

# TIFR Hyderabad High Field NMR Facility: Inaugural Symposium

13<sup>th</sup>-14<sup>th</sup> February 2018, TIFR Hyderabad

13 <sup>th</sup> Feb 2018		
Time	Speaker	Title
09.30 – 09:45	P. K. Madhu	Introduction
	V. Chandrasekhar	Inaugural remarks
09:45 – 10.30	Christian Griesinger	Dynamic and kinetics measured by NMR: Recognition and droplets
10.30 – 11.15	<b>Opening ceremony of the facility</b>	
Session Chair: S.P.Sarma		
11.15 – 11:45	K. V. R. Chary	Structure of a Ca <sup>2+</sup> -binding protein from <i>Entamoeba histolytica</i> and its involvement in trophozoite proliferation regulation
11:45 – 12:15	Vipin Agarwal	Selective <sup>1</sup> H- <sup>1</sup> H recoupling: An attempt to measure quantitative proton distances in fully protonated solids
12:15 – 12:45	Yusuke Nishiyama	Sensitivity enhancement in solid-state NMR by reducing the repetition delay
12:45 – 14:00	<b>Lunch</b>	
Session Chair: Christian Griesinger		
14:00 – 14:30	Neel Bhavesh	Novel RNA binding property of cyclophilin A
14.30 – 15:00	Sujoy Mukerjee	Correlation between thermodynamic stability and conformational entropy in gain-of-function mutants of p53's DNA binding domain.
15:00 – 15:30	Pramodh Vallurupalli	Methyl <sup>1</sup> H multi-quantum experiments to characterize $\mu$ s-millisecond protein dynamics
15:30 – 16:00	<b>Tea/Coffee Break</b>	
Session Chair: K.V.Ramanathan		
16:00 – 16:20	Kshama Sharma	MAS solid-state NMR: Asynchronous recoupling and multiple data acquisition
16:20 – 16:40	Mukul Jain	REDOR modifications to measure strong one-bond dipolar couplings from slow to very fast MAS frequencies
16:40 – 17:00	G. Gopi Krishna	A data centric algorithm for amino acid residue identification and protein backbone assignment
17:00 – 17:15	<b>Break</b>	
Session Chair: Narayanan Kurur		
17:15 – 17:45	K. Takeda	Electro-mechano-optical detection of NMR
17:45 – 18:15	Monu Kaushik	Dynamic nuclear polarization for sensitivity enhancement in NMR
18:15 – 18.45	G. Rajalakshmi	Low-field NMR studies using optical detection techniques
19:00	<b>Leave for Dinner</b>	
14 <sup>th</sup> Feb 2018		
Session Chair: P. K. Madhu		
09:30 – 10:00	M. McMahon	pH sensitive MRI contrast agents in nephrology
10:00 – 10:30	Eriks Kupce	AVANCE NEO – Breakthrough in multi-receive NMR technology
10:30 – 11:00	Srinivasa L Poojary	JEOL NMR Technologies: Solution-State NMR

11.00 – 11.30	Tea/Coffee Break	
Session Chair: Roberta Pierattelli		
11.30 – 12.00	Mandar Deshmukh	The mechanism of RNA-binding proteins induced post-transcriptional gene regulation
12:00 – 12.30	Ashutosh Kumar	Motion, regulation, recognition in specialized nucleosome formation
12:30 – 13:00	Kaustubh Mote	Improving resolution in proton-detected NMR experiments under fast magic-angle spinning
13:00 – 14.00	Lunch	
Session Chair: R. V. Hosur		
14.00 – 14.30	S.P. Sarma	Structure of the intrinsically disordered plant viral protein – VPg
14:30 – 15:00	Roberta Pierattelli	The heterogeneous structural behaviour of viral intrinsically disordered proteins revealed by NMR spectroscopy
15:00 – 15:10	Tea/Coffee Break	
Dharmatti Session on Evolution of NMR in India and TIFR		
Session Chair: K.V. R. Chary		
15:10 – 15:40	G. Govil	History of development of NMR in India
15:40 – 16:10	C. L. Khetrapal	Evolution of NMR in India and TIFR
16:10 – 16:40	Anil Kumar	40 years of NMR in India using superconducting magnets
16:40 – 17:10	R. V. Hosur	My NMR journey at TIFR
Closing Remarks		
Departure (Tea/Coffee)		

## Abstracts

### Dynamic and kinetics measured by NMR: Recognition and droplets

S. Prathihar<sup>1</sup>, C. Smith<sup>1,2</sup>, J. Reddy<sup>1</sup>, T.M. Sabo<sup>1</sup>, L. Wong<sup>1</sup>, L. Russo<sup>1</sup>, J. Kühn<sup>3</sup>, S. Pirkuliyeva<sup>3</sup>, D. Lee<sup>1</sup>, T.M. Sabo<sup>1</sup>, S. Ryzanov<sup>1,4</sup>, L. Antonschmidt<sup>1,4</sup>, A. Martinez Hernandez<sup>5</sup>, H.Y. Agbemenyah<sup>6</sup>, S. Shi<sup>7</sup>, A. Fischer<sup>6</sup>, G. Eichele<sup>5</sup>, D. Lee<sup>1</sup>, S. Becker<sup>1</sup>, D. Becker<sup>1</sup>, A. Leonov<sup>1,4</sup>, R. Benz<sup>8</sup>, M. Zweckstetter<sup>1,4</sup>, J. Wienands<sup>3</sup>, A. Giese<sup>7</sup>, and C. Griesinger<sup>1,4</sup>

<sup>1</sup>Dept. for NMR-based Struct. Biology, Max-Planck Institute for Biophysical Chemistry; <sup>3</sup>Institute of Cellular and Molecular Immunology, Georg August University of Göttingen, Göttingen; <sup>4</sup>DFG-Center for the Molecular Physiology of the Brain, Göttingen; <sup>5</sup>Genes and Behavior Dept., Max-Planck Institute for Biophysical Chemistry, Göttingen; <sup>6</sup>European Neuroscience Institute Göttingen; <sup>7</sup>Center for Neuropathology and prion research, LMU, Munich, Germany; <sup>8</sup>Jacobs University of Bremen, Germany

Kinetics of protein dynamics will be discussed on examples of folded and unfolded proteins (1). The kinetics can be measured down to life times of states of 300 ns with dedicated hardware. By that fast processes in proteins can be studied label free. These techniques are applied to the measurement of recognition dynamics of protein protein complex formation based on a new formalism described in ref. (2). Examples are given on the complex formation of ubiquitin with an SH3 domain.

