# Nu Biophysical Society presents

# From Molecules to Behaviour

## MBU In-house Symposium 2014

## Abstract Booklet

**Molecular Biophysics Unit**

**Indian Institute of Science**

**Bangalore 560012**

**India**

# Table of Contents

## Molecular Biophysics

Molecular Biophysics is an interdisciplinary science encompassing Biology, Physics, Chemistry, Engineering, Math and Computation. Integrating theory and experiments, it aims at understanding biological systems at various levels. Understanding the physical and chemical principles underlying life forms has been the impetus for many scientists and this has propelled this field over the decades.

## Molecular Biophysics Unit at the 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 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 research publications exceeds 1000. Seven faculty members have received prestigious S.S. Bhatnagar prize, eight members are fellows of the Indian Academy of Sciences and seven members are fellows of the Indian National Science Academy.

## Areas of Research at the Unit

- Protein folding and dynamics
- Computer modeling and dynamics of biological molecules
- Unusual DNA structure 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

# MOLECULAR BIOPHYSICS UNIT In-House Symposium

*Life Sciences at the intersection of Physics, Mathematics and Chemistry*

**Program** **Date**: 14 June, 2014 **Venue**: Faculty Hall

**8:45 – 9:00 AM Chairman's Address Prof. RaghavanVardarajan (MBU, IISc)**

**9:00 – 10:00 AM Plenary Lecture 1 Dr. Vatsala Thirumalai, NCBS**

**Title: Mind the gap: Gap junctions and neural circuit assembly in larval zebrafish**

**Student Talks (Session 1) 10:00 AM – 10:45 AM Chairpersons: Nilesh Aghera & Sufyan Ashhad**

| | | |
|---|---|---|
| 10:00 – 10:15 | Krishnayan Basuroy | Prof. P. Balaram's group |
| 10:15 – 10:30 | Karuna Dixit | Prof. Siddhartha P. Sarma's group |
| 10:30 – 10:45 | M. Saranya | Dr. Rahul Roy group |

**Tea Break and Poster Session 1 (10:45 AM - 11:30 AM)**

**Student Talks (Session 2) 11:30 Noon – 12:45 PM Chairpersons: Anurag Pandey & Thyageshwar C**

| | | |
|---|---|---|
| 11:30 – 11:45 | S M Arif | Prof. M. Vijayan's group |
| 11:45 - 12:00 | Debanjan Dasgupta | Prof. Sujit K. Sikdar's group |
| 12:00 – 12:15 | Kumar Perinbam | Prof. B. Gopal's group |
| 12:15 – 12:30 | Geeta Deka | Prof. M. R. N. Murthy's group |
| 12:30 – 12:45 | Deivanayaga Barathy V | Prof. K. Suguna's group |

**Lunch Break: Main Guest House (12:45 PM – 2:00 PM)**

**Student Talks (Session 3) 2:00 PM - 3:15 PM Chairpersons: Mamta Bangera & Ashutosh Gulati**

| | | |
|---|---|---|
| 2:00 - 2:15 | Asmita Gupta | Prof. Manju Bansal's group |
| 2:15 - 2:30 | Anindita Das | Dr. Rishikesh Narayanan's group |
| 2:30 - 2:45 | Chetana Baliga | Prof. R. Varadarajan's group |
| 2:45 - 3:00 | Richa Mudgal | Prof. N. Srinivasan's group |
| 3:00 - 3:15 | Saurabh Yadav | Prof. Surolia |

**Tea Break and Poster Session 2 (3:15 PM – 4:00 PM)**

**Student Talks (Session 4) 4:00 PM – 5:00 PM Chairpersons: Rustam Ali & Indra Mani Sharma**

| | | |
|---|---|---|
| 4:00 – 4:15 | Soma Ghosh | Prof. Saraswathi Vishveshwara's group |
| 4:15 – 4:30 | Hitesh Verma | Dr. Jayanta Chatterjee |
| 4:30 – 4:45 | Mahavir Singh | Mahavir Singh |
| 4:45 – 5:00 | Sunanda Margrett | Prof. Dipankar Chatterji |

**5:00 – 6:00 PM Plenary Lecture 2 Prof. Rohini Balakrishnan, CES, IISc**

**Title: Acoustic communication in crickets: Behavioural ecology to biophysics**

**Awards and Vote of Thanks (6:00 PM– 6:15 PM)**

# Plenary Lecture Abstracts

Plenary Lecture 1

# Mind the gap: Gap junctions and neural circuit assembly in larval zebrafish

**Dr. Vatsala Thirumalai**

National Centre for Biological Sciences
NCBS-TIFR, GKVK, Bangalore.

---

Gap junctions are cytoplasmic continuities between cells, through which ions, second messengers and small molecules can be exchanged. They are formed by assemblies of connexin proteins in vertebrates. Of the several known isoforms of connexins, Connexin 36 (Cx36) in mammals and Cx35 in teleosts are predominantly expressed in the nervous system. Using immunohistochemical methods in zebrafish, we show that Cx35 is first seen in the commissures and the optic tectum at 2dpf. Other regions like the cerebellum acquire it later at 4-5dpf. Immunoreactivity was mostly punctate and localized in fiber tracts, neuropilar areas and on somata. In the cerebellum, we localized Cx35 puncta on Purkinje neurons using cell-specific markers. In the hindbrain, the Mauthner neuron was labeled at club endings and in the axonal cap region. To test the function of Cx35 during development, we designed and microinjected splice-blocking morpholino antisense oligonucleotides specific for Cx35 and validated that Cx35 mRNA was mis-spliced. I will discuss results of our recent experiments in the cerebellum and hindbrain, which implicate Cx35 in glutamatergic synaptogenesis and neuronal activity.

Plenary Lecture 2

# Acoustic communication in crickets: Behavioural ecology to biophysics


## Prof. Rohini Balakrishnan

Centre for Ecological Sciences
Indian Institute of Science
Bangalore 560 012


---

Many animal groups such as crickets, frogs and birds use long-distance acoustic signals for mate attraction. The senders are typically males and the 'intended' receivers are females of the same species. Each species typically has a unique acoustic signal by which it may be identified at a distance. Females use spectral and temporal properties of the acoustic signal to recognize and then locate calling males of their species, who are potential mates. In most natural environments, a large number of acoustically communicating species co-exist and signal simultaneously, giving rise to the conspicuous dawn chorus of birds and the dusk chorus of crickets and frogs. This raises the question of how males and females of each species manage to communicate with each other in the face of high levels of masking interference and signal degradation. Over the past ten years, my research group has worked on an assemblage of acoustically communicating species of crickets and katydids in the tropical evergreen forests of Kudremukh National Park in the Western Ghats. The aim is to examine sender and receiver strategies for communication in the complex, noisy acoustic environment of the dusk chorus. In this context, signal structure, signal degradation and signaller behaviour have been examined for evidence of sender strategies to avoid masking interference and maximize high-fidelity information transfer. Receiver strategies are being examined in terms of auditory mechanics, physiology and behaviour. Another important evolutionary force determining signal structure and signaller behaviour is predation: I will describe some recent work examining the role of bat predation in shaping cricket communication systems. I will also illustrate the importance of biophysical measurements of structures involved in sound production, transmission and reception for an understanding of the ecology and evolution of acoustic communication systems.

# Student Talk Abstracts

# The $C_{11}/C_9$ Mixed Helices with Alternate Directionality Hydrogen Bond in the Crystal Structures αβ of Hybrid Peptides

**Krishnayan Basuroy, Vasantham Karuppiah, Narayanaswamy Shamala and Padmanabhan Balaram**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *pb@mbu.iisc.ernet.in*

---

Helices are most abundant secondary structures in proteins and α-polypeptides. The two major helix types, $3_{10}$ and α-helices, in α-polypeptides, are characterized by intramolecular hydrogen bonds that run in the same direction and have hydrogen bonds of the type $C{=}O_i\ldots HN_{i+n}$ (n = 3 for the $3_{10}$ helix and n = 4 for the α-helix) which require orientation of peptide units in the same direction. Helices with mixed hydrogen bond directionality in which alternating C=O…HN hydrogen bonds run in opposite directions are unprecedented in the structural chemistry of α-polypeptides. While studies in solution provide strong evidence for the occurrence of mixed helices in oligo-β-peptides and hybrid αβ sequences, limited information is currently available in the crystalline state.

