2nd India-Japan Workshop on Magnetic Resonance

Date: Monday, 9th December – Tuesday, 10th December 2019

Venue: Tata Institute of Fundamental Research, Sy. No. 36/P, Gopanpally, Serilingampally, Hyderabad -500 107

Program

Monday, 9th December 2019	
Time	**Speakers**
9:15-9:30	***Opening remarks by Director TIFR Hyderabad and Director NMR Division, Riken***
Session Chair: B. Jagdeesh	
9:30-11:00	Yoshitaka Ishii, Tokyo Institute of Technology & Riken, Yokohama ***Sensitivity-Enhanced Protein Solid-state NMR using Ultra-fast MAS and Structural Studies of Alzheimer's Amyloid-β*** Pramodh Vallurupalli, TIFR, Hyderabad ***Double Resonance CEST Spectroscopy: A New Tool to Study Multi-State Protein Conformational Exchange*** Mandar V Deshmukh, CCMB, Hyderabad ***Understanding the mechanism of small RNA mediated gene regulation in higher eukaryotes***
11:00-11:30	Coffee/Tea Break
Session Chair: Yoshitaka Ishii	
11:30-13:00	Yusuke Nishiyama, Jeol Resonance and Riken, Yokohama ***Electron and NMR Nano-Crystallography*** Kristhoff Grohe, Bruker Biospin, Germany ***Towards exact distance restraints in solid-state NMR for determination of structure and dynamics of insoluble proteins*** Kaustubh Mote, TIFR, Hyderabad ***Rapid data collection at slow and moderate MAS frequencies without increasing probe duty cycles***
13:00-14:00	Lunch
Session Chair: Tomoyasu Aizawa	
14:00-15:30	K. Takeda, Kyoto University ***In situ solid-state measurements of a magnetically oriented microcrystal suspension / Up-conversion of radio-frequency NMR signals to an optical regime using a membrane transducer*** Rajalakshmi, TIFR, Hyderabad ***Optical detection of Spins Polarisation*** Yoh Matsuki, Osaka University ***Mesoscale Sample Domain Selection by 460 GHz-700 MHz DNP NMR using Closed-Cycle Helium MAS and Dual Gyrotron***
15:30-16:00	Coffee/Tea Break
Session Chair: Kanchan Garai	
16:00-18:00	Soumya De, IIT Kharagpur ***Identification of rigid segments with important biological functions in intrinsically disordered regions of proteins by solution NMR spectroscopy*** Navratna Vajpai, Biocon Biologics Ltd – RND, Bangalore ***High-resolution HOS characterization of biologics***

	Srinivasan L Poojary, JEOL India Ltd. ***Latest Developments and New features from JEOL NMR Technologies*** Sebastian Wegner, Bruker Biospin, Germany ***Automation in solid state NMR, a comprehensive look at CPMAS and ICONNMR***
18:30	Leave for Dinner
Tuesday, 10th December 2019	
Session Chair: Abani Bhuyan	
9:00-11:00	Tomoyasu Aizawa, Hokkaido University ***Development and application of novel overexpression systems for NMR analysis of antimicrobial peptides*** Ashutosh Kumar, IIT Bombay ***Mechanism of recognition between Plasmodium falciparum and Human sumoylation machinery*** Bharathwaj Sathyamoorthy, IISER, Bhopal ***Application of optimized heteronuclear NMR methodology towards characterizing sensitivity limited DNA duplexes containing epigenetic modifications*** Ranabir Das, NCBS, Banaglore ***Molecular mechanism of how deamidation by Shigella silences the host immune response***
11:00-11:30	Coffee/Tea Break
Session Chair: Yusuke Nishiyama	
11:30-13:30	P.K. Pujari, BARC, Mumbai ***Understanding Phase Behaviour of Nanoconfined Liquids: An NMR and Positron annihilation spectroscopic Approach*** Yasuto Noda, Kyoto University ***Toward In-situ/Operand High-Resolution Solid-State NMR*** Vivek Polshettiwar, TIFR, Mumbai ***Noble Metal and Ligand Free Nanocatalysts to Convert CO_2 to Fuel and Plastic Waste to Chemicals*** T. N. Narayanan, TIFR, Hyderabad ***Importance of in situ Probes in Electrochemical Experiments***
13:30-14:30	Lunch
Session Chair: Aprotim Mazumdar	
14:00-15:00	Jun Kikuchi, Riken Yokohama ***NMR Data Science Approach for Fishery Products and Aquatic Ecosystem*** Neeraj Sinha, CBMR, Lucknow ***Improving survival predictability and biological insight through NMR based metabolomics of Acute Respiratory Distress Syndrome (ARDS)***
	Vote of Thanks (P.K. Madhu)

Titles and Abstracts

Guidelines for Talks

1) The typical duration of the talk should be 22-25 minutes with 5-8 minutes for discussions and questions.
2) You are free to use your own system (Mac windows and Linux are equally fine)
3) Thunderbolt, VGA and HDMI connectors will be available for connecting computers to the projector.