The role of partially disordered proteins in two fields of research is investigated. One is the adaptor protein SLP65 which interacts with CIN85 (3). The two proteins are essential for B cell activation. The protein is found to be mainly unstructured and its various segments entertain different functions

or interact with membranes, SH3 domains and forming coiled coils. Based on the structures, a molecular lego will be described that reduces the SLP65/CIN85 interaction to its absolutely necessary essentials. The two proteins can perform phase separation which is related to function. Finally, a surprising link between neurodegeneration and cancer can be identified. (4).

#### REFERENCES:

- (1) C. SMITH ET AL. PROC. NATL. ACAD. SCI. USA. 113, 3296-74 (2016); C. SMITH ET AL. ANGEW. CHEM. INT. ED. 54, 207-10 (2015)
- (2) F. PAUL T.R. WEIKL, PLOS COMP. BIOL. 12, E1005067 (2016)
- (3) M. ENGELKE ET AL. SCIENCE SIGNALING: 7 (339) RA79 (2014); J. KÜHN ET AL. SCIENCE SIGNALING: 9 (434) RA66 (2016)
- (4) E. TURRIANI, ET AL. PROC. NATL. ACAD. SCI. USA 114, E4971-E4977 (2017)

## Structure of a $\text{Ca}^{2+}$ -binding protein from *Entamoeba histolytica* and its involvement in trophozoite proliferation regulation

Deepshikha Varma,<sup>1,2</sup> Aruna Murmu<sup>4</sup>, Alok Bhattacharya<sup>4</sup> and K.V.R. Chary<sup>1,2,3</sup>

<sup>1</sup>Tata Institute of Fundamental Research, Mumbai

<sup>2</sup>TIFR Center for Interdisciplinary Sciences, Hyderabad

<sup>3</sup>Indian Institute of Science Education and Research, Berhampur

<sup>4</sup>School of Life Sciences, Jawaharlal Nehru University, New Delhi

The protozoan parasite *E. histolytica* encodes twenty-seven  $\text{Ca}^{2+}$ -binding proteins (CaBPs) suggesting that the organism has an intricate and extensive  $\text{Ca}^{2+}$ -signaling system. The structural and functional characterization of some of these CaBPs studied so far revealed their predominant role in phagocytosis and endocytosis. However, not all amoebic CaBPs are involved in phagocytosis and endocytosis. One of these CaBPs (abbreviated as EhCaBP6) is mainly localized in the nucleus and present at the microtubule end and at the intercellular bridge with the microtubules during cytokinesis. Further, the increased expression of EhCaBP6 has been correlated with a significant increase in the number of microtubular structures suggesting that this protein may be involved in the regulation of chromosome segregation and cytokinesis in *E. histolytica*. In other organisms, calmodulin (CaM) plays a role of a major signal-transducing factor, through which  $\text{Ca}^{2+}$  concentrations are regulated during the cell cycles. Although CaM activity has been biochemically shown in *E. histolytica*, the presence of a typical CaM like protein is not known till date.

In an attempt to understand the structural and functional similarity of EhCaBP6 with CaM (if any), we have determined the 3D solution NMR structure of EhCaBP6, and identified one unusual, one canonical and two non-canonical cryptic EF-hand motifs. The  $\text{Ca}^{2+}$ -binding EF-I and EF-III pair with the cryptic EF-II and EF-IV, respectively, to form a two-domain structure similar to CaM. The observed structural similarity between EhCaBP6 and CaM, despite their low sequence identity, suggested that they might be having similar functions during the cell cycle. Exploring this hypothesis, we investigated the localization and interaction of EhCaBP6 with microtubules at different stages of the cell cycle using confocal imaging, immuno-precipitation and co-sedimentation studies. Further, we investigated the role of EhCaBP6 in the regulation of amoebic cell cycle specifically by facilitating DNA synthesis and transition from G1 to S phase. The downregulation of EhCaBP6 has been shown to affect cellular proliferation, DNA synthesis and cytokinesis. Overall our results, including structural inferences, showed the importance of EhCaBP6 in the regulation of cell proliferation in *E. histolytica*, a feature not observed in other parasites.

## Selective $^1\text{H}$ - $^1\text{H}$ recoupling: An attempt to measure quantitative proton distances in fully protonated solids

Vipin Agarwal

TIFR Centre for Interdisciplinary Sciences (TCIS),  
Tata Institute of Fundamental Research,  
Sy. No. 36/P, Gopanpally, Ranga Reddy District  
Hyderabad -500 107, India

Nuclear Magnetic Resonance spectroscopy of protons in fully protonated solids has always remained a formidable challenge. In recent past, techniques such as fast magic angle spinning or/and homonuclear decoupling schemes have been employed to improve resolution in the proton spectrum. Experiments to quantitatively measure proton-proton distances still remain elusive mainly due to the presence of strong and multiple proton-proton dipolar couplings. In this presentation we propose a new MAS solid-state NMR technique to selectively recouple the dipolar interactions between two protons. We show in a small molecule system that quantitative distances of upto few angstroms can be selectively measured despite the presence of other strongly coupled protons.

