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Perspectives in Magnetic Resonance

Advances in biological NMR circa WWMR 2010 in Florence

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ABSTRACT

We summarize the advances and breakthroughs of 'biological' NMR that were presented at the Joint EUROMAR 2010 - 17th ISMAR Conference – aka 'World Wide Magnetic Resonance 2010' in Florence, Italy.

1. Introduction

This perspective is an attempt to capture the state of 'biological' NMR as presented at the joint EUROMAR 2010 – 17th ISMAR conference – aka 'World Wide Magnetic Resonance 2010' in Florence, Italy. By its very nature this review is limited in scope since it focuses only on one meeting. In our opinion, however, WWMR 2010 did provide excellent coverage of the field and was well attended. On top of that it was impossible for us to cover everything,[1] taking into consideration number of presentations affiliated with bioNMR (72 talks and 289 posters out of 208 and 712, respectively) and the number of parallel sessions (six, sometimes seven).

From the outset we felt that there was a real desire from the researchers to try to solve genuine biological problems, and we attempt to capture the advances and breakthroughs that were presented. It was amazing, for example, to see other biological data (even poly acrylamide gels!) presented at an NMR meeting to support the activity and physiological relevance of the samples being studied. We categorize our discussion under the following topics and associated questions:

- Research having an impact on health and disease.
  - Are we making progress?
- Biological solid-state NMR.
  - Powerful new technology or only for specialized systems?
- Membrane proteins.
  - Finally coming of age using a combination of solution and solid-state NMR?
- G-protein-coupled receptors – a clearly important example.
  - Breakthroughs in a really tough system?
- Hybrid structural methods.
  - Successful application to large and multi-component systems?
- The use of paramagnetic spins and the interplay between NMR and EPR.
  - New applications of old technologies?

Of course the above divisions are arbitrary; for example, much of the biological solid-state NMR and GPCR sections discuss membrane proteins. Also it is difficult to predict the future of biological NMR, and it will be interesting to see how these areas develop.

2. Research having an impact on health and disease

Several examples illustrate how NMR can be used to help solve critical problems having an impact on health and disease such as viral infection and protein misfolding diseases.[2]

Bax [1] presented structural studies of sticky fingers of influenza virus, the full length HAfp fusion peptide essential for entry of the virus into the host cell. NMR experiments performed in DPC micelles showed a structure with tight helical hairpin packing stabilized by inter helical hydrogen bonds. They found no evidence from ^{15}N relaxation analysis to indicate a structural transition with lower pH, contrary to previous predictions. However, RDC measurements showed that a mutant undergoes large amplitude

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[1] One obvious omission is the wide-ranging research of the meeting organizer, Professor Ivano Bertini, which was not highlighted in plenary presentations.

[2] All presentations are identified by the laboratory PI. References are to the meeting abstract book which can be downloaded at http://www.cerm.unifi.it/wwmr2010/files/Book.pdf.