During the course of studies of $(\alpha\beta)_n$ hybrid peptides containing the $\beta^{2,2}$-disubstituted residue, $\beta^{2,2}Ac_6c$ (1-aminomethylcyclohexanecarboxylic acid), we observed formation of the mixed $C_{11}/C_9$ structures, where αβ segments form forward direction $C{=}O_i\ldots HN_{i+3}$ intramolecular hydrogen bonds and the βα segments form reverse direction $C{=}O_i\ldots HN_{i-1}$ hydrogen bonds. In these examples α-residues adopted semi-extended poly-proline ($P_{II}$ : $\phi \approx -60^o$, $\psi \approx 120^o$ / $P_{II'}$: $\phi \approx 60^o$, $\psi \approx -120^o$) like conformations and β-residues adopted the backbone conformation $\phi \approx 90^o$, $\theta \approx 60^o$, $\psi \approx -90^o$.

We will present the conformational analysis of mixed helical structures observed in some of the crystal structures of $(\alpha\beta)_n$ hybrid peptides and discuss the Interconversion between $C_{11}$(unidirectional helix) and $C_{11}/C_9$ (alternate directionality helix) structures.

# Structural studies of *Sesbania Mosaic Virus* (*SeMV*) VPg (Viral Protein Genome linked) in the free and bound state

**Karuna Dixit, H. S. Savithri and Siddhartha P. sarma**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *karuna@mbu.iisc.ernet.in*

---

*Sesbania mosaic virus* (*SeMV*) is a plant virus that belongs to the genus *sobemovirus* and infects *Sesbania grandiflora*. The length of the genus is ~ 4 Kb. The genome has four open reading frames of which ORF2 codes for two polyproteins. Polyprotein processing is a major strategy employed by both animal and plant viruses to generate more than one functional protein. In SeMV, the N-terminal protease domain coded by ORF2 mediates the processing. On processing, it releases protease, VPg, P10, P8 and a RNA dependent RNA polymerase (RdRP). The uridylated VPg (Viral Protein Genome linked) is attached to the 5' end of the RNA and serves as template for RNA synthesis. Polyprotein processing requires the protease domain to be fused with VPg protein. In isolation, the VPg proteins are known to be intrinsically disordered. Here we have used NMR spectroscopic methods to study the state of the VPg protein in the free state as well as in an intermolecular complex with the protease domain. The results of our studies will be presented.

# Probing viral genome evolution by single RNA sequencing

**M. Saranya[1], Vaseef Razvi[2], Katsu Shiroguchi[3], Guru Prasad[4], Rahul Roy[1, 2]**

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
[2]Department of Chemical Engineering, Indian Institute of Science, Bangalore 560 012
[3]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[4]Translational Health Science and Technology Institute (THSTI), Gurgoan, India

E-mail: *saranya@nanobiophotonics.org*

---

RNA viruses propagate as quasispecies of closely related genotypes in the host. This helps the virus in rapid adaptation, drug resistance and expansion. Sequence diversity and virulence of a viral species is regulated by the host selection pressures at different levels of its life cycle. Ability of a particular variant in a genotype to propagate rapidly compared to others, is due to its inherent genetic modifications. Our main objective is to define such genetic contributions of the full-length virus genome to its infectivity, which we term as "infectivity fitness". To probe the sequence-infection relationship, we are developing a method that would give us the full-length sequence of each individual viral RNA molecules and thereby provide the tools to track the sequence variations in a viral population. A complete sequence landscape of the viral pool will help explain the contribution of various mechanisms like recombination and mutation to viral genome evolution. In addition, full-length viral sequences will allow us to correlate the inherited elements such as co-evolving residues to functional changes in viral constituents.

# Conformational selection and ligand binding in *Mycobacterium tuberculosis* uracil-DNA glycosylase (*Mt*UNG)

**Arif S M,[1] Geethanandan K,[1] Mishra P,[1] Surolia A[1] , Varshney U[2] and Vijayan M[1]**

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore.
[2]Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore.

E-mail: *smarif@mbu.iisc.ernet.in*

---

Uracil-DNA glycosylase excises uracil from a single or double stranded DNA arising either from deamination of cytosine or incorporation of dUMP during replication. We report here the structures of the complexes of *M. tuberculosis* uracil-DNA glycosylase (MtUNG) with uracil and its fluoro, chloro, nitro, amino and thio analogues. The protein is known to exist in a closed conformation when bound to DNA and in an open conformation in the absence of DNA. DNA binding is also associated with movements in the C-terminal region in the second domain. Although DNA is bound to the protein in none of the present structures, some of them exhibit closed conformation while others remain in the open conformation. Variability in the C-terminal region in the second domain is also observed among the structures. Thus, *Mt*UNG appears to present an interesting case of conformational selection. Uracil and all the analogues bind to the protein with reasonable but varying affinity. The compounds exhibit two modes of binding. The locations of the uracil moiety in the two cases are related by a two-fold symmetry passing through N3 and C6. The existence of the two modes can be largely explained in terms of steric interactions. Further examination of the structures is in progress.

# Calcium permeable AMPA receptor dependent long lasting plasticity of intrinsic excitability in fast spiking interneurons of the dentate gyrus decreases inhibition in the granule cell layer

**Debanjan Dasgupta, and Sujit Kumar Sikdar**

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012.
E-mail: *debanjanmona@mbu.iisc.ernet.in*

---

The local fast-spiking interneurons (FSINs) are considered to be crucial for the generation, maintenance and modulation of neuronal network oscillations especially in the gamma frequency band. Gamma frequency oscillations have been associated with different aspects of behaviour. But the prolonged effects of gamma frequency synaptic activity on the FSINs have not been explored previously. Using whole cell current clamp patch recordings, we observed a sustained decrease of intrinsic excitability in the FSINs of the dentate gyrus (DG) following repetitive synaptic stimulations of the mossy fibers at 30 Hz (gamma bursts). Interestingly, when the gamma bursts were paired with membrane hyperpolarization, the decrease in excitability following the induction protocol accentuated further, while it was attenuated when the gamma bursts were paired with membrane depolarization. Paired pulse ratio measurement of the synaptic responses did not show significant changes during the experiments. However, we observed post-synaptic calcium rise associated with the induction protocols. Interestingly, the maximum and the minimum increase occurred during pairing of gamma bursts with membrane hyperpolarization and depolarization respectively. Including a selective blocker of calcium-permeable AMPA receptors (CP-AMPARs) in the bath significantly attenuated the calcium rise and blocked the membrane potential dependence of the calcium rise in the FSINs, suggesting their involvement in the observed phenomenon. Chelation of intracellular calcium blocked the expression of plasticity. Blocking HCN channel conductance or CP-AMPARs during the experiment also forbade the long lasting expression of the plasticity. Simultaneous dual patch recordings from FSINs and synaptically connected putative granule cells (GCs) confirmed that the decreased intrinsic excitability in the FSINs accompanied decreased strengths of inhibitory post-synaptic potentials in the GCs. Experimentally constrained network simulations using NEURON predicted increased spiking in the GC owing to decreased input resistance in the FSIN. We hypothesize that the plasticity in the FSINs induced by local network activity may serve to increase information throughput into the downstream hippocampal subfields besides providing neuroprotection to the FSINs.

# Structural insights into the role of the *Bacillus subtilis* reductase YwfH (BacG) in tetrahydrotyrosine synthesis

**P Kumar and Gopal B**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *pkumar@mbu.iisc.ernet.in*

---

The synthesis of the di-peptide antibiotic bacilysin involves the sequential action of multiple enzymes in the *bac* operon. YwfH (also referred to as BacG) catalyzes the stereo-selective reduction of $H_2HPP$ (dihydro-hydroxyphenylpyruvate) to $H_4HPP$ (tetrahydro-hydroxyphenylpyruvate) in this biosynthetic pathway. YwfH is a NADPH dependent reductase that facilitates the conjugate addition of a hydride at the $C^4$ olefin terminus of $H_2HPP$. Here we describe the structure of YwfH in three conformational steps- the apo form, an apo-like conformation and the NADPH complex. YwfH is structurally similar to other characterized short-chain dehydrogenase/reductases despite marginal sequence similarity. The structures of YwfH in different conformational states provide a rationale for the ping-pong reaction mechanism. The identification and role of the residues in the catalytic tetrad (K113-Y117-S155-N158) in proton transfer was examined by mutational analysis. Together, the structures and biochemical features reveal synchronized conformational changes that facilitate co-factor specificity and catalysis of $H_4HPP$ formation en-route to tetrahydrotyrosine synthesis.

