Nuclear Magnetic Resonance and BioMolecules (NMRBM)

International Symposium in honor of late Prof. HS Atreya

Date : 08-11-2020 (Sunday)
Time: 09.30 am-04.30 pm IST

About Prof HS Atreya :

Prof. Hanudatta Sastry Atreya obtained his five years Integrated Master of Science in Chemistry in 1997 from Indian Institute of Technology, Bombay and his PhD in Molecular Biophysics from Tata Institute of Fundamental Research (TIFR) in 2002 under the supervision of Prof. KVR Chary and Prof. G. Govil. Following postdoctoral research with Prof. Tszyperski at the State University of New York at Buffalo, Dr. Atreya has joined NMR Research Centre, IISc as an assistant Professor in 2006. Prof. Atreya focused during his research career on using NMR spectroscopy for studying proteins and peptides, NMR methodologies and relaxation studies, metabolomics, protein-nanomaterial interaction studies using NMR and recently started working on artificial intelligence for reconstruction of NMR spectra. Prof. Atreya continued as a Professor of NMR Research Centre, IISc till his last moment in July-2020.

About the symposium:

Prof. Atreya was among the well-known Protein-NMR spectroscopists who always contributed to the field through his innovative ideas and incorporating interdisciplinary fields of research. Beside being a great scientist, Prof. Atreya was a dedicated mentor and supervisor for his students. As a mark of respect and honour to our teacher, students of Prof Atreya are organizing a one-day symposium 'Nuclear Magnetic Resonance and BioMolecules (NMRBM)' on Sunday, 8th of November, which coincides with his birthday.

Speakers

Prof. Ray Norton
Dr. Haribabu
Dr. Monalisa S.
Dr. Thirupathi R
Prof. KVR Chary
Dr. Shivanand P.
Prof. Annapoorni R.
Prof. Satish K. Adiga
Dr. Gitanjali A.
Prof. Anirban Bhunia
Dr. Sulakshana PM
Dr. Varsha B.
Shankararama Sharma R

Registration link:

https://forms.gle/Hs6kiD18xLkd2exA6

Organizers

Prof. N Suryaprakash, Chairman, NMRRC, IISc, India
Students of Prof. Atreya, NMRRC, IISc , India
Dr. Kirtimaan Syal, BITS-Pilani, Hyd, India

For doubts, kindly contact:
ksyal@hyderabad.bits-pilani.ac.in
zmohanta17@gmail.com
somnath.mondal@inserm.fr

Schedule of NMRBM (08.11.20, 09:30-17:00 IST)

09:30-09:45 am	**Welcome address and Introduction, Prof. N. Suryaprakash, Chairman, NMRRC, IISc, Bangalore** **Session I Chair: Prof. TN Guru Row, SSCU, IISc**	
09:45-10:15 am	Prof. Ray Norton, Monash Univ.	Intrinsically disordered protein antigens: opportunities for vaccine development against major pathogens
10:15-10:45 am	Dr. Haribabu A., HMS, Boston	Sensitivity vs Resolution: A case for low-gamma nuclei
10:45-11:05 am	Dr. Monalisa S., NIH, USA	Conformational dynamic study in RNA and Proteins by NMR
11:05-11:25 am	Dr. Thirupathi, Univ. of Michigan	Polymer Lipid-Bilayer Nanodiscs
Session II Chair: Prof. BJ Rao, IISER Tirupati		
11:45-12:15 pm	Prof. KVR Chary, TIFR/IISERBPR	My Journey with Atreya
12:15-12:35 pm	Dr. Shivanand P., UTMDACC, USA	Towards virtual colonoscopy: targeted molecular MRI of colorectal cancer by hyperpolarization
12:35-01:05 pm	Prof. Annapoorni R., MRDG, IISc	Role of the central metabolic regulator AMPK in cancer metastasis
Session III Chair: Prof. RV Hosur, TIFR, Mumbai		
02:00-2:30 pm	Prof. Satish K. Adiga, SMU	Non-invasive embryo selection in ART: how far from reality?
02:30-02:50 pm	Dr. Gitanjali A., St. Jude, USA	NMR studies of a self-assembling intrinsically disordered polypeptide
02:50-03:20 pm	Prof. Anirban Bhunia, Bose Inst.	Designing of Antimicrobial Peptides - From Laboratory to Transgenic Plant
Session IV Chair: Dr. Sasikumar, Aurigene, Bangalore		
03:40-04:10 pm	Dr. Sulakshana PM, IIT-R	Mechanistic insights into the specific NF-kB dimer formation
04:10-04:30 pm	Dr. Varsha B., Jain Univ.	Understanding Nanoparticle–protein corona by solution NMR Spectroscopy
04:30-04:50 pm	Shankararama Sharma R, IISc	Deep Learning for Reconstruction of NUS Spectra of Complex Metabolic Samples
04:50-05:00 pm	Reminiscence	HSA lab students, colleagues and other students