## Sensitivity enhancement in solid-state NMR by reducing the repetition delay

Yusuke Nishiyama

JEOL REOSNANCE Inc., Musashino, Akishima, Tokyo 196-8558, Japan & RIKEN CLST-JEOL  
Collaboration Center, Tsurumi, Yokohama, Kanagawa 230-0045, Japan

As nuclear spin systems are typically well isolated from the lattice, nuclear spins preserve coherence long period, giving fruitful information to NMR spectra. On the other hand, it takes long to recover to the thermal equilibrium state, resulting in long  $T_1$  relaxation time. This significantly reduces throughput of NMR measurements. Since the spin system should be close to the thermal equilibrium in the most NMR measurements, relaxation delay  $t_{rd}$  with an order of  $T_1$  should be inserted between consecutive scans. Here we propose methods to reduce  $t_{rd}$  (a) by enhancing the  $^1\text{H}$ - $^1\text{H}$  spin diffusion during  $t_{rd}$  at very fast MAS conditions (typically  $> 60$  kHz) [1] and by applying flipback pulse both for (b) moderate ( $\sim 10$  kHz) and (c) very fast MAS ( $> 60$  kHz) conditions. (a) The  $^1\text{H}$   $T_1$  relaxation time is no longer uniform at very fast MAS conditions because of suppression of  $^1\text{H}$ - $^1\text{H}$  dipolar interactions. This results in very long  $T_1$  for spectrally or spatially isolated  $^1\text{H}$  nuclei. In this case,  $^1\text{H}$ - $^1\text{H}$  recoupling which enhances  $^1\text{H}$ - $^1\text{H}$  spin diffusion reduces the optimal  $t_{rd}$ . (b) Although flipback pulse is widely used in CPMAS experiments at moderate MAS rate, it is not trivial to find the optimal  $t_{rd}$ . Here, we introduce a simple way to find the optimal  $t_{rd}$ . [2] (c) The flipback works well even at very fast MAS rate. This allows to apply flipback pulse right after CP rather than after  $^1\text{H}$  decoupling, improving the sensitivity further. [3]

### REFERENCES:

- 1) Y.Q. YE, M. MALON, C. MARTINEAU, F. TAULELLE, Y. NISHIYAMA, J. MAGN. RESON. 239 (2014) 75-80.
- 2) N.T. DUONG, J.R. YARAVA, J. TREBOSC, Y. NISHIYAMA, J.-P. AMOUREUX, IN PREPARATION.
- 3) Y.Q. YE, C. MARTINEAU, F. TAULELLE, Y. NISHIYAMA, IN PREPARATION.

## **Novel RNA binding property of cyclophilin A**

Neel Sarovar Bhavesh

International Centre for Genetic Engineering and Biotechnology (ICGEB),  
Aruna Asaf Ali Marg, New Delhi 110 067 India.

Cyclophilins are highly conserved, ubiquitous in nature and known as peptidyl prolyl isomerases (PPIases), playing major role in proline *cis-trans* isomerization. Initially, cyclophilin A was identified as a protein having very high affinity for immunosuppressive drug cyclosporin A (CsA) from bovine thymocytes. Cyclophilins are reported to perform multiple function in eukaryotic system like modulating cell division, transcriptional regulation, protein trafficking, cell signaling, pre-mRNA splicing, molecular chaperone mechanism and stress tolerance, mitochondrial apoptosis and also play a role in virus replication. We have recently identified and characterized a novel RNA binding property of cyclophilins using cyclophilins from different sources like human and fungus. Key residues involved in the RNA recognition were identified with the help of solution-state NMR spectroscopy and showed that the residues involved in RNA binding are conserved across the species. Our data on specificity of RNA recognition conclusively established that cyclophilin A acts both as a molecular chaperone helping in correct folding and functioning of different interacting proteins as well as RNA binding and stabilization of the complex in association with different RNA binding proteins or individually. Specific binding of CypA to GU-rich RNA also indicates the possibility of its role in stability of mRNA transcripts as ~70% of cellular transcripts carry UG-rich region in their 3'-UTR as well as exon-intron junctions. Further we have found higher expression levels of the protein under salt stress conditions in the fungus, indicating its active role to counter salt stress. We also demonstrated for the first time a direct evidence of countering osmotic stress tolerance in transgenic plant by genetic modification using a *P. indica* gene.

## **Correlation between thermodynamic stability and conformational entropy in gain-of-function mutants of p53's DNA binding domain**

Sujoy Mukerjee

Structural Biology and Bioinformatics Division,  
Indian Institute of Chemical Biology.

4. Raja S. C. Mullick Rd., Kolkata, West Bengal, India. 700 032.

Mutations in p53's DNA binding domain (p53DBD) lead to abrogation of its DNA binding ability and are associated with ca. 50% of cancers. While many of the oncogenic mutations change the protein-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 a combination of NMR and MD simulations, we found interesting differences in the backbone dynamics of mutants for both categories, in contrast to the wild type. Chemical shift perturbation analysis revealed important differences in tertiary structural changes that may be associated with these mutations, although secondary chemical shift analysis of backbone spins indicated negligible change in secondary structure. Moreover we found that the fast timescale derived conformational entropy of these mutants are correlated with their thermodynamic stabilities that have been previously reported using urea denaturation studies.



