Structural Biology Meeting 2019

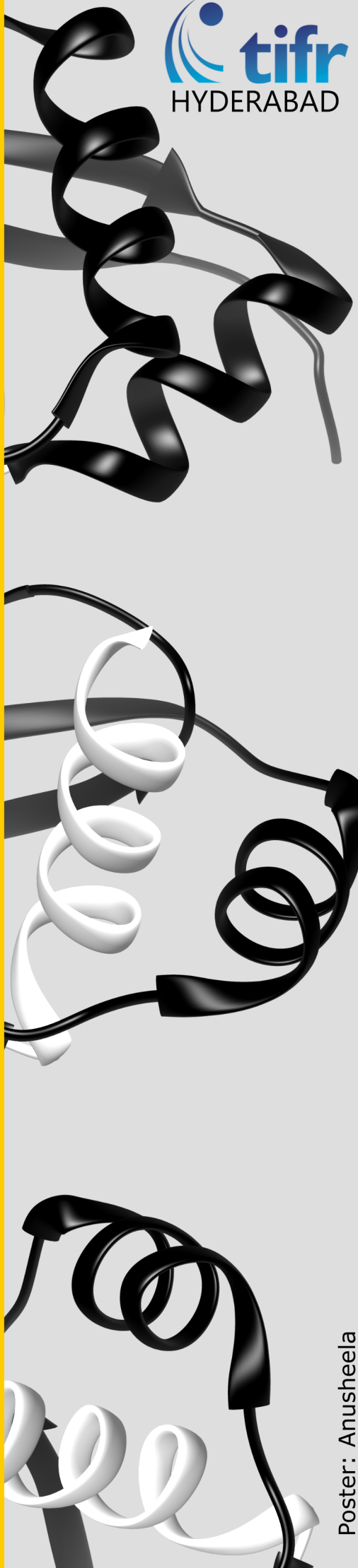
tifr HYDERABAD

21 August, 2019
Venue: TIFR Hyderabad

A specialised structural biology meeting to encourage collaboration among DAE labs. This meeting will cover various aspects of structural biology- from structures to dynamics using various experimental techniques, X-ray Crystallography, NMR Spectroscopy, Cryo-Electron Microscopy, Fluorescence Spectroscopy etc.

Speakers include:

1) Vinay Kumar (BARC)
2) Vinothkumar Kutti Ragunath (NCBS)
3) Arati Ramesh (NCBS)
4) Ranabir Das (NCBS)
5) Anjana Badrinarayanan (NCBS)
6) Vipin Agarwal (TIFRH)
7) Kaustubh R. Mote (TIFRH)
8) P. K. Madhu (TIFRH)
9) Pramodh Vallurupalli (TIFRH)
10) Kanchan Garai (TIFRH)
11) Kalyaneswar Mandal (TIFRH)



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Poster: Anusheela

Structural Biology Meeting

August 21st 2019

TIFR Hyderabad

Time	Speaker	Title/Event
Session-1 (9:00 to 11:30 AM) Chair: V Chandrasekhar (TIFRH)		
9:00-9:15 AM	Sandip/VC	Welcome
9:15-10:05 AM	Arati Ramesh (NCBS)	Structure to signaling: Regulating metal-homeostasis through RNA
10:05-10:55 AM	Vinay Kumar (BARC)	Structure-function studies of bio-technologically important proteins
10:55-11:30 AM	Kanchan Garai (TIFRH)	Real time monitoring of amyloid aggregation using single-molecule fluorescence techniques
11:30-11:45 AM		Coffee
Session-2 (11:45 AM to 1:25 PM) Chair: Abani K. Bhuyan (University of Hyderabad)		
11:45 AM -12:35 PM	Vinothkumar K R (NCBS)	Structures of macromolecules by electron cryomicroscopy
12:35-1:25 PM	Vipin Agarwal (TIFRH)	TBA
1:25-2:10 PM		Lunch
Session-3 (2:10 PM to 4:25 PM) Chair: Jagannath Mandal (TIFRH)		
2:10-3:00 PM	Anjana Badrinarayanan (NCBS)	Bacterial DNA repair – what happens when the war is over?
3:00-3:35 PM	Kalyaneswar Mandal (TIFRH)	Mirror-image proteins: novel protein inhibitors of natural protein-protein interactions
3:35-4:25 PM	Kaustubh Mote (TIFRH)	Improving the throughput for the NMR based structural biology of membrane proteins
4:25-4:40 PM		Coffee
Session-4 (4:40 PM to 6:45 PM) Chair: Surajit Sengupta (TIFRH)		
4:40-5:30 PM	Ranabir Das (NCBS)	Protein engineering by domain-swapping
5:30-6:20 PM	Pramodh Vallurupalli (TIFRH)	Understanding the Conformational Dynamics of Proteins in Solution
6:20 PM	P K Madhu (TIFRH)	Closing Remarks
6:45 PM		Leave for Dinner at OTM