Sensitivity-Enhanced Protein Solid-state NMR using Ultra-fast MAS and Structural Studies of Alzheimer's Amyloid-β

Yoshitaka Ishii[1,2]

1) School of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan

2) NMR Science and Development Division, RIKEN SPring-8 Center, RIKEN, Yokohama, Japan

This work involves two separate topics on our on going progress of protein SSNMR methods using ultra-fast MAS and solid-state NMR (SSNMR) applications to amyloid proteins. First, we discuss resolution and sensitivity enhancement in ^{1}H-detected biomolecular SSNMR under ultra-fast magic angle spinning (UFMAS) conditions (≥ 80 kHz) in a high magnetic field (^{1}H frequency: 750-900 MHz). [1,2] Our data on protein microcrystal GB1 and amyloid-β (Aβ) fibril show that traditionally time-consuming 3-5D biomolecular SSNMR is feasible for signal assignments and structural examination of proteins in a nano-mole-scale with this approach. Our discussion will include drastic sensitivity enhancement by novel polarization-transfer schemes and other methods for multi-dimensional SSNMR using ultra-fast MAS. We briefly introduce our nation-wide effort to construct a 1.3 GHz NMR at RIKEN.

Second, we examine structures, kinetics, and functions of amyloid-β using solid-state NMR (SSNMR). Increasing evidence suggests that formation and propagation of misfolded aggregates of 42-residue Aβ42, rather than the more abundant 40-residue Aβ40, provokes the Alzheimer's cascade. Our group recently presented the first detailed atomic model of Aβ42 amyloid fibril based on SSNMR data.[3] The result revealed a unique structure that was not previously identified for Aβ40 fibril. Based on the results and additional SSNMR data, we discuss how amyloid fibril structures affect "prion-like" propagation across different Aβ isoforms, including WT Aβ40 and E22G pathogenic mutant of Aβ40.[4] We also discuss SSNMR-based structural analysis of toxic spherical assembly of Aβ, including one that was identified from brains affected by AD.[5] The results provide insight into amyloid misfolding of Aβ42 in Alzheimer's disease.

References:

(1) Wickramasinghe, N.et al. Nat. Methods 2009, 6, 215.
(2) Ishii, Y.et al. J. Magn. Reson. 2018, 286, 99.
(3) Xiao, Y.et al. Nat. Struct. Mol. Biol. 2015, 22, 499.
(4) Yoo, B.et al. JACS 2018, 140, 2781.
(5) Parthasarathy, S.et al. JACS 2015, 137, 6480.

Double Resonance CEST Spectroscopy: A New Tool to Study Multi-State Protein Conformational Exchange

Pramodh Vallurupalli

TIFR, Hyderabad

Proteins are dynamic molecules adopting different conformations in solution. Despite the importance of protein dynamics to function, studying exchange between multiple

conformational states remains a challenge because sparsely populated states cannot be detected by conventional experiments. CEST NMR experiments can detect minor states with lifetimes between 5 and 200 milliseconds populated to just 0.5% that are in exchange with a visible state. However when exchange occurs between three or more states, the exchange mechanism of the process being studied cannot be deduced from regular CEST data alone and the role that the detected minor states play in the process being studied remains unknown. For example in folding studies it is often impossible to distinguish between on-pathway folding intermediates and off-pathway minor conformers using just regular CEST data or using data from other techniques. To study multi-state exchange, we have developed a double resonance CEST experiment in which resonances from two minor states simultaneously irradiated. This new experiment is used to study the folding of T4 lysozyme. The molecule exchanges between the dominant native state and two minor states, the unfolded state (U) and a second minor state (B), each populated to only ~4%. Regular CEST does not provide the folding mechanism but double resonance CEST clearly shows that the protein can fold directly from the unfolded to the native state without going through the minor state B.

Understanding the mechanism of small RNA mediated gene regulation in higher eukaryotes

Mandar V. Deshmukh

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

In higher eukaryotes, 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 manner.

