with minimal perturbation of its donor and acceptor capability. Thus, we targeted the solvent exposed site of the Pin1 WW domain, a small β-sheet protein, and replaced individual backbone amide bonds with thioamide, a better H-bond donor. Circular dichroism spectroscopy data show that all the O to S (amide to thioamide) mutated variants fold into the same β-sheet structure as the wild type. Further thermodynamic studies of O to S mutated protein variants revealed that solvent shielded hydrogen bonds as opposed solvent exposed ones has greater stabilizing effect. With a single atom substitution, we could achieve around 15 ºC enhancement in thermal stability (T_M) compared to the wild type. Thus, this simple strategy via a single atom substitution (O to S) of the peptide bond can not only be a great tool to understand the folding kinetics and thermodynamics of proteins but also to engineer thermodynamically stable variants via native chemical ligation.

Flexibility and Structure of the DNA Flanking a Consensus Motif Significantly Impact Transcription Factor Binding

Venkata Rajesh Yella[1], Devesh Bhimsaria[2], Debostuti Ghoshdastidar[1], José A. Rodríguez-Martínez[2], Aseem Z. Ansari[2] and Manju Bansal[1]

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Spatial and temporal expression of genes is essential for maintaining phenotype integrity. Transcription factors (TFs) modulate expression patterns by binding to specific DNA sequences in the genome. Along with the core binding motif, the flanking sequence context can play a role in DNA-TF recognition. Here, we employ high-throughput *in vitro* and *in silico* analyses to understand the influence of sequences flanking the cognate sites in binding of three most prevalent eukaryotic TF families (Zinc finger, homeodomain, and bZIP). *In vitro* binding preferences of each TF toward the entire DNA sequence space were correlated with a wide range of DNA structural parameters, including DNA flexibility. Results demonstrate that the flexibility of flanking regions modulates binding affinity of certain TF families. Furthermore, DNA duplex stability and minor groove width play an important role in DNA-TF recognition but differ in how exactly they influence the binding in each specific case. Our analyses also reveal that the structural features of preferred flanking sequences are not universal, as similar DNA-binding folds can employ distinct DNA recognition modes.

Structural and functional characterization of a small Heat Shock Protein (sHSP18.8) from *Entamoeba histolytica*

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Small heat shock proteins (sHSPs) belong to the class of ATP-independent molecular chaperones. Several reported studies showed that sHSPs are linked to many diseases. The expression levels of sHSPs get upregulated under stress conditions like high temperature, extreme pH etc. Under such situations, cellular proteins start to unfold leading to aggregation and loss of activity. sHSPs prevent the aggregation and thus help in maintaining protein homeostasis in the cell.

Only a few high-resolution crystal structures have been reported so far for sHSPs. The subunits of all sHSPs share a structurally conserved central domain called the α-crystallin domain (ACD). ACD is flanked on either side by unstructured N- and C terminal regions varying in length and sequence. ACD helps in the formation of dimers which are the basic building blocks of sHPS, while the N- and C- terminal regions are responsible for chaperonic activity and oligomerization.

There were no structural reports on sHSPs of parasitic protozoa. We are currently working on sHSPs from various protozoa with an aim to functionally and structurally characterize them. We have crystallized and determined the tetrameric structure of one of the small heat shock proteins sHSP18.8 from *Entamoeba histolytica* to a resolution of 3.4 Å. Solution studies show that oligomerization is concentration dependent and the higher oligomer dissociates into smaller oligomers under extreme pH conditions. We also characterized the chaperonic function of sHSP18.8 through NdeI activity and lysozyme aggregation assays.

Structural analysis of Cystathionine Beta Synthase of *Mycobacterium tuberculosis* by cryo-electron microscopy

Ishika Pramanick[1], Anil Kumar[1], Parijat Bandyopadhyay[2], Somnath Dutta[1], Amit Singh[2]

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Mycobacterium tuberculosis (Mtb) is the causative agent of one of the deadliest infectious disease known to man, tuberculosis. *Mtb* has a remarkable ability to persist inside the hostile environment of human phagocytes encountering ROS, RNS, low pH and nutrient starvation. The role of sulfur metabolites generated using cysteine as a precursor protects *Mtb* from oxidative stress. The major pathway in eukaryotes for synthesizing cysteine is the transsulfuration pathway. The significance of transsulfuration pathway in *Mtb* remains poorly understood. This pathway consists of two enzymes, cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE). CBS is the first enzyme of this pathway and catalyses the production of cystathionine from serine and homocysteine. Cystathionine is then subsequently utilised by CSE to produce cysteine. CBS activity is dependent upon pyridoxal phosphate which acts a cofactor for this enzyme and CBS is allosterically activated by S-adenosyl methionine (SAM). *In silico* analysis revealed the presence of orthologs of CBS and CSE in *Mtb*. Structural analysis of CBS and CBS treated with SAM will help to understand the conformational changes resulting from SAM binding. In future, these structural insights may enable us to develop specific inhibitors of *Mtb* CBS.

CBS was expressed in *E.coli* BL21(DE3) and purified by Ni-NTA affinity chromatography followed by SEC. The protein identity was confirmed by MALDI-MS. Native and SAM treated CBS were visualized by negative staining EM and homogenous particle distribution was observed. We proceeded for high resolution structure determination of CBS by cryo-electron microscopy and we have a 3.7 angstrom map. 3D model reconstruction of native and SAM treated CBS clearly indicates the conformational differences. Further, we also docked Crystal structure in the Cryo-EM density map.

Mechanistic insights into pleckstrin-homology domain (PHD) activity in dynamin mediated fission

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Dynamin, a large multi-domain GTPase, catalyzes membrane fission in a highly regulated manner [1]. Classical dynamin associates with phosphatidylinositol-4,5-biphosphate (PIP2) lipids through its centrally located pleckstrin-homology domain (PHD). Interestingly, PHD is known to be a dispensable domain as fission can take place even in its absence [2]. However, the rate of fission slows down manifold in absence of PHD [3]. In this work, we have combined advance sampling methods (Metadynamics), molecular dynamics based Highly Mobile Membrane Mimetic (HMMM), all-atom (AA) and coarse-grained (CG) simulations to explore the molecular basis of PHD interactions with membranes. We report molecular-level insights into the possible role of PHDs as catalysts in dynamin-induced membrane fission during synaptic vesicles recycling. Using Metadynamics-based free-energy calculations, we extract the docking geometry of PHD with inositol lipid, and identify the participating residues in membrane association. With the implementation of HMMM simulations, we capture the orientation variability in PHD during membrane association which appears to be an inbuilt property of PHD. Our AA-MD simulations unravel the effect of different lipid compositions on protein-membrane association, which corroborates well with experiments [2]. The effect of mutations on membrane association captured in AA-MD simulation also agrees with experiments [2,4] and further provides molecular-insights behind the experimental observations. We also find that PHDs make the membrane more pliable for fission as revealed from bending modulus calculations on the membrane. Currently, we are working on the comparison of obtained simulation data with CryoEM data for establishing what we term as "the orientation selection" of PHD membrane association [5].

References:

[1] Faelber, K *et al*. Nature. **477** , 556-60 (2011)
[2] Dar, S *et al*. MBoC. **28** , 152-160 (2017)
[3] Shnyrova , A *et al*. Science. **339**,1433-6 (2013)
[4] Ramachandran, R *et al*. MBoC. **25** , 879-90 (2014)
[5] Hinshaw J.E *et al*. Cell Reports **8 ,** 734-42 (2014)

The first structural and related study on an archeal lectin with an unusual quaternary association

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Much of our earlier studies on lectins have been on those from plants. The work subsequently extended to include microbial lectins. It was then realized that no lectin has been unambiguously identified yet from archea. A genomic search led to the identification of several lectins from archea. One of them, that from *Methanococcus voltae A3*, *mevolectin,* has been cloned, expressed and purified. Crystallographic studies on three forms of this lectin show that it has an unusual quaternary association with seven-fold symmetry. Solution studies on the protein have also been carried out. The results of Dynamic Light Scattering (DLS) are ambiguous. However, SEC-MALS and gel filtration studies unambiguously indicate the molecule to be a heptamer. Further crystallographic and solution studies of the lectin are in progress. With the identification of lectins in archea and experimental studies on one of them, it has been demonstrated that lectins exist in all three domains of life. It would also appear that lectins evolved to the present form before the three domains diverged. The observation of an unusual quaternary association in *mevolectin*, adds an interesting dimension to the study.