# Methyl $^1\text{H}$ multi-quantum experiments to characterize $\mu\text{s}$ -millisecond protein dynamics

Pramodh Vallurupalli

TIFR Centre for Interdisciplinary Sciences (TCIS),  
Tata Institute of Fundamental Research,  
Sy. No. 36/P, Gopanpally, Ranga Reddy District  
Hyderabad -500 107, India

CPMG experiments are now routinely used to characterize the millisecond timescale dynamics of protein molecules between a visible dominant major state and ‘invisible’ minor state(s) with millisecond (ms) lifetimes. However, new methods need to be developed to complement and overcome the limitations of the existing methods. The methyl groups in proteins consist of three equivalent protons providing an opportunity to develop  $^1\text{H}$  multi quantum (MQ) experiments to study the dynamics of proteins. Newly developed methyl  $^1\text{H}$  MQ CPMG experiments and 2D experiments where methyl  $^1\text{H}$  MQ coherence is evolved in the indirect dimension will be presented. These experiments are more sensitive to chemical exchange than the traditional single quantum experiment and can be used to detect and reconstruct the spectra of minor states with lifetimes as low as 50  $\mu\text{s}$ .

## MAS solid-state NMR: Asynchronous recoupling and multiple data acquisition

Kshama Sharma<sup>1</sup>, Johannes Hellwanger<sup>2</sup>, Kaustubh R. Mote<sup>1</sup>, Matthias Ernst<sup>2</sup>, P. K. Madhu<sup>1,3</sup>

<sup>1</sup>TIFR Centre for Interdisciplinary Sciences, Hyderabad 500 075, India

<sup>2</sup>Physical Chemistry, ETH Zurich, Vladimir-Prelog- Weg 2, 8093 Zurich, Switzerland <sup>3</sup>Department of Chemical Sciences, TIFR, Colaba, Mumbai 400 005, India

Solid-state NMR is a very flexible and powerful technique for elucidation of geometry and dynamics information on a variety of samples. However, there is still need to overcome sensitivity and resolution aspects along with the necessity to carry out multidimensional experiments in a short span of time. In order to overcome these challenges, we have made use of two approaches.

The structural investigation techniques rely on the recoupling of various interactions such as dipolar coupling. The use of symmetry-based dipolar-recoupling sequences has shown great success in determining geometrical constraints. First approach involves the improvement of recoupling efficiency of one of the symmetry-based dipolar- recoupling sequence for determination of dipolar coupling under MAS. We have shown that an asynchronous implementation<sup>1</sup> of R26<sup>114</sup> (a symmetry-based dipolar recoupling sequence) outperforms the rotor-synchronised and super-cycled version of R26<sup>114</sup> especially for spin pairs with small dipolar couplings and large chemical-shift anisotropies. Fig 1. represents the experimental transfer efficiency (DQ- transfer efficiency) for synchronous (red) and asynchronous R26 (blue and green) as a function of RF amplitude.

Secondly, to speed up the data acquisition process, pulse sequences that implement sequential acquisition strategies on one and two radio radiofrequency channels with a combination of proton and carbon detection to record multiple experiments under MAS have been coded<sup>2</sup>. We show that complementary 2D experiments such as CxHx, NHN and 3D experiments such as NCaHa and

CaNHN can be combined in a single experiment to ensure time savings of 40%. These experiments can be done under fast or slow- moderate spinning frequencies aided by windowed  $^1\text{H}$  acquisition. Fig 2. represents DARR and NHH experiments acquired sequentially using 2A2E2R (2A: two direct acquisitions, 2E: two indirect evolutions and using 2R: two receivers).

## **REDOR modifications to measure strong one-bond dipolar couplings from slow to very fast MAS frequencies**

Mukul G Jain<sup>1</sup>, G. Rajalakshmi<sup>1</sup>, Kaustubh R. Mote<sup>1</sup>, Matthias Ernst<sup>2</sup>, Vipin Agarwal<sup>1</sup>, P. K. Madhu<sup>1,3</sup>

<sup>1</sup>Tata Institute of Fundamental Research, Hyderabad, India.

<sup>2</sup>Physical Chemistry, ETH Zurich, Zurich, Switzerland.

<sup>3</sup>Tata Institute of Fundamental Research, Colaba, Mumbai, India.

Rotational-Echo DOuble Resonance (REDOR) is a heteronuclear dipolar recoupling experiment which readily estimates the dipolar coupling between two nuclei. Numerous implementations of this experiment can be found in material science, biochemistry, and NMR crystallography. But the experiment performs badly when probing stronger couplings (tens of kHz) at slow/intermediate MAS frequencies. We propose a few modified REDOR sequences to probe stronger couplings from low to very high MAS frequencies. These findings open up possibilities for application on strongly coupled systems at slow and intermediate MAS frequencies and also extending all the way to the currently achievable frequencies of 120 kHz.

### REFERENCES:

- 1) GULLION, T.; SCHAEFER, J. J. MAGN, RESON. 1989, 81, 196.
- 2) DUSOLD, S; SEBALD, A. ANNU. REP. NMR SPECTROSC. 2000, 41, 185.
- 3) JAIN, M. G.; RAJALAKSHMI, G.; EQUBAL, A.; MOTE, K. R.; AGARWAL, A.; MADHU, P. K. J. CHEM. PHYS. 2017, 146, 244201.