To understand the origin and necessity of the evolutionary divergence in RNAi, we have defined the functional roles of RDE-4 in *C. elegans* as well as DRB4 in *A. thaliana* in the last few years. We are currently exploring the structure-function relationship in DRB2, DRB3, DRB5, and DRB7.2 in *A. thaliana* and R2D2 in *D. melanogaster* using solution structure, biochemistry, and dynamics studies. Subsequently, we anticipate assembling a ternary complex of Dier:dsRBP and corresponding initiator dsRNA for structural and mechanistic studies. So far, our results imply a fine balance in seemingly conserved and highly homologous systems that are tuned to alter the fate of the small RNA mediated gene silencing. Surprising heterogeneity in the structure and function of dsRBPs suggests that the process of RNAi initiation is unique for each organism and is dependent on the step-wise assembly of the Dicer, dsRBP, and the trigger small RNA.

Electron and NMR Nano-Crystallography

Yusuke Nishiyama
JEOL RESONANCE Inc. and RIKEN

Understanding hydrogen-bonding networks in nanocrystals and microcrystals that are too small for X-ray diffractometry is a challenge. Although electron diffraction (ED) or electron 3D crystallography are applicable to determining the structures of such nanocrystals owing to their strong scattering power, these techniques still lead to ambiguities in the hydrogen atom positions and misassignments of atoms with similar atomic numbers such as carbon, nitrogen, and oxygen. Here, we propose a technique combining ED, solid-state NMR (SSNMR), and first-principles quantum calculations to overcome these limitations. The rotational ED method is first used to determine the positions of the non-hydrogen atoms, and SSNMR is then applied to ascertain the hydrogen atom positions and assign the carbon, nitrogen, and oxygen atoms via the NMR signals for ^{1}H, ^{13}C, ^{14}N, and ^{15}N with the aid of quantum computations. This approach elucidates the hydrogen-bonding networks in L-histidine and cimetidine form B whose structure was previously unknown.

References:

[*1] C. Guzmán-Afonso,† Y.-l. Hong, † H. Colaux, H. Iijima, A. Saitow, T. Fukumura, Y. Aoyama, S. Motoki, T. Oikawa, T. Yamazaki, K. Yonekura, Y. Nishiyama*, Nat. Commun. 10 (2019) 3537. DOI: 10.1038/s41467-019-11469-2*

Towards exact distance restraints in solid-state NMR for determination of structure and dynamics of insoluble proteins

Kristhoff Grohe

Bruker Biospin, Germany

Fast-magic-angle-spinning solid-state NMR is a developing technique for determination of protein structure and dynamics. As a drawback, proton-proton correlations usually lead to rough estimation of internuclear distances. Restraints derived out of these distances only allow for the determination of reasonable structures, if they are available in high numbers. This represents a serious hurdle for high- resolution structure elucidation.

On this front, analogous to the ‘‘exact-NOE’’ concept in solution, we demonstrate an approach that yields accurate distance information with minimal analytical effort, by using an interrelated data analysis strategy. As such, solid-state NMR protein structures with improved precision and accuracy are achievable, for any given number of obtainable contacts.

Further, if the errors of internuclear distance-restraints deceed motional amplitudes, these can be used to determine directional protein dynamics.

Rapid data collection at slow-moderate MAS without increasing probe duty cycles

Kaustubh R. Mote

TIFR Hyderabad

Multiple sequential acquisition experiments are a promising avenue for speeding up data acquisition in the solid-state. These experiments are applicable at all MAS frequencies, and can result in time savings of a factor 2 or more. However, the main drawback of this strategy, especially at slow-moderate MAS frequencies (10-25 kHz) is that they require an increase in

the probe duty cycle by a factor 1.5-2.0. This increase is mostly due to increased decoupling duration on the proton channel, and is thus undesirable for samples susceptible to RF-induced heating. In fact, the recycle delay many a times optimized to reduce this RF-induced heating, and will thus negate any benefits from multiple acquisition, unless low-E or E-free probes are used. In this talk, I will present strategies at slow-moderate MAS frequencies that will allow some of the developments in multiple sequential acquisition experiments to be adapted in a way that will not increase the duty cycle at slow-moderate MAS. These pulse sequences will primarily give through-space correlations and will be useful in assigning as well collecting distance restraints for analysing protein structures. The extension of these experiments to dimensions > 2 will also be presented. These experiments are expected to speed up both assignments and structure calculations for small-medium sized proteins at slow-moderate MAS frequencies.

In situ solid-state measurements of a magnetically oriented microcrystal suspension / Up-conversion of radio-frequency NMR signals to an optical regime using a membrane transducer

Kazuyuki Takeda

Kyoto University, Kyoto, Japan

1. A crystal placed in a magnetic field is acted by a torque arising from the anisotropic bulk magnetic susceptibility. Under modulated rotation of microcrystals suspended in a viscous liquid medium around an axis perpendicular to the static field, the individual microcrystals can be aligned in the same direction, forming a Magnetically Oriented Microcrystal Suspension (MOMS). Solid-state NMR of the MOMS gives spectra similar to those of a single crystal. We demonstrate in situ 13C CP NMR of a MOMS of L-alanine [1].