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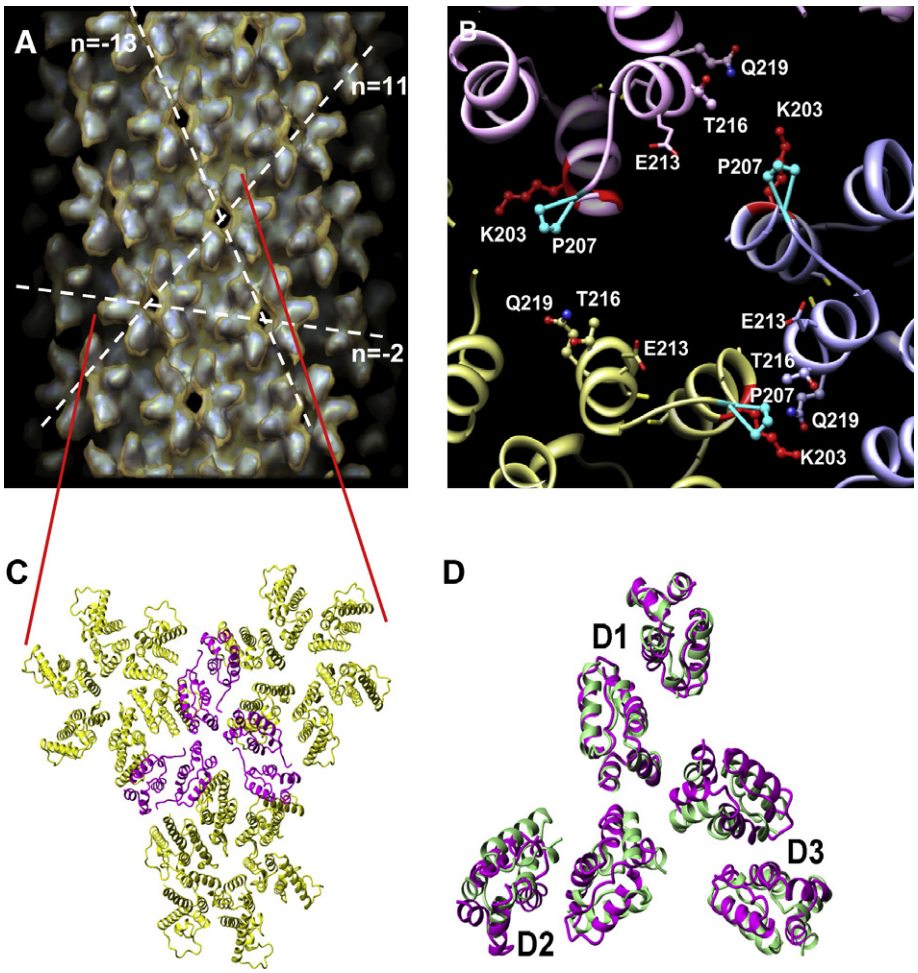

Fig. 1. Structure of the HIV-CA helical assembly and domain docking. (A) Surface rendering of the reconstructed tubular structure. Dashed lines connect hexamers in the three distinct helical arrangements denoted as $n = -2$, 11, and -13 helices; n is the Bessel order. (B) Detailed view of the threefold axis illustrating the interactions at the interface. (C) Present cryo-EM pseudoatomic structure of capsid hexamers in tubes. The NTD is shown in yellow ribbon representation and the CTD is in magenta. (D) Superposition of the CTD dimer structures at the trimer interface of the current cryo-EM model (magenta) and the 2D crystal model (pale green) (adapted from [24]).

motions, invisible by ^{15}N relaxation, where the C-terminal helix can move, resulting in a much weaker alignment tensor even while both N- and C-terminal residues remain clearly α-helical. The authors are trying to prove that this is relevant for the actual fusion mechanism, and hypothesize that the hairpin has to come apart and form a straight helix when transitioning from the semi-fusion to the final fusion stage.

Delepierre [2] addressed the molecular basis of rabies virus pathogenicity. She and her co-workers used NMR and X-ray techniques for structural studies in conjunction with other biophysical method like fluorescence spectroscopy, confocal microscopy, and analytical ultracentrifugation to study two peptides corresponding to G protein cytoplasmic domains (CytoG) from pathogenic and non-pathogenic RABV strains. These peptides were studied in complex either with serine threonine kinase (MAST) or with tyrosine phosphatase PTPN4. The authors found that single amino acid change in binding site of PDZ domain activates the apoptosis of infected neurons and allows G protein to interact not only with its usual partner, MAST, but also with another one, PTPN4.

Gronenborn [3] presented studies of inter subunit interactions critical for HIV-1 capsid function important to the life cycle of the virus. The high resolution NMR structure of C-terminal domain (CTD) of a capsid protein dimer and a cryo-EM study of the tubular assembly of capsid were presented (Fig. 1). Differences between previous X-ray structure and the present NMR structure were supported by the EM experiments. The functional importance of the new CTD–CTD interface in the protein dimer was confirmed by mutagenesis.