## **A data centric algorithm for amino acid residue identification and protein backbone assignment**

G Gopi Krishna<sup>1</sup>, Janeka Gartia<sup>1</sup>, KVR Chary<sup>1,2</sup>

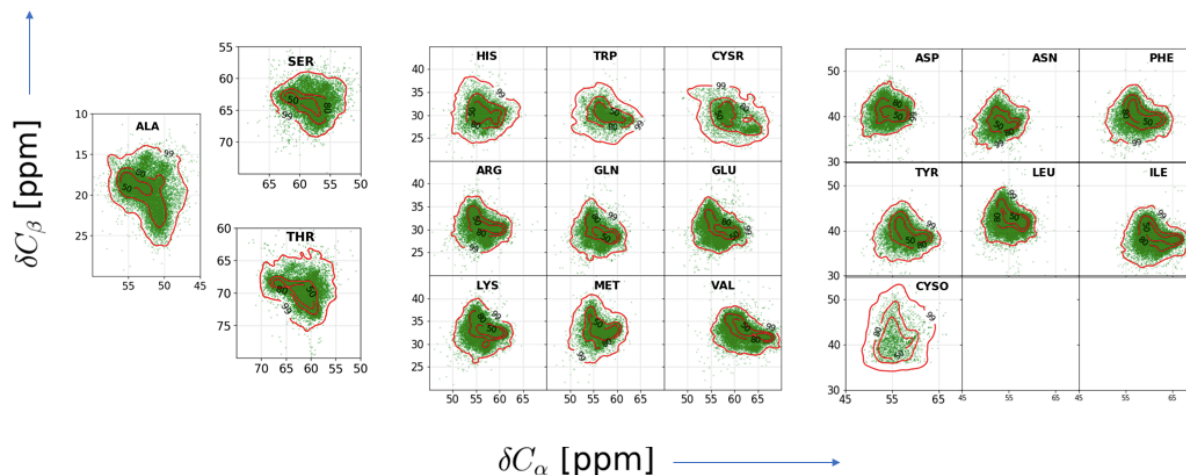
<sup>1</sup>TIFR Center for interdisciplinary sciences, Hyderabad

<sup>2</sup>Indian Institute of Science Education & Research, Berhampur

Sequence-specific resonance assignment is a prelude for the 3D structure determination of proteins using NMR spectroscopy. The automated algorithm, Tracked AuTomatic Assignments in PROteins (TATAPRO) proposed earlier utilizes the protein primary sequence and peak-lists from a pre-defined suite of triple-resonance spectra (for example CBCANH, CBCA(CO)NH, HNCO and HN(CA)CO)), which correlate  $^1\text{H}^{\text{N}}$  and  $^{15}\text{N}$  chemical shifts of a given amino acid residue with those of  $^{13}\text{CA}$ ,  $^{13}\text{CB}$ ,  $^{13}\text{CO}$ . TATAPRO algorithm is carried out in two steps. 1. Identifying dipeptides linked through a given pair of  $^1\text{H}^{\text{N}}$  and  $^{15}\text{N}$  chemical shifts as self and sequential. 2. Assigning the chemical shifts by tracking the sequences obtained by connecting the dipeptides onto the protein primary sequence.

In this presentation, I will discuss about the optimizations and enhancements we incorporated in the new version of TATAPRO algorithm. The optimization of residue identification has been implemented using a model based on kernel density estimations (KDEs). The KDEs are calculated

using distributions of data points in [ $^{13}\text{C}_\alpha$ ,  $^{13}\text{C}_\beta$ ] chemical shift spaces, shown in the figure below, with various density level contours. Introduction of HB chemical shifts further optimized the identification process by analyzing the values of KDE in a 3D space. This in turn aided us in optimizing the process of tracking in our backbone assignment procedure.



## Electro-mechano-optical detection of NMR

K. Takeda

Division of Chemistry  
Kyoto University  
Kyoto 606-8502, Japan

Signal reception of NMR usually relies on electrical amplification of the electromotive force caused by nuclear induction. Here, we report up-conversion of a radio-frequency NMR signal to an optical regime using a high-stress silicon nitride membrane that interfaces the electrical detection circuit and an optical cavity through the electro-mechanical and the opto-mechanical couplings. This enables optical NMR detection without sacrificing the versatility of the traditional nuclear induction approach. While the signal-to-noise ratio is currently limited by the Brownian motion of the membrane as well as additional technical noise, we find it can exceed that of the conventional electrical schemes by increasing the electro-mechanical coupling strength. The electro-mechano-optical (EMO) NMR detection presented here opens the possibility of mechanical parametric amplification of NMR signals. Moreover, it can potentially be combined with the laser cooling technique applied to nuclear spins.

## Dynamic nuclear polarization for sensitivity enhancement in NMR

Monu Kaushik and Björn Corzilius

Institute of Physical and Theoretical Chemistry,  
Institute of Biophysical Chemistry, and Center for Biomolecular Magnetic Resonance (BMRZ),  
Goethe University Frankfurt, Max-von-Laue-Str. 7-9, 60438 Frankfurt, Germany



Magic angle spinning (MAS) NMR is a ubiquitous technique for structural studies of biomolecules and materials. MAS NMR has been furthermore promoted by the developments in the field of dynamic nuclear polarization (DNP). This hyperpolarization method leads to sensitivity gain of two to three orders of magnitude thus making prohibitively long experiments feasible. Unique structural information can be extracted by selective enhancement of resonances when radicals embedded in a glass forming matrix act as the source of electron polarization. Organic radicals such as TOTAPOL, AMUPol, trityl, or BDPA are widely used for DNP. However, for specific applications, paramagnetic metal ions prove to be an advantageous choice. Many metalloproteins contain paramagnetic metals or can easily be doped with a paramagnetic substitute acting as an intrinsic polarizing agent. Moreover, transition metal ions serve as an excellent model for studying polarization transfer mechanisms/pathways. Here, we discuss different aspects of DNP as a method for signal enhancement and use of high spin metal ions as polarizing agents.

## **Low-field NMR studies using optical detection techniques**

G. Rajalakshmi

TIFR Centre for Interdisciplinary Sciences (TCIS),  
Tata Institute of Fundamental Research,  
Sy. No. 36/P, Gopanpally, Ranga Reddy District  
Hyderabad -500 107, 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 by a factor of 10<sup>5</sup> or more due to the reduction in the Larmor precession rate. Optical magnetometers 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 effects 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.

Low field also means that the magnetisation of the sample is very low, which will lower than NMR signal intrinsically. One way to enhance the signal is to hyperpolarise the sample. There are several methods used in the literature to create far from equilibrium polarisation, one of which is Spin Exchange Optical Pumping (SEOP) used to create hyperpolarisation in noble gases (<sup>129</sup>Xe, <sup>83</sup>Kr, <sup>3</sup>He). I will also overview the development of a Xe polariser in our laboratory.

## **pH sensitive MRI contrast agents in nephrology**

M. McMahon

Department of Radiology and Radiological Sciences  
Johns Hopkins School of Medicine

Acid-base homeostasis is an essential process for maintaining proper pH levels in the body. This balance helps preserve normal cell metabolism and function. Chemical Exchange Saturation

Transfer (CEST) is a contrast mechanism with great potential for creating images of pH. This is due to the large amplification of the CEST probe signal and the saturation frequency specificity of the contrast. This study aims to explore the potential of CEST imaging for detecting injuries to kidneys, a key organ for controlling the acid-base balance in the body. Three different types of CEST pH probes were investigated: iopamidol, Imidazole-4,5-dicarboxyamides (I45DCs) and salicylates. Iopamidol and I45DCs possess two labile protons enabling measurement of pH using the two labile proton frequency ratiometric method while salicylates generally possess one labile proton requiring usage of a ratiometric power method to create pH maps. We will demonstrate that these probes can be used to detect several different types of kidney injuries and discuss the advantages and disadvantages of the various probes.

## AVANCE NEO – Breakthrough in Multi-Receive NMR Technology

Helena Kovacs<sup>1</sup>, Ēriks Kupče<sup>2</sup>

<sup>1</sup>Bruker BioSpin AG, CH-8117 Fällanden, Switzerland

<sup>2</sup>Bruker UK Ltd., Banner Lane, Coventry, UK

E-mail: [helena.kovacs@bruker.com](mailto:helena.kovacs@bruker.com)

NMR experiments involving multiple receivers provide a unique way of increasing the sensitivity and information content of data recorded in a given period of time [1-4].

AVANCE NEO represents the latest generation in the AVANCE series product line. The electronics is based on a ‘transceive’ principle, meaning each NMR channel has both transmitter and receiver capabilities. Thus, each channel is its own independent spectrometer with the full RF generation, transmission and receive infrastructure.

This architecture provides high flexibility and allows the implementation of multi-receive experiments in a straight-forward manner. Examples of multiple receiver operation are shown in routine analysis of small molecules and structure elucidation of biomolecular systems. The design of the experiments and the optimal applications for multiple receivers are discussed.

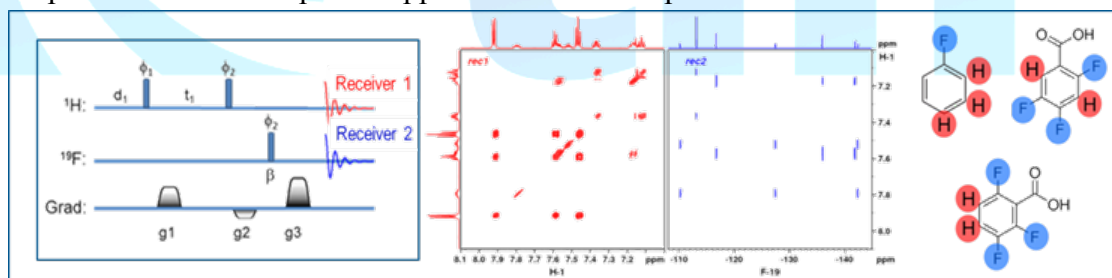


Fig. 2D H-H COSY and F-H COSY spectra of a mixture of three <sup>19</sup>F-labelled aromatic compounds recorded in parallel using the pulse sequence shown.

### REFERENCES

- 1) R. FREEMAN, Ē. KUPČE, IN MODERN NMR APPROACHES TO THE STRUCTURE ELUCIDATION OF NATURAL PRODUCTS (EDS. A. J. WILLIAMS, G. E. MARTIN, D. ROVNYAK), ROYAL SOC. CHEM. 1, 117-145, (2016)
- 2) Ē. KUPČE, NMR WITH MULTIPLE RECEIVERS, EMAG. RES. 4, 721-732 (2015)
- 3) H. KOVACS, Ē. KUPČE, PARALLEL NMR SPECTROSCOPY WITH SIMULTANEOUS DETECTION OF <sup>1</sup>H AND <sup>19</sup>F NUCLEI, MAGN. RESON. CHEM. 54, 544-560 (2016)
- 4) A. VIEGAS, T. VIENNET, T.-Y. YU, F. SCHUMANN, W. BERMEL, G. WAGNER, M. ETZKORN, UTOPIA NMR: ACTIVATING UNEXPLOITED MAGNETIZATION USING INTERLEAVED LOW-GAMMA DETECTION, J. BIOMOL. NMR 64, 9-15 (2016)

## JEOL NMR Technologies: Solution State NMR

Srinivasa L Poojary

JEOL NMR, Delhi

JEOL is a leading manufacturer of scientific instruments; our primary goal is to provide the best possible products, technologies, and support that will enable customers to advance their work. JEOL is continuing making the most sophisticated state-of-the-art NMR spectrometer and probe designs. The JNM-ECZ NMR spectrometer is our new NMR system that fully incorporates the latest digital and high frequency technologies. Incorporation of unique design of Transceiver, multisequencer RF architecture which enable the high complex pulse program with fast, overlapped, high power pulses for solution and solid state NMR experiments. This spectrometer in combination with robust high sensitive probe technologies makes users to get the excellent results. User friendly advanced software, and smaller dimension, efficient magnet system is adding on to scientific contribution to the NMR researcher.

## The mechanism of RNA-binding proteins induced post-transcriptional gene regulation

Mandar V. Deshmukh

CSIR - Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad

How do RNA-binding proteins effect post-transcriptional gene regulation? Our group explores the role of regulatory proteins which bind to a variety of RNA molecules and effect post-transcriptional gene regulation.

In prokaryotes, the gene regulation is manifested by a set of long non-coding RNA which are globally regulated by Hfq and specifically controlled by Crc, RapZ etc. Our lab has derived the solution structure of Crc (~ 32 kDa) using solution NMR techniques. We find that Crc is divergently evolved from AP endonucleases and regulates lncRNA using a non-canonical RNA binding surface.

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, e. g., in *C. elegans* and *H. Sapiens*, only one Dicer and one or two dsRBDs are found to dictate both siRNA and miRNA biogenesis. Whereas, organisms like *D. melanogaster* have two separate sets of Dicer:dsRBPs for executing siRNA and miRNA pathway. On the contrary, *A. thaliana* requires four Dicers and four dsRBPs to accomplish the small RNA pathway in a unique and highly controlled fashion. Sequence analysis of Dicer and dsRBPs show a variety of differences, e. g., the linker between RDE-4 dsRBDs *C. elegans* is composed of ~70 amino acids, whereas, DRB4 *A. thaliana* contain linker of 8 amino acids. Additionally, several prominent changes in the key features of dsRBDs, C-terminal regions, dimerization etc. can be further noticed.

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 DRB4 and DRB2 *A. thaliana* using solution structures and complementary assays. Further, <sup>15</sup>N backbone relaxation and CPMG experiments are used to probe functionally relevant dynamics in dsRBDs. Moreover, relative inter-domain orientation determined through PREs and SAXS provides a vivid picture on the role of dsRBPs in

the RNAi initiation. 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.

## **Motion, regulation, recognition in specialized nucleosome formation**

Ashutosh Kumar

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

Centromeres in most eukaryotes are identified by the formation of specialized nucleosome(s) where the Histone 3 (H3) is replaced by a unique variant CENP-A (Centromeric protein-A). Histone variant CENP-A<sup>Cse4</sup> is required for kinetochore assembly and is an epigenetic marker for centromere identity in budding yeast. The regulation of the cellular levels of Cse4 is primarily achieved by ubiquitin-mediated proteolysis. However, the structural transitions needed for centromere localization and its interaction with regulatory components are poorly understood. Using various biophysical techniques we showed that soluble Cse4 can exist in a ‘closed’ conformation, inaccessible to various regulatory components. We also characterized its interaction with obligate partner H4, which stabilizes Cse4, ensuring an ‘open’ state that will lend itself to proteolysis and allow interaction with kinetochore proteins. Our dynamic model shows allosteric effect of H4 binding on Cse4 N-terminus, which is required for interaction with centromeric components. The specific requirement of H4 binding for the conformational regulation of Cse4 suggests a novel structure-based regulatory mechanism for Cse4 localization and prevention of premature kinetochore assembly.

## **Improving resolution in proton-detected NMR experiments under fast magic-angle spinning**

Kaustubh R. Mote

TIFR Centre for Interdisciplinary Sciences (TCIS),  
Tata Institute of Fundamental Research,  
Sy. No. 36/P, Gopanpally, Ranga Reddy District  
Hyderabad -500 107, India

Proton detection in solid state has been brought to the forefront due to the advances in magic-angle spinning technologies, and MAS frequencies > 60 kHz can now be routinely accessed. However, it is observed that the proton linewidths in fully protonated samples are still more than an order of magnitude higher than in solution NMR. This is a limiting factor for the study of proteins, and perdeuteration is indispensable at 60 kHz MAS in order to fully assign and characterize these. One way to improve upon these is the application of homonuclear decoupling sequences on proton that will reduce the linewidth. However, under fast MAS in isotopically enriched samples, the presence of heteronuclear couplings negates a large fraction of the improvements in linewidths that is achieved by homonuclear decoupling. Here, I will discuss a strategy to simultaneously apply homonuclear and heteronuclear decoupling on isotopically enriched samples to recover this lost

resolution. An improvement in resolution by a factor of 1.5-2 as compared to MAS + heteronuclear decoupling alone will be shown.

## **Structure of the intrinsically disordered plant viral protein – VPg**

Siddhartha P. Sarma

Molecular Biophysics Unit,  
Indian Institute of Science  
Bangalore, India

In plant viruses, the Viral Protein genome - linked (VPg) is an intrinsically disordered protein that is essential for virus production and virulence. Essential to the virulence of viruses is the biochemical mechanism of poly-protein processing, a strategy for production of multiple proteins from a single polypeptide by proteolysis. VPg is essential for this poly-protein processing through its interaction with the partner protease. Furthermore, VPg interacts with several proteins and takes part in such varied activities as transcription, translation, gene silencing suppression and phloem loading of the virus. The number of protein partners and the clustering of the various interactions centring around it, the biological importance for some of these interactions and the intrinsically disordered state of the protein allude to VPg being a hub protein that controls many processes leading to viral production and spread. The conformational transitions of intrinsically disordered proteins are of current interest for understanding virulence, pathogenesis and disease in plants, livestock and humans and also to understand how these proteins fold. The three-dimensional structures of an intrinsically disordered VPg from the plant virus Sesbania mosaic virus in the free and protease bound forms have been determined by isotope - edited and isotope - filtered multidimensional solution NMR spectroscopic methods. The thermodynamics of the binding - induced folding association of the VPg protein with its cognate protease have been determined. The results of these studies will be presented.

## **The heterogeneous structural behaviour of viral intrinsically disordered proteins revealed by NMR spectroscopy**

Roberta Pierattelli

Magnetic Resonance Center (CERM) and Department of Chemistry “Ugo Schiff”, University of Florence,  
Via L.Sacconi 6, 50019 Sesto Fiorentino (Florence), Italy

The importance of local flexibility in determining the function of proteins has been recognized long ago and also widely scrutinized. If the extent of local flexibility is taken to its extreme conditions it leads to completely random coil behaviour of a polypeptide chain, indicated as intrinsic disorder, through a wide variety of intermediate cases both in terms of extent of mobility or in terms of protein stretches involved.

Many examples of intrinsically disordered proteins (IDPs) appeared in the literature showing how their structural plasticity and intrinsic flexibility can be key features to enable them to interact with a variety of different partners and to adapt to different conditions. These properties provide functional advantages to IDPs enabling them to play key roles in many regulatory processes and their function has also been related to several diseases<sup>(1)</sup>. IDPs are extensively used by viruses to infect healthy cells since, in virtue of their small genomes able to code only a limited number of proteins, they need economic ways to interfere with the host<sup>(2)</sup>. This is the case of the small-DNA



human viruses, which encode versatile molecular hubs able to engage in an extremely complex network on intermolecular interactions and hijack cell regulation.

The general properties of IDPs cannot be captured in ordered crystals, preventing them to be suitable targets for crystallographic studies. Thus, nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in their investigation, being the only method that allows the atomic-level description of their structural and dynamic features in solution. The high flexibility has several consequences on the NMR spectroscopic parameters that, if properly handled, can give precious information<sup>(3)</sup>.

We present here the high-resolution characterization of the protein E7 from one of the most dangerous variants of the Human Papilloma Virus (HPV)<sup>(4)</sup> and of the Adenovirus early region 1A protein (E1A) from Human Adenovirus (HAdV)<sup>(5)</sup>. These proteins are essential for productive viral infection in human cells and a vast amount of biologically relevant data are available on their interactions with host proteins. We will illustrate how NMR spectroscopy can help in describing the importance of intrinsic disorder to encode in a relatively short polypeptide many functional modules.

## History of the development of NMR in India

Girjesh Govil

Tata Institute of Fundamental Research, Homi Bhabha Road, Navynagar, Mumbai 400005

In my talk, I will describe the development of NMR in India. The activities in India started as early as 1953. Srinivas Shridhar Dharmatti set up a group at TIFR, devoted to high resolution and solid-state NMR. Putcha Venketashwarlu started working on high resolution NMR at Aligarh Muslim University around the same time. A. K. Saha and T. P. Das at the Saha Institute of Nuclear Physics wrote one of the first books on NMR entitled “*Theory and Applications of Nuclear Induction*”. One of the first notable publication was “<sup>59</sup>Co chemical shifts in inorganic complexes”. Mention may also be made to the contribution made by TIFR to AEET (now called BARC) in analysing samples of D<sub>2</sub>O that is used as coolant in Nuclear Reactors, for their purity. However, support to NMR in early years was small. Several laboratories built their own spectrometers for solid state NMR. Pandit Jawahar Lal Nehru formally inaugurated the TIFR facility in 1961. The first summer school on “Magnetic Resonance” was organized by TIFR in 1965. The first MRI instrument was purchased by NIMHANS by Brigadier Laxmipati. Today, hundreds of spectrometers are available for work in Chemistry, Solid State Physics, Biological Molecules and cells and whole body Imaging. NMR is also used by Industries. Indian authors have written about 50 books. A society called AMRS was established. Subsequently, this was replaced by NMRS. I will illustrate some of these aspects during the presentation.

## Evolution of NMR in India and TIFR

C.L. Khetrpal

Centre of Biomedical Research

Sanjay Gandhi Post Graduate Institute of Medical Sciences Campus

Lucknow - 226 014

“Evolution of NMR in India and TIFR”, the theme of this session and “Evolution of NMR world wide” are in fact synonymous essentially because of Professor S.S. Dharmatti. The activities in India started from TIFR and the traditions have been maintained during the past over 6 decades.

So, India has rich traditions in NMR and contributions of the Indian Scientists in this field cannot be ignored. The Indian Scientists have been associated with discoveries leading to all chemical, biological and medical applications of NMR. This will be highlighted and demonstrated with the hope that the traditions are maintained - a challenge for the future generations.

## **40 years of NMR in India using superconducting magnets**

Anil Kumar

Indian Institute of Science  
Bangalore

Abstract

### **My NMR journey at TIFR**

Ramakrishna V. Hosur

Department of Chemical Sciences  
Tata Institute of Fundamental Research  
Homi Bhabha Road, Mumbai 400005

My NMR research at TIFR began with the establishment of the National Facility for High Field NMR at TIFR in 1983, when the first 500 MHz solution state NMR spectrometer of the country was commissioned. I had the initial training at the 270 MHz NMR spectrometer commissioned at IISc Bangalore in 1978 and also during the two years (March 1981- March 1983) of my post-doctoral stint at ETH Zurich. During the past three and half decades, my research has spanned several aspects of biomolecular NMR, which includes, development of NMR methods, determination of solution structures of nucleic acids and proteins, development of computer algorithms for data analysis and modeling, study of protein folding pathways, study of intrinsically disordered proteins, to list the broad themes. Many important advances have been made and there have been several original contributions. Scaling methods in 2D NMR, novel techniques for protein NMR, distance geometry algorithm, TANDY for nucleic acid structure, AUTOBA for protein backbone assignment, elucidation of self - association pathways of some proteins into mega-Dalton size assemblies are some of the high lights. In recent years I have also embarked on characterizing interaction of important biological systems with herbal preparations. This talk will highlight these accomplishments.