2. In what we call Electro-Mechano-Optical NMR, or EMO NMR [2-3], radio-frequency NMR signals are up-converted to an optical regime using a high-stress silicon nitride membrane that interfaces the NMR-probe circuit and an optical cavity. A metal layer coated on the membrane serves both as an electrode of a capacitor and a mirror of an optical cavity, so that the nuclear induction signal is transcribed to the vibration of the membrane through the electro-mechanical coupling. In turn, the displacement of the membrane modulates the light in the optical cavity. In this way, optical NMR detection is realized without sacrificing the versatility of the traditional nuclear induction approach. We show demonstrations of EMO NMR as well as our current efforts toward its extension, including:

- New design and fabrication of a compact rf-to-light transducer that would fit inside the bore of a superconducting magnet, and

- Development toward combination of EMO with MRI.

References

[1] R. Kusumi, H. Kadoma, M. Wada, K. Takeda, and T. Kimura, Journal of Magnetic Resonance (2019) (in press); doi: 10.1016/j.jmr.2019.106618.
[2] K. Takeda, K. Nagasaka, A. Noguchi, R. Yamazaki, Y. Nakamura, E. Iwase, J.M. Taylor, K. Usami, Optica. 5 (2018) 152; doi:10.1364/OPTICA.5.000152.
[3] Y. Tominaga, K. Nagasaka, K. Usami, K. Takeda, J. Magn. Reson. 298 (2019) 6; doi:10.1016/j.jmr.2018.11.003.

Optical detection of Spins Polarisation

Rajalakshmi

TIFR, Hyderabad

Atomic systems have been shown to be sensitive probes for local spin polarisation. Recent advances in technology have enabled optical atomic magnetometry techniques to attain sensitivities comparable to those of Super-conducting Quantum Interference Devices (SQUID). Atomic magnetometers are cryogen free and are used for diverse application from fundamental physics tests to geophysical and biomedical measurements. Atomic magnetometers that detect the nuclear polarisation by non-inductive means are also ideal sensors for low field NMR, where the signal sensitivity of pick coils is reduced by the lower larmor frequency of the nucleus. The optical rotation of a linearly polarised probe laser beam passing through a gas of alkali atoms that have beam spin polarised by optical pumping, is a sensitive measure of the local magnetisation changes. In our group we have implemented a Rb optical magnetometer, using linear and nonlinear Faraday effects. We employ techniques based on the concept of weak measurement to enhance the sensitivity of the magnetometer to optical rotation and are able to measure changes of 0.6nT in the local dc field. We are currently improving the system to reach pT sensitivity required to study NMR at near zero fields.

Mesoscale Sample Domain Selection by 460 GHz-700 MHz DNP NMR using Closed-Cycle Helium MAS and Dual Gyrotron

Yoh Matsuki

Institute for Protein Research, Osaka University

Sensitivity of magic-angle spinning (MAS) solid-state NMR has been dramatically improved by the advent of high-field dynamic nuclear polarization (DNP) techniques through numerous discussions and breakthroughs made for improving the signal enhancement factor. Beyond the discussions on the sensitivity gain, we here propose two new methods to pursuit hitherto under-explored curiosity: a method to confine the hyperpolarization for spatially selective observation of mesoscale sample domain, and a method that enables quantitation of absolute ^{1}H polarization amplitude and its spatial distribution around a radical molecule (polarizing agent). The **former method** utilizes our unique dual-gyrotron setup and its ability to switch the microwave frequency back and forth over the range of 0.7 GHz in synchrony to the RF pulses [2]. Each microwave frequency is set to excite the positive or negative DNP effect in turn, producing a sort of "polarization wave" in space around the radical molecule. The **latter method** is based on the use of a closed-cycle helium MAS system for ultra-low temperature DNP [3, 4], enabling the total sensitivity gain exceeding a factor of 1000 at T = 30 K and B_0 = 16.4 T. In such a case, the high-order spin-correlated term ($2I_zS_z$) in the quasi-equilibrium spin density operator grows in a significant amplitude and, as we show, is observable separately from the lowest-order Zeeman term (S_z) for the polarization quantitation. The method does not require evaluation of "microwave-off" signal as well as un-doped reference sample, and is also unaffected by the quenching and depolarization effects, providing an

accurate and efficient way for the polarization quantitation [1]. Potential applications will also be discussed.

