NMR Meets Biology 5

tifr HYDERABAD ncbs National Centre for Biological Sciences Tata Institute of Fundamental Research

This iteration of the meeting will focus on the state-of-the-art solid- and solution-state NMR methods to tackle challenging issues in biology, as well as hyperpolarization techniques.
There will be dedicated tutorial sessions on the basics of solid-and solution-state NMR spectroscopy, as well as introduction to topics such as the study of dynamics in solution state and the determination of protein structures.

December 05 -11, 2022
Malligi Hotel, Hospet,
Karnataka, India

SPEAKERS

Daniel Huster (Leipzig University, Germany)
Malcolm Levitt (University of Southampton, UK)
Matthias Ernst (ETH Zurich, Switzerland)
Yusuke Nishiyama (Riken, Jeol, Japan)
Amir Goldbourt (Tel Aviv University, Israel)
Danila Barskiy (University of Mainz)
Tobias Hett (University of Bonn)
Asif Equbal (NYU Abu Dhabi)
Sheetal Jain (IISc Bangalore)
Ashok Shekhar (IISc Bangalore)
Bharathwaj Satyamoorthy (IISER Bhopal)
G. Rajalakshmi (TIFR Hyderabad)
Kalyan Chakraborti (Krea University)
Mandar Deshmukh (CCMB Hyderabad)
Neeraj Sinha (CBMR Lucknow)
Marimuthu Krishnan (CCNSB, IIIT-Hyderabad)
Ashutosh Kumar (IIT Bombay)
T. Ajithkumar (NCL, Pune)
K. V. Ramanathan (IISc Bangalore)
Raghunathan Ramakrishnan (TIFR Hyderabad)
Samanwita Pal (IIT Jodhpur)
Chandan Singh (BHU, Varanasi)
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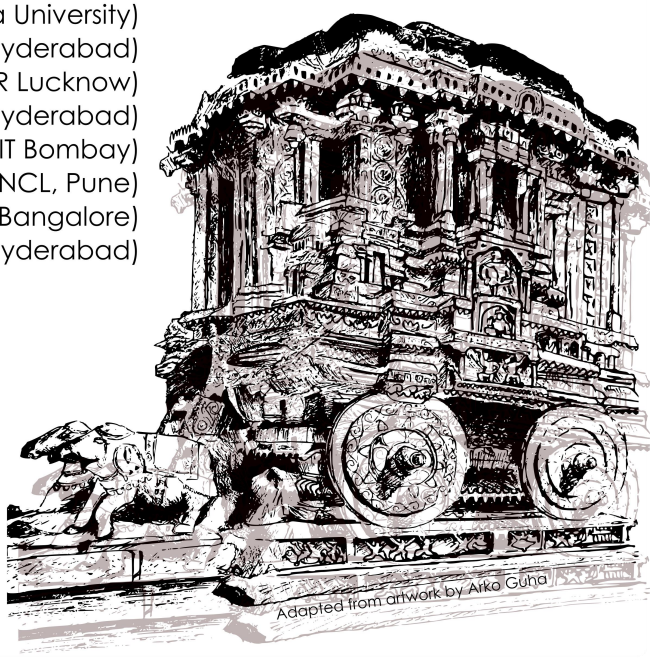

Book of Abstracts

Research Talks

Solid-state NMR structural virology of filamentous bacteriophage viruses

Amir Goldbourt, Tel Aviv University, Israel

(6th Dec, 14:50-15:30)

Filamentous bacteriophages infect bacterial cells that possess a pili organelle. They are highly-symmetric one-micron long viruses containing a single-stranded DNA wrapped by several thousand copies of a mostly-helical coat protein termed gVIIIp. During replication, when the non-structural gene V protein (gVp) reaches a critical concentration, it attaches to the replicative-form DNA and blocks replication by creating a large protein-ssDNA complex that signals the assembly of a new particle. In recent years we managed to solve the atomic-resolution structures of two intact filamentous phage, M13 (with solid-state NMR) and IKe (with cryoEM), and detect common structural assembly motifs. Dynamic properties studied by chemical-shift-anisotropy recoupling methods suggest a highly rigid helical coat with a mobile DNA interface. Recently we solved, using solid-state NMR, the structure of the pre-mature phage that is made of a circular ssDNA molecule wrapped by gVp. Unlike the structural coat protein gVIIIp, gVp is composed mostly of beta strands and a high percentage of loops and disordered regions; it is significantly more dynamic than gVIIIp, and the structure of the entire gVp-ssDNA complex shows that it undergoes a very large conformational change upon binding to the DNA, while losing several additional secondary structure motifs. These modifications facilitate the binding mechanism and promote cooperative binding in the assembly of the gVp-ssDNA complex.

Rhomboid-Catalized Intramembrane Proteolysis Requires Hydrophobic Matching with the Surrounding Lipid Bilayer

Daniel Huster
University of Leipzig, Germany

(6th Dec, 15:30-16:20)

Membrane thinning of the rhomboid GlpG has been proposed to reduce the hydrophobic mismatch between the enzyme and its surrounding lipid environment. Here, we directly show that the membrane environment of the rhomboid influences the velocity of substrate cleavage. We first measure the impact of GlpG on the hydrophobic thickness in phosphatidyl-choline membranes of varying thickness, where the rhomboid only marginally alters the surrounding membrane. However, in an E. coli relevant lipid mix of phosphatidyl-ethanolamine and phosphatidylglycerol, a decrease in hydrophobic thickness of -1.1 Å per leaflet is observed. The cleavage velocity of GlpG is highest in DMPC followed by POPC, POPE/POPG and DLPC, while in the thickest membranes (DPPC/cholesterol) enzyme function is abolished. This suggests that an optimal window of membrane thickness (between ~24 – 26 Å) exists while headgroup specificity does not seem to be decisive for protein function. We infer from these results that the lipid environment can fine-tune GlpG function. By adjusting membrane thickness, for instance through dynamic domain formation, the cell can regulate membrane protein function.