Structure of *E.coli* HigBA toxin-antitoxin system and its implications for promoter DNA and ribosome binding

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Toxin-Antitoxin (TA) systems are prokaryotic genetic modules that are involved in several processes such as plasmid maintenance, phage defense, control of stress response and bacterial persistence. Type II TA systems consist of a protein antitoxin, which directly binds and inhibits its cognate protein toxin. Both antitoxin and toxin are part of the same operon and antitoxin regulates expression of the operon by directly binding to the promoter region, while the toxin acts as corepressor in some TA families. The antitoxin in free form and in TA complex is shown to have different binding affinity to the promoter region. Unlike most of the type II TA systems, the HigB toxin gene in the operon precedes the antitoxin HigA gene. HigB is a ribosome-dependent endoribonuclease, which only cleaves translating mRNAs. The binding of antitoxin, HigA to toxin, HigB does not mask the active site of the toxin, hence the probable mechanism of toxin inhibition by antitoxin is blocking its interaction with ribosome. However, the reasons for ribosome dependent activity of toxin, the mechanism of mRNA cleavage and the mode of promoter regulation in HigBA family remain elusive. In this study, we addressed these aspects through biophysical approaches. We have determined the crystal structure of HigBA complex at 2.1Å resolution showing that the complex exists in heteroterameric (HigA2B2) complex. Using stoichiometric EMSA assays and SEC-MALS analysis, we have confirmed the stoichiometry and differential binding of HigA and HigBA with the promoter sequences. Through computational modelling approaches, we have modelled the HigA2B2 complex with promoter DNA and HigB with the ribosome structure. Further, studies are underway to unravel the structural basis of endoribonuclease activity of HigB and expression regulation of HigBA operon.

Efficient Phase Coding And Excitability Robustness Within The Degeneracy Framework

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Hippocampal place cells encode space using both rate and temporal codes. Whereas a rate code is defined by a marked increase in the neuronal firing rate, the associated precession of firing relative to the hippocampal theta rhythm characterizes a phase code. We explored phase coding from the perspective of the efficient coding hypothesis that relates neuronal representations to information maximization. We approached this question within the framework of degeneracy, a principle that states that disparate structural components could yield similar functional properties. We built a conductance-based phase coding model, defined and quantified the efficiency of a phase code within an information theoretic framework, and employed this framework to assess neuronal characteristics that would maximize encoding efficiency. We recruited a multi-parametric stochastic search strategy to generate numerous models that span a wide range of intrinsic parameters to mimic the inherent channel heterogeneities and to avoid problems associated with parametric bias. We assessed the efficiency of spatial information (from *within* a single place field) transfer through phase precession in these models. We found that comparable levels of high-efficiency information transfer could be achieved across these models with strikingly disparate intrinsic properties, thereby establishing the expression of degeneracy in efficient phase coding. Finally, we imposed additional constraints with reference to electrophysiological measurements of CA1 pyramidal neuron excitability. These resultant models displayed both phase coding efficiency and robust intrinsic excitability signatures, with distinct parametric combinations yielding similar functionality. Together, our results unveil significant degeneracy in the ability of a neuron to concurrently achieve a highly efficient encoding system and maintain robustness in intrinsic electrophysiological signatures.

Suppression of epileptic activity by lactate through GIRK channel in subicular pyramidal neurons

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Epilepsy is a neurological disorder characterized by unprovoked recurrent seizures. Many evidences suggest that the subiculum in hippocampus plays a significant role in initiation and propagation of epileptiform activity. Lactate acts as a neuroprotective agent against several kinds of brain damage including ischemic, excite-toxic, and mechanical insults. During epileptic seizures increased cerebral metabolic activity results in excess lactate formation which reaches upto 5-6 mM in the brain. We studied the effect of lactate on subicular pyramidal neurons after induction of epileptogenesis using 4AP-0Mg^{2+} in an *in vitro* epilepsy model in rats. We found that application of 6 mM lactate after epileptic induction reduced spike frequency and hyperpolarized the resting membrane potential of subicular pyramidal neurons in whole cell patch clamp experiments in acute hippocampal slices. Hydroxycarboxylic acid receptor 1(HCA1) is present in many regions of the brain and it gets activated with 6 mM lactate. We hypothesized that the effect of lactate on the electrophysiological properties of subicular neurons might be through HCA1 receptors. Immunohistochemical studies confirmed the expression of HCA1 in subicular pyramidal neurons. To investigate the signaling mechanism involved in mediating the effect of lactate, a specific HCA1 agonist- 3, 5-dihydroxybenzoate was used which revealed similar reduction in spike frequency. Electrophysiological recordings showed the involvement of Giβγ in the lactate induced mechanism. Further, our voltage-clamp experiments with Tertiapin-Q, a blocker of GIRK current confirmed that lactate application results in outward GIRK current. Our findings suggest a new role of lactate as neuroprotectant acting *via* HCA1receptor to activate GIRK channels in subicular pyramidal neurons.

Activation of TRIF pathway by lysozyme induces neuropathic pain

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Inflammation is a defense response of the body towards infection or injury which localizes and eliminates the injurious agents and promotes clearance of cellular debris leading tissue repair and regeneration. Chronic inflammation, on the other hand, is a sustained and damaging inflammatory condition far beyond the tissue regeneration process. Hence, chronic inflammation by itself can lead to further tissue damage. Pain is one of the cardinal features of inflammation. However, chronic pain, which outlasts the healing period is a disease state originating from altered neuronal activity. Injury to the nervous tissues and associated neuroinflammatory condition leads to neuropathic pain, a major debilitating condition affecting about 30% adults worldwide. Toll like receptors (TLRs) play a key role in the neuro-immune and neuro-glial interactions during neuropathological conditions including chronic pain. Out of all the TLRs, TLR4 is of great interest as it is expressed by glial cells, immune cells and by primary sensory neurons of the dorsal root ganglion of the peripheral nervous system which are involved in neuroinflammation and pain pathology. Activation of TLR4 during sterile nerve injuries is still not completely understood. Importantly, activation of neuronal TLR4 and its effects on the pathophysiology of neuropathic pain is not known. Previous work from our lab has shown that lysozyme activates TLR4 during nerve injury. TLR4 activation may lead to activation of either MyD88 or TRIF or both pathways. Our current work shows that lysozyme mediated activation of TLR4 leads to activation of TRIF pathway in neurons resulting in pain in animal models.

Are protein-protein interaction partnerships always conserved in evolution?

Sneha Vishwanath[1], Deepa Jain[2], and Narayanaswamy Srinivasan[1]

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The binding partners of homologous proteins are believed to be evolutionarily related. Indeed, this assumption forms the basis for prediction of interacting proteins referred to as interologs approach. Here, we investigated the validity of this assumption using structural information of protein-protein complexes. We analysed the datasets of known 3-D structures of protein-protein complexes, organised in terms of evolutionary relationships among participating proteins. Surprisingly, we noted that in majority of the protein-protein complexes with a pair of homologues involved in the two complexes, the binding partners are evolutionarily unrelated. In other words, it appears that divergently evolved homologous proteins bind to proteins that are, in most cases, non-homologous. This unexpected observation could have resulted due to the limited repertoire of the 3-D structures of protein-protein complexes while the dataset of known protein-protein complexes is much bigger. In order to address this concern, the entire analyses were performed on a dataset of known protein-protein complexes and results turned out to be still the same as obtained using a dataset of known 3-D structures. Detailed analyses of specific cases with unconserved binding partners of homologous proteins were carried out to understand the reason for interaction of unrelated proteins with homologous binding partners. Our learning from the analyses of these case studies is that the partnerships are unconserved due to the absence of the homologous binding partner in the organism concerned or incompatible physicochemical features at the binding interface of homologous protein pairs. The observation of non-conversation of protein-protein interaction during evolution calls for re-visit of the belief that homologues of two proteins that are known to interact commonly interact with each other.

Exploring modes of RNA remodeling by the ZIKV NS3 helicase

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Flaviviruses are positive strand RNA viruses that replicate within mammalian host cells using two primary enzymes, the Non-Structural (NS) 5, that codes for a methyl transferase and the RNA polymerase domain and the NS3 protease-helicase protein. Flaviviral NS3 helicase is a DExH RNA helicase and is a potential druggable candidate for virus inhibition.

In the present work, we have focused on characterizing the Zika virus (ZIKV) NS3 helicase – RNA substrate interactions. Using fluorescence-based spectroscopic assays, we measured the kinetics of RNA binding and unwinding by the ZIKV NS3 helicase. Our results show that the ATP:ADP ratio modulates helicase activity, with excess ADP lowering the fraction of unwound RNA. We next explored the underlying mechanism for modulation of helicase activity. Here, we report that the ZIKV NS3 helicase can efficiently "anneal" low concentrations of ssRNA, in addition to the canonical RNA unwinding activity. We examined whether transient oligomerization of RNA bound ZIKV NS3 helicase could sequester RNA thus promoting RNA annealing or annealing can be regulated by the coupling between ATP binding and RNA binding sites. Our preliminary results suggest transient oligomerization by NS3 helicase as the possible route to RNA annealing promoted by the helicase.

Finally, I will discuss the biological relevance of the dual and counteracting activity of ZIKV NS3 helicase in the context of Flavivirus replication.