# Structural studies to elucidate the catalytic mechanism of Diaminopropionate ammonia lyase

**Geeta Deka[1], Shveta Bisht[1], M. R. N. Murthy[1], H.S. Savithri[2]**

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
[2]Department of Biochemistry, Indian Institute of Science, Bangalore 560 012

E-mail: *geetadeka@mbu.iisc.ernet.in*

---

Diaminopropionate ammonia lyase (DAPAL) is a non-stereo specific fold-type II pyridoxal 5' phosphate (PLP) dependent enzyme that catalyzes the conversion of both D/L isoforms of the nonstandard amino acid Diaminopropionate (DAP) to pyruvate and ammonia. DAP is important for the synthesis of nonribosomal peptide antibiotics such as viomycin and capreomycin. Structural studies on *Ec*DAPAL bound to a reaction intermediate (aminoacrylate) suggested that the enzyme follows a two base mechanism, where Asp120 and Lys77 function as general bases to abstract proton from D-DAP and L-DAP respectively. To understand the reaction mechanism structural and biochemical characterization of active site mutants Asp120 (Asp120Asn/Ser/Thr/Cys) and Lys77 (Lys77His/ Thr/Ala/Val) of EcDAPAL has been carried out. Reduction of catalytic efficiency (Kcat/Km) of D120N EcDAPAL for D-DAP by 140 folds and presence of the uncatalyzed ligand in external aldimine form at the active site in the crystal structure suggested that Asp120 indeed abstracts proton from D-DAP. Lys77, which was speculated to be important for proton abstraction from L DAP; however seemed to be crucial for PLP binding only. Presence of non-covalently bound PLP in the L77W mutant and occurrence of both the ketoenamine, enolimine forms of internal aldimine in L77R mutant provided an in depth insight into the unique chemistry of internal aldimine formation in PLP dependent enzymes. Thus, these studies provide deeper insights into the reaction mechanism of *Ec*DAPAL, representing the overall reaction mechanism followed by several other fold-type II PLP pendent enzymes.

# Crystal structure of an aspartic proteinase-like domain from *Mycobacterium tuberculosis*

**Deivanayaga barathy V, Sushanth Kumar, Muraleedhara Reddy T, Kaza Suguna**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *barathy@mbu.iisc.ernet.in*

---

A search in the genome of *M. tuberculosis* showed a weak similarity to the HIV aspartic proteinase sequence. This region corresponds to the C-terminal domain of a PE family protein in M. tuberculosis (Rv0977). A search with the sequence of this domain revealed the presence of similar domains in two other proteins of *M. tuberculosis*, Rv1983 and Rv2519. The presence of two signature motifs, DTG and DSG, of aspartic proteinases in the full sequence of this domain encouraged us to take up further studies on this domain. Previous reports identifying the protein as a surface antigen and our findings on the occurrence of similar domains in two other PE proteins of *M. tuberculosis* indicated that these domains probably play an important role in infecting the host. The crystal structure of one of the domains showed that it has a pepsin-like fold and the catalytic site architecture of known aspartic proteinases. However, no proteolytic activity was detected. The size of the molecule is intermediate to eukaryotic pepsins and the homodimeric retroviral pepsins. A close examination of the binding site revealed subtle differences when compared to the active enzyme structures. Appropriate mutations of some of the residues in this region to convert it to an active enzyme did not make it active. Multiple sequence alignment of *M. tuberculosis* aspartic proteinases shows that the critical substrate binding residues of Rv2519 are similar in nature to those in pepsins. Hence, it is likely that Rv2519 could be an active aspartic proteinase.

# Molecular basis for nucleocytoplasmic transport of tRNA by Exportin-t

**Asmita Gupta, Senthilkumar Kailasam and Manju Bansal**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *asmita@mbu.iisc.ernet.in*

---

Export of mature, fully processed tRNA molecules from nucleus to cytoplasm is carried out by Exportin-t (Xpot) protein in mammals (Los1p homologue in *S.cerevisiae).* It belongs to a family of nuclear import/export proteins called Karyopherins, which are formed of a repeating motif of 35-40 amino acids called HEAT repeats. The export and subsequent release of tRNA cargo by Xpot involves Ran protein and GTP hydrolysis in the cytoplasm which is associated with Ran. Despite the availability of crystal structures of nuclear and cytosolic forms of Xpot, the molecular details regarding the sequential events leading to tRNA release and conformational change by Xpot remains unclear. We studied a range of molecular complexes including free Xpot protein and intermediate state complexes bound either to Ran or tRNA, to illustrate gross structural motions in Xpot after cargo release and identified various molecular determinants responsible for cargo binding. We employed classical all atom molecular dynamics simulation methods to study all the molecular complexes involving Xpot. The overall conformational change in Xpot due to cargo release was attributed to a highly fluctuating C-terminal region. The identity of residues in GTP and GDP bound states of transporter protein was also established, besides analyzing the change in the molecular electrostatic surface potential of Xpot during conformational change after tRNA release. The energetics of complete and intermediate complexes were also studied in order to illustrate differential binding affinities of Xpot in presence or absence of its specific cargo and directionality of the overall transport process.

# Neuronal ion channels and their spatial localization regulate feature selectivity in single neurons

**Anindita Das and Rishikesh Narayanan**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *anindita@mbu.iisc.ernet.in*

---

An outstanding question in the quest to understand information encoding in our brain is what features neurons extract from the multi-dimensional sensory input that they receive from the external world. Memory encoding requires processing and integration of information from multiple sensory cues, a feat achieved by the well-organized circuitry of the hippocampus, a brain region critically involved in learning and memory. Pyramidal neurons in the hippocampus are prodigious computational devices that transform tens of thousands of afferent synaptic inputs into action potentials that encode critical features about these inputs. However, little is understood on how the biophysical properties of single neurons and the voltage-gated ion-channels (VGIC) that reside on their plasma membrane aid in this information transformation. In this talk, I will present a quantitative approach to addressing this lacuna employing what is known as the spike triggered average (STA), a measure of the specific input features that a neuron selectively responds to with an action potential. The approach involves the quantification of several STA-derived measures to assess the role of VGICs in altering the features that neurons respond to. Through the course of the talk, I will present several lines of evidence that demonstrate that VGICs and their specific localization along the neuronal membrane can radically alter the specific input features that neurons respond to. These results demonstrate that the biophysical properties of a neuron play a critical role in information encoding by regulating the specific features that a neuron is selective to.

# Rational design of cold sensitive phenotypes

**Chetana Baliga[1], Kajari Mondal[1], Antara Bhattacharjee[1], Sandipan Majhi[1], K. VijayRaghavan[2] and Raghavan Varadarajan[1]**

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
[2]National Centre for Biological Sciences, TIF, Bangalore.

E-mail: *varadar@mbu.iisc.ernet.in*

---

Cold sensitive (cs) mutants behave like loss-of-function mutants at temperatures below the restrictive temperature but display wild type phenotypes at higher temperatures and are useful tools to probe gene function *in vivo*. They are rarer than temperature sensitive (ts) mutants and the molecular mechanisms responsible for cs phenotypes are poorly understood. Herein, we propose a method for rational design of cs mutants. It is known that loss of function mutants can often be rescued by overexpression [Bajaj *et al*, 2008]. We have previously shown that it is possible to accurately predict buried residues solely from amino acid sequence [Varadarajan *et al*, 1996] and to engineer destabilizing mutants at predicted, buried positions [Chakshusmathi *et al*, 2004]. We hypothesize that destabilized and/or partial loss-of-function mutants can be converted to cs ones by increasing their expression level selectively at high temperature. In order to test this hypothesis, 45 known ts mutants of the *E.coli* toxin CcdB were overexpressed at 37oC while maintaining basal levels at 30oC. In all cases, this resulted in a cs phenotype, with activity at 37 oC and no activity at 30oC. In addition, previously isolated partial loss-of-function mutants of yeast Gal4 were expressed in *S. cerevisiae* under the control of a heat inducible promoter. Three of the four mutants tested showed cs phenotypes. We also designed buried site mutants in Yeast Trp1 and Ura3 proteins and screened them for cs behavior. Finally, the four Gal4 mutants were individually cloned into a *Drosophila* P element vector under control of an eye specific GMR promoter containing Heat Shock Elements (HSEs). Transgenic *Drosophila* lines were generated, crossed to UAS reporter lines and progeny were screened for reporter gene expression at multiple temperatures. In three cases, cs phenotypes were observable. These data demonstrate that cs phenotypes need not result from complex, temperature-dependent mutational effects. Instead, many partial loss-of-function mutants can be converted to cs ones by either increasing their expression level or activity at high temperatures; or by decreasing the same at low temperatures.