Computational NMR spectroscopy using Quantum Chemistry Big Data

Raghunathan Ramakrishnan
Tata Institute of Fundamental Research Hyderabad, India

(6th Dec 16:45-17:15)

Quantum Chemistry Big Data is a research theme spanning various aspects associated with datasets computed using first-principles modeling, their storage, and their analysis to discover knowledge. The MolDis repository (https://moldis.tifrh.res.in/index.html) under development at TIFR Hyderabad aims to provide a publicly accessible analytics platform for Big Data of computed molecular/materials properties. Presently massively large datasets are being generated for a multitude of domains of application. This talk will give an overview of the MolDis initiative and discuss the critical role machine-learning algorithms play in this project. In particular, we will focus on works based on the QM9 dataset[1], one of the largest datasets comprising calculated equilibrium structures, and properties of 133,885 synthetically feasible small organic molecules. Recently, isotropic shielding of over 0.8 million C atoms of the QM9 molecules has been calculated using high-throughput quantum chemistry calculations. For the resulting QM9-NMR dataset[2], we present results for the prediction transferability of machine learning models with popular local structural descriptors. We also discuss the transferability of the models trained on QM9 molecules to larger drug molecules, discuss related activities carried out elsewhere, and comment on planned future activities towards data-driven modeling of NMR parameters of organic solids.

[1] Quantum chemistry structures and properties of 134 kilo molecules, Raghunathan Ramakrishnan et al. Scientific Data 1 (2014). DOI: https://doi.org/10.1038/sdata.2014.22

[2] Revving up 13C NMR shielding predictions across chemical space: Benchmarks for atoms-in-molecules kernel machine learning with new data for 134 kilo molecules, Amit Gupta, et al. Machine Learning: Science and Technology, 2 (2021) 035010. DOI: https://doi.org/10.1088/2632-2153/abe347

Natural abundance DNP enhanced ^{13}C – ^{13}C correlation spectroscopy of bone tissue biomaterial

Neeraj Sinha
CBMR, Lucknow, India

(6^{th} Dec 17:15-17:45)

Natural abundance ^{13}C – ^{13}C correlation spectroscopy for bone tissue like biomaterials are challenging due to low sensitivity. Dynamic Nuclear Polarization (DNP) based method are showing promising results for this important class of biomaterials where isotopic enrichments are difficult to achieve. We present a method for obtaining a dynamic nuclear polarization (DNP)-enhanced double - quantum filtered (DQF) two-dimensional (2D) dipolar ^{13}C-^{13}C correlation spectra of bone-tissue material at natural ^{13}C abundance. DNP-enhanced DQF 2D dipolar ^{13}C-^{13}C spectra were obtained using a few different mixing times of the dipolar-assisted rotational resonance (DARR) scheme and these spectra were compared to a conventional 2D through-space double-quantum (DQ)-single-quantum (SQ) correlation spectrum. Applications of this correlation scheme has resulted in better assignments of collagen residues and short/long range contacts in bone extra cellular matrix.

Dealing with membranes: In search for perfect home for membrane proteins

Chandan Singh, BHU, Varanasi, India

(6th Dec,17:45-18:15)

Nuclear magnetic resonance (NMR) spectroscopy is a great tool to study membrane proteins in native environment. The different membrane systems have evolved in search of a better and more native system from micelle to liposomes and nanodiscs now. Presently liposomes are used as membrane system to study membrane proteins by using solid state NMR and nanodiscs are used in solution NMR. Many pathogenic bacteria belong to gram negative group of bacteria. To mimic the membrane environment of gram-negative bacterial cell wall further modification of membrane systems such as liposomes and nanodiscs required addition of lipopolysaccharides to the membrane systems. However, the membrane systems are still evolving, and it has reached up to isolating membranes directly from the bacteria along with the membrane protein of interest and studying it in perfectly native environment. The processes involving preparation and utilization of these membrane systems are particularly useful for the people working on structural and functional aspects of membrane proteins.

Processing with Wavelet Transforms in NMR for Signal Enhancement and Removal of Artefacts

K. V. Ramanathan
Indian Institute of Science, Bangalore, India

(6^{th} Dec, 18:30-19:00)

Use of Wavelet Transform has become popular in recent years in a variety of signal processing applications such as noise reduction, data compression, time-frequency analysis and image analysis. Here we present representative applications of wavelet transform to process spin noise and NMR spectra and illustrate the advantages that result from the adoption of the method. Spin noise is naturally present in samples and can be recorded without using r.f. excitation [1]. Several conventional signal enhancement techniques used in NMR would fail to produce the desired S/N enhancement in the case of spin noise spectra. On the other hand, we observe that the use of Discrete Wavelet Transform (DWT) is one of the simple and convenient ways of increasing the signal of the correlated nuclear spin noise in comparison to the uncorrelated random noise, thus significantly reducing the time required to obtain a spin noise spectrum [2]. In the talk, optimum combinations of wavelets and thresholding methods will be presented along with application to both 1H and 13C spin noise spectra.