Structural studies on MazEF9 toxin-antitoxin system from *Mycobacterium tuberculosis*

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Toxin-antitoxin (TA) systems, unusually abundant in *Mycobacterium tuberculosis,* contribute to its ability to persist in a drug-tolerant latent state. In Mtb, the MazEF TA system belongs to the Type II class, wherein, under favourable growth conditions, the protein antitoxin (MazE) binds to its cognate toxin (MazF) forming an inactive complex, that dissociates in response to stress, thereby allowing the toxin to affect protein synthesis and consequently, bring about growth arrest.

Our interest lies in determining the structures of the MazF9 toxin, the MazE9 antitoxin, and the MazEF9 complex, using nuclear magnetic resonance spectroscopy.

Like in other TA systems, the MazE9 antitoxin protein is intrinsically disordered in the C-terminal region, which is expected to bind to and inactivate the toxin. This prompted the synthesis of the N terminal del(1-42) deletion mutant(MazE9c), to overcome issues associated with the large size of the complex. Proton NMR spectra of the full length MazE9 shows limited chemical shift dispersion in the amide and aliphatic regions. Low intensity resonance lines are observed close to 0.0 ppm, indicating the possibility of conformational exchange between the disordered and ordered forms. MazF9 on the other hand, shows several resonance lines at chemical shifts > 8.8 ppm and < 0.4 ppm, indicating a well structured protein. Backbone assignments have been made for MazE9c using triple resonance NMR experiments. The monomeric C terminal disordered tail of MazE9 antitoxin, along with the MazF9 toxin will be used to reconstitute the complex *in vitro*. This will make structural studies by NMR more feasible.

Protein Nanoparticle display of influenza stem immunogens

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Influenza virus is a human pathogen leading to frequent respiratory illness. Hemagglutinin (HA), the primary surface glycoprotein on Influenza virus has always been an attractive target for vaccine design. We recently designed a 'headless' HA stem immunogen pH1HA10-foldon, where Foldon is a trimerization domain from phage T4 fibritin. The HA stem is derived from the H1N1 A/California/04/2009 isolate (PDB-3LZG). In a recent study, Ferritin nanoparticles which self-assemble to 24-mers were engineered to display repetitive arrays of full-length HA and an HA mini stem that induced a strong protective immune response (Kanekiyo et al, 2013). We therefore engineered a fusion of pH1HA10-Foldon with Ferritin as the antigen display platform. Since Ferritin can self-assemble into a 24-mer, we also removed the Foldon trimerization domain to generate the final construct pH1HA10 fused to Ferritin. This was biophysically characterized. The same immunogen was fused to various other nanoparticle platforms such as MSDPS-2(12-mer) (PDB-2Z90), Lumazine Synthase(60-mer) (PDB-1HQK), E2p(60-mer)(PDB-1B5S) and Encapsulin (180-mer)(PDB-4PT2). SEC-MALS and negative stain images of such particles have revealed that it is possible to successfully fuse our construct pH1HA10 to each of these different sized nanoparticle platforms. Binding studies to stem directed antibodies further confirmed the conformational integrity of these fusion constructs. These nanoparticle fusions provide improved antigen stability and will likely increase immunogenicity. This will be shortly tested in mouse immunization and challenge studies.

Student Posters

Insights into the DNA binding nature of AT-rich interaction domain of BAF250a

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SWI/SNF chromatin remodeling complexes contain a subunit belonging to AT rich interacting domain (ARID) containing family of proteins through which they are recruited to nucleosomal target DNA with high affinity, allowing the transcriptional activation of genes. In higher eukaryotes, SWI/SNF complexes contain two or more mutually exclusive ARID containing subunits e.g. BAF200 in the PBAF sub-complex, while BAF250a and its paralogous BAF250b in BAF-A and BAF-B sub-complexes respectively. The ARID belongs to a family of highly conserved DNA binding domain, however it's exact contribution in SWI/SNF functions and DNA binding specificities has not been well established. Here we have probed the structure and interaction of BAF250a ARID with different dsDNA sequences to understand its DNA binding properties. A comprehensive biophysical study, using NMR spectroscopy and ITC methods revealed complex nature of ARID–DNA interactions. The thermodynamic signatures of BAF250a ARID with AT rich sequences are distinct from GC rich sequences. We observed that for a 12mer dsAT DNA, the binding proceed with a negative enthalpy change, while in case of 12mer dsDC DNA it is endothermic at low temperature while it reverses to exothermic at higher temperature. Using NMR chemical shift perturbation experiments, we have identified DNA binding residues on ARID and mapped them on to the ARID structure. CSPs under different salt concentration revealed residues that were perturbed distinctly for AT-rich sequences compared to the GC-rich sequences. Further, we have generated the NMR data driven HADDOCK models of ARID-DNA complexes, which are further validated using mutational and computational approaches.

Structural insights into the specificity and catalytic mechanism of mycobacterial nucleotide pool sanitizing enzyme MutT2

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Mis-incorporation of modified nucleotides, such as 5-methyl-dCTP or 8-oxo-dGTP, in DNA can be detrimental to genomic integrity. MutT proteins are sanitization enzymes which function by hydrolyzing such nucleotides and regulating the pool of free nucleotides in the cytoplasm. Mycobacterial genomes have a set of four MutT homologs, namely, MutT1, MutT2, MutT3 and MutT4. Mycobacterial MutT2 hydrolyzes 5m-dCTP and 8-oxo-dGTP to their respective monophosphate products. Additionally, it can hydrolyze canonical nucleotides dCTP and CTP, with a suggested role in sustaining their optimal levels in the nucleotide pool. The structures of *M. smegmatis* MutT2 and its complexes with cytosine derivates have been determined at resolutions ranging from 1.10 Å to 1.73 Å. The apo enzyme and its complexes with products (dCMP, CMP and 5m-dCMP) crystallize in space group $P2_12_12$, while those involving substrates (dCTP, CTP and 5m-dCTP) crystallize in space group $P2_1$. The molecule takes an α/β/α sandwich fold arrangement, as observed in other MutT homologs. The nucleoside moiety of the ligands is similarly located in all the complexes, while the location of the remaining tail exhibits variability. This is the first report of a MutT2-type protein in complex with ligands. A critical interaction involving Asp116 confers the specificity of the enzyme towards cytosine moieties. A conserved set of enzyme-ligand interactions along with concerted movements of important water molecules led provide insights into the mechanism of action.

Understanding the mechanism of Action of Antimicrobial Peptides

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Multi drug resistance bacteria represents a global health challenge that results in increased mortality rates. Antimicrobial peptides serve as potential alternatives to the conventional antibiotics in treating drug-resistant bacteria. These peptides are found to be rich in positively charged residues such as lysine and arginine. However, the role of such positively charged functional groups in disrupting bacterial membrane have not been systematically explored. Analysis of the beta hairpin antimicrobial peptide database, we observed clusters of arginine residues both at the termini and at the turn region. To further gain an insight on the role of such arginine clusters, we chose Polyphemusins as our model system. We systematically mutated Arginine to Ornithine, thereby replacing the guanidinium functional group with an amine functional group without compromising on the charge of the peptide. MIC experiments demonstrated that removal of guanidinium group from the termini clusters led to loss of the antibacterial activity of the peptide but it did not hamper its membrane permeabilizing property. Next, we did membrane depolarization that showed similar binding of peptides towards membrane. Biophysical characterization through NMR and CD showed β-hairpin nature of the library with minimum structure perturbation. Further, CD in SDS micelle provided a qualitative view of the differential interaction of peptides. The peptides were also found to be aggregating at pH 7.4 as compared to pH 3.8 as can be validated from both ^{1}H spectra as well as CD spectra.

Structural studies on *M. tuberculosis* argininosuccinate lyase and its liganded complex provide insights into its catalytic mechanism.

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Argininosuccinate lyase (ASL) from *M. tuberculosis* catalyses the reversible breakdown of argininosuccinate into arginine and fumarate. It is a 470-amino acid long protein known to form tetramers in its quaternary association. The precise understanding of the catalytic mechanism followed by this family of proteins has been hindered by the lack of competent substrate or product bound structures. Here we report the crystal structures of *Mt*ASL in an unliganded form and its complex with the substrate/product at 2.2 Å and 2.7 Å, respectively. The unliganded and liganded proteins crystallise in the orthorhombic space group $P2_12_12_1$ with two and one molecule in their asymmetric units, respectively. Movement of the highly conserved SS-loop and the concurrent movement of a region of C-domain is observed upon complexation. Of the four active sites in the liganded molecule, three were found to contain substrate/intermediate molecules, while one contained the hydrolysed products. We, therefore, have a detailed picture of the active site and this has enabled the precise location of the substrate and product binding along with clear identification of the catalytic residues. In light of this, the catalytic mechanism has been revisited. In addition to this, structure solution in the presence of an uncommon crystal defect, lattice translocation defect, will be briefly discussed.