References

*[1] T. Sugishita, Y. Matsuki and T. Fujiwara, SSNMR **99**, 20-26, (2019)*
*[2] Y. Matsuki et al., J. Magn. Reson. **264**, 107-111, (2016)*
*[3] Y. Matsuki et al., J. Magn. Reson. **259**, 76-86, (2015)*
[4] Y. Matsuki and T. Fujiwara, in "High-Frequency Dynamic Nuclear Polarization NMR," Chapter 5: Cryogenic Platforms and Optimized DNP Sensitivity, eMagRes 7 9-24 (2018)

Identification of rigid segments with important biological functions in intrinsically disordered regions of proteins by solution NMR spectroscopy

Soumya De[1], Snigdha Maiti[1], Bidisha Acharya[1], Bharat Manna[2], Amit Ghosh[2]

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Intrinsically disordered regions (IDRs) are ubiquitously present in eukaryotic proteins. Although their biological significance is well appreciated, the underlying mechanism explaining how these disordered regions facilitate protein-protein interactions, and thus regulate protein function, is still obscure. We propose a rigid segment model of IDRs. We present a NMR-based method of determining residue-wise flexibility of IDRs and show that they consist of rigid segments, which are functionally important.

We have characterized the dynamics of several HOX transcription factors from *Drosophila* and *Human*. We measured residue-wise flexibility from fast time-scale (ps-ns) NMR relaxation experiments and found that the flexibility of residues in the disorder regions vary significantly. IDRs have short stretches (~5-7) of relatively rigid amino acids that were linked by stretches of flexible amino acids. Using NMR titration experiments we further show that the identified rigid segments specifically interact with a partner protein that is known to modulate function of the HOX transcription factors. Furthermore, using MD simulations we find that the rigid segments explore less conformational space in the Ramachandran map as compared to the other neighboring residues in the disordered region. We reasoned that the restrained conformational sampling of these rigid residues should decrease the loss in conformational entropy during their interactions with binding partners and result in sequence specific interaction. Overall, we present a NMR-based method to identify functionally important segments in the intrinsically disordered regions of proteins.

High-resolution HOS characterization of biologics

Navratna Vajpai

Biocon Biologics Ltd – RND, Bangalore

Traditional higher order structure (HOS) characterisation techniques, though routine, provide information on global picture of a biological product. When manufacturing '*bioequivalents'* or '*biosimilars'*, any subtle differences in the protein structure especially in the vicinity of its binding partners could be alarming. My talk will cover importance of high resolution

techniques such as NMR and/or HDX-MS, which need to be implemented at an early stages of the drug development to circumvent these future challenges.

Latest Developments and New features from JEOL NMR Technologies

Srinivasa L. Poojary

JEOL INDIA PVT LTD

JEOL is well known in the field of scientific analytical instruments. We are continuing to make the most sophisticated state-of-the-art FT NMR spectrometers with highly efficient probes suits for customer requirements. The JNM-ECZ NMR spectrometer is our new NMR system that fully incorporates the latest digital and high frequency technologies. Our new probe Royal HFX has a unique feature to switch between single and dual tune mode on the HF ($^{1}H/^{19}F$) coil, which make the user to perform triple resonance ($^{1}H/^{19}F/X$) experiments even with the 2ch console without compromising on its performance. Such triple resonance experiment (e.g. ^{13}C $\{^{1}H, ^{19}F\}$) is inevitable for the accurate structural analysis on the fluorine compounds.

In this talk we discuss about JEOL's latest developments and New features of ECZ Series NMR Spectrometer, JEOL's Delta V5.3 NMR Software. There are many interesting tools and advance processing method added to the software to enhance the usability of instrument and helps the users to easy processing of NMR Data will be discussed.

Automation in solid state NMR, a comprehensive look at CPMAS and ICONNMR

Sebastian Wegner

Bruker Biospin, Germany

Traditionally solid state NMR has been seen as a tool which needs a lot of manual interaction from the user in order to achieve good experimental results and therefore solid state NMR has a touch of mystery for many users, this is true for the not especially dedicated users as well as for the specialists.

These challenges are not only related to the NRM instrument itself – might it be manual tuning and matching of probes or the pulse sequences itself – but also already in the sample preparation and the general lack of automation possibilities in solid state NMR.

During this contribution we will give an overview of the different new option available in probe hardware and also about the software components, which are in the meantime available to make solid state NRM "easy" and to move forward towards a level of automation for solid state CPMAS experiments which are so far only known from liquid state NMR applications.

Mechanism of recognition between *Plasmodium falciparum* and Human sumoylation machinery

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

Department of Biosciences & Bioengineering, 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 genus Plasmodium. *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.