In Fourier-transform NMR, a strong free induction decay (FID) signal tends to result in large baseline artifacts, including baseline offset and baseline distortion that are sometimes much larger than small peaks, making data interpretation difficult. As a facile and efficient means of baseline correction of the NMR spectrum, we have explored the utility of the DWT conjugated with spline functions. At the same time, the algorithm allows the possibility of filtration of the signal leading to the removal of the high frequency components, thus achieving base line correction and S/N enhancement simultaneously. The method of Wavelet Transforms has also been applied to suppression of t1 noise in 2-dimensional NMR spectroscopy and the results have been demonstrated for the case of NOESY spectra where spectral artifacts seriously affect the quality of the results obtained.

Insights into the structure and pharmacological action of sucralfate from ^{27}Al solid- and liquid-state NMR

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T.G.Ajithkumar

Central NMR Facility and Physical/Materials Chemistry Division, CSIR-National Chemical Laboratory, Dr HomiBhabha Road, Pune 411008, India

(6th Dec, 19:00-19:30)

The digestive disease, Gastro-oesophageal reflux cause irritation and discomfort to the stomach lining due to acid production. For an effective relief from this disease, the amount of acid produced must be decreased or the rate at which the stomach remove the acid must be increased. Antacids and acid suppression therapies are used to treat this condition. In addition, cytoprotective agents are also very effective since they protect the stomach lining by covering the ulcerated region and inhibit further attack from the acid. There are several drugs in the market, which has sucralfate as the active pharmaceutical ingredient (API), which are effective for gastric and duodenal ulcers. The sucralfate complex contains a positively charged aluminium hydroxide and negatively charged sucrose octasulphate, and when it reacts with acid, ionizes as aluminium ions and sucrose octasulphate ions. The sucrose octasulphate anion act as the binding site for the protein present in the stomach, forming a paste preventing mucosal damage. This mechanism of action of sucralfate, based on its structure has not been studied in detail, considering that at least two forms of this API is available in the market and there are no reports on the different forms of sucralfate and their difference in the pharmacological action, to the best of our knowledge. Considering that for every API, it is important to identify and analyze the form which has effective pharmacological action, we have carried out the characterization of the two forms of sucralfate by 27Al solid- and liqud- state NMR. The acid neutralization behavior and the rate at which the aluminum releases from the two forms which could affect the pharmacological action are analysed and compared.

References

[1] K. Ochi, "Chemistry of Sucralfate," Sucralfate, pp. 47–58, 1995, doi: 10.1007/978-0-585-32154-7_5.

[2] Y. Dastagiri Reddy, D. Dhachinamoorthi, and K. B. Chandra Sekhar, "Preparation and characterization of Sucralfate suspension containing different suspending agents for improving suspendability," Int. J. Res. Pharm. Sci., vol. 5, no. 3, pp. 171–177, 2014.

[3] D. VAIRA et al., "Gastric retention of sucralfate gel and suspension in upper gastrointestinal diseases," Aliment. Pharmacol. Ther., vol. 7, no. 5, pp. 531–535, 1993, doi: 10.1111/j.1365-2036.1993.tb00129.x.

[4] M. Ricky Wayne, "A Role for Pre-Polymerized Sucralfate in the Management of Erosive and Non-Erosive Gastroesophageal
Reflux Disease," J. Clin. Gastroenterol. Treat., vol. 6, no. 1, 2020, doi: 10.23937/2469-584x/1510072.

Accessing motional order-parameters in non-deuterated proteins at the magic-angle spinning frequency of 100 kHz

Pravin P. Taware, Mukul Jain, Vipin Agarwal, P. K. Madhu, and
Kaustubh R. Mote

Tata Institute of Fundamental Research Hyderabad, India

(6^{th} Dec, 19:30-20:00)

Protein function is often controlled by conformational changes that occur on a wide range of time scales. In the solid-state, measuring heteronuclear dipole-dipole couplings, especially the one-bond ^{13}C-^{1}H and ^{15}N-^{1}H can give direct insight into the the amplitude, and in some cases, the timescale of these motions. This coupling is often measured by experiments such as the Rotational-echo double resonance (REDOR). However, measuring the dipole-dipole couplings of heteronuclei to protons in non-deuterated systems is challenging, as the presence of homonuclear dipole-dipole couplings between protons causes spin-system dependent deviation in the REDOR dephasing profiles. Here, I will present alternatives based on REDOR variants ε-REDOR and DEDOR, that circumvent the above problem, and allow an accurate measuremet of ^{13}C-^{1}H and ^{15}N-^{1}H dipole-dipole couplings in proteins at the MAS frequency of 100 kHz.

^{14}N NMR at Fast MAS

Yusuke Nishiyama
Riken, Japan

(7^{th} Dec 18:40-19:30)

^{14}N is abundant (>99%) isotope of nitrogen. Although it is NMR active nuclei, the direct observation o f powdered solids are not straightforward due to its spin quantum number (I=1) and associated quadrupolar couplings. Fortunately, the combination of indirect detection (introduced by Bodenhausen and Gan separately) and fast MAS allows rapid observation of ^{14}N NMR. In this talk, we will share the basics and recent advances of ^{14}N NMR at fast MAS. The talk includes the practical experimental setup of fast MAS.

Disordered regions tune order in chromatin organization and function

Ashutosh Kumar
Department of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai 400076

(7th Dec, 19:30-20:00)

Intrinsically disordered proteins or hybrid proteins with ordered domains and disordered regions (collectively designated as IDP(R)s) defy the well-established structure-function paradigm due to their ability to perform multiple biological functions without a well-defined 3D structure. IDP(R)s have a unique ability to exist as a functional heterogeneous ensemble, where they adopt multiple thermodynamically stable conformations with low energy barriers between states. The resultant structural plasticity or conformational adaptability provides them with a high functional diversity and ease of regulation. Hence, IDP(R)s are highly efficient biological machinery to mediate intricate cellular functions such as signaling, gene expression, and assembly of complex structures. One such structure is the nucleoprotein complex known as Chromatin. Interestingly, the proteins involved in shaping up the structure and function of Chromatin are abundant in disordered regions, which serve more than just as mere flexible linkers. The disordered regions are involved in crucial processes such as gene repression, regulation chromatin architecture maintenance, and liquid-liquid phase separation initiation. In the talk, I will attempt to show the role of IDR in Chromatin compaction that mediates the formation of complex multiprotein networks.