Griesinger [4] presented extensive solution and solid-state NMR studies of the transformation of α-synuclein from monomer to fibril form, as a part of a translational research program using NMR to address questions in mechanistic systems neurobiology focused on Parkinson's disease. He and his colleagues addressed how information from liquid and solid-state NMR is used for drug development.[3] A new compound, anle138c, which stabilized a non-toxic dimer was identified by high throughput screening, and shown to be effective in blocking the transition to the toxic fibril form.

3. Biological solid-state NMR

Most structural data for biological systems comes either from solution NMR or X-ray diffraction methodologies. Amongst 67,131 PDB depositions in RCSB Protein Data Bank (http://www.rcsb.org) 58,161 structures were obtained by X-ray diffraction, 8489 obtained by solution NMR, 40 obtained by solid-state NMR, and 24 structures obtained by various hybrid methods. Hart-

[3] A. Giese et al. WO 2010/000372 A2, January 7 2010.

mut Oschkinat presented a compilation of the solid-state NMR structures; most obtained for microcrystalline samples of small very stable proteins, which give the best spectra. However, while solution NMR and X-ray diffraction are limited by solubility of biological material or ability to forming crystals, respectively, solid-state NMR is not limited to a particular physical state. Moreover, structure is only one of the interesting features of a biological system (such as kinetics and dynamics properties, and intermolecular interactions). Therefore, solid-state NMR appears to be an ideal tool for the study of protein systems under a wide variety of conditions such as amyloid fibrils, membrane proteins, and supramolecular structures, and may also be a complementary method to other biophysical techniques.

Akutsu [5] presented solid-state MAS NMR analysis of the 525 kDa F_0F_1 ATP synthetase to understand the driving force of this rotational catalyst. They used high-resolution two- and three-dimensional spectra for ^{13}C and ^{15}N signal assignments and magnetization transfer experiments between the protein and deuterated lipids to determine protein positions. Their rotational resonance spectra gave evidence for a ring structure, which was not consistent with the current *Escherichia coli* subunit c ring mode, but consistent with the sodium ATP synthetase F_0 subunit c – ring crystal structure.

Riek [6] presented use of solid-state MAS NMR along with electron microscopy, confocal microscopy and X-ray diffraction to illustrate the relationship between 3D structures and properties of functional amyloids and the functional prions. These 'functional amyloids' play a role in hormone storage; this type of packing has the highest concentration, hence is the most efficient. Solid-state NMR spectra of hormone amyloids show very sharp lines, which is most likely a result of well-organized structure. He also presented spectra of the functional prion HET-s of the fungus *Podospora anserine*, which is believed to limit the spread of viral DNA by inducing limited cell death if these HET-s prions interact with a fungus which has the HET-S protein (HET-s and HET-S are natural polymorphic variants of the same protein but HET-S lacks prion-forming ability *in vivo*).

Oschkinat [7] presented solid-state MAS NMR, SAXS and X-ray scattering study on small heat shock protein αB-crystallin, which acts as an ATP-independent chaperone. Dysfunction of this protein leads to several diseases such as cataracts in the eye lens, multiple sclerosis, cardiomyopathies, and Alzheimer's disease. This work lead to an understanding of oligomer assembly and heterogeneity on a molecular level. He also presented solid-state NMR spectra reflecting the movement of kinesin on microtubules.

An interesting approach in drug design and discovery was presented by Watts [8], who made the provocative statement that a large fraction of ligand structures were incorrect (Fig. 2). By isotopically labeling selective parts of ligands, and monitoring the differential dynamics upon binding and dipolar recoupling between specific labels, they were able to resolve details of the bound structure in the absence of the target structure. This method is useful especially for study embedded membrane proteins in natural membrane fragments or in reconstituted complexes.