Structure to signaling: Regulating metal-homeostasis through RNA

Arati Ramesh

NCBS-TIFR, Bangalore

My lab's interests are centered around RNA structure. Three broad areas that we are investigating are: 1) Identifying bacterial signaling pathways that are mediated by RNA structures. This includes signaling through metabolite-binding ribsowitches as well as protein-RNA complexes, 2) Design of RNA based sensors that can be used as probes for metabolites in the cell, and 3) understanding the role of RNA structure in fundamental RNA-driven processses such as translation.

Our broader interest in identifying natural RNA elements that function in cell signaling has recently led us to discover a novel class of RNAs that are likely to function as specific metal-sensors in bacteria. From mammals to bacteria, all cells require iron. Iron directly controls fundamental cellular process that involve redox. Iron is essential for ATP synthesis through oxidative phosphorylation where it occupies the electron transfer centers of respiratory chain complexes. For most cells, iron-dependent enzymes are essential and required to survive oxidative stress. In most pathogenic bacteria, the ability to sequester iron from the host determines its pathogenicity. Despite this pivotal role across biology, how intracellular iron is sensed, what are the sensors at play is not known. In eukaryotes, metabolic enzymes such as aconitase have been implicated to moonlight as iron-sensors. In bacteria, apart from a few iron-sensing transcriptional factors, it is unclear how iron may be sensed. Our collective findings suggest two modes of RNA based iron regulation- 1) that is independent of protein factors, and comes from direct iron binding to RNA via an iron-responsive riboswitch and 2) a variation on the theme of moonlighting metabolic enzymes that is widepread in bacteria. Our broader goal is to uncover the structural and functional mechanisms by which intracellular iron is sensed by structurally conserved RNAs. We seek to identify key players involved, determine their mechanisms of action, tying in RNA-mediated iron sensing with cellular redox states.

Structure-function studies of bio-technologically important proteins

Vinay Kumar

Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre Mumbai

We have recently been focussing on structural and functional studies of bio-technologically important proteins; solar-energy absorbing phycobiliproteins of marine cyanobacteria, a disulphide oxido-reductase (FrnE) of *Deinoccocus radiodurans*, and mosquito-larvicidal BinAB proteins from *Lysinibacillus sphaericus*. Crystal structures of three important component proteins (allophycocyanin, phycocyanin and phycoerythrin) reveal quaternary structure of phycobiliproteins, provided insight into direction of energy flow and their evolution [**1,2**]. Structural and functional studies of drFrnE 'trapped' in different redox states provided clues for the critical role of C-tail residues in regeneration of enzyme active-site during catalytic cycle and its cytoplasmic localization. 'True' orthologs of drFrnE were found in several eubacterial phyla and, interestingly, all of these groups apparently lack functional glutaredoxin system [**3**]. The talk shall also focus on *Lysinibacillus sphaericus* BinAB toxin that is responsible for high mosquito-larvicidal activity of several *L. sphaericus* strains used world-wide for controlling the mosquito population. We have 'rationally' engineered/modified the toxic component (BinA) that displays much higher larvicidal activity as compared to the combination of BinA/BinB components [**4**]. Further, we have characterized BinA receptor (Cqm1) protein, elucidated its high-resolution crystal structure, probed toxin-receptor interactions by small angle neutron scattering, and proposed the likely mechanism of intracellular toxicity of BinAB proteins [**5,6**].

1. Kumar et al.,Photosynth Res. 2016;129:17-28.
2. Sonani et al. Sci Rep. 2019;9:9863.
3. Bihani et al. Antioxid Redox Signal. 2018;28:296-310.
4. Sharma et al. Bioconjug Chem. 2017;28:410-418.
5. Sharma et al. Insect Biochem Mol Biol. 2018;93:37-46.
6. Sharma et al. J Invertebr Pathol. 2018;156:29-40.