Developments in fast magic-angle spinning and automation

Jochem Struppe and Kristoff Grohe
Bruker, Switzerland

Proton Line Width in MAS Solid-State NMR

Matthias Ernst
Physical Chemistry, ETH Zürich, Vladimir-Prelog-Weg 2, 8093 Zürich, Switzerland

(8th Dec, 14:50-15:40)

To obtain resolved proton spectra in solids under magic-angle spinning (MAS) NMR, there are two options. One can either spin as fast as possible in order to spin out the homonuclear dipolar coupling or one can use slow to moderate MAS and in addition homonuclear decoupling of the protons. Both methods lead to line width in the order of 0.5 ppm in fully-protonated samples. We have been investigating the residual line width in such strongly-coupled proton spin systems under MAS only and under homonuclear decoupling. In the talk, I will discuss limitations to the line width under FSLG decoupling for direct observation as well as under spin-echo conditions. For direct detection, the distribution of effective chemical shifts due to static rf-field inhomogeneity limits the resolution. Under spin-echo conditions, the modulation of the radial rf-field inhomogeneity seems to limit the achievable line width especially for high rf-field amplitudes. In order to restrict the sample in terms of rf-field inhomogeneity, B1-field selective pulses have been used. This provides a more flexible and easy to use restriction of the sample volume than a physical restriction of the sample. They are implemented as resonant pulses with a spin-lock field using standard chemical-shift selective pulses like I-BURP or eSNOB. Homonuclear-decoupled spectra under spin-echo conditions often show additional oscillations that can be reduced by implementing a double echo instead of a single echo. We show that such oscillations can be explained by a single-spin model that takes into account effective fields generated by the chemical-shift offset and pulse imperfections. Components of the effective field along the direction of the refocusing pulse lead to modulations of the echo that can in some cases significantly be reduced by a double echo. The appearance of such echo modulations in single-spin systems is surprising and has, to the best of our knowledge, not yet been described in the literature.

Lie Algebra and Long-Lived States

Malcolm Levitt
School of Chemistry, University of Southampton, United Kingdom

(8th Dec, 15:40-16:30)

Long-lived states (LLS) are nuclear spin configurations that are protected against major relaxation mechanisms. These states exhibit relaxation times that may exceed the conventional spin-lattice relaxation T1 by large factors, and in some cases, by more than an order of magnitude. For example, a molecular system has been demonstrated which supports a LLS with a relaxation time constant exceeding one hour in room-temperature solution. A useful concept in theory of long-lived states is the commutant. Consider a set of spin operators. The commutant of that set is a second set of operators, all of which commute with all members of the first set. Similarly, the bicommutant is a third set of operators, all of which commute with all members of the second set. To the best of our knowledge, magnetic resonance theory has not exploited the concepts of commutant and bicommutant before, at least not by name. In the context of long-lived states, suppose that the spin Hamiltonian always consists of a linear combination of a certain set of operators. The spin dynamical Lie algebra may be generated by evaluating all commutators between members of this set, and then all commutators between those operators, and so on, until no new operators can be generated by commutation. The long-lived states are members of the commutant of the spin dynamical Lie algebra. The routines LieAlgebra, Commutant, and Bicommutant are now included in SpinDynamica software, and may be used to predict the long-lived states of a spin system under arbitrary circumstances. These concepts will be illustrated by examples and diagrams.

Heteronuclear NMR Spin-lattice Relaxation: A tool to analyse dynamics of molecules in solution

Samanwita Pal
Indian Institute of Technology Jodhpur

(8^{th} Dec 16:45-17:15)

Heteronuclear NMR relaxation methods are used extensively to analyze biomolecular dynamics in condensed phases mostly by investigating carbon and nitrogen spins of the biomolecules. On the other hand small molecular systems provide varied options of heteronuclear spins to exploit such as ^{2}H, ^{7}Li, ^{17}O, ^{19}F, ^{31}P to name a few. These set of nuclei represent both dipoles and quadrupoles having interesting relaxation behaviors that offer gamut of information related to dynamics of small molecules in solution as well as in solids. In this talk we will try to discuss solution dynamics of three different molecular systems: firstly aqueous fluoroalcohol solution of i) bee venom peptide melittin1 and, ii) macrocyclic carbohydrate cyclodextrin2 and secondly iii) aqueous electrolyte solution of alanine. The former two cases were analyzed through solvent ^{19}F and ^{2}H spin-lattice relaxation while the latter case was investigated using ion-solute/solvent ^{1}H and ^{7}Li spin-lattice relaxation measurements. In each case the relaxation mechanism of the heteronuclear spins is laid-out carefully to extract information relevant to understand the nuances of dynamic processes present in these systems.