Baldus [9] presented recent progress on membrane transport systems including the chimeric KcsA-Kv1.3 potassium channel and the nuclear pore complex protein Nsp1. They identified pore lining residues of the potassium channel, studied the effect of protonation and channel dynamics, and proposed model structures for the activation/inactivation gate. For the nuclear pore complex, they identified specific transient hydrophobic interactions between Phe and methyl side chains as well as intermolecular β-sheets between spacer regions in the 62 kDa repeat domain of the yeast nuclear pore complex protein. They also showed relationship between protein structure and dynamics in the permeability barrier of nuclear pore complexes. Their results are important to understand structural aspects of nucleo-cytoplasmic exchange and also provide an explanation of dependence between molecular trafficking and amyloidosis.

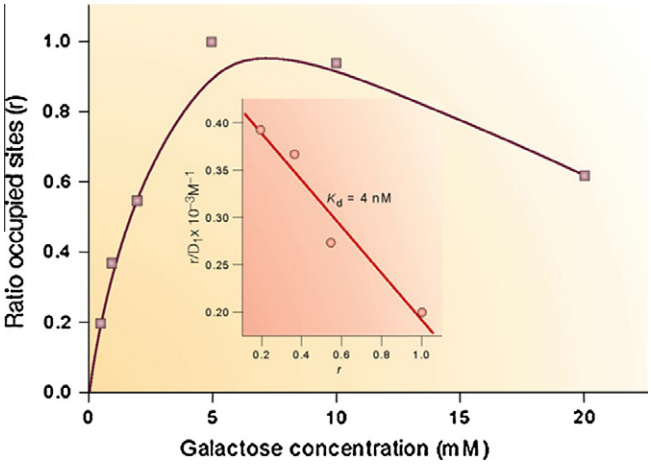

Fig. 2. Determining K_d for ligands. The NMR spectral line height for a ^{13}C-label, weakly binding ligand increases as the concentration of ligand is added to a fixed amount of target protein. The fractional ratio of bound ligand is specifically selected for using NMR methods. By suppressing the NMR spectrum from isotropic, unbound ligand, and only observing bound ligand, an equilibrium K_d is determined directly (adapted from [25]).

4. Membrane proteins

Membrane proteins constitute a large and biologically important family of proteins – almost a quarter among all known proteins in the cell. Thus the determination of the 3D structures of membrane proteins in environments reflecting their natural milieu is important in understanding many biological processes such as signal transduction, transportation, enzymatic catalysis, apoptosis, secretion and many others. X-ray studies are limited because of difficulties in the crystallization of membrane proteins due to their lipid dependence. NMR is potentially the ideal technique for investigation of the structural and dynamic properties of membrane proteins.

Veglia [10] presented combination of solution and solid-state NMR techniques used to characterize both the ground and excited states of phospholamban, which is a membrane protein inhibitor of SERCA. He demonstrated a correlation between the amount of the excited site of this protein and its efficacy, and suggested that tuning the structural dynamics could be used to generate better candidates for gene therapy in the regulation of heart disease.

Marassi [11] showed results of solid-state NMR experiments in oriented bilayers and solution NMR experiments in weakly oriented micelles performed on the *E. coli* outer membrane protein OmpX. She described NMR structures of membrane proteins integral to the cell envelopes of *Mycobacterium tuberculosis*, the causative agents of tuberculosis, and *Yersinia pestis*, the causative agent of plague. She demonstrated that *M. tuberculosis* protein Rv0899 adopts a mixed α/β-structure and not a transmembrane β-barrel such as OmpX.