Development and application of novel overexpression systems for NMR analysis of antimicrobial peptides

Tomoyasu Aizawa

Faculty of Advanced Life Science, Hokkaido University, 060-0810 Sapporo, Japan

The production of large numbers of targets is a key step in three-dimensional structural studies on peptides and proteins. For example, NMR studies using stable isotope-labeled recombinant peptides/proteins are among the most powerful means of studying peptide and protein structures. Many protein expression systems have been established to produce recombinant proteins. Moreover, a wide variety of strategies have been developed to improve the yield of recombinant proteins. One such strategy, fusion protein systems have generally been used to improve protein folding and solubility, yielding biologically active products. However, these approaches are not always successful. In particular, it is very difficult to produce target peptides and proteins that are lethal to host cells and/or are easily degraded in soluble form.

We present here a novel method of facilitating the expression level in *E. coli* of a recombinant peptide that is difficult to express in conventional production systems. We demonstrated that coexpression of the aggregation-prone protein remarkably enhanced the target peptide's

expression level. It seems that overexpression of the partner protein protects the target peptide from proteolytic degradation by forming insoluble inclusion bodies, thus accounting for the higher observed yields. Importantly, this method can isolate the target peptide from the partner protein by simple affinity chromatography, and it never requires chemical or enzymatic cleavage of the fusion protein. These advantages make our new method sufficiently effective and cost-efficient for large-scale production.

Application of optimized heteronuclear NMR methodology towards characterizing sensitivity limited DNA duplexes containing epigenetic modifications

Manjula Jaisal[1], Yingxu Fan[2], Yi Xue[2], Bharathwaj Sathyamoorthy[1]

[1]Indian Institute of Science Education and Research, Bhopal, India, [2]Tsinghua University, Beijing, China;

NMR spectroscopy has witnessed tremendous boost following the advent of $^{13}C/^{15}N/^{2}H$-isotopic labeling methodologies, innovative pulsing methods and the introduction of cryogenic probes that have enabled studying biomolecules with multidimensional NMR at low concentrations. However, samples that cannot be prepared with isotope enrichment or at high concentrations with natural isotopic abundance are difficult to study. This commonly arises with modified DNA samples, such as lesion or epigenetic modifications, that are subjected only to ^{1}H-^{1}H homonuclear based two-dimensional (2D) NMR studies.[1] In this study, we have optimized 2D heteronuclear experiments exploiting sparse sampling[2] and processing[3] methods to obtain ^{13}C-^{1}H and ^{15}N-^{1}H chemical shifts and scalar/residual dipolar couplings (RDCs). The optimized experiments are applied to study epigenetic modifications such as 5-methyl cytosine (^{m}C) and its oxidized counterparts (hydroxymethyl and formyl; ^{hm}C and ^{f}C) in duplex DNA. Chemical shifts and RDCs were obtained for DNA duplexes with sample concentrations as low as 100 μM at natural isotopic abundance (~1 μM [^{13}C] and ~400 nM [^{15}N]) within a total duration of 1 week that would have otherwise taken 4 weeks. In conjunction with molecular dynamics simulations, the results from this study[4] point towards no discernable changes to the structural and dynamic landscape of the epigenetically modified, contrasting to observations for DNA lesions[5] that occur on the Watson-Crick face of the nucleobase.

References

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2. *Hyberts SG, et. al, Journal of the American Chemical Society, 132, 7, 2145-2147 (2010)*
3. *Kazimierczuk K and Orekhov V, Angewandte Chemie-International Edition, 50 (24), 5556-5559 (2011)*
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Molecular mechanism of how deamidation by *Shigella* silences the host immune response

Priyesh Mohanty[1], Rashmi[1], Batul Ismail Habibullah[1], Arun G S[1] and Ranabir Das[1,2]

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The deamidase OspI from enteric bacteria *Shigella flexneri* deamidates a glutamine residue in the host ubiquitin-conjugating enzyme UBC13 and converts it to glutamate (Q100E). Consequently, its polyubiquitination activity in complex with the RING-finger ubiquitin ligase TRAF6 and the downstream NF-κB inflammatory response is inactivated. The precise role of deamidation in inactivating the UBC13/TRAF6 complex is unknown. We report that deamidation inhibits the interaction between UBC13 and TRAF6 RING-domain ($TRAF6^{RING}$) by perturbing both the native and transient interactions. Deamidation creates a new intramolecular salt-bridge in UBC13 that competes with a critical intermolecular salt-bridge at the native UBC13/$TRAF6^{RING}$ interface. Moreover, the salt-bridge competition prevents transient interactions necessary to form a typical UBC13/RING complex. Repulsion between E100 and the negatively charged surface of RING also prevents transient interactions in the UBC13/RING complex. Our findings highlight a mechanism where a post-translational modification perturbs the conformation and stability of transient complexes to inhibit protein-protein association.