# Design of artificial proteins to aid detection of related natural proteins

**Richa Mudgal[1], Ramanathan Sowdhamini[2], Nagasuma Chandra[3], Narayanswamy Srinivasan[4*] and Sankaran Sandhya[3]**

[1] IISc. Mathematics Initiative , Indian Institute of Science, Bangalore.
[2] National Center for Biological Sciences, Bangalore.
[3] Department of Biochemistry, Indian Institute of Science, Bangalore.
[4] Molecular Biophysics Unit, Indian Institute of Science, Bangalore.

E-mail: *rmudgal@mbu.iisc.ernet.in*

---

With the advent of high-throughput methods for genome and proteome sequencing, the need for efficient and sensitive tools for functional annotation of proteins is -increasing. In the absence protein structures, sequence similarity is a key factor in detecting remote relationships. However sequence-based search methods are rendered less effective due to the non-uniform dispersion of the protein sequence space. Here, we describe a computational approach to purposefully fill-in the void and sparse regions between two related protein families through directed design of protein-like 'linker' sequences. Protein families are represented as multiple profiles and related protein domain families are aligned using a profile-profile alignment method, AlignHUSH. Where reliable alignments were achieved, a Roulette wheel-based method was used to design 3,611,010 artificial sequences corresponding to 374 SCOP folds. Using 3024 queries with known distant evolutionary relationships, we demonstrate that such designed sequences when integrated with natural protein sequences in databases and employed in sequence-based remote homology searches, show improved fold coverage and up to 66% in the number of correct evolutionary relationships discovered, with an average increase of 15.6% at significantly low error rates (1.7%). Although sequences could not be designed between some families, designed sequence between other families within the fold aided in detecting 373 difficult relationships. Additionally, artificial sequences when plugged-into other generic sequence databases such as Pfam also empowers remote homology detection and fold recognition.

Reference:

R. Mudgal, R. Sowdhamini, N. Chandra, N. Srinivasan & S. Sandhya (2014) J. Mol. Biol., 426, 962-979.

# Investigating the modulators of Neuropathic Pain

**Saurabh Yadav and Avadhesha Surolia**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *saurabhy@mbu.iisc.ernet.in*

---

Nociception has evolved as a mechanism to avoid a potentially hazardous stimulus and thus helpful in the survival of the organism. In general, pain enhances the sensitivity of the injured tissue, against noxious stimulus, thereby protecting the injured area from further injuries and thus helpful for the healing process. Physiological pain, hence is generally transient (acute pain) in nature. Chronic pain may occur as a result of pathological chronic inflammation (inflammatory pain) or lesions to the nervous system (Neuropathic pain). Neuropathic pain arises due to nerve lesions of primary sensory neurons of the central or peripheral nervous system and is associated with dysesthesia , hyperalgesia, and mechanical allodynia being one of the most distinctive feature of neuropathic pain.

Molecular mechanism behind neuropathic pain has not been fully understood, although various inflammatory mediators, ion channels (like voltage gated Na, K, and Ca channels), intracellular signaling molecules (like MAPK, CaMK, ERK, PKA, cAMP, ERK, CREBP , NFkB etc) etc. have been reported to play a role in its pathogenesis. NFkB is one of the key regulators of cellular transcription, and homeostasis. It was known to play a very important role in inflammatory pain, however its role in neuropathic pain has been discovered recently. In the past few years there has been many reports showing that siRNA mediated as well as pharmacological inhibition of p65 subunit of NFkB leads to alleviation of neuropathic pain. However the mechanism of regulation of the levels of p65 subunit itself is not known. We tried to study the cellular pathways involved in regulation of p65 expression. Our data shows that a whole new pathway involving ILK1 as an intermediate plays a role in the regulation of p65 expression in neuronal cells and modulate their firing properties which may contribute to the pathophysiology of neuropathic pain.

# Investigating the mechanism of *iron dependent Repressor* (*ideR*) activation and DNA binding

**Soma Ghosh, Nagasuma Chandra, Saraswathi Vishveshwara**
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
E-mail: *soma@mbu.iisc.ernet.in*

Metalloproteins form a major class of enzymes in the living system and are involved in critical biological functions such as catalysis, redox reactions and as "switches" in signal transductions. Irondependent repressor (ideR) is a metal sensing transcription factor that regulates free iron concentration in *Mycobacterium tuberculosis*. Crystal structures of ideR bound to DNA are available and indicates the formation of a *'dimer-of-dimer'* complex, with the two dimers binding to opposite sides of the DNA double helix. Apart from regulating iron homeostasis, ideR is also known to promote bacterial virulence. Various experiments have suggested that ideR exists as a monomerdimer equilibrium, with the equilibrium shifting towards the dimer in the presence of the metal. The two metallated dimer, in-turn binds to DNA and facilitates its biological function. In this study, we seek to understand the role of iron in ideR activation and DNA binding at the atomistic level. This is performed at two levels, a) shift in monomer-dimer equilibrium, and b) interaction of ideR with DNA. Molecular dynamics (MD) was employed to obtain the ensemble structures in the presence and absence of metals, which were then analyzed to study the overall structural variation between the two states and hence the influence of iron. Free energy landscapes of the different ligand bound systems were identified and compared. Details at the atomistic levels were investigated using hydrogen bonds and protein structure networks [PSN/PDG] and further quantified using free energy [MMPBSA] calculations.

The results show significant variation between the metallated and the nonmetallated systems. At the level of monomers, iron strongly influences the flexibility of the structure and intradomain movements, which in turn promotes dimerization and DNA binding. Mode of dimerization in the absence and presence of iron and how that influences DNA binding has also been thoroughly discussed.

Perhaps, the most striking results are obtained from the simulations of the ideR-DNA complex in the absence of metals. As compared to the metallated systems, the protein molecules are seen to move away from the DNA in the absence of metals. Such drastic changes in the ideR-DNA interactions not only provides molecular insights about the role of iron, but also about the mechanism of DNA binding and unbinding. These results would be presented in details in the talk. Further, the possible role of iron as an allosteric effector in ideR function would also be discussed. Finally, we propose a model describing the sequence of events that govern ideR binding to DNA in the presence of iron.

# Multiple thionation of cyclic peptides

**Hitesh Verma, Jayanta Chatterjee**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *hiteshvrm32@gmail.com*

---

Cilengitide and Aplidine are N-methylated cyclic peptides, which are in clinical trials phase III against several cancers. Extensive research on N-methylation of biologically active cyclic peptides revealed that in spite of subtle loss in bioactivity, N-methylation remarkably improves the pharmacological properties of cyclic peptides. Thus, by the substitution of a 'H' atom with 'CH3' group in amide bonds dramatically alters the physicochemical and pharmacological properties of cyclic peptides.

Thioxo peptide bond (thioamide) (–ψ[CS–NH]–) represents an isosteric replacement of the peptide bond in which the carbonyl oxygen is replaced with a sulfur atom, with a slight change in electron distribution in the ground state, which have been used in studies of biologically active peptides. The thioamide NH is a stronger hydrogen bond donor than the amide NH, while the sulfur is a weaker hydrogen bond acceptor than the amide oxygen. With this in mind we will employ a rational approach of multiple thionation of cyclic peptide backbone and study the impact of multiple thionation on the physicochemical and pharmacological properties of cyclic peptides.

# Identifying key residues of the Ferroxidation Processes in *Mycobaterium smegmatis* DNA binding Proteins under Starvation

**Sunanda Margrett Williams**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *swilliams@mbu.iisc.ernet.in*

---

DNA binding proteins under starvation (Dps) are dodecameric nano-compartments for iron detoxification and storage in bacterial cells. In addition, they characteristically bind DNA and protect it from free radical damage by bypassing the Fenton reaction. The mechanism by which these proteins belonging to the ferritin protein family oxidize and store iron is an area of active research. Here, we use X-ray crystallography to determine the structures of co-crystals of iron and Dps and dissect the changes in these ligand bound form with respect to the apo-protein. These studies have been done in the second mycobacterial Dps protein from *Mycobacterium smegmatis*, MsDps2. Also, we have mapped a route for iron atoms from its entry in the protein shell to the ferroxidation centre, by mutations and interaction analysis of a conserved arginine residue. The mutants generated by these substitutions were found be increasing unstable and dissociate from the dodecamer in the presence of iron. The iron-loaded proteins of low, medium and high iron-bound forms on analysis, were found to exhibit asparate residues with alternate conformations of which some could be directly linked to the active sites of ferroxidation and iron entry. Here, we demonstrate that the increased flexibility of the aspartates in presence of iron facilitate the movement of iron from entry site to the ferroxidaton site. We also propose that the dps-like interface of these proteins function as an entry point for chloride ions to the protein shell to neutralize iron. The ferritin-like (iron entry point) and dps-like trimeric interfaces in these proteins exhibit many similarities to cation and anion channel proteins, respectively.