Triangulating Mn(II) Insertion in $Cs_2NaBiCl_6$ Doped Double Perovskite using Magnetic Resonance Spectroscopy

Sheetal Jain
SSCU, Indian Institute of Science, Bangalore, India

(8^{th} Dec,17:15-17:45)

Metal halide double perovskite materials are gaining increasing attention for optoelectronic applications. Transition metal-doped double perovskites are emerging as lead-free, stable semiconducting materials with tailorable optical bandgaps. One candidate material is Mn(II)-doped Cs2NaBiCl6, where the influence of Mn-insertion on the chemical structure is poorly understood due to low Mn loading. A comprehensive three-pronged strategy involving solid-state nuclear magnetic resonance (NMR), high field dynamic nuclear polarization (DNP), and electron paramagnetic resonance (EPR) spectroscopies to identify the location of Mn(II)-insertion in these materials will be presented. Complimentary EPR and NMR measurements will be shown to confirm that the cubic structure is maintained with Mn(II) incorporation at room temperature. Moreover, Paramagnetic Relaxation Effect (PRE) and hyperfine sublevel Correlation (HYSCORE) EPR spectroscopy results will be discussed that indicate that Bi-Mn exchange is preferred on doping. Finally, Endogenous DNP NMR measurements from Mn(II) → ^{133}Cs (<30 K), revealing that the Mn(II) is homogeneously distributed within the double perovskite phase, will be shown.

Spatiotemporal Resolution of Conformational Changes in Biomolecules by Pulsed Electron-Electron Double Resonance Spectroscopy

T. Hett, T. Zbik, R. Seifert, N. Brenner, H. Grubmüller, U.B. Kaupp, O. Schiemann

Clausius Institute of Physical and Theoretical Chemistry, University of Bonn, Germany

Proteins are highly dynamic biomolecules that can undergo ligand-induced conformational changes, thus often playing a crucial role in biomolecular processes. For an in-depth understanding of protein function, the conversion of one conformational state into another has to be resolved in space and time. Pulsed electron-electron double resonance spectroscopy (PELDOR/DEER) in combination with site-directed spin labelling (SDSL) is a powerful tool for obtaining distributions of interspin distances in proteins [1, 2]. It allows for measurements with angstrom precision, but it cannot directly determine the time scale and the mechanism of the conformational change. However, coupling PELDOR with rapid freeze-quench techniques adds the time axis to the distance distribution and thus permits studying conformational changes with temporal resolution.

Here, we show that the combination of Microsecond Freeze-Hyperquenching (MHQ) [3] and PELDOR resolves ligand-triggered conformational changes in proteins on the angstrom and microsecond time scale. It allows taking snapshots along the trajectory of the conformational change by rapid quenching within aging times of 82-668 µs, and it is applicable at protein amounts down to 7.5 nmol (75 µM, 100 µL) per time point. We applied MHQ/PELDOR to the cyclic nucleotide-binding domain (CNBD) of the MloK1 channel from *Mesorhizobium loti*, which undergoes a conformational change upon binding of cyclic adenosine monophosphate (cAMP). We observed a gradual population shift from the *apo* to the *holo* state on the microsecond time scale, but no distinct conformational intermediates (Fig. 1a, b). [4]

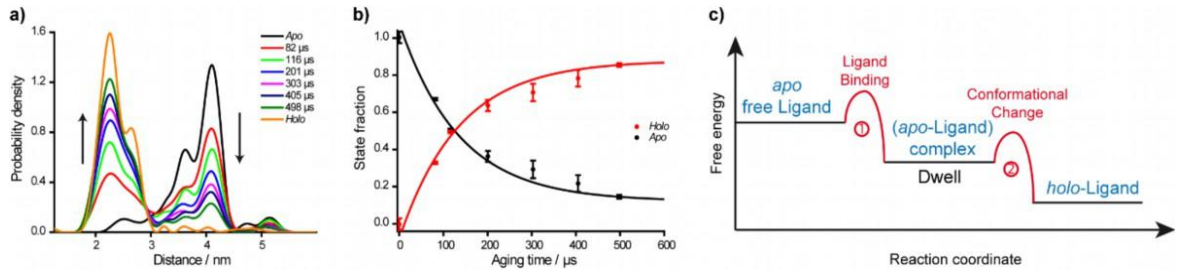


Figure 1: *a) Interspin distance distributions obtained at different aging times and b) the corresponding fractions of apo and holo state. c) Free-energy profile of the ligand-induced conformational change.*

Corroborated by measurements of ligand-binding kinetics and molecular dynamics (MD) simulations, we interpret the data in terms of a dwell time distribution. The transitions across the free-energy barriers (Fig. 1c) i.e., ligand binding and the conformational change, are on the nanosecond time scale and thus below the time resolution of the MHQ device. However, the dwell time of the *apo* state in complex with the cAMP ligand is in the microsecond range and can be monitored by MHQ/PELDOR. [4]

Literature: [1] A.D. Milov *et al.*, *Fiz. Tverd. Tela* **1981**, *23*, 975-982. [2] G. Jeschke, *Annu. Rev. Phys. Chem.* **2012**, *63*, 419-446. [3] A.V. Cherepanov *et al.*, *Biochim. Biophys. Acta* **2004**, *1656*, 1-31. [4] T. Hett *et al.*, *J. Am. Chem. Soc.* **2021**, *143*, 6981-6989.

Zero to Ultra low field NMR using atomic magnetometers

G. Rajalakshmi
Tata Institute of Fundamental Research Hyderabad, India

(8^{th} Dec, 18:30-19:00)

Zero- to ultralow-field nuclear magnetic resonance (ZULF NMR) provides an alternative to standard high-field NMR to study spin systems in a regime dominated by inter-/intra-nuclear spin-spin interactions. Pioneering experiments in this field were done by Pines and coworkers. In these experiments dipole coupling in powdered solid samples were studied by overcoming the line broadening caused by the random orientation of the dipoles with respect to the applied magnetic field. Modern ZULF experiments are performed on solution sample and typically detect the j-couplings using atomic magnetometers. The fT sensitivity and few 100 Hz bandwidth of commercial atomic magnetometers make them ideally suited for j-spectroscopy. In solid samples, however, the dominant dipole interactions are in the 1-30 KHz regime. We have developed an atomic magnetometer that has pT sensitivity but a effective bandwidth of about 40 kHz. We present our progress towards implementing ZULF NMR studies using our magnetometer.