Conformational heterogeneity of c-terminal domain elicit poly-modal sensitivity in TREK-1 channels: Insights from replica exchange solute tempering simulations

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The mystery of protein folding was believed to be bound within the thermodynamic tenet that a protein folds into a single energetically favorable structure suitable to perform its function. However, the discovery of intrinsically disordered proteins (IDPs) and metamorphic proteins proves an exception to this 'one protein – one-structure – one function' paradigm. These proteins attain multiple conformations, as directed by their stimulants, to perform multiple functions.

TREK-1, a two pore domain potassium channel functions in response to stimuli as diverse as bilayer stretch, pH, temperature, change in lipid composition, lactate, second messengers, and cytoskeleton binding. This 'poly-modal recognition' of TREK-1 is controlled by its c-terminal domain (CTD), the structure of which is believed to be of metamorphic in nature. Here, we utilize a novel computational approach called replica exchange with solute tempering (REST) to explore the conformational ensemble of TREK-1-CTD.

Our results suggest that the CTD indeed adopts multiple conformations that stay in a dynamic equilibrium. In explicit water, the proximal part of CTD adopts predominantly helix/coil structure with occasional transition to beta sheet. Whereas, the distal part of CTD exhibits a range of beta-sheet conformations with 2-9 beta strands. Associating the conformations to the stimuli recognition warrants further studies to be executed under different stimulus.

Structural and biochemical studies of *Bacillus subtilis* BacF in bacilysin biosynthesis

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Bacilysin is one of the antibiotics synthesized non-ribosomally by *Bacillus subtillis* which acts against a wide range of bacteria and fungi. BacF is a fold type I pyridoxal-5-phosphate (PLP) dependent enzyme involved in Bacilysin biosynthesis pathway. BacF catalyse a transamination reaction which is a rate limiting step in Bacilysin biosynthesis pathway due to its stereospecificity. The crystal structure of *Bacillus subtillis* BacF has been determined in its native as well as ligand bound (L-Tyrosine, Glycine) forms. The structure reveals the presence of PLP in the form of internal aldimine inside the active site pocket. The ligand (L-Tyrosine) bound structure suggests the geometry of the active site residues and their respective role in catalysis. In one of the crystal structure of BacF obtained after soaking with the substrates (L-Phenyl alanine and 4-Hydroxy phenyl pyruvate) reveals the presence of the product (L-Tyrosine) inside the enzyme active site pocket indicating that the crystallization of a functionally active enzyme. Thereby this study can help to understand Bacilysin biosynthesis pathway

Rethinking hydrogen bond and the influence of local environment in protein stability.

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Hydrogen bonds play a crucial role in maintaining the protein structure and its importance cannot be overemphasized. The energy of a hydrogen bond contributes around 5-6 kcal/mol for the isolated bond to 0.5-1.5 kcal/mol for proteins in solution. However, this contribution is context dependent and is rather difficult to assess. Although few approaches have been used to study the contribution of hydrogen bonds by replacing amide bond of interest in a protein with an ester or an olefin moiety, but this mutation eliminates a H-bond donor or both H-bond donor and acceptor. Our goal is to gain a better understanding of hydrogen bond strength with minimal perturbation of its donor and acceptor capability. Thus, we targeted the solvent exposed site of the Pin1 WW domain, a small β-sheet protein, and replaced individual backbone amide bonds with thioamide, a better H-bond donor. Circular dichroism spectroscopy data show that all the O to S (amide to thioamide) mutated variants fold into the same β-sheet structure as the wild type. Further thermodynamic studies of O to S mutated protein variants revealed that solvent shielded hydrogen bonds as opposed solvent exposed ones has greater stabilizing effect. With a single atom substitution, we could achieve around 15 ºC enhancement in thermal stability (T_M) compared to the wild type. Thus, this simple strategy via a single atom substitution (O to S) of the peptide bond can not only be a great tool to understand the folding kinetics and thermodynamics of proteins but also to engineer thermodynamically stable variants via native chemical ligation.

Possible effects of synonymous mutations on mRNA structure

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CcdA is the antitoxin component of the E. coli type II toxin- antitoxin system, ccdAB. CcdB is the cognate toxin, involved in F- plasmid maintenance and bacterial drug tolerance. It is typically bound to CcdA. When free, CcdB causes cell death by poisoning DNA Gyrase. A natively unfolded region of CcdA can rejuvenate Gyrase by actively dissociating it from CcdB, and thus help in bacterial cell survival. We have observed that synonymous mutations at multiple positions in CcdA result in a loss of function phenotype, leading to cell death. In the present study, the structures of such ccdRNAs with single synonymous codons were studied by inline probing. The mutants studied were the inactive mutants T8_ACU and L16_CUG, which caused bacterial cell death and the double mutant, T8_ACU/L16_CUG, where the synonymous codons mutually suppressed the inactive phenotype, resulting in bacterial cell survival. We probed a 150 nucleotide *in vitro* transcribed RNA covering a region of 24bps upstream the translation start site till the amino acid 41 of the CcdA gene containing T8_ACU, L16_CUG and T8_L16 synonymous codons and the corresponding WT ccdRNA by In-line probing. We observed that the putative Shine-Dalgarno sequence is unstructured in all the mutants. The individual inactive mutants show gain of secondary structure in the general vicinity of the respective mutations which is lost in the corresponding double mutant, suggesting that changes in RNA structure in inactive mutants likely affect translation of the ccdA gene, in turn affecting the ratio of the ccdA and ccdB gene products.

Probing Structures and binding specificities of MazE and MazF proteins from *Mycobacterium tuberculosis*

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The *M. tuberculosi*s genome harbours nine TA systems that are members of the mazEF family, unlike other prokaryotes which have only one or two. The mazEF operon encodes the labile antitoxin MazE and the stable toxin MazF. Each *M. tuberculosi*s MazF toxin (MazF-mt1 to MazF-mt9) appears to recognise and cleave a unique RNA sequence which is also structurally distinct. Although the overall tertiary fold of these MazFs are predicted to be similar, it is unclear how they then recognize very structurally different RNAs. We have used SEC-MALS to probe the oligomerization status of the individual toxins, antitoxins and their respective complexes. Nano-DSF was carried out to further understand the relative stabilities of the above molecules, employing intrinsic tryptophan or tyrosine fluorescence. Finally, binding studies have been carried out using MST to understand the relative affinities between the cognate and non-cognate toxin–antitoxin partners. We also describe the preliminary steps of a simple and efficient method for accurate mapping of protein-protein interaction sites of the mazEF TA systems. This method involves screening a panel of purified cognate proteins or peptides (toxin/antitoxin) against a panel of chemically masked single cysteine mutants of its interacting partner displayed on the surface of yeast cells. Such libraries would have much lower diversity than those generated by saturation mutagenesis, simplifying library generation and data analysis. Further, because of the steric bulk of the masking reagent, labeling of virtually all exposed epitope residues will result in loss of binding and buried residues will be inaccessible to the labeling reagent. The binding residues are deciphered by probing the loss of binding to the labeled cognate partner by flow cytometry.

Structural and mechanistic insights into T7 secretion system ESX-1 AAA+ ATPase protein EccA1

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EccA1 is a vital component of type VII secretion system (T7SS) which is involved in transport of important virulence factors. The T7SS machinery is ~1500 kDa complex composed of a membrane pore and associated proteins including membrane-associated and cytosolic ATPases that are thought to provide the energy for transporting protein cargoes across the membrane. The most intensely studied region, ESX-1, includes the Rv3868 gene encoding the AAA+ ATPase EccA1 which has been shown to interact with CFP-10: ESAT-6 heterodimer as a substrate.

EccA1 is surprisingly also involved in the optimal synthesis of mycolic acids, integral cell-envelope lipids. EccA1 mutants showed decreased in vivo virulence and intracellular growth. Hence, two mycobacterial virulence hallmarks, ESX-1-dependent protein secretion and mycolic acid synthesis, are critically linked via EccA1.

EccA1 protein was expressed and purified by Ni^{++} NTA affinity purification followed by size exclusion chromatography. MALDI TOF analysis confirmed the identity of the protein. The protein showed salt concentration dependence and showed high hexameric populations at low salt condition as revealed by size exclusion chromatogram. Circular dichroism was used to assess the structural changes on binding with ATP. Preliminary bioinformatics studies revealed that Rubisco activase is the closest homolog to EccA1, sharing 46% identity to C-terminal domain of EccA1. Negative stain EM revealed that EccA1 alone forms amorphous chain like structure. In presence of ATP, it forms long fibrillar structure. However, in presence of both ATP and its substrate, it forms hexameric ring like structure as revealed by negative stain 2D class averages. This behaviour is very similar to its closest homolog Rubisco activase. Presently, we have collected preliminary cryo EM images by Talos Arctica 200kV (IISc Bengaluru) and we are optimising the conditions for cryo EM. The unravelling of mechanism of transport of substrates by EccA1 and its structural elucidation will pave the way for better understanding of this pathway which remains to be deciphered.