Real time monitoring of amyloid aggregation using single-molecule fluorescence techniques to determine the rate constants of the microscopic processes

Kanchan Garai

TIFR Hyderabad

Elucidation of the underlying mechanisms of amyloid aggregation of proteins is critical in understanding the development of many neurodegenerative diseases. Knowles and co-workers have suggested a simple reaction mechanism whereby soluble monomers self-assemble to fibrillar forms via both primary nucleation and secondary nucleation processes followed by elongation of the nuclei. The secondary nuclei may be catalysed at the surface of the existing fibrils or may arise due to fragmentation of the fibrils. However, determination of the rate constants of the various microscopic processes using ensemble measurements are difficult. We have used two single-molecule sensitive techniques, viz, cuvette-FCS and total internal reflection fluorescence microscopy (TIRFM) for real time monitoring of amyloid aggregation. Analysis of the fluorescence bursts of thioflavinT due to aggregation of amyloid-β (Aβ) in Cuvette-FCS allows measurements of concentration of the fibrils (P(t)), size distribution of the fibrils and total aggregation (M(t)) both under quiescent conditions and in presence of stirring. Thus, cuvette-FCS allows characterization of the
early aggregates and direct measurement of the elongation rate constant. Additionally, we have established a TIRFM based approach to visualize time dependent growth of the individual amyloid fibrils. While both the techniques provide estimation of the rate constants of elongation and the secondary nucleation, TIRFM provides measurements of the heterogeneity of the various rate constants. Furthermore, TIRFM enables characterization of the parallel pathways of aggregation. Global fitting of the carefully performed ensemble experiments using a large set of concentrations of Aβ have been used earlier to determine the rate constants. However, ability to measure both P(t) and M(t) at the same time in the single molecule experiments makes the measurements of the rate constants easy and robust. For example, we find that while kinetics of the overall aggregation depend highly on sample handling, the elongation rate constant remains almost invariable. Taken together, single molecule measurements of amyloids can be used to evaluate individual rate constants of both primary and secondary processes of amyloid aggregation.

Structures of macromolecules by electron cryomicroscopy

Vinothkumar K.R.

NCBS-TIFR, Bangalore.

Electrons and electron microscopy have the power to image individual atoms. In the study of inorganic materials, resolutions better than 1 Ångstrom are routinely achieved. However, this requires high electron dose and radiation damage by the electron beam means that structure determination of biological molecules requires averaging multiple molecular images. Together with radiation damage, electron beam-induced movement and the need for higher signal to noise are limiting factors for biological specimens and the resolutions that was achieved by CryoEM until recently has remained low.

During the last few years, there has been enormous progress in the determination of three-dimensional biological structures by CryoEM, allowing maps to be obtained with higher resolution and from fewer images than required previously. This is due principally to the introduction of a direct electron detector that has 2- to 3-fold higher detective quantum efficiency (DQE) than available previously, and to the improvement of the computational algorithms for image processing. Using selected biological molecules involved in bioremediation, I will describe how these advances result in high-resolution structures of proteins in understanding their function.

TBA

Vipin Agarwal

TIFR Hyderabad

Bacterial DNA repair – what happens when the war is over?

Anjana Badrinarayanan

NCBS-TIFR, Bangalore.

Maintenance of genomic integrity is essential for the faithful propagation of life. Cells in all domains of life are under the constant threat of DNA damage from a variety of internal and external factors and are capable of fixing the same via dedicated repair mechanisms. As part of this process, cells also stop cell cycle progression so as to facilitate repair and ensure that daughter cells inherit intact chromosomes upon division. While the steps of cell cycle arrest are well-studied, how cells exit this state is unclear. This is particularly important as successful repair also has to result in the re-entry of cells into the cycle of replication and division. In this talk, I will share our recent insights into the dynamics of cellular recovery from DNA damage in bacteria using a time-resolved, quantitative live cell imaging approach. I will highlight the robust principles of cell size maintenance in this perturbed system and discuss probable mechanisms by which cells coordinate DNA repair with chromosome segregation and asymmetric cell division to produce fit daughter cells that tend to be devoid of damage.