# Student Poster Abstracts

# Population shift mechanism opens up new druggable hotspot in Pregnane X Receptor

**Aneesh Chandran, Saraswathi**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *aneeshcna@mbu.iisc.ernet.in*

---

The Pregnane X Receptor (PXR), a member of the nuclear receptor superfamily, regulates the expression of proteins involved in xenobiotic metabolism. PXR gets activated after binding promiscuously to a wide variety of compounds with different geometrical and chemical features. The most common clinical implications of such promiscuous ligand binding and PXR activation is the occurrence of adverse drug-drug interactions and also the onset of early stage metabolism of drugs. Therefore, understanding the molecular basis of promiscuity in ligand binding to PXR is crucial for the development of effective PXR antagonists. In this study, we have performed molecular dynamic (MD) simulations and analysis of protein structure networks of different ligand bound systems to reveal the ligand induced subtle changes in PXR. MD simulations revealed the existence of two different conformational states, 'breathin' and 'breathe-out'. Ligand binding events shift this conformational equilibrium towards 'breathe-out' state. Computational solvent mapping has identified a new druggable site whose opening-closing mechanism directly correlates with shift in conformational population. Structure-based virtual screening has identified new lead compounds, which lock this druggable pocket in its open-state. MD simulations further revealed that the presence of lead compound in allosteric site disrupts the native PXR-ligand interaction and thereby destabilize the PXR-ligand complex formation.

# Comprehending structure, function and interactions across host and pathogen proteomes using sequence-profiles: Implications in target identification for *Mycobacterium tuberculosis* H37Rv

**Gayatri Ramakrishnan[1,3], Nagasuma R. Chandra[2] and Narayanaswamy Srinivasan[3]**

[1]Indian Institute of Science Mathematics Initiative, [2]Department of Biochemistry,
[3]Molecular Biophysics Unit Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

---

An imperative step towards perceiving the biology of an organism is to comprehend its functional repertoire. In the context of a pathogen, protein structure and function recognition becomes a prerequisite to understand the intricateness in host-pathogen interactions. The availability of the genome sequence of *Mycobacterium tuberculosis* H37Rv has played a major role in its functional characterization, but the emergence of multidrug and extensively drug-resistant strains necessitates further advances in understanding pathogenesis and its underlying complexity. Curtailing the incompleteness in previously annotated proteomes, we report an enriched structural and functional characterization with the use of sensitive profile-based remote homology and fold recognition algorithms which comprehend ~95% of the *M. tuberculosis* proteome. The results obtained served as guiding tools for prediction and analysis of transient interactions across host and pathogen at domain level. 39 domain family interactions across host and pathogen could be identified which encompass 83 *M. tuberculosis* proteins and ~1000 human proteins. Additionally, transient interactions across host and pathogen were also derived with the help of known transient protein-protein complexes.

Crucial factors such as functional importance of a protein, its role in pathogenesis and its essentiality in growth and survival of the pathogen play key role in its prioritization as a potential drug target. Established on the grounds of drug repositioning, we report a target identification methodology based on exploration of the evolutionary relationship between targets of known FDA-approved drugs and *M. tuberculosis* proteins, wherein the FDA-approved drugs are initially subjected to a filter to eliminate the drugs known to act on human proteins. Such a withdrawal from the analysis minimizes the chances of obtaining an anti-target as well as enriches the credibility of potential drug targets identified in *M. tuberculosis*. In retrospect, a total of 134 FDA-approved drugs were identified which could be repositioned for 56 potential targets. Protein-ligand docking studies were also performed to ensure reliability and comprehensiveness of the targets identified.

# Development of Selective Protein Phosphatase-1 Inhibitor

**Ishita Chakraborty, Jayanta Chatterjee**

Peptide Chemical Biology Lab, Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

---

Reversible phosphorylation of proteins is one of the most important regulatory mechanisms controlling protein activity. Protein kinases phosphorylate protein whereas protein phosphatases dephosphorylate. Protein phosphatases PP1 and PP2A belong to PPP phosphatases, a family of serine/threonine phosphatases. They are closely related in sequence and structure and display overlapping substrate specificity. PP1 and PP2A account for more than 90% of all serine/threonine dephosphorylations in eukaryotic cells. However, there are no chemical tools to specifically block the activity of PP1 in cells. Thus, we aim to develop a cell permeable selective inhibitor of PP1 on the basis of a regulatory protein called Inhibitor-2 that specifically inhibits the activity of PP1 in a cell cycle dependent manner.

# Gaussian Network Model study of Protein Kinases: Relationship between sequences, structural fluctuations and functions

**Raju Kalaivani and Narayanaswamy Srinivasan**
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
E-mail: *kalaivani@mbu.iisc.ernet.in*

Protein Kinases (PKs) are a large and diverse family of enzymes, that phosphorylate ~30% of all cellular proteins and are implicated in cancer upon mutations. Despite a conserved structural scaffold in PKs, how do kinase specific regulatory mechanisms function? Although structural conformations correspond to functional states, what mechanistic aspects of the conformations enable the activity/inactivity of the kinase? How do structural motifs achieve specific modules of function? To this end, we investigated the structures of PKs using Gaussian Network Model (GNM) based Normal Mode Analysis (NMA). 1. We analyzed the GNM perceived fluctuations of PK structures of varying catalytic competence. The fluctuations in the active and inactive states were not identical. Systematic differences were found in the fluctuations: the inactive state fluctuates more than the active state. This was true of different structures of the same kinase, individual kinase pairs and a population of kinases. Consequently, we predicted that the conformational energy of a PK would be unfavourable in the inactive state than in the active state, and proved the same using free energy calculations. Interestingly, the higher fluctuations in the inactive state were contributed specifically by αC helix, αG helix and activation loop regions, which are implicated in the conformational switch from active to inactive forms and in protein-protein interactions. 2. Fluctuations of PKs as a property of their relatedness were studied. Closely related PKs within the same subfamily showed higher conservation in dynamics than distantly related PKs. Dynamics of Src and EGFR, homologous PKs having contrasting mechanisms of regulation, were studied. Using only the GNM perceived fluctuations, we could identify regions of regulation and protein-protein interaction unique to each of them. 3. GNM fluctuations were assessed for information about the functional sites within a PK. We characterized the fluctuations of different functional motifs within the PK. Integrating this information with evolutionary conservation scores, we were able to predict/recall the functional sites with specificity and sensitivity > 0.93. In summary, (a) GNM perceived fluctuations co-vary with sequence, structure, function information and fluctuation differences provide an insight about kinase-specific functional attributes; (b) Fluctuations are sensitive to conformational changes (active<->inactive). αC helix, αG helix and activation loop contribute to the fluctuation difference during the switch; (c) Since fluctuation patterns are characteristic and function dependent, they can reliably predict/recall functionally specialized regions in a PK structure.

# Molecular basis of feedback regulation of pppGpp synthesis

**Kirtimaan Syal[1], Himanshu Joshi[2], Vikas Jain[2]**

[1]Molecular Biophysics Unit,Indian Institute of Science
Bangalore 560 012
[2]Department of Biological Sciences, IISER, Bhopal

---

Under stress conditions, bacteria give stringent response via signaling cascades which help in their survival. (p)ppGpp synthesis by $Rel_{MSM}$ has been recognized as the key process in stringent response. $Rel_{MSM}$ can synthesize as well as hydrolyze the (p)ppGpp. The C-terminal half of $Rel_{MSM}$ has been shown to contain two domains viz TGS and ACT, which are involved in the regulation of its activity. We have earlier demonstrated that an intramolecular crosstalk is responsible to achieve this regulation and involvement of (p)ppGpp is indicated. So we suggested that the auto-regulation of synthesis by (p)ppGpp could be achieved by binding of the latter to the C-terminal domain (CTD) of enzyme. The azido derivatives of (p)ppGpp have been synthesized for studying their direct interaction with the protein. We cross linked these molecules with Rel CTD protein (that contain only the TGS and ACT domains). Our data clearly showed that pppGpp carrying the azido group interacts and binds to Rel CTD. The selective binding of pppGpp with Rel CTD has been further evaluated by carrying out the ITC titration. Binding site of pppGpp in $Rel_{MSM}$ is identified by mass spectrometry. The azido labeled molecule has been cross-linked to Rel CTD and the complex is subjected to trypsin digestion followed by mass spectrometry. Mutational analysis has been done in search of critical residues and further synthesis assays are carried out to measure the activity. We found that (p)ppGpp auto-regulates its own synthesis and involved in change in conformation of enzyme resulting in switching of synthesis to hydrolysis activity.