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Stochastic model to probe infection heterogeneity and fitness function of Hepatitis C Virus

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Hepatitis C virus (HCV) infection is a global health problem. It is reported that about 2 million of the world population is affected annually and most of those infected develop chronic liver disorder. HCV belongs to the family of positive sense single stranded RNA (+ssRNA) viruses, *Flaviviridae*. Due to the nature of genome, the same substrate, +ssRNA, is required for translation, replication and virus assembly. In addition, virus induces re-organization of host membranes, to form vesicular structures where viral genome replicates. We propose a cellular model that includes these viral processes, to describe infection due to +ssRNA viruses.

A system of coupled ordinary differential equations was used to model the time evolution of various viral molecular species in the cell. Key assumption used in the model: viral genome and polymerase interact to form replication complex, but the number is limited by the host membrane that can be re-organized. This allowed us to build a more concise, mean-field model (compared to existing ones) which quantitatively captures the experimental reports [1]. However, being a deterministic mean field model, it fails to capture infection heterogeneity arising due to low copy number of viral products (~1 to 100) inside the cell during the start of the infection. We observe that random fluctuations in this stochastic regime can significantly alter the viral output.

Using a stochastic model framework and parameters from mean field model, we aim to quantify infection properties like viral infectivity, number and diversity of viral progenies. Such analyses can provide novel insights about (i) viral fitness function(s) and evolutionary trade-offs imposed on the virus, and (ii) how virus evolves effective strategies to sustain infections.

Keywords: +ssRNA viral dynamics, replication compartments, mean field model, stochastic model, viral fitness.

Reference:

[1] Aunins, Thomas R., et al. "Intracellular hepatitis C modeling predicts infection dynamics and viral protein mechanisms." *Journal of virology* (2018): JVI-02098.

Interaction interfaces in toxin-antitoxin systems: Probing relationships among paralogues to evaluate potential for cross-reactivity and understanding the mode of antitoxin action

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Type II toxin-antitoxin systems (TAs) are two-gene modules consisting of protein toxins and antitoxins. Under normal growth conditions, they occur as complex but during adversities, lone toxins bind to cellular targets that are either RNA or enzymes like DNA gyrase, triggering survival responses in bacteria. We employed sequence and structure-based analyses to study 1) the relationships within 80 TA systems in *M. tuberculosis* and evaluate their potential for cross-reactivity 2) the mechanism by which an *E. coli* antitoxin reverts the activity of its cognate toxin. When clustered, TAs group into distinct sub-clusters based on their ability to relate to each other. Over 30 toxins, could relate to 40 other toxins, indicating that many are paralogous. An analysis of the alignments within each toxin sub-clusters showed similarities in their core domains and target-binding sites. Interestingly, evaluation of antitoxin alignments for each sub-cluster showed conservation in their DNA-binding sites. We extended our study to the interfaces between toxins and antitoxins to specifically identify a set of features common to each paralogous group, which can be used a) to design a peptide to modulate TA interaction b) to guide computational modelling of complexes for other TA systems. With this approach, TA complexes were built for 6 non-trivial cases in *M. tuberculosis*. The availability of these models will form the basis to support our ongoing studies of sequence and structural similarities at the predicted interface to explore cross-reactivity amongst the paralogous pairs. Moreover, our studies also identified and computationally characterized the interfaces of two hitherto unknown novel TA systems in *M. tuberculosis* which have also been validated by our bench-lab collaborators. Finally, we have employed normal-mode analysis on the *E. coli* CcdB-CcdA TA system to unveil the mechanism by which binding of CcdA at its interface on CcdB can have substantial effect on the gyrase binding interface.

DNA-damage inducible protein 1 (Ddi1) from protozoans

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DNA damage inducible protein1 (Ddi1) is a multi-domain protein that belongs to the ubiquitin receptor family of proteins, which are generally involved in proteasome-mediated proteolysis of ubiquitinated proteins. It has been found to be involved in various cellular processes.

Interestingly, in AIDS patients receiving HAART (Highly Active Antiretroviral Therapy), it was observed that the treatment also offered protection against parasitic diseases, like leishmaniasis to some extent. Subsequent studies showed that this effect was due to the HIV-PR inhibitors used as drugs present in HAART which target the aspartic retroviral protease-like domain of Ddi1 present in the parasites. This has opened up a new possibility to develop potent inhibitors which can specifically block the active site of Ddi1, thus curing the disease to some extent. A structural understanding of the protease domain is necessary to develop effective inhibitors. Also, structural insights from Ddi1 in complex with known HIV-PR inhibitors will add to the basic understanding of the exact binding modes of the inhibitors to the protein and will help in developing more potent inhibitors.

Structure of the protease domain of Ddi1 from *Leshmania Major* has been determined in our laboratory and its interaction with HIV-PR inhibitors has been established. We are also working on Ddi1 from other pathogens like *Entamoeba histolytica*, *Trypanosoma cruzi* and others.

Characterisation of putative global suppressors in the CcdB toxin from *E. coli*

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Global suppressors are mutations in a protein that can suppress the effects of a number of individual, loss of function, point mutations located in diverse regions of the structure. However, the mechanism(s) responsible for such suppression are not understood. The experimental system of the current study is a 101-residue homo-dimeric protein CcdB (Controller of Cell Death protein B), important in F-plasmid maintenance in *E. coli.* Exhaustive second-site saturation mutagenesis libraries were constructed in the background of certain loss of function, Parent Inactive Mutants (PIMs) which were then displayed on the yeast surface and screened to identify the suppressors by FACS. Three residues R10, E11 and S12 on an exposed loop region of the protein were identified as distal suppressors. The aim of the present study was to experimentally validate whether these mutations can suppress the folding and stability defects of the known PIMs. We have carried out *in vivo* activity assays of the PIMs in the background of the suppressors as a function of repressor and activator concentrations, and measured their solubilities *in-vivo* also. Further we have also measured the relative stabilities of the above mutants using a Thermal Shift Assay. The global suppressors improve thermal stability of the inactive mutants as well as of the wild type protein. The data suggest that such suppressor mutations improve protein foldability and solubility *in-vivo*, as well as thermal stability *in-vitro*. Future studies will probe the effects of these mutations on folding kinetics and folding mechanisms.

Structural and Biophysical Characterisation of Mycobacterial MazEF6 TA Complex

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M. tuberculosis possesses a large number of Toxin-Antitoxin (TA) systems that are known to confer multidrug tolerance, regulate biofilm formation and arrest cellular growth under conditions of stress inside the host cell. They provide the bacterium the ability to persist in a non-replicating state. In the case of the Maz TA systems, structures are known for MazF3,4,6 and 9. Structural analysis of homologous proteins from other bacterial species indicate that the C-terminal regions of the MazE (antitoxin) proteins are intrinsically disordered and helps the protein bind to the cognate toxin. The antitoxin dimerizes via its N-termini. Solving the structure of the toxin bound to the antitoxin can lead to the structural basis of inactivation of the toxin. While the mRNA bound form can give a detail account of its endoribonuclease activity. We have undertaken structural and biophysical studies of MazEF6 TA system. The MazE6 protein exhibited discernible temperature dependence of chemical shifts of residues in the C-terminal region. A strategy was devised to study the structure and conformation of N-terminal deletion mutant (D1-46, MazE6c) of the antitoxin in the free and toxin bound forms. Near complete sequence specific assignments for backbone 1H, 13C and 15N nuclei of the protein in the free form have been obtained from triple resonance experiments. Analysis of secondary chemical shifts and {1H}-15N heteronuclear NOE data indicate that MazE6c is structurally disordered. Superposition of spectra of MazE6 and MazE6c show that the C-terminal region is disordered in the full length protein too. MazE6c shows increase in chemical shift dispersion upon binding with MazF6 suggesting gain in secondary structure content. Structure calculation of MazEcF6 complex is underway.

hnRNPA1 mediated specific recognition and unfolding of DNA G-quadruplexes

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hnRNPA1 is a modular ribonucleoprotein with two RNA Recognition Motifs (RRMs) (collectively called the UP1), an RGG-box, and a C-terminal region that contains the nuclear localization sequence. It is involved in RNA transport, trafficking, alternative splicing, and telomere DNA maintenance. hnRNPA1 interacts with ss and G-quadruplex DNA as well as RNA structures and also possesses DNA G-quadruplex unfolding activity. Using both X-ray and NMR spectroscopy methods, the UP1 domain has been studied previously for its structure and interaction with the DNA and RNA substrates in detail. The crystal structure of UP1 with single-stranded telomeric DNA has been solved, where two protein molecules were shown to form a complex with the single stranded DNA in an anti-parallel conformation. In solution, however, the two RRMs interact with each other and the relative orientations of them have been shown to resemble the nucleic acid bound form rather than the free form. The G-quadruplex unfolding mechanism of the hnRNPA1 is not fully understood. In this study, using CD, fluorescence, and NMR spectroscopy and ITC methods, the interaction and unfolding of telomeric DNA G-quadruplexes by hnRNPA1 domains was studied. Structure specific interaction of hnRNPA1 with DNA G-quadruplexes has been observed. Mutation studies have also been carried out to confirm these results. These results provide insight into the recognition, affinity, and destabilization of DNA G-quadruplex structures by hnRNPA1. Our results provide a comprehensive picture of hnRNPA1 interaction with telomeric G-quadruplex structures.