Prof. Raymond S Norton (09:45-10:15 am)

Intrinsically disordered protein antigens: opportunities for vaccine development against major pathogens

Intrinsically disordered proteins are highly abundant in the malaria parasite Plasmodium and other important human pathogens. Merozoite surface protein 2 (MSP2) is one such antigen from P. falciparum that is highly abundant on the surface of merozoites and elicits a protective immune response in humans. Our recent analysis of antibody recognition of disordered antigens shows that these antigens make excellent vaccine candidates.

Crystallographic and NMR studies showed that recognition of a conserved N-terminal epitope from MSP2 by the mAb 6D8 is incompatible with the membrane-bound conformation of this epitope, suggesting a mechanism by which parasite MSP2 escapes recognition by 6D8. Intriguingly, NMR also identifies transient, strain-specific interactions between the 6D8 mAb and regions of MSP2 beyond the conserved epitope. Even though these interactions are transient, they nonetheless modulate the binding of these epitopes, either as peptides or full-length antigens, to the antibody.

At the other end of the protein, the conserved C-terminal region of MSP2 is recognised by mAbs 4D11 and 9H4, with 4D11 binding to merozoites much more strongly than 9H4. A crystal structure of 4D11 Fv bound to the linear epitope NKENCGAA reveals the possible conformation of the C-terminal region of MSP2 on the parasite. These results underpin ongoing efforts to not only optimise recombinant MSP2 constructs but also develop peptide-based antigens as malaria vaccine candidates.

Dr. Haribabu A. (10:15-10:45 am)

Sensitivity vs Resolution: A case for low-gamma nuclei

15N detection never had the limelight in bimolecular NMR due to the inherent low sensitivity compared to 1H/13C-detected experiments. The low gyromagnetic ratio of 15N results in favorable relaxation properties and it is the slowest relaxing nucleus in protein NMR, which results in narrow linewidths. With the advancement in data collection using Non-Uniform Sampling and improvement in cryoprobe technology, sensitivity should no longer be seen as a number, rather the ability to see the weakest resonance of interest. This talk will introduce a suite of 15N-detected 2D and 3D experiments, discuss their advantages and application in studying Intrinsically Disordered Proteins (IDPs) and large systems.

Dr. Monalisa S. (10:45-11:05 am)

Conformational dynamic study in RNA and Proteins by NMR

In solution biologically active molecules like RNA, enzymes are not static; they are continuously undergoing motions over a range of timescales and playing important role in their function. These motions vary from fast ps time scale to slow time scales of the order of few seconds. NMR is the only techniques that can capture a very broad range of time scale motions at an atomic resolution level. I will be talking about the findings of these conformational motions by different NMR relaxation experiments with example to a RNA named add Riboswitch and an enzyme called DNA Polymerase beta (Polb). We will also see how the presence of ligand alters these biological motions in different regions further emphasizing the importance of the conformational dynamics in RNA and proteins.

Dr. Thirupathi Ravula (11:05-11:25 am)

Polymer Lipid-Bilayer Nanodiscs

Membrane proteins play important roles in a variety of cellular functions and also on the pathology of many diseases. High-resolution structural and functional studies on these membrane bound proteins are severely limited by the difficulties associated with their solubilization and reconstitution without a loss of their function. Polymer-based nanodiscs are valuable tools in biomedical research that can offer a detergent-free solubilization of membrane proteins maintaining their native lipid environment. In this presentation, I will discuss about the design,synthesis, demonstration of nanodiscs formation, magnetic-alignment, and feasibilities of solution and solid-state NMR experiments. Our results demonstrate the first use of magnetic-alignment behavior of lyotropic liquid crystalline polymer macro-nanodiscs (> 20 nm in diameter) for solid-state NMR studies on membrane proteins and also as a novel alignment medium for the measurement of RDCs using high resolution NMR. 31P, 14N, 2D SLF, TROSY-HSQC, natural-abundance 17O NMR experimental results will presented. The easy preparation of macro-nanodiscs, divalent metal ions, high stability against pH, and high homogeneity are the key advantages for studies to investigate a wide range of molecular systems including natural products, proteins and RNA.

Prof. KVR Chary (11:45-12:15 pm)

My journey with Atreya

I remember Atreya as one of the finest craftsmen of NMR spectroscopy in India, a brilliant scientist, and an exceptionally kind human being, who had been an institution of knowledge, inspiration and wisdom to many in the field. I want to share some of our finest memories which were also instrumental in the evolution of a great scientist.

Dr. Shivanand Pudakalakatti (12:15-12:35 pm)

Towards virtual colonoscopy: targeted molecular MRI of colorectal cancer by hyperpolarization

Colorectal cancer is a 4th most prevalent cancer amongst men & women and 2th leading cause of cancer-related mortality. This an aggressive and initially insidious lethal disease that develops relatively symptom-free. The absence of early symptoms and lack of a reliable screening test has created a critical need for developing a new noninvasive imaging strategy for colorectal cancer early detection. The overarching goal of research project is to develop a non-invasive Magnetic Resonance Imaging (MRI)-based *in vivo* molecular imaging modality to detect colorectal cancer at an early stage with high sensitivity and specificity. The high sensitivity is be achieved by hyperpolarized (HP) Silicon particles (SiPs) and nanoparticles (SiNPs) by Dynamic Nuclear Polarization (DNP) that leads to over 10,000 fold sensitivity enhancement. In contrast to the hyperpolarization of carbon -13 nucleus, wherein MR signals are lost within a few minutes, hyperpolarization of ^{29}Si produces MR signals with a characteristic decay time of >1 hour. The high specificity is achieved by functionalizing SiNPs with an antibody. For the detection of colorectal cancer at early stage we are employing mucin-1 (MUC-1) as receptor molecule which is a membrane glycoprotein overexpressed in colorectal cancer tumors. In this study described, we are functionalizing SiNPs with anti-MUC1 antibody, *214D4* for real-time MR based molecular imaging application in colorectal cancer.

Prof. Annapoorni Rangarajan (12:35-01:05 pm)

Role of the central metabolic regulator AMPK in cancer metastasis

A leading cause of cancer-associated deaths is due to the spread of cancer cells by metastasis. This requires cell-detachment from the extracellular matrix (ECM) at the primary site of cancer initiation, intravasation into the blood vessels, followed by extravasation and colonization of secondary organ sites. However, ECM-detachment triggers cell death, termed as anoikis, in normal epithelial cells. Therefore, overcoming anoikis is a prime requisite for metastasis. One key aspect of this problem is the distinction between the cellular states of matrix-attached and matrix-deprived cancer cells. Work done in my laboratory has identified a novel role for AMP-activated protein kinase (AMPK) – an energy sensor and central metabolic regulator – in governing the cellular state of matrix-detached cells and help overcome anoikis. Further, using an in vitro transformation model, we have identified elevated levels of proline in matrix-detached tumorigenic cells using Nuclear Magnetic Resonance (NMR) spectroscopy, and a role for AMPK in the same. Our work proposes targeting AMPK, and AMPK-dependent metabolic alterations, as a novel mechanism to prevent/delay metastasis.

Prof. Satish Kumar Adiga (02:00-2:30 pm)

Non-invasive embryo selection in ART: how far from reality?

Despite the numerous developments and frequent use, Assisted Reproductive Technology (ART) is experiencing relatively low success rate due to inability to evaluate the developmental competence of the preimplantation embryos derived *in vitro*. Although, morphological assessment has considered as 'gold standard' and remarkably improved ART success over the past 40 years, its subjectivity and physiological irregularities in embryos with identical morphology made the embryo selection a challenging task. Besides, the conventional microscopic evaluation of morphology does not address the embryonic genetic integrity. Time lapse monitoring (TLM) is being implemented as an adjunct tool to morphological evaluation of embryos. Noninvasive monitoring of preimplantation embryo metabolism has emerged as one of the prime candidates in the pursuit of alternate and improved means of embryo selection. Numerous technologies have been established to study early embryonic metabolism and to identify metabolites that can be used as biomarkers to determine implantation potential, genetic integrity, aneuploidies, and embryo viability. The presentation discusses the challenges and opportunities in using metabolomics approach as non-invasive markers for embryo selection.

Dr. Gitanjali Asampille (02:30-02:50 pm)

NMR studies of a self-assembling intrinsically disordered polypeptide

Intrinsically disordered proteins (IDPs) are of prodigious interest due to their diverse biological functions such as cell signalling and cellular organization. They exhibit mysterious structural dynamics. We describe and illustrate a self-assembling IDP; the IDP of interest forms a higher order nanostructure (Nanotube) via disulphide formation. This redox responsive nanotube is ultra-stable over a wide range of temperature and shows enhanced cell specific interactions by virtue of its functional motif i.e. RGD (Arg-Gly-Asp). Extensive characterization of the nanosystem is performed and its functional efficacy in cancer cell targeting and tissue engineering has been evaluated. Here, we focus on real time monitoring of self-assembly using SDS-PAGE and two-dimensional NMR spectroscopy experiments emphasizing on the initial time points of oligomerization to observe the intermediates. Disulphide formation during self-assembly has been investigated. Structural and dynamic aspects of the self-ordering process have been studied as a function of time. We have successfully explained the molecular motions at different time scales. A detailed discussion of the observation of protein oligomer signatures and respective changes in the spectral profile has been provided.

Prof. Anirban Bhunia (02:50-03:20 pm)

Designing of Antimicrobial Peptides - From Laboratory to Transgenic Plant

Understanding the mechanisms of biological processes requires precise knowledge of the three- dimensional structures of the executor molecules such as proteins, bioactive peptides and others. Atomic-resolution structures of well-folded proteins or complexes can be obtained from X-ray crystallography. However, a large number of proteins or domains of large proteins (e.g., in signaling cascades) and bioactive peptides (e.g., antimicrobial peptides) appear to be dynamic, thus limiting the application of X-ray-based methods. On the other hand, gaining insights into such molecular systems at the atomic level is possible using nuclear magnetic resonance (NMR) spectroscopy. To this end, we have determined the three-dimensional structure of a de novo designed non-toxic, non-hemolytic and bacterial cell selective antimicrobial peptide from the active fragment of Dengue Virus fusion peptide in complex with lipopolysaccharide (LPS), the major component of the outer-membrane of gram negative bacteria, using solution-state NMR methods. LPS is essential for bacterial survival by establishing an efficient permeability barrier to exogenous compounds, and is also primarily responsible for sepsis/septic shock syndromes associated with serious Gram-negative bacterial infection. Thus, the design of novel antimicrobial peptides (AMPs) and the determination of their three-dimensional structures in LPS is useful for the development of more potent antibacterial and antiendotoxic peptides for the treatment of human and plant pathogens.

Dr. Sulakshana Mukherjee (03:40-04:10 pm)

Mechanistic insights into the specific NF-kB dimer formation

NF-kappaB family of transcription factors is vital to immune response in vertebrates. The family members function as dimers formed among its members in various combinations with RelA-p50 being the most abundant dimer physiologically. In my talk I will show that the stability of the individual NF-kB dimer is fine-tuned in such a manner that the formation kinetics of a specific dimer is regulated. This kinetics of specific dimer formation can be modulated through fine-tuning of thermodynamic stability of individual NF-kBs.

Dr. Varsha B. (04:10-04:30 pm)

Understanding Nanoparticle–protein corona by solution NMR Spectroscopy

The nanoparticle surfaces, especially the metal nanoparticles, interact readily with biomolecules including proteins. On coming in contact with the biological fluids, the nanoparticle surface is covered by proteins forming a corona. The nanoparticle with protein corona is the true identity of nanoparticle in living system. Nanoparticles can affect the confirmation and function of proteins leading to active or passive targeting of nanomedicine designed for specific reason. Therefore it is important to understand protein corona composition, stoichiometries and binding rate of protein on nanoparticle surface.

In the present study we explored the ubiquitin corona on surface of gold nanorods with aspect ratio of 2. The solution NMR spectroscopy and other techniques revealed that ubiquitin formed a multilayer corona on the gold nanorod surface. The other techniques employed in addition to NMR studies include UV-Visible spectroscopy, dynamic light scattering (DLS), zeta potential measurements and transmission electron microscopy (TEM). The constant increase in hydrodynamic radius of gold nanorods was observed from DLS measurements with increase in ubiquitin concentration. Additionally the zeta potential values decline with increased protein concentration as, gold nanorods surface is positively charged with zeta potential value of 39 mV, indicating negative surface of protein is interacting. The higher hydrodynamic and solution NMR spectroscopy results suggest multilayer ubiquitin corona formation. The detailed investigations of mechanism of biding and nanoparticle binding site on protein at residue level is in progress.

Shankararama Sharma R (04:30-04:50 pm)

Deep Learning for Reconstruction of NUS Spectra of Complex Metabolic Samples

High-resolution NMR spectra in 2D and higher dimensions consume a lot of time to acquire. It takes a few hours to days sometimes. Non-Uniform Sampling (NUS) is a method suggested to reduce the time of acquisition. Such data needs to be specially processed, to recreate the complete spectrum.

Deep learning with its wide range of applications is proving to be a practical solution to problems. Metabolic NMR spectra can typically have tens to hundreds of peaks with varying intensities. The data is complex as well as voluminous. Here, we shall look at applying deep learning for the reconstruction of NUS spectra. The model is trained on synthetically created samples and tested on practical data at different sampling percentages and schedules. This model is then compared with the existing methods like hmsIST and SMILE.