NMR Meets QC

Anil Kumar
Department of Physics, Indian Institute of Science, Bangalore, India

(8^{th} Dec, 19:00-19:30)

In this talk I will describe how nuclear spins can be treated as Qubits. Introduce the idea of pseudo-pure states and how various Gate operations and Quantum Algorithms can be implemented. In particular implementation of Logic Gates by one- and two-dimensional NMR, implementation of Quantum Algorithms such as Deutsch-Jozsa and Grover's search algorithm; non-destructive discrimination of Bell-States and finally prove "Quantum No-Hiding Theorem" by NMR.

Fast Side Chain Motions in Proteins: Hidden Regularity and Response to Perturbations

Marimuthu Krishnan
CCNSB, IIIT-Hyderabad, India

(8th Dec, 19:30-20:00)

The heterogeneous fast side-chain dynamics of proteins play crucial roles in molecular recognition and binding. Site-specific NMR experiments quantify these motions by measuring the model-free order parameter on a scale of 0 (most flexible) to 1 (least flexible) for each methyl-containing side-chain of proteins. The talk will focus on the hidden regularity and universal features of these fast side chain motions. NMR order parameter-based statistical methods to characterize the response of fast side-chain fluctuations to ligand binding and to changes in the local nonbonded interactions will be discussed.

NMR studies of Ubiquitin signaling

Ranabir Das
National Centre for Biological Sciences, Bangalore, India

(9th Dec, 18:30-190:00)

Many bacterial pathogens secrete effector proteins in the host cell during infection. Often these effectors are enzymes that modify eukaryotic host proteins with posttranslational modifications. Such modifications help the pathogen modulate the host cellular environment conducive to its replication. Recently, enzymatic deamidation has emerged as a common posttranslational modification utilized by a broad range of bacterial pathogens of both plants and animals to modulate the functions of host proteins. Deamidation replaces an amide group with a carboxylate group at the side chain of glutamine and asparagine amino acids. Therefore, it converts glutamine and asparagine to glutamic acid and aspartic acid, respectively. Deamidation is an irreversible process that increases the mass of the target protein by 1Da, increases the negative charge of the target protein, and releases ammonia. Non-specific deamidation can occur spontaneously with the age of the protein. Enzymatic deamidation is absent in the eukaryotic host but is a valuable tool for bacteria to control the host cellular environment. We will discuss some recent results that reveal the atomistic details underlying how deamidation changes the structure and interactions of host proteins.

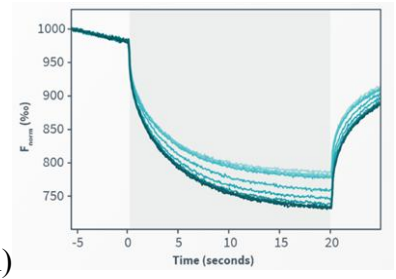
Spectral shift and TRIC Technologies overcome various limitations in the characterization of challenging molecular interactions

Ruchika Dadhich
NanoTemper Technologies, India

(9th Dec, 19:00-19:30)

The road to the successful characterization of challenging molecular interactions is filled with roadblocks often imposed by the limitations of the widely established biophysical methods — like dependence on a surface immobilization, high sample consumption, buffers constraints, or molecular mass difference of interacting partners. There is a clear need for a biophysical technology that gives scientists the solution they need to finally study challenging interactions that involve molecules like cell surface receptors, small molecular ligands, PROTACs, AAVs, liposomes, RNAs, DNA, or other complex molecules.

The new Monolith — with Spectral Shift and TRIC technology excels precisely at the measurement of these demanding interactions, independently of buffer composition. TRIC (temperature-related intensity change) technology allows for the quantification of molecular interactions between a target and ligand by detecting changes in fluorescence intensity while a temperature gradient is applied over time. On the other hand, Isothermal Spectral Shift utilizes the phenomenon of small shift in the emission spectrum of a fluorescently labeled target as it binds to a ligand. It's a well-known fact that this happens because of the changes in the local environment of the fluorophore. The detector exploits this phenomenon by performing ratio metric measurements at two different emission wavelengths of a labelled target in the presence of various concentrations of a ligand. Both the methods ensure reduced time in assay development, towards obtaining highly reproducible data.

A) 

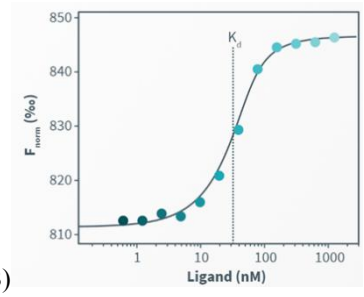
B) 

Figure 1. The affinity constant (Kd) is calculated from a fitted curve (B) that plots normalized fluorescence (A) against concentration of ligand.

In this talk we will share with you how the biophysical principles of both these methods can be harnessed to obtain reliable and meaningful binding affinity data (binding constant, stoichiometry and thermodynamic parameters). We will also discuss a set of examples where successful characterization of challenging interactions was achieved with high-quality data.