Functional annotation of Putative *fad*E9 as Isobutyryl-CoA dehydrogenase in *Mycobacterium tuberculosis*

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Members of the Acyl-CoA dehydrogenase (ACADs) family of enzymes play a crucial role in cholesterol and steroid catabolism and are widely studied in the oldest known human pathogen, *Mycobacterium tuberculosis* (*Mtb*). However, there is a paucity of information on ACADs involved in branched chain amino acid catabolism. Here we characterized one of the putative ACAD enzyme, *fad*E9, as "Isobutyryl CoA Dehydrogenase (IBDH)" using a combined computational and experimental approach, guided by homology modeled structural information, affirming its role in valine catabolism. Multiple sequence alignment and phylogenetic analysis place it in a separate cluster from a recently identified family of *α2β2*-heterotetramer ACADs in *Mtb*, based on the position of the conserved Arg247 and catalytic Glu368 residues. The conserved Arg247 was predicted to play an essential role at the center of H-bonding network of reaction centre and was confirmed by the reduced activity of R247K mutant. Thus, in addition to the finding of an architecturally distinct *α2β2*-heterotetramer amongst ACADs, these studies also highlight the differences between *Mt*IBDH, *fad*E9 from the other ACADs that are involved in cholesterol and steroid catabolism of *Mtb*.

Exploring CH-π 'hydrogen bond' in the context of protein stability and engineering protein-ligand interaction

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Three dimensional structure of a protein is stabilized by coordination and interplay between a number of weak non covalent interactions (NCIs) like hydrogen bond, van der Waals interaction, salt bridge, cation-π, X(C/N/O)H-π interaction, etc. CH-π interaction is one such type of weak, non canonical hydrogen bond like force where the aliphatic or aromatic –CH group and the aromatic π system of amino acids, falling within some specific geometrical cut-offs, act as the hydrogen bond donor and acceptor respectively. Each CH-π hydrogen bond is associated with ~1.5-2.5 kcal/mol stabilization energy. In order to understand this subtle but vital force, we first assessed the role of CH-π interaction in the strands of a well established β hairpin model system. A series of substitutions were done at two non-hydrogen bonded positions in the strand and their free energy contributions to the overall foldedness of the peptides were calculated. D-Pro-Gly (type II' turn) motif was kept constant across all the analogs. From this study we could find the energetically favorable cross-strand donor acceptor pair (Leu-Trp). There was -1.12 kcal/mol gain in free energy in this variant as compared to the wild type. The principle technique used for this analysis was NMR. Further we wanted to explore how this interaction plays a role in tuning the stability of the miniprotein Pin1 WW domain. Hence, we targeted a favorable solvent exposed site on the protein for incorporating various donor-acceptor pairs (Leu-Trp, Ile-Trp, Val-Trp) and monitored the stability profiles through thermal denaturation. Finally, we aim to design potent activators of the enzyme Protein Phosphatase–I utilizing this interaction *i.e.* by engineering suitable donor-acceptor pair between the protein and its ligand.

Mutational Studies of Toxin-Antitoxin Systems

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A growing body of evidence has shown that synonymous mutations within the gene can significantly affect protein function due to various mechanisms. Our lab has shown that even single synonymous mutants have a drastic effect on phenotype, at least in the system studied, CcdA. Since the CcdAB system has an observable phenotypic read-out of cell death versus cell growth, we attempt to generate a directed synonymous mutant library of all the positions of the c*cdB* gene to study the effect of single-site synonymous mutants in the operon context with respect to survival of mutants in resistant vs sensitive strain. A resistant strain is a strain resistant to toxin activity whereas a sensitive strain is a strain sensitive to toxin activity. To study the mutational sensitivity of ~1600 single site mutants generated by saturation mutagenesis in the native operon context, we subcloned a CcdB saturation mutagenesis NNK library into its native operon

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Structural analysis of LnmI domain of leinamycin biosynthesis gene cluster of *Streptomyces atroolivaceus*

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Polyketides and nonribosomal peptides, including polyketide–nonribosomal peptide hybrids, are the secondary metabolites produced by the different microorganisms. These polyketides exhibit broad biological activities, and include some of the most important clinical drugs. While remarkably diverse in structure, the biosynthesis of these natural products is atleast conceptually, featuring modular polyketide synthases (PKSs), nonribosomal peptide synthetases (NRPSs), or PKS–NRPS hybrids.

The complex and highly functionalized molecular scaffolds of polyketides, nonribosomal peptides, or hybrids are derived from simple building blocks, for example, acyl-CoAs, amino acids, or both, which are activated, incorporated, and further modified as needed by dedicated PKS and NRPS domains and modules within the biosynthetic machinery. So by applying the combinational biosynthesis, we can engineer the biosynthetic machinery of every family of natural products for the production of novel analogs.

Leinamycin is one of these natural products which was first isolated from *Streptomyces atroolivaceus* S-140 in 1989, features a unique 1,3-dioxo-1,2-dithiolane moiety that is spirofused to an 18-membered macrolactam ring. Upon reductive activation in the presence of cellular thiols, LNM exerts antitumor activity by an episulfonium ion-mediated DNA alkylation, a mode of action that is unprecedented among all DNA-damaging natural products. Therefore, LNM has been pursued as a promising anticancer drug.

The NRPS domain of LnmI was successfully expressed and purified by Ni^{++} NTA affinity chromatography followed by size exclusion chromatography.LC-MS analysis confirmed the identity of the protein. Preliminary results by negative staining show a homogenous population of particles. We are presently working on generating 2D reference free class averages by EMAN and RELION. Simultaneously, we are trying to optimize the conditions for cryo EM which will help us to get insights into the mechanism of this novel pathway of leinamycin biosynthesis. The unravelling of architecture, organisation and mechanism of LnmI domain of leinamycin biosynthesis from Streptomyces atroolivaceus S-140 by cryo electron microscopy will assist in designing bioengineering approaches to understand the mechanistic insight into this novel pathway.

Dissection of protonation sites for antibacterial recognition and transport in QacA, a multi-drug efflux transporter

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QacA is a drug:H^+ antiporter (DHA2) with 14 transmembrane helices, that renders antibacterial resistance to methicillin-resistant *Staphylococcus aureus* (MRSA) strains, with homologues in other pathogenic organisms. It is a highly promiscuous antiporter, capable of H^+-driven efflux of a wide array of cationic antibacterial compounds and dyes. Our study, using a homology model of QacA, reveals a group of six protonatable residues in its vestibule. Systematic mutagenesis resulted in identification of D34 (TM1), and a cluster of acidic residues in TM13 including E407 and D411 and D323 in TM10, as being crucial for substrate recognition and transport of monovalent and divalent cationic antibacterial compounds. The transport and binding properties of QacA and its mutants were explored using whole cells, inside-out vesicles, substrate-induced H^+ release and microscale thermophoresis. We identify two sites, D34 and D411 as vital players in substrate recognition while E407 facilitates substrate efflux as a protonation site. We also observe that E407 plays a moonlighting role as a substrate recognition site for dequalinium transport. These observations rationalize the promiscuity of QacA for diverse substrates. The study unravels the role of acidic residues in QacA with implications for substrate recognition, promiscuity and processive transport in multidrug efflux transporters, related to QacA.

Biochemical characterization of *Mycobacterium tuberculosis* LexA and structural studies on its C-terminal segment

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LexA is a protein involved in SOS response. The protein from *M. tuberculosis* and its mutants have been biochemically characterized and the structure of its catalytic segment determined. The protein is made up of an N-terminal segment, which includes the DNA binding domain, and a C-terminal segment encompassing much of the catalytic domain. The two segments are defined by a cleavage site. The full-length LexA, the two segments and point mutants including two involving changes in active site residues were cloned and purified. The wild-type protein autocleaves at basic pH while the mutants do not. The wild-type and the mutant proteins dimerise and bind DNA with equal facility. The C-terminal segment also dimerises, with a tendency to form tetramer as well. Attempts to crystallise the N-terminal segment were not successful, while the C-terminal segment readily crystallised. Crystals obtained from attempts involving the full-length protein and its mutants contained only the C-terminal segment involving the catalytic core and a few residues preceding it, in a dimeric or tetrameric form, indicating protein cleavage during the long period involved in crystal formation. Modes of tetramerisation of the full-length protein similar to those observed for the catalytic core are feasible. A complex of *Mt*LexA with *M. tuberculosis* SOS box could be modelled, wherein the mutual orientation of the two N-domains is different from that in the *Ec*LexA-DNA complex. These results represent the first thorough characterization of *Mt*LexA and lend definitive information on its structure and assembly. They also provide leads for further exploration of this important protein.