# Relation between phenotype, *in-vivo* solubility, and stability for single-site mutants of CcdB

**Arti Tripathi, Kritika Gupta, Pankaj Jain, Ajai J Pulianmackal, Prasanth Kumar, Raghavan Varadarajan**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *kritika@mbu.iisc.ernet.in*

---

A quantitative understanding of mutational effects on protein structure, function and stability is an important goal of many protein engineering studies. Saturation mutagenesis is a useful tool to understand the relation between genotype and phenotype. Using the 101 amino acid, homo-dimeric protein CcdB as a model, the effect of mutations on protein activity, stability and folding was studied. CcdB acts as a toxin in *E.coli* by binding to DNA Gyrase and killing the cells. Of the ~ 1600 mutants generated using Site-Saturation Mutagenesis tools (Bajaj et al, 2008, Jain & Varadarajan, 2014), 80 single-site mutants of CcdB with varying activities were previously purified and characterized. The correlation between *in-vivo* activity, solubility and *in-vitro* stability was examined and the effect of mutations on phenotype, protein folding and stability was rationalized. In a cell, the ability of a mutant to fold to the native state is affected by many parameters that include the crowded environment of the cell, folding assistance by various chaperones that buffer mutational effects on protein stability, and quality control mechanisms which are involved in degradation and removal of mis-folded proteins from a cell. These factors might be responsible for the less than perfect correlation between *in-vitro* stability and *in-vivo* activity.

The soluble fraction can comprise both of the folded protein which is active and soluble aggregates/partially mis-folded protein which is inactive. To study the relation between *in-vivo* activity and solubility, *in-vitro* activity of selected mutants was studied by monitoring binding of the soluble fraction of the cell lysate to gyrase using SPR. *In-vitro* activity correlated well with the *in-vivo* phenotype, even for those mutants for which the correlation between the *in-vivo* activity and solubility was low.

Effect of ATP-independent chaperones on folding was studied for a few selected mutants .When the cellular proteostasis machinery was perturbed by either over-expression or depletion of ATP-independent chaperones, change in the activity of many mutants was observed. Most mutants showed an increase in *in-vivo* activity and solubility upon mild over-expression of either Trigger factor or SecB chaperone indicating an increase in the folded fraction of the protein which is active.

This study thus helps in understanding mutational effects on protein stability, activity and folding and provides useful correlates for protein design.

# Deciphering Novel Functions of (p)ppGpp and c-di-GMP in *Mycobacterium smegmatis* Physiology

**Kuldeepkumar Ramnaresh Gupta**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *kuldeep@mbu.iisc.ernet.in*

---

In this work, we have attempted to find out various physiological functions that are mediated by second messengers (p)ppGpp and c-di-GMP in *Mycobacterium smegmatis*. To this end, we have made use of wild-type *M. smegmatis* and its isogenic derivatives Δ*rel* and Δ*msdgc1*, which lack (p)ppGpp and c-di-GMP, respectively. These second messengers have already been shown to mediate long-term survival under nutrient deprivation in *M. smegmatis.* We have used a recent technology, called Phenotype Microarray (PM), to search for novel functions mediated by aforementioned second messengers. PM analysis of wild-type and its isogenic derivatives Δ*rel* and Δ*msdgc1* showed that knockout strains are growing better in antibiotics. Paired t-test showed that the growth difference between the knockouts and the wild-type was statistically significant. PM data was corroborated by determining the minimum inhibitory concentrations (MICs). The MICs for knockouts were generally higher than those for wild-type. We propose that this apparent increase in the MICs may be due to changes in cell wall structure of knockouts due to lack of (p)ppGpp and c-di-GMP in these strains. We found that knockouts had altered colony morphology and reduced sliding motility and were defective in biofilm formation. Analysis of various cell wall fractions isolated from both planktonic and biofilm cultures showed that amounts of Glycopeptidolipids (GPLs) and Polar lipids were reduced in the knockouts compared to that of wild-type. These results indicate that second messengers (p)ppGpp and c-di-GMP may be involved in regulating cell wall properties in *M. smegmatis*.

# Conservation of conformational changes upon binding same ligand in lipocalin protein family

**B. Lakshmi[1, 2], G. Archunan[2], N. Srinivasan[1]**

1. Molecular Biophysics Unit,Indian Institute of Science, Bangalore 560 012
2. Department of Animal Science, Bharathidasan University, Tiruchirappalli, India

E-mail: *lakshmi@mbu.iisc.ernet.in*

---

Generally, in protein families, the functionally important residues are conserved resulting in the conservation of overall function of the homologous proteins. When a protein binds to a small molecule, often the protein undergoes structural changes. The nature of structural changes can be subtle, rearrangement of atoms or even large scale rigid body movements. In this paper we address the question of extent of conservation of conformational changes in homologous proteins upon binding to the same ligand. The current study has been conducted by considering the family of lipocalins. The family of lipocalins consists of sequentially diverse small extracellular proteins that have the ability to bind and transport various small molecules. We have analyzed a dataset of available crystal structures corresponding to 35 lipocalins bound to 9 different ligands. The nature and extent of conservation of structural changes in lipocalins upon binding to the same ligand have been analyzed. Interestingly the extent of structural change is not same among homologous lipocalins upon binding to the same ligand. Detailed analysis reveals that both the local conformational change and the conformational change in the ligand interacting regions are not well conserved in homologous lipocalins. Our results suggest that extreme caution has to be exercised in modeling conformational changes, upon ligand binding, on the basis of 3-D structures of homologues and their complexes.

# Display of HIV1 gp120 fragment immunogens on Qβ virus like particles

**Mansi Purwar [1], Pranveer Singh[1], Sanchari Bhattacharyya [1], Celia C. La Branche[2], David C. Montefiori[2], Jon Pokorski[3], M.G. Finn[3] and Raghavan Varadarajan[1]**

1.Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
2. Department of Surgery, Duke University, Durham, North Carolina 27705
3. Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, USA

E-mail: *varadar@mbu.iisc.ernet.in*

---

b12, one of the few broadly neutralizing antibodies against HIV-1, binds to the CD4 binding site (CD4bs) on the gp120 subunit of HIV-1 Env. We have previously reported design of an *E.coli* expressed small fragment of HIV-1 gp120, b122a, which display about 70% of the b12 epitope. b122a bound b12 with high affinity as well as sera from rabbits primed with b122a showed higher breadth of neutralization in a TZM-bl assay against Tier2 and Tier3 viruses. However, as these immunogens were only partially structured as assessed by CD and protease resistance, we attempted stabilization of b122a by engineering additional disulfides. A disulfide mutant b122a1-293-448 showed 15 fold higher affinity for b12 compared to b122a. This was further used for rabbit immunization studies. Virus like particles or VLPs resemble intact virions in terms of size and presentation of antigens, however, they are non-pathogenic, non-replicative and hence are safe for administration. In this work, we attempted to display various gp120 fragment proteins on the surface of Qβ virus like particles to make chimeric VLPs. The fragment proteins were displayed either by conjugation of the purified protein on the surface of the VLPs using click chemistry or directly displaying the protein as a fusion to the coat protein of the phage. The particles were purified and used for rabbit immunization studies with and without adjuvant and boosted with gp120. Titers against gp120 were highest for the group primed with b122a1-293-448 displayed on the particle, and were similar for sera from groups with b122a fragment conjugated to the particle both in the presence and absence of adjuvant. However, the group with adjuvant showed higher titers against the priming immunogen. Though sera did not neutralize Tier 2 viruses, sera from particles displaying b122a1-293-448 and b122a with adjuvant showed higher neutralization titers for tier 1 viruses as compared to other groups. Competition binding assays with b12 also showed that sera from these groups contained significantly higher amounts of antibodies directed towards the CD4 binding site than sera from group primed with empty particles and boosted with gp120.