Understanding Phase Behaviour of Nanoconfined Liquids: An NMR and Positron annihilation spectroscopic Approach

P. K. Pujari

BARC, Mumbai

Nanoconfined liquids play important role in many processes ranging from cellular behavior to microelectronic devices with potential applications in the area of catalysis, interfacial adhesion, lubrication and drug delivery. Nano-confinement significantly affects physical and thermodynamic properties of liquids. This includes nature of phase transition (first order or continuous), direction of shift in freezing/melting point, origin of hysteresis and structural and dynamical properties. The difference in the behavior of confined liquid than its bulk counterpart is attributed to the effect of finite size, reduced dimensionality and surface forces. An in-depth understanding on the behaviour of liquids confined in nanodomains is important to utilize the properties of liquids not only for technological applications but design of new materials. The present talk summarizes our recent results on phase transition behavior of liquids confined in different nanodomains. The multidimensional approach utilizing different techniques especially 1H Solid State NMR and positron annihilation spectroscopy enabled us to understand the fundamental aspects behind the observed phase transition behavior of liquids under the effect of confinement.

Toward In-situ/Operand High-Resolution Solid-State NMR

Yasuto Noda

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Solid-state NMR is one of powerful tools to analyze the local structure and dynamics of materials nondestructively. Recent newly technologies such as ultra-fast MAS and DNP have improved the sensitivity and extend the usefulness more and more. Another possible branch of development may be thought of as in-situ or operand measurements exploiting the nondestructive property and selective observation in conjunction with pulse sequences and isotope labelling.

In the meeting, a talk will be given about our development research toward in-situ or operand high-resolution solid-state NMR for contribution to material science and device developments. Firstly, a microcoil MAS probe will be introduced, which is a small electro circuit composed of a microcoil and capacitors and mounted on a conventional MAS module.[1] Next, a disk MAS probe developed based on the microcoil MAS system will be presented, which allows to us measure high-resolution NMR spectra of thin-films nondestructively.[2] Finally, a probe will be described which allows to measure the functional materials under fast MAS with given gas species without exposing the sample to the air in the process. The probe can be combined with the disk MAS system, and thus is expected to open the door to in-situ or operand high-resolution NMR for functional materials and devices.

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Importance of *in situ* Probes in Electrochemical Experiments

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The talk will be discussing two challenges in our recent electrochemical experiments, which unveil the importance of *in situ* probes during the reactions. The first one is on heterogeneous catalysis and the latter is on batteries, where we are studying high ionic conductivity solid polymer electrolytes (SPEs) for solid state devices.

In recent works we showed that supporting ions such as lithium (Li^+) can transiently modify the electrode-electrolyte interfaces and hydrogen evolution reaction (HER) on different metals is found to be modified with concentration of lithium salt [1-2]. The response of different metals vary, for example, on gold Li^+ augmented the HER process while that is suppressed on platinum. The effect of Li^+ ions is tested on variety of metals and the studies show that Li ions may modify the metal-hydrogen binding energy, which in turn affects the overall HER process. The role of Li^+ is found to be completely transient during the reaction since the post-electrode analyses did not show any signature of Li^+ or its other forms. Such an engineering technique will be highly beneficial for other reduction reactions as well, where we showed that ambient N_2 reduction to ammonia is possible using Li^+ containing electrolytes. We showed a reasonably high Faraday efficiency electrochemical ammonia (~12%) production with low applied potentials. Direct evidence towards the role of Li^+ is still lacking [2].

Secondly, SPEs are highly sought after due to their importance in safer batteries. Our recent experiments show that Poly(ethylene oxide) (PEO)- lithium perchlorate ($LiClO_4$) based well

researched SPEs can be modified with Polydimethylsiloxane (PDMS), where PDMS itself can not promote Li^+ transport [3-4]. The mixture of PEO-PDMS has a better ion transport efficacy, and molecular dynamics based studies show that PDMS helps for the segmental dynamics of PEO. Direct evidence towards the augmented dynamics of PEO is still not obtained though dielectric relaxation studies give evidences for the enhanced dynamics of PEO-$LiClO_4$ system in comparison to pristine PEO. Such dielectric studies on polymer mix will be highly complicated. Nevertheless, such novel SPEs are important for the next generation electronics and their conductivity values need to be reached on par with the liquid counter parts, where understanding the mechanism is highly important in designing such novel SPEs.