Measuring Bacterial Cellular Heterogeneity

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Traditional bulk experiments fail to record cellular heterogeneity which may arise due to intrinsically stochastic gene expression patterns. This phenomenon is particularly noted in promoter systems at low inducer concentrations, leading to generation of sub-populations with different levels of gene products.

To probe this phenomenon, we study a fast degrading version of GFP, which is better in capturing protein dynamics as compared to the long lived wild-type version. The fd-gfp gene was cloned under the arabinose inducible promoter of the arabinose operon (*araBAD)* which is reported to be a tightly regulated and modulated system. It is hence commonly used for controlled exogeneous gene expression in a series of expression vectors (pBAD). Single cell studies have shown that the arabinose expression vectors have an all-or-none response at intermediate levels of induction [1]. Initially attributed to the heterogeneity in the expression of the transporter AraE [2], this phenomenon has now been noted at intermediate arabinose concentrations in non-arabinose metabolizing cells, with a constitutively expressed transporter.

While the cells showed heterogeneity in their GFP levels, bulk level mRNA quantification showed similar transcript levels between GFP-positive and GFP-negative cells (Chetana Baliga, unpublished data).

To determine whether the observed protein heterogeneity stems from transcript level, which are not detected in bulk experiments, we decided to adopt a single molecule RNA quantification technique, smRNA-FISH wherein multiple Cy5 labelled probes are used against the same mRNA, with simultaneous GFP imaging to estimate RNA and protein levels in the same cell.

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Quantifying degeneracy and complexity in membrane lateral organization: Lattice Model of Lipid Bilayers

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Rich structural complexity of bio-molecules gives rise to degeneracy of great functional relevance [1]. Here we focus on degeneracy in lateral organization of the membrane, which plays a key role in processes such as signal transduction, pathogen intake and assembly of protein complexes.

Variety of lateral organization on the membrane surface occur due to preferential segregation and clustering of certain types of lipids and proteins due to their differential inter- and intra-molecular interactions. In this work, we explore the degeneracy in organization of membrane using tools from simple statistical mechanics theories. We develop a physics-based Hamiltonian for membrane organization using long atomistic trajectories on systems that exhibit liquid-ordered and liquid-disordered (L_o/L_d) coexistence. The three atomistic membrane systems with their fractional composition, chosen for this work, are listed below: (i) DPPC/DOPC/Chol (0.37/0.36/0.27) (ii) PSM/DOPC/Chol (0.43/0.38/0.19) (iii) PSM/POPC/Chol (0.47/0.32/0.21). These systems have very different molecular-level substructures and unique Lo/Ld interface boundaries [2,3].

We evolve the Hamiltonian, written as a function of degree of non-affinity in topological rearrangement of the lipids ($\chi 2$) [4], using Monte Carlo (MC) algorithm to recapitulate the lateral organization in the above-mentioned AA systems [5,6]. We find that PSM/POPC/CHOL system, which is physiologically more relevant amongst the three compositions, has the highest degeneracy.

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Stabilizing HIV-1 Envelope glycoprotein and novel Cyclic Permutant gp120 immunogens of Clade A,B and C

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A vaccine for HIV-1 is long sought but remains elusive till date. The envelope protein gp160 of HIV-1 is the most immunogenic component on the viral envelope due to its abundance. The inherent metastability of the Env protein makes it unfeasible to use the Env protein as a immunogen in triggering broadly neutralizing antibodies. We sought to stabilize the ectodomain of HIV-1 Env gp140 by consensus sequence and structure guided Rosetta computational thermostabilization. Three designs were predicted to be thermostabilizing and were used for biophysical and antigenic characterization. One of the engineered immunogens had improved yields and thermal stability without altering the antigenic properties of the native ectodomain protein BG505SOSIP.664 gp140. In addition, the results from our previous work on the novel cyclic permutant gp120 trimers in clade B JRFL motivated us to extend this novel design strategy. We are testing the design applicability in two of the dominant strains of HIV-1 clade A BG505 and clade C 16055. All the three clades of cyclic permutant gp120 immunogens have shown similar thermal stability of ~73°C and binding to various broadly neutralizing antibodies, including quarternary epitope specific bNAb PGT145,PGDM1400. These promising immunogens are being tested for immunogenicity in small animal immunizations.

Specificity of folds in protein-protein interactions

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Specificity in the interaction between proteins is determined by the spatial and temporal expression of proteins in the cell and the chemical and geometrical complementarity of the surfaces of the proteins. Based on the analysis of 3-D structures of protein-protein complexes available in the protein data bank (PDB), we propose that a protein domain that adapts a particular fold is selective in its interaction with protein(s) of certain folds. Only 0.1% of the theoretical possible associations between folds are observed in the dataset of protein-protein complexes with known structure. Some folds (~39%) are observed to interact only with a protein domain of the same fold, irrespective of the sequence identity between the interacting protein domains. 16% of the folds interact with more than five folds and termed as gregarious folds. The folds that are observed to be non-compatible in the protein-protein association studies are observed to be enriched in the dataset of proteins known not to interact with each other. Similar trends were seen for fold-fold interactions derived from BioGRID, a sequence database of protein-protein interactions and the PDB dataset; implying that observations in PDB are not due to the limited number of structures available for protein-protein complexes. These findings suggest that the specificity between proteins can be adjudged based on their folds and like the tertiary structure of proteins, folds of protein assemblies are limited. In addition, every fold-fold interaction uses a finite structural space for relative spatial orientation of interacting folds. The deductions from this work on specificity of folds in protein-protein interactions and preferred orientation of folds in the protein assemblies will find use in partitioning large-scale datasets of putative protein-protein interactions for true and possible protein-protein associations with clues to their potential orientation with respect to each other as well.

"Old Drugs, New Tricks": Drug Repurposing approach to identify new Anti-candida agents

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The current public health burden caused by invasive fungal infections, especially by *Candida albicans,* is becoming an unmet medical threat due to emergence of anti-fungal drug resistance. The problem is further aggravated by limited availability of safe anti-fungal drugs. Indeed, this calls for search of novel and safe anti-fungal agents. Concomitantly, several research groups have sought to improve treatment via different strategies such as combinatorial therapy or boosting host immune response through vaccines. In the current work, we have exploited an *in-silico* drug-repurposing strategy to identify potential anti-candida agents from the existing repertoire of FDA-approved drugs by means of comparative analyses of targets of approved drugs and proteins of *C. albicans.* We identified 26 approved drugs with anti-fungal potential that can be repurposed against 31 protein targets of *C. albicans.* Through chemical similarity search, we identified 33 additional set of approved drugs that may have an inhibitory effect on *C. albicans.* Our analyses suggest that while some of the drugs identified in this study could be used as polypharmacological agents, few others may serve as effective tools for combinatorial therapy in the treatment of infections caused by *C. albicans*. The drug-target associations identified in this work using a chem-bioinformatics approach have the potential to be carried forward for further experimental investigations related to anti-fungal drug repurposing and development pipeline.

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Molecular mechanism behind inactive phenotype of synonymous mutations in CcdA

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While studying the phenotypic effects of single point mutations in the bacterial antitoxin, CcdA, several synonymous mutations exhibited inactive phenotype in *E coli*. To decipher the molecular basis of the detrimental effects of synonymous substitutions, the *ccdA* gene was expressed on yeast surface and binding with cognate partner, CcdB was studied by FACS. Result suggested that proteins, expressed from synonymous mutation carrying constructs, bind CcdB similarly as wild-type, in yeast surface display system and therefore should be functional. On the contrary, CcdA with non-synonymous mutations in active residues showed slightly decreased CcdB binding. This indicates that the inactive phenotype observed for synonymous mutants in the operon context is not due to alteration of protein structure or function. The level of functional protein or the ratio of CcdA: CcdB in cell could be a more important determinant of phenotype. A study of relative amounts of the proteins *in vivo* demonstrated perturbation in the CcdA:CcdB ratio in case of inactive synonymous mutants with respect to wild-type, which would explain the inactive phenotype. Analysis of levels of CcdA and CcdB segments of the mRNA revealed that synonymous CcdA mutations result in high levels of mRNA for both the gene segments, though the ratio remains close to one as for the wild-type. This likely results from the altered structures in the mutated mRNAs that alters the relative translation efficiency of each gene in the polycistronic ccdAB operon, ultimately lowering the ratio of expressed CcdA and CcdB proteins in cell.