# New Structural forms of a mycobacterial adenylyl cyclase Rv1625c

**Nikhil G Bharambe[1], Deivanayaga V Barathy[1], Rohini Matto[2], Sandhya S Visweswariah[2], Kaza Suguna[1]**

1. Molecular Biophysics Unit, 2. Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560 012

E-mail: *nikhilb@mbu.iisc.ernet.in*

---

Rv1625c is one of the 16 adenylyl cyclases encoded in the genome of *Mycobacterium tuberculosis*. Rv1625c exists predominantly as a monomer with a small amount of dimer in solution. It was shown earlier that the monomer was active and the dimeric fraction was inactive. Both fractions of Rv1625c-Wt crystallized as head-to-head inactive domain swapped dimers as opposed to a head-to-tail dimer seen in other functional adenylyl cyclases. About half the molecule is involved in the extensive domain swapping. The strain created by a serine residue located on a hinge loop and the crystallization condition might have led to this unusual domain swapping. The inactivity of the dimeric form of Rv1625c could be explained by the absence of the required catalytic site in the swapped dimer. A single mutant of the enzyme was also generated by changing a phenylalanine predicted to occur at the functional dimer interface to an arginine. This single mutant exists as a dimer in solution but crystallized as a monomer. Analysis of the structure showed that a saltbridge formed between a glutamate residue in the N-terminal segment and the mutated arginine residue hinders dimer formation by pulling the N-terminal region to the dimer interface. Both the structures reported here show a change in the dimerization arm region which is involved in the functional dimer formation. We conclude that the dimerization arm along with other structural elements like the N-terminal region and certain loops are vital for determining the oligomeric nature of the enzyme which in turn is required for its activity.

# High-conductance states and *A*-type $K^+$ channels are potential regulators of the conductance-current balance triggered by HCN-channel expression

**Poonam Mishra and Rishikesh Narayanan**

Cellular Neurophysiology Laboratory, Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *mpoonam@mbu.iisc.ernet.in*

---

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are non-specific cation channels. An increase in HCN-channel conductance reduces excitability of a neuron by decreasing its input resistance, whereas the consequent increase in an inward current depolarizes the membrane, propelling a unique conductance-current balance triggered by HCN-channel expression. We employed morphologically realistic, conductance-based models of hippocampal neurons to explore certain aspects of this conductance-current balance. First, motivated by the properties of *A*-type $K^+$ current, we asked if this current could nullify the effect of HCN channels on neuronal resting membrane potential (RMP). We found that the somatodendritic gradient of depolarized RMP induced by the introduction of HCN channels was significantly reduced by the insertion of a physiologically relevant gradient in *A*-type $K^+$ channels. Next, motivated by the well-established modulation of neuronal excitability by high-conductance states observed under *in vivo* conditions, we asked if the conductance-current balance triggered by HCN-channel expression could be altered by the presence of background synaptic activity. To do this, we inserted thousands of excitatory and inhibitory synapses spanning the somatodendritic arbor, with their distributions constrained by experimental observations. We measured the efficacy of HCN channels, independently and in conjunction with other channels, in altering RMP and input resistance when the neuron received randomized synaptic bombardments through variable numbers of excitatory, inhibitory or balanced excitatory-inhibitory synaptic inputs. We found that the average RMP was predominantly regulated by the impinging synaptic drive and the impact of HCN channels on input resistance was severely weakened under high-conductance states. Our results suggest that the debate on the role of HCN channels in altering excitability should encompass physiological and pathophysiological neuronal states under *in vivo* conditions and the spatiotemporal interactions of HCN channels with other ion channels expressed along the dendritic arbor.

# CLAP - An efficient alignment-free method to classify and establish functional relationships among multi-domain

**Prachi Mehrotra[1,2], Vimlakany G Ami[3] and Narayanaswamy Srinivasan[2]**

[1]Indian Institute of Science Mathematics Initiative
[2]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
[3]Bharathidasan University, Trichirapalli.

E-mail: *prachim@mbu.iisc.ernet.in*

---

The voids in the sequence and function space can be potentially filled using computational techniques that directly link the sequences to function. Although several sequence comparison methods exist, many fundamental and computational limitations arise, especially in multi-domain proteins. The reason for the limitation is that the existing methods employ an alignment-based approach focusing on single domains in isolation. On the contrary, proteins are constantly regulated by the accessory and tethered domains, which contribute to the overall function. To this end, we have developed an alignment-free method to compute Local Matching Scores (LMS) using frequency distribution of fixed length patterns between two sequences.

Instead of globally aligning the sequences, LMS matches sequence patterns of length 5 amino acids and then scores them based on evolutionary substitution frequencies. Thus, it will accurately relate the sequences even if domain shuffling, duplication or circular permutation events have occurred. The normalized scores are hierarchically clustered using minimum variance method and the quality of clusters is assessed. As there are no gold standards for full-length protein sequence classification, we resorted to Gene Ontology and domain architecture based similarity measures to assess our LMS based Classification of Proteins (CLAP). CLAP is freely available as a web-server at http://nslab.mbu.iisc.ernet.in/clap/

Two test data-sets were chosen, one with tyrosine phosphatases and the other one with SH3 domain containing proteins from Pfam (sequence database version 27.0). The phosphatases family helped to ascertain whether our method performs equally well as the other alignment-based approaches, in the case of single domain proteins. SH3 proteins on the other side occur mostly as multi-domain in nature. Using CLAP, domain architecturally pure clusters with higher functional relevance were obtained. CLAP is ~ 7 times faster than alignment-based methods. Thus our method is instrumental in providing a biologically meaningful clustering of any given set of proteins, utilizing only the sequence information.

# Influence of the AgrC-AgrA complex in the response time of *Staphylococcus aureus* quorum sensing

**Rajasree K, Fasim A, Sandeep SK, Arakere G, Gopal B**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *rajasree@mbu.iisc.ernet.in*

---

The *Staphylococcus aureus* agr quorum sensing system plays a major role in the transition from the persistent to the virulent phenotype. *S. aureus* agr type (I-IV) strains are characterized by mutations in the sensor domain of the histidine kinase AgrC and differences in the sequence of the secreted auto-inducing peptides (AIP). Here we demonstrate that interactions between the cytosolic domain of AgrC ($AgrC_{Cyto}$) and the response regulator domain of AgrA ($AgrA_{RR}$) dictate the spontaneity of the cellular response to AIP stimuli. The crystal structure of $AgrC_{Cyto}$ provided a basis for a mechanistic model to understand AgrC-AgrA interactions. This model enabled an analysis of the biochemical and biophysical parameters of AgrC-AgrA interactions in the context of the conformational features of the AgrC-AgrA complex. This analysis revealed distinct sequence and conformational features that determine the affinity, specificity and kinetics of the phosphotransfer reaction. This step, that governs the response time for transcriptional re-engineering triggered by an AIP stimulus, is independent of the *agr* type and similar for agonist or antagonist stimuli. These experimental data could serve as a basis to validate simulations of the quorum sensing response and for strategies that employ the agr quorum sensing system to combat biofilm formation in *S. aureus* infection.

# Structural And Mechanistic Insights Into Splicing Factor Sf3b Complex

**Rakesh Ramachandran[1, a], Agnel Praveen Joseph[2, b], Ramachandra M Bhaskara[3, a], N. Srinivasan[4, a]**

[a.] Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012
. [b]. National Centre for Biological Sciences, GKVK, Bellary Road, Bangalore

E-mail: *rakesh@mbu.iisc.ernet.in*

---

Spliceosome SF3b complex is an important component of the U2 snRNP as well as U11/U12 di-snRNP. The human SF3b complex consists of seven components – p14, SF3b49, SF3b145, SF3b155, SF3b10, SF3b130 and SF3b14b. A cryo-EM density map of SF3b complex was obtained at a resolution of 9.7 Å by Golas *et. al.* in 2003[1]. The protein p14 mainly consists of an RNA – recognition motif (RRM) domain and is known to interact with the branch point adenosine. X-ray and NMR structures have been solved for this protein in complex with an N-terminal fragment of SF3b155. This is the only protein-protein complex structure known in the SF3b complex at the atomic level.