Measuring hydroxyl exchange rate constants in glycans using saturation transfer and relaxation dispersion NMR methodology

Ashok Sekhar
Molecular Biophysics Unit, Indian institute of Science, Bangalore

(9th Dec, 19:30-20:00)

Glycan-protein and glycan-glycan interactions mediate a number of key cellular processes such as cell adhesion and the immune response. The dynamic hydrogen bonding network formed by the hydroxyl groups in free glycans is replaced by hydrogen bonds with partner molecules and the specificity of these hydrogen bonds facilitates molecular recognition. An important parameter that characterizes hydroxyl groups is the rate constant at which they exchange with water. Hydroxyl exchange rate constants report on the structure and dynamics of the environment surrounding the hydroxyl group as well as on whether the hydroxyl is participating in an intramolecular or intermolecular hydrogen bond. In this presentation, I will detail our efforts at measuring the hydroxyl exchange rate constants in sugars such as sucrose, cellobiose and maltose as well as on the glycans defining the blood group antigens. Since glycan hydroxyl exchange rate constants are fast, we use a combination of 13C-based saturation transfer and relaxation dispersion NMR methods applied in concert with the two-bond H/D isotope effect to characterize hydroxyl exchange. Our results show that several factors including the accessibility of the hydroxyl group govern the rate at which it exchanges with the solvent. Our results also suggest that low hydroxyl exchange rate constants may indicate the presence of transient intra and intermolecular hydrogen bonds formed by the glycan molecule.

DNP with Boltzmann-polarized and hyper-polarized electron spins

Asif Equbal
NYU Abu Dhabi

(10th Dec, 14:00-14:50)

Dynamic nuclear polarization (DNP) has tremendous potential to revolutionize magnetic resonance spectroscopy and imaging by combining the best of NMR (resolution) and EPR (sensitivity). There have been significant developments in the last two decades, but we are still far from reaching the full potential of DNP. In the first part of my talk, I will discuss the evolution of our understanding of the DNP mechanism under MAS (rotating sample) using persistent electron spins; polarized to their Boltzmann equilibrium. The second half of my talk will focus on DNP using optically polarized electron spins which can transfer ultra-high optical polarization to nuclear spins via electron spins. I will talk about recent progress and challenges in realizing optically-activated DNP.

Hyperpolarized NMR Spectroscopy of Small Biomolecules on a Bench

Danila A. Barskiy
Helmholtz Institute Mainz, GSI Helmholtz Center for Heavy Ion Research GmbH, 55128 Mainz, Germany
Institute of Physics, Johannes Gutenberg-Universität, 55128 Mainz, Germany

(10th Dec, 14:50-15:30)

In my talk, I will outline the latest developments in our Lab regarding hyperpolarization of small biomolecules using chemistry-based approaches: PHIP (parahydrogen-induced polarization), SABRE (signal amplification by reversible exchange) as well as CIDNP (chemically induced dynamic nuclear polarization). We are focusing on enhancing NMR signals from small biomolecules including alcohols (methanol, ethanol) [1], aminoacids (glycine, tryptophane, methionine), sugars (glucose) [2], amines (ammonia, dopamine) among others with the goal of detecting them using 13 C and 15 N benchtop NMR spectroscopy (magnetic fields of 1-2 T). Since nuclear polarization is generated without using large magnetic fields, sensitivity of benchtop NMR detection is sufficient for observing chemicals at millimolar concentration, for some molecules at natural isotopic abundance of 13 C and 15 N nuclei. In addition, I will show how chemical information can be extracted from samples using "unconventional" detection schemes at ultralow magnetic fields (<100 nT) using atomic magnetometers [3]. Combination of novel signal generation and detection concepts may find utility for analyzing biological samples in situations when the use of conventional high-field approaches is impossible or hard to implement.

[1] Van Dyke et al., Sci. Adv., 2022, DOI: 10.1126/sciadv.abp9242.
[2] Alcicek et al., ChemRxiv, 2022, DOI: 10.26434/chemrxiv-2022-vzr8f.
[3] Put et al., Anal. Chem., 2021, DOI:10.1021/acs.analchem.0c04738.

Time shared CPMG relaxation dispersion approaches for simultaneous backbone and side-chain dynamics in proteins

Jithender Reddy
CSIR-IICT, Hyderabad, India

(10th Dec, 15:30-16:00)

Conformational dynamics of proteins on milli- to micro-second (ms - μs) time scale plays crucial role in deciphering their folding-structure-function relationship. Both, backbone, and side chain dynamics are essential to understand their structural and/or functional importance. So far, different Relaxation Dispersion (RD) based NMR experiments were designed for understanding each of these dynamics in differently labelled samples.

Herein we describe new pulse sequences using time shared (TS) approach on Carr–Purcell–Meiboom–Gill (CPMG) RD experiment to derive both backbone (amide) and sidechain (methyl) dynamics simultaneously using single (U- 15 N, methyl- 13 CHD 2) sample. One for 15 N-amide / 13 C-methyl and another for 1 H-amide/ 1 H-methyl CPMR-RD profiles. These profiles directly explain whether both the dynamics are happening in synergy. This strategy is time effective and provides dynamics of different sites to accurately identifying the conformational process/es happening in the system.

Using a fast-folding α/β topology 62-residue protein, gpW, we show that both amide and methyl kinetics can be accessed using single experiment. Both dynamics are synergistically show that native folded state interconverts with a transiently formed, sparsely populated second state, where only β-hairpin is unfolded. Our results clearly show that this process is driven by hydrophobic interaction between β-hairpin and the α-helices.