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Noble Metal and Ligand Free Nanocatalysts to Convert CO_2 to Fuel and Plastic Waste to Chemicals

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Active and stable metal-free heterogeneous catalysts for CO_2 fixation are required to reduce the current high level of carbon dioxide in the atmosphere, which is driving climate change. In continuation of our work in the field of nanocatalysis,[1-11] recently we showed that defects in nanosilica (E'-centres, oxygen vacancies, and non-bridging oxygen hole centres) convert CO_2 to methane with excellent productivity and selectivity. Neither metal nor complex organic ligands were required, and the defect alone acted as catalytic sites for carbon dioxide activation and hydrogen dissociation and their cooperative action converted CO_2 to methane. Unlike metal catalysts, which become deactivated with time, the defect-containing nanosilica showed significantly better stability. Surprisingly, the catalytic activity for methane production increased significantly after every regeneration cycle, reaching more than double the methane production rate after eight regeneration cycles. This activated catalyst remained stable for more than 200 h. Detailed understanding of the role of the various defect sites in

terms of their concentrations and proximities as well as their cooperativity in activating CO_2 and dissociating hydrogen to produce methane was achieved.

In 2nd part of my talk, I will present the synthesis and application of a new class of material, called "Amorphous Zeolites (AmZe)", which possesses Brönsted acidic sites like in zeolites and textural properties like ASAs. AmZe efficiently converted a range of waste plastics to hydrocarbons at a significantly lower temperature. The catalytic activity and selectivity of AmZe was then investigated by conventional and DNP-enhanced solid-state NMR studies to provide molecular-level understanding of the distinctive Brönsted acidic sites of these materials.

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NMR Data Science Approach for Fishery Products and Aquatic Ecosystem

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Ecosystem services are important for human life as well as sustainability of biological diversity. As the economic growth, the homeostasis degradation of environments and ecosystems, such as enormous consumption and pollution, is a serious issue toward future sustainability of human society. Along with the development of analytical instruments and data science technology in recent years, it has become possible to search for important factors by extracting features of molecular complexity of biological samples and environmental samples[1)]. The analytical approach targeting such a wide range of molecules needs to develop a database with scalable web environment[2)]. Environmental homeostasis can be evaluated by data mining of analytical parameters from natural samples, such as environmental water[3)].

Firstly, I introduce ecoinformatics approach for integrated analysis of natural fishes, sediments and water[4)]. Moreover, I will demonstrate that the fishery products, such as fish muscles can be also evaluated metabolic profiles of analytical big-data[5,6)], and their machine-learning approach[7-9)]. Namely, quality control is essential in modern industry, including fishery and food industries. In these cases, benchtop NMR might potentially innovate the quality control process, identifying storage-based and fermentation-originating metabolic changes[10)]. As we have demonstrated previously, SENSI approach can overcome weak S/N ratios and enhance peak separation for the benchtop NMR data[11-13)]. Moreover, NMR has the tremendous advantages of reproducibility and inter-institution convertibility because the observed parameters such as chemical shifts and J values, are physical quantities; in addition, it is possible to refer to standard data measured by high-field NMR.

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Improving survival predictability and biological insight through NMR based metabolomics of Acute Respiratory Distress Syndrome (ARDS)

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Acute Respiratory Distress Syndrome (ARDS), as characterized by the onset of clinically significant hypoxemia and diffuse pulmonary infiltrates, has been a challenge to the critical care physicians due to high death toll rate. Categorization of the severity of ARDS is based on degree of hypoxemia enumerated by partial pressure of oxygen to the fraction of inspired oxygen (PaO2/FIO2) ratio and chest X-ray. ARDS diagnostic criteria is based on Berlin definition and classified as mild ARDS (P/F between 200-300), moderate ARDS (P/F between 100-200), severe ARDS (P/F between <100). Due to complex etiology of ARDS, efforts are required to apply system biology tools to understand disease progression and to improve survival prediction. In this direction, we have applied nuclear magnetic resonance (NMR) based metabolomics to understand heterogeneous biology of ARDS. The NMR spectroscopy of mini – bronchoalveolar lavage fluid (mBALF) was optimised and several small molecular weight metabolites were identified which are indicator of lung pathology. NMR spectroscopy of human serum samples helps in identifying the metabolites associated with ARDS severity. Further sub classifying the progression, outcome and the metabolites contributing to pulmonary and non-pulmonary causes of ARDS, mBALF and serum samples were being used in larger sample size for which initial model was tested with respect to control which showed good separation and accuracy. The sensitivity and specificity of individual serum metabolites and mBALF metabolites as resultant serum and mBALF

endotypes were used further to determine their clinical predictability when combined with clinical APACHE and SOFA score. The accuracy increased to AUROC 1 indicating the clinical relevance of the above-determined metabolic endotypes. Pathway analysis of serum endotype and mBALF endotype predictive of mortality gave important metabolic pathway symbolic of ARDS correlated changes in metabolism.