Mirror-image proteins: novel protein inhibitors of natural protein-protein interactions

Kalyaneswar Mandal

TIFR Hyderabad

All proteins found in nature are inherently chiral and made up of *L*-amino acids. By contrast, a protein made from *D*-amino acids will fold to form the mirror image of the natural protein with the same amino acid sequence. There is no biochemical machinery exists in nature to synthesize *D*-proteins; therefore, it can only be realized using synthetic chemistry. A properly engineered mirror-image protein molecule would have near optimal properties as a therapeutic. A small *D*-protein will resist proteolytic degradation, will be less immunogenic and will have better tissue penetration compared to conventional antibody therapeutics. We set out to apply a unique combination of 'chemical protein synthesis' and 'mirror image phage display' to systematically identify *D*-protein inhibitors of disease related protein-protein interactions. As a proof of concept we applied this technique to develop a mirror-image protein antagonist of vascular endothelial growth factor (VEGF-A), and demonstrated the binding by determining the first ever crystal structure of the hetero chiral protein complex by racemic crystallography. Later, we extended this concept to find inhibitors of red blood cell invasion by malaria parasites.

Improving the throughput for the NMR based structural biology of membrane proteins

Kaustubh R. Mote
TIFR Hyderabad

Membrane proteins form a critical area of structural biology given their outsized relevance in clinical studies. NMR spectroscopy, in particular, solid state NMR is particularly suited to the study of membrane proteins as it can give direct insights into their function arising from their dynamic nature. These proteins are however, challenging to study by NMR due to three main reasons: (i) Poor heterologous expression of these proteins makes their production in a form suitable for NMR studies difficult, (ii) poor sensitivity in NMR experiments due to low packing fractions necessitated by the presence of lipids and hydration, and (iii) poor resolution due to repeated presence of a relatively high degree of similar secondary structural elements. In addition, these proteins also tend to be more susceptible to degradation during the course of lengthy NMR experiments due to the inherent instability of sample components. I will discuss these issues within the context of a particular class of membrane proteins - the secondary active transporters and present our work which takes steps in the direction of alleviating some of them. In particular, I will present the use of a new fusion protein system for large-scale over expression of membrane proteins in E. coli, and the development of ways to combine experiments in solid-state NMR with minimal increase in the amount of deleterious radiofrequency pulses that the sample is subjected to. Taken together, these synergistic developments will enable a systematic application of advanced solid-state NMR techniques to decipher the functioning of these proteins.

Protein engineering by domain-swapping

Ranabir Das

NCBS-TIFR, Bangalore.

Rational design of protein-protein interactions can be used to build supramolecular assemblies capable of performing both biological and bio-inspired functions. The structural complexity of proteins necessitates the optimization of each design approach in a protein-specific manner. Hence, universal strategies of protein design are rare. We seek to devise new rules and strategies that can be universally applied for protein design. Domain swapped proteins are useful for oligomer assembly, encode novel functions and evolution of novel protein folds. We will discuss design of universal motifs for producing domain swapped dimers by truncating and substituting beta-turns in proteins. Such universal motifs are in high demand for protein engineering.

Understanding the Conformational Dynamics of Proteins in Solution

Pramodh Vallurupalli

TIFR Hyderabad

Despite the importance of protein dynamics to function, studying exchange between different conformational states remains a challenge because sparsely populated states cannot be detected by conventional experiments. Even less is known about how the proteins cross the barrier between different conformational states. Using newly developed NMR experiments it is now possible to detect minor conformational states with lifetimes between ~50 μs and ~200 ms populated to as low as 0.5% so long as they are in exchange with a visible state. As the spectra of these invisible minor states can be reconstructed very accurately it is even possible to obtain atomic resolution structures of the protein in these conformational states. I will describe how these methods are being used to study the conformational exchange of T4 lysozyme variants between different compact conformers and to understand the folding mechanism of the protein. Even though T4 lysozyme and its mutants have been studied for several years, the new NMR experiments have provided several new and surprising insights into the different conformational exchange processes. Using CPMG NMR experiments an atomic resolution model of a minor conformer with a lifetime of ~1 ms populated to just ~3% was obtained while CEST NMR experiments clarified the folding mechanism of T4 lysozyme. I will also describe how we are using the experiments to obtain insights into the role that water plays in activating protein molecule to cross the free energy barrier between different states and to obtain structural information about the conformations that the protein molecule adopts while crossing the barrier.