Bacterial small heat shock protein

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Bacteria survive in diverse environmental stresses such as fluctuations in pH, temperature, nutrient scarcity, etc. These specific stress conditions trigger adaptive responses and upregulate expression of certain genes like Heat Shock Proteins (HSP). Among HSPs, the most prominent ones, which have been detected in virtually all types of organisms are the small Heat Shock Proteins (sHSPs).

sHSPs are a family of ATP-independent molecular chaperones with molecular masses ranging from 15 to 42 kDa. They are oligomeric stress proteins characterized by an α-crystallin domain (ACD) surrounded by a highly variable N-terminal arm and a flexible C-terminal extension. They are usually polydisperse and change oligomeric organization and size on exposure to stress and when interacting with substrate. They are often polydisperse existing in a variety of oligomeric forms with different abundance reflecting a high dynamism in quaternary structure as a result of rapid and extensive subunit exchange between oligomers. Mutations in sHSPs are associated with a variety of severe diseases, including myopathies, dystrophies, and cataracts.

Pathogenic bacteria like *Staphylococcus aureus* experience stress from their initial moment of contact with the host. *S. aureus* has a single sHSP and to understand its role we have cloned and purified the sHSP and carried out biophysical and biochemical characterization. They form high molecular weight oligomers and display high structural heterogeneity and dynamics in response of changing conditions. Substrates such as lysozyme, citrate synthase and malate dehydrogenase are used to understand the anti-aggregations activity of this protein.

Clusters and Networks in Alpha/Beta Hydrolases

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Alpha/beta hydrolases are well known for their diversity in function. Neither very less experimental information nor their poor sequence similarity could stop appreciating them. It is also known that the superfamily can be finely divided into subfamilies based on the sequence or structural homologs. Apart from this division it is also possible to treat and view them as clusters and networks based on their structural, functional and other significant features when they are mapped to information from multiple studies and databases.

We analyzed the superfamily and domains connecting diverse information such as SCOPe, Gene Ontology, PDB, CATH, EC number etc. We discovered some members of the superfamily/family come together based on the properties they share with their peers. We also followed a top-down and bottom-up analyses of the family/domain/members and we discovered the behavior is observed both at inter and intra levels.

We further expanded our thoughts to view the concept and information as networks/nodes/edges. We discovered some domains/properties acting as hubs among them that share the information with large number of domains. The clusters and the networks vary based on information or property analyzed. Information treated as edges are given weightage based on the level and shared density.

We also cluster and derive networks based on the disease associated with the members of the superfamily. The disease biology approach can provide better information about the superfamily and the domains based on this approach. Based on the integrated approach it is possible to view the diverse information associated with the superfamily as clusters and networks. Clusters and networks can provide better insight about the superfamily and the domains to delve into their details. Viewing the diverse information as clusters and networks are more convenient for the human eye instead of viewing them as tables and databases.

Structural and functional studies on Omega subunit of RNA polymerase

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Silent mutations are mutations that affect the DNA and RNA information, but the encoded protein sequence remains the same due to the degeneracy of genetic code. Till recently, silent mutations were considered to be of no importance to protein structure and activity. However, several recent studies have shown the relevance of silent mutations in alteration of protein structure and function. Omega (ω) is the smallest subunit of RNA polymerase and is encoded by the *rpoZ* gene. It is a 91 amino acid containing protein and is intrinsically disordered. To elucidate the function of ω, *Sarkar et al*. generated several dominant lethal mutants of the protein. This included a mutant ω6 carrying a N60D mutation, which was shown to be helical. ω6 containing RNAP was defective in transcription initiation due to its effect on plasticity of RNAP. Surprisingly, they also discovered a silent mutant ω9 which was helical and dominant lethal. So, we hypothesized that the change from an intrinsically disordered to an ordered structure might be responsible for the lethal phenotype. We attempt to address how despite of having the same amino acid sequence, this silent mutant acquired a higher helical content using CD. *In vitro* transcription assay showed that structured mutants like ω6, ω9 and ω7 were transcriptionally defective. Unstructured conformation of ω is therefore important for RNAP function. Furthermore, ITC studies would provide an estimate of binding affinity of the silent mutants to RNAP, and cryo-electron microscopy model of RNAP containing ω silent mutants would provide a better insight on the structural functional relationship of ω subunit in RNAP.

Deriving Functional Networks from Structural Normal Mode Analysis: Flaviviridae NS3 as a case study

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Flaviviridae NS3 protein harbors a SF2 RNA helicase domain that hydrolyses ATP to fuel RNA unwinding. Functional coupling between active sites of the helicase, viz. the ATPase and RNA binding sites drives enzyme's unwinding efficiency. The current work is a comparison of NS3 helicases from Hepatitis C Virus, Dengue Virus and Zika virus using dynamical analysis of protein contact network. Normal mode analysis (NMA) can capture large structural deformations of proteins, corresponding to time-scales relevant to biological processes (e.g. RNA-protein binding). Using the NMA derived trajectories, weighted energy contact networks (ECN) were produced for the NS3 helicase proteins. Betweenness centrality, a robust network measure was used to identify key residues that are likely to play an important role in cross talk between ATP- and RNA-binding sites. Our study finds the ECN to be significantly different between the NS3 helicases among the viruses potentially alluding to the differences in the RNA interaction and unwinding activity. We propose a unique node and edge betweenness measure for a given paired set of vertices in a network, wherein the vertices represent residues defining the biologically active sites. Though the network structure across modes varies, inter-site betweenness centrality is conserved across all normal modes. This correlated motion could be responsible for strong cross talk between the sites. Using network-based analysis, we compare various features of the Flaviviridae NS3 such as local substructure, the effect of the NS3 protease on helicase function, and the categorizing mutations that may disrupt helicase activity.

Structural studies of antibiotic efflux transporter NorC from *Staphylococcus aureus*

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NorC of *Staphylococcus aureus* is a 14-transmembrane containing transporter protein belonging to major facilitator superfamily. Transporter proteins are integral membrane proteins that regulate the movement of biomolecules across the plasma membrane and play a key role in driving and regulating physiological processes that are essential for maintaining cellular homeostasis.

NorC was reported to be involved in providing multi-drug resistance to methicillin resistant *S. aureus* (MRSA) strains against quinolone class of drugs. Our work aims to elucidate the structure of this transporter and undertake its functional characterization. Integral membrane transporters are challenging molecules to crystallize given the high levels of conformational heterogeneity and minimal solvent-exposed surface area that impedes formation of lattice contacts, a prerequisite for crystal formation.

To overcome this hurdle, we have used single chain Indian camelid antibody (ICab) as crystallization chaperone.

In this work, we cloned and expressed NorC in *E.coli* and successfully purified the protein. We immunized an Indian camel with NorC and isolated NorC-specific ICabs from the cloned antibody library using yeast surface display platform, and expressed those in *E.coli*. Further, we purified the ICabs and characterized their binding with NorC using ITC. To gain structural insights on ICab, we crystallized one of them and determined its structure. We then used this ICab as a crystallization chaperone and successfully crystallized NorC-ICab complex. The crystals diffracted to a resolution of 4.25 Å. Our current efforts are focused on improving the resolution of this complex.

Structural studies on MazEF9 toxin-antitoxin system from *Mycobacterium tuberculosis*

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Toxin-antitoxin (TA) systems, unusually abundant in *Mycobacterium tuberculosis,* contribute to its ability to persist in a drug-tolerant latent state. In Mtb, the MazEF TA system belongs to the Type II class, wherein, under favourable growth conditions, the protein antitoxin (MazE) binds to its cognate toxin (MazF) forming an inactive complex, that dissociates in response to stress, thereby allowing the toxin to affect protein synthesis and consequently, bring about growth arrest. Our interest lies in determining the structures of the MazF9 toxin, the MazE9 antitoxin, and the MazEF9 complex, using nuclear magnetic resonance spectroscopy.Like in other TA systems, the MazE9 antitoxin protein is intrinsically disordered in the C-terminal region, which is expected to bind to and inactivate the toxin. This prompted the synthesis of the N terminal del(1-42) deletion mutant(MazE9c), to overcome issues associated with the large size of the complex. Proton NMR spectra of the full length MazE9 shows limited chemical shift dispersion in the amide and aliphatic regions. Low intensity resonance lines are observed close to 0.0 ppm, indicating the possibility of conformational exchange between the disordered and ordered forms. MazF9 on the other hand, shows several resonance lines at chemical shifts > 8.8 ppm and < 0.4 ppm, indicating a well structured protein. Backbone assignments have been made for MazE9c using triple resonance NMR experiments. The monomeric C terminal disordered tail of MazE9 antitoxin, along with the MazF9 toxin will be used to reconstitute the complex *in vitro*. This will make structural studies by NMR more feasible.