Insights into the endogenous inverted-repeat siRNA (endo-IR) pathway by the organization of the DRB4:DRB7.2 complex in plants

Mandar Deshmukh
CSIR-CCMB, Hyderabad

(Dec 10th Dec, 16:00-16:30)

The presence of noncoding yet transcribable inverted repeat sequences throughout the eukaryotic genome plays a vital role in genome stability and regulation of transposable elements, mutations, and diseases. In plants, the dsRNA Binding Proteins (dsRBPs), DRB7.2 and DRB4, together sequester long endogenous inverted-repeat siRNA (endo-IR) precursors that are natural substrates of Dicer-like protein3 (DCL3), thus stalling the endo-IR mediated gene regulation. Earlier, in vivo studies demonstrated that the mutation in DRB4 or DRB7.2 enhanced the production of DCL3-dependent 24 nt endo siRNAs. To understand how DRB7.2 and DRB4 interact with each other to sequester the dsRNA, we have identified the minimal interaction domains of DRB4 and DRB7.2, derived their solution structures, and determined the crystal structure of the complex. Interestingly, we found that the interacting domains exhibit significant conformational heterogeneity individually but adopt a stable fold only in the presence of the interacting partner. Further, using a series of biochemical and biophysical assays, we propose a functional model for the DRB7.2 and DRB4 association, explaining their role as the complex for sequestering endo-IR precursors.

Characterizing Sequence-Specific Conformations of Duplex DNA

Bharathwaj Satyamoorthy
IISER Bhopal, India

(Dec 10th Dec, 17:00-17:30)

DNA achieves its function by interacting with various proteins facilitating critical biomolecular transactions. Protein interaction with DNA is perceived to be carried out by two mechanisms: (a) sequence readout from the major groove and (b) shape readout3 that is based on subtle differences in the double helix. It remains an open question whether the differences that arise in shape is a consequence of the protein binding or is an inherent ability of the DNA to sample various sequence-specific conformations conferring protein recognition modes. Sequence-specific differences evade reliable characterization by crystallography due to packing artifacts that bias the polymorphic folding landscape of DNA. On the other hand, Nuclear Magnetic Resonance (NMR) spectroscopy is an apt technique for obtaining atomistic details of duplex DNA providing both tertiary structure and conformational dynamics across a wide timescale. However, a thorough and systematic study on how primary sequence affects tertiary structure of DNA has not been undertaken. While $^{13}C/^{15}N$-chemical shifts-based methods have paved the way for reliable and accurate conformational characterization of proteins and RNA,5 their application to DNA is uncharted. To address the same, we apply solution-state NMR spectroscopy to investigate forty dodecamer sequences sampling all possible trinucleotide steps across the canonical Watson-Crick base pairs. The trinucleotide steps were positioned in pseudo palindromic and "unique" sequences to tease apart effects across these relevant contexts. We observe that the measured NMR parameters (such as 15N/13C/1H chemical shifts and homo-/heteronuclear scalar couplings) effectively report on subtle differences across the trinucleotide steps. Data suggests that the two nearest neighbors confer local conformational preferences. A machine learning approach applied here provides an avenue to predict chemical shifts and sequentially assign resonances for DNA solely from primary sequence. In this talk, I shall present the first step towards obtaining sequencespecific conformations of DNA duplexes using NMR parameters and machine learning.

A litmus test using high-power relaxation dispersion for classifying recognition mechanisms of transiently binding proteins

Kalyan Chakrabarty
Krea University, India

(10th Dec, 17:30-18:00)

Protein-binding partner recognition is critical for all biological functions, and yet, delineating its mechanism is challenging, especially when recognition happens within microseconds [1,2]. We present a novel theoretical and experimental framework to distinguish between two-state vs three-state binding, including conformational selection and induced fit [3], based on straightforward kinetic experiments using NMR high-power relaxation dispersion [4], sensitive to single-digit microseconds. The novel framework predicts that conformational selection prevails on ubiquitin's paradigmatic interaction with the SH3c domain from an adapter protein [5]. We then reveal the residues that engage in the conformational selection mechanism using molecular dynamics simulations and Markov state modeling [4]. The novel framework is robust and expandable for implementation in other binding scenarios with the potential to show that conformational selection might be the design principle of the hubs of interaction networks.

Exploring the Free Energy of Surface of Proteins using CEST NMR Experiments

Pramodh Vallurupalli
Tata Insititute of Fundamental Research Hydebarad, India

(10^{th} Dec, 18:00-18:30)

Protein molecules interconvert between multiple states and these exchange events play important roles in their function and misfunction. Often the states that are involved are invisible to traditional biophysical methods, complicating their analysis. The Chemical Exchange Saturation Transfer (CEST) NMR experiment that was originally conceived a half century ago [1] was only relatively recently further developed [2] for studies of sparse states in biomolecules that are in slow exchange with a visible major state. In this talk I will discuss a recent development that now extend the ability of CEST experiments to probe exchange between multiple states over multiple timescales considerably. By including in an analyses of CEST data the linewidths of minor state peaks, we show that it now becomes possible to detect invisible states with extremely low populations (~0.1%) and a wide range of lifetimes (~10^{-5} to ~10^{-1} s) [3]. Using this strategy we discovered that the A39G FF domain folds from the unfolded state through two intermediates via a branched pathway to the folded state on a volcano shaped free energy surface.

H-MAS Update

Ago Samosan
Tallinn University of Technology

(10th Dec, 18:30-19:00)

Inverse detection in solids is arguably among the most important developments in recent history of NMR. We shall present some basic technical details, regarding rotor driving, and various speed-S/N classes currently available. We also illustrate how resolution in 1H dimension reveals couplings to ^{14}N and correlated chemical shift dispersions. e.g. the latter provides insight to morphology of cellulose.