The current work aims to get insights into the structure of SF3b complex by using integrative structure modelling techniques guided by the SF3b cryo-EM density map. From our modelling efforts we were able to localize the components of the SF3b complex in the cryo-EM density map and delineate its molecular architecture. The structural coverage of the complex was increased by fitting either partial or complete structures for some of the other components. This was done by taking advantage of the new homologous structures solved for the components as well as advances in both protein structure prediction techniques as well as cryo-EM based modeling techniques such as flexible fitting.

We also used the patterns of evolutionary conservation observed at the surfaces of protein structures known to interact with other proteins as an additional restraint in our integrative structure modelling approach to increase the accuracy of the model. The functional significance at the protein-protein and protein-RNA interaction level for each of the components involved in the SF3b complex was also studied. Hence we are also able to provide a mechanistic understanding of SF3b Complex from the structural context and its implications for further understanding of both splicing as well as the diseases such as cancer caused by defects in some of the proteins of the complex.

**Reference:**

1. Golas, M.M., Sander, B., Will, C.L., Luhrmann, R., and Stark, H. (2003). Molecular architecture of the multiprotein splicing factor SF3b. Science *300*, 980-984.

# Active dendrites regulate the spatiotemporal spread of signaling microdomains in a hippocampal model neuron

**Reshma Basak and Rishikesh Narayanan**

Cellular Neurophysiology Laboratory, Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *reshmab@mbu.iisc.ernet.in*

---

Calcium is a key component responsible for the conversion of incoming neuronal signals from the electrical to the biochemical realm. Calcium ions accomplish this by differentially activating several downstream signaling pathways, some of which have been implicated in mediating neuronal plasticity. Given this critical importance of cytosolic calcium to several physiological processes, it is essential to constrict the spatiotemporal spread of the calcium signal, which is tightly regulated by several extrusion mechanisms in the cytoplasm and on the plasma membrane. Here, we consider the calcium-calmodulin-dependent protein kinase II (CaMKII) pathway and quantitatively assess the spread of synaptically-driven microdomains of calcium and its downstream molecules. To simplify the morphological and computational complexity of the analyses, we employed a four-cylinder model, and used Markovian kinetics to assess the role of dendritic voltage-gated ion channels (VGIC) on the spatiotemporal spread of signaling in the CaMKII pathway. We found that the molecules downstream of calcium had progressively higher spatiotemporal spread compared to calcium, with phosphorylated CaMKII (pCaMKII) displaying the largest spread as a consequence of the positive feedback mechanism related to the autophosphorylation of CaMKII. Next, to study the role of active dendrites in the regulating signaling microdomains, we took up two subthreshold dendritic VGICs, the *A*-type potassium channels (KA) and *T*-type calcium channels (CaT). We independently varied the membrane conductance of either channel and quantified its impact on the microdomain spread of calcium and pCamkII. Whereas an increase in KA conductance resulted in a reduction in the spatial spread and amplitude of the calcium signal and a more pronounced reduction in pCaMKII, increasing the CaT conductance induced increases in the spreads and the amplitudes. Finally, we also noted that the impact of both channels on the spreads and the amplitudes (especially of pCaMKII) were significantly higher when synaptic stimulation evoked spikes. Our study demonstrates that active dendrites could play a critical role in steering the spatiotemporal spread of signaling molecules, thereby acting as routing instruments that regulate signaling specificity.

# Structural comparison and normal mode analysis of protease-inhibitor complexes suggest high retention of interface structure and flexibility of inhibitor proteins

**Sneha Vishwanath and N.Srinivasan**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

---

During evolution, interface residues of protein-protein complexes have been observed to be conserved due to functional as well as structural constraints. But some cases have been reported in the literature, where interface residues show high sequence variability at the interface and one such example is protease inhibitors. Here we analyze a dataset of protease-inhibitor complexes of known three dimensional structures. The interface residues of the proteases in the dataset show high sequence conservation with 47% of them being highly conserved. On contrary, interface residues of inhibitors show high sequence divergence with only 24% of them being highly conserved which is on par with the extent of conservation of non-interacting solvent exposed residues. Moreover, two residues interacting with each other in a complex can be conserved to different extent; for example an interface residue which is conserved throughout evolution can interact with a residue which is highly variable. The residues forming side chain – side chain interactions were observed to show high variability while residues forming main chain – main chain interactions were observed to be conserved. On comparing the structure of complexes at C-alpha level, the interfacial region of both proteases and inhibitors were observed to be structurally conserved. But the interface region of inhibitors were observed to be highly flexible and the interface region of proteases were observed to be highly rigid, as measured using normal mode analysis.

Thus from our observations, we can conclude that asymmetry is observed in the evolution of interface residues of proteases and its corresponding interacting inhibitors. Conservation of residues involved in main chain – main chain interactions and the conservation of the structure of the interfacial region, irrespective of sequence variability, indicate the importance of backbone conformation for the interaction between proteases and inhibitors.

We believe that the higher flexibility of the inhibitor interface residues is the reason behind accommodation of the sequence variability. Thus we argue here that high sequence variability and high flexibility allows inhibitors to interact with many different proteases but the conserved backbone limits its reactivity to only few or confining to mainly a specific family of proteases.

# C terminal CGNR Zinc finger protein: A novel activator of transcription in *Mycobacterium smegmatis*

**Subho Ghosh**

Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012

E-mail: *subho@mbu.iisc.ernet.in*

---

Zinc finger proteins have at least one compact domain stabilized by one zinc atom. Since the discovery of TFIIIA as the 1st zinc finger domain protein, many zinc finger domain proteins have been discovered in all three domains of life and they have been implied in various biological functions. C terminal CGNR zinc finger domain containing proteins are present in the genome of many non-pathogenic bacteria and have not been studied so far. Msmeg_0118 is such a protein in *Mycobacterium smegmatis*. This has been found to be a zinc and pH dependent DNA binding protein and also interact with RNA polymerase. This protein has a zinc dependent tetrameric form. This protein is expressed in log phase. On over expression of this protein in *Mycobacterium smegmatis* colony morphology, sliding motility and biofilm formation are affected. Invitro single round transcription with this protein has shown an increase in amount of transcript. This work elucidates the function of a novel zinc finger protein.

# Active spines and dendrites regulate spine neck impedance and perturb their indirect estimate along with calcium-handling mechanisms

**Sufyan Ashhad and Rishikesh Narayanan**

Cellular Neurophysiology Laboratory, Molecular Biophysics Unit,
Indian Institute of Science, Bangalore 560 012
E-mail: *soofy@mbu.iisc.ernet.in*

Most excitatory inputs onto neurons of the central nervous system impinge upon specialized post-synaptic compartments called spines. Anatomically, these are tiny protrusions from the dendritic branches comprising of a narrow neck (<0.1 µm diameter and ~1 µm length) attached to a bulbous head (~1 µm diameter). These anatomical features endow these structures with the ability to amplify synaptic excitatory post-synaptic potentials (EPSPs) within the spine head, while causing unidirectional attenuation of EPSPs when they travel from the spine head towards the parent dendrite. Spine neck impedance (SNI) is a quantitative measure of this electrical transformation across the spine neck, and of the role of spines in neuronal information processing. As a consequence of several experimental constraints resulting from their small size, direct measurement of SNI and its regulators have been infeasible. Furthermore, estimates of SNI from indirect methods, some of which image changes in spine calcium levels and infer that as a direct readout of spine voltage, have inherent assumptions that might not be valid. In this study, we employed biophysically constrained, conductance-based, morphologically realistic neuronal models endowed with detailed calcium handling mechanisms to explore the role of voltage-gated ion channels (VGIC) on the direct measurement and indirect estimate of SNI. We found that the presence of *A*-type $K^+$ channels led to a significant reduction in the SNI, in a manner that was dependent on the synaptic strength and the consequent local EPSP amplitude. Furthermore, the presence of *A*-type $K^+$ (KA) channels also resulted in an overestimate of SNI obtained through the indirect method, owing to the dependence of calcium transients on KA channels. The presence of HCN channels, on the other hand, had minimal effect on the direct measurement of SNI, but resulted in an underestimate of SNI obtained through the indirect method. Finally, we also found that the indirect estimate of SNI was heavily modulated by various calcium handling mechanisms with the density of sarcoplasmic endoplasmic reticulum pump being the most significant among parameters that influenced the estimate of SNI. Our results suggest that sub-threshold VGICs and their plasticity could act as gain modulators for spine signals, thereby critically influencing neuronal integration and output while contributing to encoding and storage of the incoming information. Our results also emphasize the need to account for the presence and localization of VGICs and calcium-handling mechanisms in interpreting indirect estimates of SNI.

NOTES: