NMR Meets Biology 4
Khajuraho, India
Dec 16-21 2018
Book of Abstracts

Preface

The NMR Meets Biology series of meetings was started as an avenue for researchers working towards solving biological problems using NMR spectroscopy to discuss the past, present and especially the future of the technique in this ever changing field. The strategy has been to include tutorial sessions along with research talks that allow for a open discussion of all aspects of NMR and biology, for both the expert and the beginning NMR spectroscopists. The informal atmosphere, comparatively small number of attendees and focus on the balance between teaching and research are the key aspects of this meeting. This meeting was first held in April 2014 in Goa, India. The second iteration was held in Allepey, Kerala (2016), and the third in Grossbothen in Germany (2017). This iteration of the meeting is being from December 16th to 21st 2018 in the Clarks Hotel in Khajuraho, a historic city in the Indian state of Madhya Pradesh noted for its exemplary temple architecture, now a UNESCO World Hertiage site. The meeting will focus on the state-of-the-art solid- and solution-state NMR methods to tackle challenging issues in biology. There will be dedicated tutorial session on relaxation methods in NMR and their use to study dynamics. The meeting will have several brain-storming sessions on the future prospects, with respect to the methods and systems.

We hope that the informal environment will encourage an organic exchange of ideas and that the teaching and tutorial sessions will help students, beginners and advanced, to better understand the intricacies of NMR as a technique and apply it to thier own research problems.

Organising Committee

P. K. Madhu, TIFR Hyderabad
Vipin Agarwal, TIFR Hyderabad
Kaustubh R. Mote, TIFR Hyderabad
Neel Sarovar Bhavesh, ICGEB, New Delhi
Ashutosh Kumar, IIT Bombay

Contents

Preface **iii**

Research Talks: December 16 **1**

Introduction (***P. K. Madhu***) 1

Relaxation in NMR experiments (***Beat Meier***) 1

Solid-state NMR method development at TIFR Hyderabad (***Vipin Agarwal***) 1

Biophysical insights into the role of membrane dynamics in diseases (*Manjari Mishra, Ruchika Dadhich, Adhyapak Pranav, Jaladhar Matao,* ***Shobhana Kapoor***) 1

Research Talks: December 18 **3**

Solid-state NMR investigations of hepatitis B virus structural proteins (***Anja Bockmann***) 3

Small molecule-based targeting of AML1-ETO oligomerization as a potential strategy for anti-leukemia therapy (***Mohanraj Gopalswamy***[1], *Tobias Krger, Benedikt Frieg, Tao Zhang, Alexander Metz, Luitgard Nagel-Steger, Manuel Etzkorn, Holger Gohlke*) 3

Studying conformational dynamics of proteins in the solution state (***Pramodh Vallurupalli***) 4

Small RNA mediated gene silencing in plants: Lessons for knock-down in gene therapeutics (***Mandar V. Deshmukh***) 4

Probing Protein Dynamics using NMR, H/D Exchange, and Atomistic Simulations (***Harish Vashisth***) 5

Protein aggregation investigated by NMR spectroscopy (***Bernd Reif***) . . 5

Research Talks: December 19 **7**

NMR spin relaxation measurements and MD simulations reveal tuning of conformational entropy in oncogenic mutations of p53's DNA binding domain. (***Sujoy Mukherjee***) 7

Elucidating the mechanism of interaction of Hsp70 with client proteins (***Ashok Sekhar***[1]*, Algirdas Velyvis, Guy Zoltsman, Rina Rosenzweig, Guillaume Bouvignies Lewis E. Kay*) 7

Dynamical modes in RNA binding protein allow for shape-dependent RNA recognition (*Harshad Paithankar,* ***Jeetender Chugh***) 8

Applications of NMR spectroscopy in studying self-assembly and membrane interaction of Amphiphilic Ionic Liquids (*Sandeep Kumar, Navleen Kaur, Gourav Kumar,* ***Venus Singh Mithu***) 9

Research Talks: December 20 **11**

Probing membrane protein ground and conformationally excited states using dipolar- and J-coupling mediated MAS solid-state NMR experiments (***Tata Gopinath***) 11

Sensitivity Enhancement via Triply Compensated Pulses for High-Field Spectrometers (***V. S. Manu****, Gianluigi Veglia*) 11

Influence of aromatic side-chains in biomolecular structure and dynamics (***Bharathwaj Sathyamoorthy***) 12

Structural basis for the increased microtubule localization of EB1 by GTP and SxIP (*Shine A.,* ***Vinesh Vijayan***) 13

NMR Study of Biomaterials From Bone Protein Structure to Minerals in Mechanically Different Bones (*Shani Hazan, Irina Matlahov, Alex Kulpanovich, Artyom Semionov, Taly Iline-Vul, Keren Keinan-Adamsky, Paul Zaslansky , Ron Shahar,* ***Gil Goobes***) 13

NMR Based Metabolomics Mirroring Heterogeneous Biology of Acute Respiratory Distress Syndrome (ARDS) (***Neeraj Sinha***) 14

Molecular mechanism of RNA recognition by SR1 protein from Plasmodium falciparum (*Akshay Kumar Ganguly, Garima Verma,* ***Neel Bhavesh***) 15

Research Talks: December 21 **17**

Singlet-state NMR experiments with pairs of nearly equivalent nuclear spins (*Kirill F.Sheberstov, Bogdan A. Rodin, Alexey S. Kiryutin, Christian Bengs, Joseph T. Hill-Cousins, Lynda J. Brown, Richard C. D. Brown, Giuseppe Pileio, Malcolm H. Levitt, Alexandra V. Yurkovskaya,* ***Konstantin L. Ivanov***[1]) 17

Developments in Dynamic Nuclear Polarisation (***Songi Han***) 18

Optical magnetometry techniques for lowfield NMR (***G. Rajalakshmi***) . 18

Photo-CIDNP in liquid and solid state (***Jorg Matysik***) 18

Structural basis of recognition between Plasmodium falciparum and Human sumoylation machinery (*Jai Shankar Singh, Vaibhav Kumar Shukla, Mansi Gujarati, Ram Kumar Mishra,* ***Ahutosh Kumar***) . . 19

Specific nucleic acid recognition sites by RNA binding proteins (***Santosh Kumar Upadhyay***) 19

A link between the DIPSHIFT and REDOR experiments (***Kaustubh R. Mote***) 20

Research Talks: December 16

Introduction

16 Dec
16:30hrs

P. K. Madhu
Tata Institute of Fundamental Research, Hyderabad, India

Relaxation in NMR experiments

16 Dec
17:00hrs

Beat Meier
ETH Zurich, Switzerland

Solid-state NMR method development at TIFR Hyderabad

16 Dec
18:10 hrs

Vipin Agarwal
Tata Institute of Fundamental Research, Hyderabad, India

Biophysical insights into the role of membrane dynamics in diseases

16 Dec
18:40 hrs

Manjari Mishra, Ruchika Dadhich, Adhyapak Pranav, Jaladhar Matao, **Shobhana Kapoor**
Department of Chemistry, Indian Institute of Technology Bombay, Mumbai, India

Cell membrane possesses an astounding diversity of lipids and uses them for orchestrating processes such as cellular signaling, membrane domain organization, and dynamicsin addition to their structural roles. Clustering of protein and lipids within lipid bilayer defines how cell utilizes spatiotemporal organization of lipids to regulate biological functions by patterning them to form structural heterogeneities like micro- and nano-domains and rafts. Plasma membrane-the first point of interaction with pathogens-with its array of raft domains serves as the hot spot for strategizing immune surveillance programs. Membrane remodeling and raft coalence enables receptors oligomerization emminent for the adaptive immune response (e.g. CD4, CD8) and immune recongition (e.g. TLR4). Pathogens are evolutionary-compelled and articulate raft hijackers subverting various host-scrutinized signaling

events by co-opting raft-associated pathways for their entry, survival and infection mostly using proteins as virulent effectors. Mycobacterium Tuberculosis (Mtb) serves as an epitome of how lipidsnext to proteinscan be utilized as central effectors in pathogenesis underpinning the dynamic interplay in host-pathogen interactions. Aptly, mycobacteria use substantial amount of its genome to synthesis atypical lipid molecules with humungous long (C60-C90) and branched carbon chain such as surface glycolipid mannose-capped lipoarabinomannan (man-LAM), phosphotidyl-myo-inositol capped LAM (PILAM), trehalose dimylcoate (TDM), Sulfoglycolipid-1 (SL-1), and PDIM. These decorated lipids are unique to Mtb and not found in mammalian counterparts, peaking the interest in exploring how mammalian membranes respond to such lipid effector molecules. The molecular and cellular mechanisms of their interaction specifically with host cell membranes and subsequent effect on the downstream membrane-associated signaling are not well understood. Here we characterized the biophysical, nanomechanical and cell biological properties of live THP-1 macrophage cell membranes upon Mtb SL-1 lipid interaction using two-photon imaging, atomic force microscopy and spectroscopy and cell biology. Taken together, we probed various aspects of host membrane-viurlent lipid interactions to generate a framework for improving our understanding of the role of virulent lipids in hijacking the host cell membrane and manipulating the same to commandeer host membraneassociated signaling.

Research Talks: December 18

Solid-state NMR investigations of hepatitis B virus structural proteins

17 Dec
16:30 hrs

Anja Bockmann
CNRS/University of Lyon, France

Small molecule-based targeting of AML1-ETO oligomerization as a potential strategy for anti-leukemia therapy

17 Dec
16:50 hrs

Mohanraj Gopalswamy[1], Tobias Krger, Benedikt Frieg, Tao Zhang, Alexander Metz, Luitgard Nagel-Steger, Manuel Etzkorn, Holger Gohlke
[1]Institute for Pharmaceutical and Medicinal Chemistry

Acute myeloid leukemia (AML) is a malignant disease of immature myeloid cells and the most prevalent acute leukemia among adults. AML is characterized by the chromosomal translocation t(8:21), which generates the oncogenic fusion protein AML1-ETO (Eight twenty one, encoded by RUNXT1). The nervy homology region 2 (NHR2) domain of ETO mediates homo-tetramerization of AML1-ETO, and this oligomerization is essential for oncogenic activity. Therefore, we aim to inhibit tetramerization of NHR2 by a highly affine and specific small-molecule inhibitor, a new therapeutic agent suppressing RUNX1/ETO oncogenic activity and, thus, exerting an anti-leukemic effect. Previously, we have identified hot spot residues that are essential for the tetramerization of NHR2 [1] followed by the identification of the inhibitor 7.44 using structure-based virtual screening [2] 7.44 shows in ex vivo and in vivo activity [3]. However, a biophysical characterization of the mode of action is missing.

Here, we show that the heterologously expressed NHR2 domain forms a stable tetramer as determined by analytical ultracentrifugation and size exclusion chromatography. Biophysical assays based on micro scale thermophoresis and differential scanning fluorimetry demonstrate the binding of 7.44 to NHR2, witha dissociation constant in the range of lower micromolar affinity. CD spectroscopy demonstrates that the NHR2/7.44 complex is thermally less stable than the free NHR2 tetramer. Furthermore, identification and mapping of the binding epitopes was achieved using saturation transfer difference (STD) and multidimensional NMR experiments.

Our results suggest that 7.44 could serve as a lead structure to guide the development of structurally related compounds with increased binding affinity, improved bioavailability, and enhanced anti-leukemic effects.

References

[1] Wichmann, C., et al. (2010) Blood, 116, 306-313

[2] Metz, A, et al., (2013) J Chem Inf Model, 23; 53, 9, 2197-202.

[3] Schanda, J., et al., (2017) Haematologica, 102, e173

---

17 Dec
17:20 hrs

Studying conformational dynamics of proteins in the solution state

Pramodh Vallurupalli

Tata Institute of Fundamental Research, Hyderabad, India

---

17 Dec
17:40 hrs

Small RNA mediated gene silencing in plants: Lessons for knock-down in gene therapeutics

Mandar V. Deshmukh

CSIR - Centre for Cellular and Molecular Biology, Hyderabad, India

In higher eukaryotes, the RNA interference (RNAi) uses two key enzymes, Dicer and Argonaute, which are assisted by a variety of multiple dsRNA binding domains (dsRBDs) containing proteins (dsRBPs) to regulate RNA mediated gene silencing. A seemingly conserved pathway of RNAi exhibits significant heterogeneity across organisms, by recruiting uneven numbers of enzymes and their partner proteins. For example, A. thaliana requires four Dicers and seven dsRBPs to accomplish the small RNA pathway in a unique and highly controlled fashion. To understand the origin and necessity of the evolutionary divergence in RNAi, we have defined the functional roles of RDE-4 C. elegans as well as DRB2, DRB3, DRB4, DRB5 and DRB7.2 in A. thaliana using solution structures and complementary assays. The backbone dynamice in ns-ps and ms-s timescale were used to probe functionally relevant dynamics in these dsRBDs. Results elaborate on the divergence in seemingly conserved and highly homologous proteins implying a fine balance in which subtle changes can make or break the small RNA mediated gene silencing in plants. The results further exemplify that the process of RNAi initiation is unique for each organism and is heavily dependent on step-wise assembly of the Dicer, its partner proteins, and the trigger small RNA. Surprising heterogeneity in structure and function of these dsRBPs imply their application in therapeutics towards selective knock-down in gene therapy.

Probing Protein Dynamics using NMR, H/D Exchange, and Atomistic Simulations

17 Dec
18:10 hrs

Harish Vashisth

Department of Chemical Engineering, University of New Hampshire, USA

The sequences and three-dimensional structural folds of homologous proteins often fail to explain many striking differences in their functional characteristics, which are often attributed to their dynamics in the solution state. The differences in dynamics of closely-related proteins present significant hurdles for structure-based design of small molecules targeting such proteins. In this talk, I will present results from experimental and simulation studies that detail the interplay of protein dynamics and their role in affecting protein-protein interfaces in a set of signaling proteins, that modulate signaling in G-protein coupled receptors. Specifically, I will present data from NMR as well as hydrogen- deuterium exchange mass spectrometry (HDX-MS) analysis that when combined with molecular dynamics (MD) simulations provide information on chemical shift perturbations on inhibitor binding and exchange rates at the resolution of single residues. These results will highlight the importance of using integrated computational and experimental approaches to gain insights into conformational repertoire of large proteins and their interfaces with other proteins

---

Protein aggregation investigated by NMR spectroscopy

17 Dec
18:50 hrs

Bernd Reif

Technical University Munich, Germany

Many (>25) diseases are associated with deposition of insoluble protein aggregates in different kinds of tissue. On the other hand, proteins involved in bacterial biofilm formation and hormone storage, as well as proteins contributing to formation membrane-less cellular organelles take over important functions in living organisms. The talk will focus on the structural charaterization of aggregates formed by the Alzheimer's disease A peptide, the type 2 diabetes peptide hIAPP and aggregates formed by immunoglobulin light chain proteins implicated in AL-amyloidosis. We show how small molecules such as the green tea compound epigallocatechin-gallate (EGCG) and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) interact with amyloid aggregates. Results on the interaction between misfolding proteins and molecular chaperones

such as the small heat shock protein (sHSP) B-crystallin (B) are presented. We show that MAS solid- state NMR techniques are applicable for the structural characterization of large soluble protein

complexes, in case the tumbling correlation time exceeds the rotor period, using B crystallin (600 kDa), the 20S proteasome core particle in complex with its 11S activator (1.1 MDa), and 50S ribosomal complexes as examples.

References

[1] Mainz A., et al (2015) Nature Struct Mol Biol 22, 898-905

[2] Prade E., et al. (2016) Biochemistry 55, 18391849
[3] Hora M., et al. (2017) PloS ONE 12, e0181799
[4] Rodriguez Camargo D. C. et al. (2017) Sci Rep 7, 44041

Research Talks: December 19

NMR spin relaxation measurements and MD simulations reveal tuning of conformational entropy in oncogenic mutations of p53's DNA binding domain.

19 Dec
16:30 hrs

Sujoy Mukherjee

CSIR-Indian Institute of Chemical Biology, Kolkata, India

Conformational entropy plays a key role in protein-protein and protein-ligand interactions. Changes in protein's entropy due to point mutations have been found to modulate the functional properties of many proteins. P53 is a tumour suppressor protein whose mutations have been linked to over 50% cases of cancers. Importantly, almost all the high frequency cancer causing mutations occur in the DNA binding domain of p53 and they block p53's tumour suppressing function by abrogation of its DNA binding ability. While some mutations alter protein-DNA interactions by substitution of DNA contact residues (i.e. DNA-contact mutations), others perturb the p53 structure leading to loss of DNA-protein contacts (i.e. structure mutations). Using NMR spectroscopy and MD simulations on the most clinically prevalent mutations, we show evidence that NMR order parameter-derived conformational entropy is linearly correlated with the change in free energy of denaturation for these mutants. Using a linear regression function, we also found that the experimentally derived parameters of urea-mediated equilibrium denaturation experiments can be used to predict the conformational entropy in p53 core domain mutants, thereby demonstrating a method to use these parameters as predictors of a protein's conformational entropy.

Elucidating the mechanism of interaction of Hsp70 with client proteins

19 Dec
16:50 hrs

Ashok Sekhar[1], Algirdas Velyvis, Guy Zoltsman, Rina Rosenzweig, Guillaume Bouvignies Lewis E. Kay

[1]Indian Institute of Science, Bangalore, India

The Hsp70 chaperones are protein quality control checkpoints that perform their function by modulating the conformation of their client proteins. While substrate proteins are globally unfolded in their Hsp70-bound state, it is not known whether Hsp70 traps a transient unfolded population of its substrate (the conformational selection-like (CS) holdase mechanism) or if it binds structured conformations and

unfolds them (the induced fit-like (IF) unfoldase mechanism). Here we use Chemical Exchange Saturation Transfer (CEST) and magnetization exchange NMR measurements in conjunction with methyl-TROSY and selective isotope labeling methodology to quantify the flux along the CS and IF pathways. We find that both an -helical and a β-sheet substrate interact with E.coli Hsp70 predominantly via a CS binding mode. The mode of recognition is CS-like for E.coli as well as human Hsp70 chaperones, suggesting that this pathway is conserved across evolution. Our results highlight the importance of molecular recognition in this pivotal binding event as well as the promise of NMR methodology to dissect mechanisms underlying biomolecular interactions.

References [1] Sekhar A et al., (2018) eLife, 7, e32764

---

19 Dec
17:20 hrs

Dynamical modes in RNA binding protein allow for shape-dependent RNA recognition

Harshad Paithankar, **Jeetender Chugh**

Indian Institute of Science Education and Research, Pune, India

The TAR-RNA binding protein (TRBP) targets multiple double-stranded RNAs (dsRNAs) with varying conformations via its double-stranded RNA binding domains (dsRBDs) in the miRNA biogenesis pathway. While the canonical interaction between a dsRBD and a dsRNA involves protein residues targeting the minor-major-minor grooves of RNA in succession, TRBP is often presented with non-canonical dsRNAs containing loops and bulges perturbing these interactions. To investigate whether the conformational flexibility of the dsRBD plays any role in targeting such dsRNAs with different conformations, we measured the motional modes of TRBP-dsRBD1 in the ms-s and ps-ns timescales. We show that the RNA binding region exhibited higher s timescale motions, in addition to lower s timescale motions; and an unusually low order parameter (average $S^2 = 0.6$) present along the backbone in the core structural region of the protein. Further, to correlate the binding modes with the conformational flexibility, titration of dsRBD1 with four dsRNAs of varying shapes were carried out. ^{1}H-^{15}N HSQC showed that all four RNAs bind with distinctive binding modes thereby suggesting that the intrinsic slow-dynamics in the dsRBD backbone paves way for conformational adaptation required to target dsRNAs of different shapes.

Applications of NMR spectroscopy in studying self-assembly and membrane interaction of Amphiphilic Ionic Liquids

19 Dec
17:40 hrs

Sandeep Kumar, Navleen Kaur, Gourav Kumar, **Venus Singh Mithu**

Department of Chemistry, Guru Nanak Dev University, Amritsar, India

NMR spectroscopy is a powerful technique when it comes to study the interaction of proteins/peptides with lipid membranes at atomic level. A variety of NMR methods been developed to study such systems. Interaction of Ionic-liquids with lipid-membranes is even simpler to study but still, application of NMR methodologies has been limited in this area. Design and synthesis of [Cnmim] based amphiphilic RTILs (AmILs) has emerged as a prominent research topic in the recent past owing to their tunable and diverse properties. At the same time, a substantial number of studies have questioned the green or eco-friendly nature of these liquid salts, and provide compelling evidence that cytotoxicity is caused by disruption of cell-membrane by ampiphilic [Cnmim] cations. Our aim is to design alternative AmILs which exhibit minimal cytotoxicity by studying the mechanism of toxicity induced by AmILs with molecular/atomic resolution using NMR as primary tool of investigation. In this talk, I will be discussing the application of NMR spectroscopy in conjugation with other biophysical techniques to study the:

1. Role of various biophysical parameters (binding constant, exchange rate etc.) in AmIL-membrane interaction.

2. Role of cation hydrophobicity and counter-ions on AmIL induced membrane permeabilization/disruption and cytotoxicity.

3. Distribution/Orientation of [CnMIM] cations in lipid membranes by solid-state NMR spectroscopy.

Research Talks: December 20

Probing membrane protein ground and conformationally excited states using dipolar- and J-coupling mediated MAS solid-state NMR experiments

20 Dec
16:30 hrs

Tata Gopinath

University of Minnesota, Minneapolis, USA

The intrinsic conformational plasticity of membrane proteins directly influences the magnitude of the orientational-dependent NMR interactions such as dipolar couplings (DC) and chemical shift anisotropy (CSA). As a result, the conventional cross-polarization (CP)-based techniques mainly capture the more rigid regions of membrane proteins, while the most dynamic regions are essentially invisible. Nonetheless, dynamic regions can be detected using experiments in which polarization transfer takes place via J-coupling interactions. Here, we review our recent efforts to develop single and dual acquisition pulse sequences with either 1H or 13C detection that utilize both DC and J-coupling mediated transfer to detect both rigid and mobile regions of membrane proteins in native-like lipid environments. We show the application of these new methods for studying the conformational equilibrium of a single-pass membrane protein, phospholamban, which regulates the calcium transport across the sarcoplasmic reticulum (SR) membrane by interacting with the SR Ca2+-ATPase. We anticipate that these methods will be ideal to portray the complex dynamics of membrane proteins in their native environments.

Sensitivity Enhancement via Triply Compensated Pulses for High-Field Spectrometers

20 Dec
16:50 hrs

V. S. Manu, Gianluigi Veglia

University of Minnesota, Minneapolis, USA

Protein NMR experiments at high field spectrometers are often jeopardized by the pulse bandwidth and RF inhomogeneities/mis-calibration. Using a novel optimization algorithm, we generated a new family of triply compensated RF pulses that cover larger bandwidth with superior compensation for inhomogeneities. The new pulses are significantly shorter than Gaussian cascade pulses, adiabatic pulses etc., thus alleviating pulse length related sensitivity loses such as relaxation and coupling evolution during the pulses. This high fidelity operation in short time enhances the sensitivity of NMR experiments involving bio macro molecules up to 100Here we

demonstrate the application of these new triply compensated pulses in ^{15}N-^{1}H and ^{13}-^{1}H heteronuclear correlation experiments. The Bruker standard pulses sequences are modified with new triply compensated pulses in ^{1}H and $^{13}/^{15}$N channels. The experiments were performed using ^{13}-^{15}N labelled Ubiquitin and ^{13}-^{15}N maltose binding protein (MBP). In comparison to the standard HSQC experiments, we obtained a sensitivity enhancement up to 80% for [^{1}H, ^{15}N] HSQC experiment and 100% for [^{1}H, ^{13}C] HSQC. We show that the improvement in sensitivity is proportional to the number of pulses replaced in the original pulse sequences. We anticipate that triply-compensated pulses will be crucial for improving the performance of multidimensional and multinuclear pulse sequences at ultra-high fields.

References

[1] Manu, V.S. et al. (2015), J Magn Reson, 260, 136-43.

[2] Manu V.S., et. al. (2016), J Magn Reson, 273, 40-46.

[3] Youlin Xia et al. (2017), J Biomol NMR) 69, 237.

---

20 Dec
17:20 hrs

Influence of aromatic side-chains in biomolecular structure and dynamics

Bharathwaj Sathyamoorthy

Indian Institute of Science Education and Research, Bhopal, India

Aromatic side-chains in biomolecules are active participants of diverse interactions, the most prominent being pi-pi networks in proteins, stacking and H-bonding in nucleic acids. Early studies of aromatic ring flips in hydrophobic core of bovine pancreatic trypsin inhibitor (BPTI) unraveled the molecular plasticity of proteins1. More recently, purine bases in DNA, thought previously as rigid entities within the Watson-Crick double helical framework, were observed to undergo ring flips that result in an alternate "Hoogsteen" H-bonding pattern2. This presentation will illustrate application of aromatic chemical shifts and RDCs towards characterization of biomolecules. In particular, aromatic RDCs help in assessing protein core modeling in structures and characterize high-energy conformational states accessed during ring-flip excursions in BPTI3. Also, structural and dynamic preferences of a modified duplex DNA that comprises of a single Hoogsteen base pair will be presented4.

References

[1] Wthrich K, et al., (, 50(2):265-8.

[2] Nikolova EN, et al., Nature, 2011, 470(7335):498-502.

[3] Sathyamoorthy B, et al., Chembiochem 2013,14(6):684-8.

[4] Sathyamoorthy B, et al. Nucleic Acids Research, 2017, 45(9), 5586-5601.

Structural basis for the increased microtubule localization of EB1 by GTP and SxIP

20 Dec
17:40 hrs

Shine A., **Vinesh Vijayan**

Indian Institute of Science Education and Research, Thiruvananthapuram, India

Microtubule plus end-binding protein (+TIPs) are the network of proteins which regulate the dynamics of microtubule at its growing end. Among +TIPs, the protein EB1 is a key regulator of microtubule dynamics.1 The auto-inhibitory interaction between the N and C terminal domains in EB1 has previously been shown to inhibit its ability to bind to microtubules and regulate microtubule dynamics. However, the factors that promote its microtubule regulatory activity by overcoming the auto-inhibition are less known. Here, we show that GTP plays a critical role in promoting the microtubule-targeting activity of EB1 by suppressing its auto-inhibition. We used solution NMR to show that GTP binds to the N-terminal domain of the protein, removing the autoinhibitory interaction with the C-terminal domain.2 Structural basis of EB1 activation for microtubule localization by the binding of SxIP aptamer is also elucidated.3 SxIP aptamer binds to the dimer interface in the C-terminal domain and stabilizes the monomeric EB1 leading to the removal of autoinhibition.

References

[1] Hayashi, I. et al., Molecular cell 2005, 19, 449.

[2] Gireesh, K. K. et al., Scientific reports 2018, 8, 9792.

[3] Honnappa, S. et al., Cell 2009, 138, 366.

NMR Study of Biomaterials From Bone Protein Structure to Minerals in Mechanically Different Bones

20 Dec
18:10 hrs

Shani Hazan, Irina Matlahov, Alex Kulpanovich, Artyom Semionov, Taly Iline-Vul, Keren Keinan-Adamsky, Paul Zaslansky , Ron Shahar, **Gil Goobes**

Department of Chemistry, Faculty of Exact Sciences, Bar-Ilan University, Ramat-Gan, Israel

Solid-state NMR has a vast potential to propel the study of biomaterials. In the context of bone structure, it has made major contributions to the understanding of the biominerals and the inorganic-organic interfaces comprising this excellent bio-composite material [1-9]. Yet, complexity of the mineral phases found in bones are only starting to be unveiled with many debates and paradigm shifts over the years, regarding the actual composition of bone in terms of the crystalline and recently non-crystalline phases it encompasses [1,10,11]. At the same time, the molecular regulation of material formation and repair, carried out by specially designed non-collagenous proteins, is still mostly unknown. The study of these proteins in the context of bone mineral has also been a territory less explored by MAS NMR. Here, we analyzed the same bone from two different fish which exhibit a stiff structure in one fish and an elastic bone structure in the other, formed via disparate biomineralization processes. We used various MAS NMR experiments to indicate dissimilarities between the mineral and organic phases formed in these two types of

bone. We also used ^{1} H driven ^{13}C-31P recoupling experiments to identify differences in the organic-inorganic interfaces and to tie-up the molecular properties to the macroscopic mechanical properties.To examine the other end of bone structure, i.e. its formation, we analyzed the structure of the bone protein osteocalcin when interacting with apatite mineral, in vitro. The findings from these works help us start to connect the rudimentary interaction of protein-material interactions with the final outcome it has on the complex structure of hard tissue.

References

[1] Wang, et al. (2012), Nat. Mater., 11, 724.

[2] Duer, M. J. et al. (2015) J. Magn. Reson., 253, 98.

[3] Hu, Y. Y. et al. (2010), Proc. Natl. Acad. Sci. U. S. A. 107, 22425.

[4] Zhu, P. et al. (2009), J. Am. Chem. Soc., 131, 17064.

[5] Rai, R. K. et al. (2011), J. Phys. Chem. C, 115, 14219.

[6] Nikel, O. et al. (2013), Langmuir, 29, 13873.

[7] Cho, G. et al. (2003), Science, 300, 1123.

[8] Kolodziejski, W. (2005). Solid-state NMR studies of bone. In New Techniques in Solid-State NMR (pp. 235-270). Springer, Berlin, Heidelberg.

[9] Wilson, E. E. et al. (2006), Biophys. J., 90, 3722.

[10] Matlahov, I. et al. (2015), Chem. Mater., 27, 5562.

[11] Iline-Vul, T. et al. (2015), Biomacromolec., 16, 2656.

---

20 Dec
19:00 hrs

NMR Based Metabolomics Mirroring Heterogeneous Biology of Acute Respiratory Distress Syndrome (ARDS)

Neeraj Sinha

Centre for Biomedical Research, Lucknow, India

Predisposing aetiologies in Acute Respiratory Distress Syndrome (ARDS), perpetuates to heterogeneous clinical course hampering therapeutic response. Therefore, physiological variables need to be identified by stratifying ARDS subphenotypes and endotype, to target ARDS heterogeneity. The present talk is focused to the fact that the ARDS heterogeneity arises from diverse pathophysiological changes leading to distinct ARDS endotypes characterized by perturbed biological mechanism, which can be exploited in terms of metabolic profile by metabolomics. Biological endotypes using (n = 464), mBALF and serum samples were identified by high resolution NMR spectroscopy from two clinically diagnosed ARDS subtypes grouped under mild, moderate and severe ARDS as subphenotype1 and pulmonary and extra pulmonary ARDS as subphenotype2. The identified mBALF endotypes (isoleucine, leucine, valine, lysine/arginine, tyrosine, threonine) and serum endotypes (proline, glutamate, phenylalanine, valine) in both subphenotypes by statistical analysis were tested for their reproducibility and robustness. By combining metabolic endotypes with clinical based mortality score (APACHE and SOFA) added to their predictive performance as ARDS mortality predictors. Thus, a comprehensive set of mBALF endotypes representing compartmentalized lung milieu and serological endotypes representing systemic markers of ARDS subtypes were validated. The interlinked

biological pathway of these disease specific endotype further elucidated their role as candidate biomarker in governing ARDS heterogeneous biology.

Molecular mechanism of RNA recognition by SR1 protein from Plasmodium falciparum

20 Dec
19:20 hrs

Akshay Kumar Ganguly, Garima Verma, **Neel Bhavesh**

International Centre for Genetic Engineering and Biotechnology, New Delhi, India

Alternative splicing confers a complexity to the mRNA landscape of Apicomplexans, resulting in a high proteomic diversity. The Plasmodium falciparum Ser/Arg-rich protein 1 (PfSR1) is the first protein to be confirmed as an alternative splicing factor in this class of parasitic protists. PfSR1 shows a preference towards purine-rich cognate RNA sequences. The contributions of its individual domains in RNA recognition, however, still remain an enigma. Our solution structure of the amino-terminal RNA recognition motif (RRM1) of PfSR1 in complex with ACAUCA RNA hexamer shows that RNA binding is mediated through specific recognition of a cytosine base situated 5' of one or more pyrimidine bases by a conserved tyrosine residue on the 1 strand. Using calorimetry and mapping of NMR chemical shift perturbations, we have also ascertained the purine preference of PfSR1 to be a property of the carboxy terminal pseudo-RRM (RRM2), which binds RNA non-canonically and with greater affinity compared to RRM1. Our findings show conclusive evidence of complementary RNA sequence recognition by the two RRMs, which may potentially aid PfSR1 in binding RNA with a high sequence specificity.

Research Talks: December 21

Singlet-state NMR experiments with pairs of nearly equivalent nuclear spins

21 Dec
14:00 hrs

Kirill F.Sheberstov, Bogdan A. Rodin, Alexey S. Kiryutin, Christian Bengs, Joseph T. Hill-Cousins, Lynda J. Brown, Richard C. D. Brown, Giuseppe Pileio, Malcolm H. Levitt, Alexandra V. Yurkovskaya, **Konstantin L. Ivanov**[1]

[1]International Tomography Center, Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia

Singlet-state NMR [1] is an important emerging concept potentially allowing one to overcome limitations imposed by finite relaxation times of nuclear spins. Experiments with nuclear singlet states allow one to exploit advantages of long-lived spin order, namely, of long-lived spin states (LLSs) and long-lived coherences (LLCs). LLSs exist when the symmetry of the static Hamiltonian and that of the fluctuating Hamiltonian (which causes relaxation) coincide with a consequence that such states become immune to particular kinds of relaxation and becomes long-lived [1]. An LLC is a spin coherence, which has a significantly longer lifetime than the standard single- quantum coherence corresponding to transverse magnetization. By using LLCs, one can suppress the contribution from inhomogeneous NMR linewidth and obtain much narrower lines. An important class of molecules for singlet-state NMR experiments is given by molecules containing pairs of nearly-equivalent spins [2], which are strongly coupled even at high B 0 fields (meaning that the J-coupling strength is much larger than the difference in the Zeeman interactions of the spins with the field). Efficient use of such molecules however requires design of special NMR methods for efficient generation of singlet spin order and robust excitation of singlet-triplet coherences. In this work, we present an optimization of NMR methods for magnetization-to-singlet (M2S) conversion and backward S2M conversion. The optimization utilizes constant-adiabaticity RF-pulses [3], which significantly increase the conversion efficiency. For experiments with LLCs, we designed NMR pulse sequences for optimal excitation of singlet-T transitions, which reveals itself in the increased intensity of forbidden NMR transitions. We propose to use such pulse sequences as building blocks for more complex sequences that can be exploited to excite the singlet-T 0 coherence, which is long-lived. The proposed methods have been tested by running experiments with a specially designed naphthalene derivative having two nearly equivalent ^{13}C-nuclei.

References

[1] M. H. Levitt (2012) Annu. Rev. Phys. Chem., 63, 89-105.

[2] G. Stevanato et al. (2015), Angew. Chem. Intl. Ed., 54, 3740-3743.

[3] B. A. Rodin,et al., (2018) J. Magn. Reson., 291, 14-22.

Acknowledgements: Financial support from the Russian Science Foundation (grant No. 14-13-01053) and Russian Foundation for Basic Research (grant No. 17-03-00932) is gratefully acknowledged.

---

21 Dec
15:00 hrs

Developments in Dynamic Nuclear Polarisation

Songi Han

University of California Santa Barbara, USA

---

21 Dec
18:30 hrs

Optical magnetometry techniques for lowfield NMR

G. Rajalakshmi

Tata Institute of Fundamental Research, Hyderabad, India

NMR signals are typically recorded at high magnetic fields by detecting the Larmor precession of the atomic nuclei inductively using pick up coils. The NMR spectrum is determined by the chemical shift dispersion of the atomic nuclei in such high field experiments. In contrast NMR spectrum at very low (below nT) external magnetic fields is dominated by the j-coupling interaction between the atomic nuclei in the sample. Inductive detection of signals at such low fields is made difficult as the induced emf in the pick coils is lowered due to the reduction in the Larmor precession rate. Optical magnometers that detect the nuclear polarisation by non-inductive means are ideal sensors for low field NMR as has been seen in recent experiments. The optical rotation of a linearly polarised probe laser beam passing through a gas of alkali atoms is a sensitive measure of the local nuclear polarisation, through its affects on the electron polarisation of the alkali atoms. In this presentation I will describe the efforts of our group in building a Rb optical magnetometer for detection of NMR signals.

---

21 Dec
17:15 hrs

Photo-CIDNP in liquid and solid state

Jorg Matysik

University of Leipzig, Germany

Photochemically induced dynamic nuclear polarization (Photo- CIDNP) is born by the spin-dynamics occurring within a spin-correlated radical pair during the progress of a photo-chemical reaction. Various mechanisms, as the classical radical-pair mechanism, are discssed. Examples of applications of this hyperpolarization method are presented.

Structural basis of recognition between Plasmodium falciparum and Human sumoylation machinery

21 Dec
18:10 hrs

Jai Shankar Singh, Vaibhav Kumar Shukla, Mansi Gujarati, Ram Kumar Mishra,
Ahutosh Kumar

Indian Institute of Technology Bombay, Mumbai, India

Malaria is one of the deadliest infectious diseases, affecting millions of lives annually and is caused by parasitic protozoans of the genusPlasmodium. P. falciparum (Pf) adopts various survival strategies including post-translational modifications (PTMs) to stabilize and potentiate its crucial proteins for successful infection cycle in the mosquito and human host and sumoylation is one of its important PTMs. It is known that SUMO interacts with both E1-activating (hetero-dimeric Aos1/Uba2), E2-conjugating (Ubc9) individually. Can the interaction of Pf-SUMO with E1and/or E2 have the cross-species interaction element that can be targeted? Moreover, the structure of Pf-SUMO is unknown, we present the first structure of Pf-SUMO solved using solution state NMR. The residue specific interactions of Pf-SUMO and Hs-SUMO1 with E2 enzymes and the possibilities of cross-interaction of host and parasite sumoylation machineries were identified by NMR studies. We have identified the important residues of Pf-SUMO proteins that involved non-covalently with Pf-E2 and Hs-E2 enzymes. However, no such interactions have been identified in case of Hs-SUMO1 with Pf-E2 enzyme. By using ITC and SPR, the binding affinity of Pf-SUMO and Hs-SUMO1 with respective E2 enzymes was determined. Overall, all these results emphasize that during disease condition the parasite may use the host sumoylation machinery to maintain its survival. The residues at the interacting interface that displayed prominent interactions have been mutated and identified the residues of Pf-SUMO protein, which is important for E2 enzymes selective binding. This information can be used for designing of drugs that specifically block the interface of Pf-SUMO protein during the sumoylation reaction. Additionally, we studied the dynamics of Pf-SUMO protein at different time scale motions. This information will help us in ascertaining role of protein plasticity in substrate recognition and specificity.

Specific nucleic acid recognition sites by RNA binding proteins

21 Dec
18:30 hrs

Santosh Kumar Upadhyay

CSIR-Institute of Genomics and Integrative Biology, New Delhi-110025, India

The RNA binding motif protein (RBM) are known to be an important factor for alternative splicing. The RBM24 is identified as a major regulator of alternative splicing in cardiac and skeletal muscle development. The RBM24 protein contains an RNA recognition motif (RRM) domain that presumably mediates the binding to target pre-mRNA required for regulation of the splicing patterns. We have applied NMR spectroscopy to get secondary chemical shift analysis and relaxation measurement which confirm the canonical architecture of the RRM domain of ----- type.

RBM24 directly binds to a single-stranded RNA motif present in p21 transcript. The data will allow for further atomic level studies aimed at understanding splicing regulation of target genes in heart and muscle development and investigation into a separate role of RBM in modulating mRNA stability of genes involved in the p53 tumour suppressor pathway. In addition, we have also investigated RBM20, a RNA binding protein, which regulates alternative splicing of many genes in muscle tissues along with giant titin protein. In particular, the mutation in RBM20 protein causes dilated cardiomyopathy, which results in severe heart disease. We have applied NMR and biophysical studies to understand structural and functional aspects of RBM20 and RNA recognition. The above described structural and biophysical studies uncovers fundamental principles of RNA binding proteins and hence opens new avenues in RNA cellular biology.

---

21 Dec
18:50 hrs

A link between the DIPSHIFT and REDOR experiments

Kaustubh R. Mote

Tata Institute of Fundamental Research, Hyderabad, India

Rotatinal Echo Double Resonance (REDOR) and Dipolar Chemical Shift (DIPSHIFT) are two of the experimentally robust techniques that allow the measurement of dipolar couplings between two spin-half nuclei. They can also be used to determine motion if the distance between the nuclei is known, for example, in the case of directly bonded nuclei such as ^{13}C and ^{1}H. Here, the theory behind these pulse sequences will be revisted, and it will shown that these sequences can be implemented in the same pulse-sequence by varying alternate blocks of the pulse-sequence. Experimental data will be shown to prove that DIPSHIFT can be used to determine small dipolar couplings (directly bonded ^{13}C-^{15}N nuclei) at slow-moderate MAS frequencies, a task often considered to be squarely in the domain of applicability of REDOR. On the flip side, the applicability of REDOR to measure large dipolar couplings, a task considered to be in the domain-of-applicability of DIPSHIFT, will also be revisited and explored. These developments have the potential to guide further development of these two techniques and aid their applications to a large range of problems in stuructural biology and material sciences.