Next two examples, presented by Hong [12] and Cross [13], focus on structure of the M2 proton channel from Influenza A virus and their structures with and without the antiviral drug, amantadine (Fig. 3). This structure has been the subject of controversy, as two different drug binding modes have been proposed – one with a single drug binding in the center of the tetrameric protein, and one with several drug molecules bound to the external periphery of the tetramer. Hong and co-workers used solid-state ^2H NMR of the drug as a function of concentration, and ^{13}C–^2H REDOR to

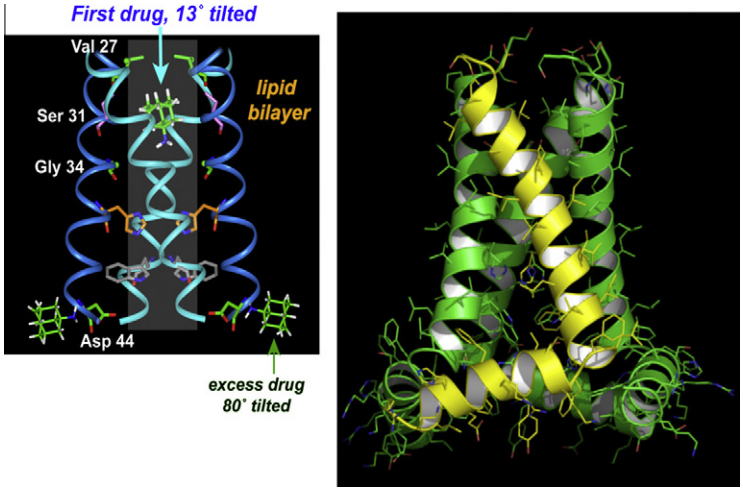

Fig. 3. Left panel: Structure of Amt-bound M2 in lipid bilayers presented by the Hong group showing at high drug concentration both a single Amt bound in the interior of the channel and multiple drug molecules bound to the external periphery of the tetramer (adapted from [26]). Right panel: The tetrameric structure of M2 (22-62) from Influenza A virus characterized in a lamellar phase lipid bilayer environment. A unique feature of this structure are the amphipathic helices, which interact with the bilayer surface, stabilize the tetrameric structure and stabilize the substantial tilt of the transmembrane helices that is essential for the functional activities. (adapted from [27]).

measure the relative occupancy of the two binding modes and well as the fraction of the drug dissolved in the lipid phase with a peptide corresponding to the transmembrane region of M2, to support the first model. Cross and co-workers determined the structure of a longer construct of the M2 protein, and demonstrated that the second drug binding mode is precluded in the structure that extends beyond the transmembrane segment.

5. G-protein-coupled receptors (GPCR)

GPCR's (also called 7TM receptors) are the largest group of transmembrane receptors and they bind to very diverse ligands: from small molecules to large proteins or lipids. Given their biological and potential pharmacological importance, we consider them separate from other membrane proteins.

Nietlispach [14] showed spectacular solution NMR spectra of the 7TM phototaxis receptor sensory rhodopsin pSRII. This was the result of extensive investigation of solution conditions over several years by his research group, and lead to high quality 3D structures with a backbone RMSD of 0.48 Å (Fig. 4). They also used paramagnetic spin labeling for mapping of hydrophobic and solvent-exposed regions of the protein and mapped the hydrophobic surface that is contacted by the aliphatic detergent side chains. Although pSRII is not formally a GPCR, he and his group also developed stable conditions for and showed solution NMR spectra of GPCRs such as the 90 kDa β-adrenergic receptor.

Stanley Opella first showed solid-state NMR experiments in aligned DMPC:Triton X-100 bicelles [15] showing significant improvement in resolution and stability due to Triton X-100. Use of this nonionic surfactant also greatly reduced the temperature dependence of aligned bilayers, resulting in fewer artifacts from sample heating in the NMR experiment. His group has developed solid-state NMR approaches focused on triple resonance methods using three membrane protein systems – the viroporins Vpu from HIV-1 and p7 from HCV, the mercury transport proteins MerE, MerF and MerT, and the G-protein-coupled receptor CXCR1. In the latter case they determined the orientation of the bound dimeric IL8 ligand to an oriented CXCR1 sample.

Ichio Shimada presented extensive biological characterization of the chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) and its interaction with the GPCR CXCR4, importance in cancer metastasis and HIV infection [16]. Once satisfied with the viability and biological activity of their preparations, they presented struc-

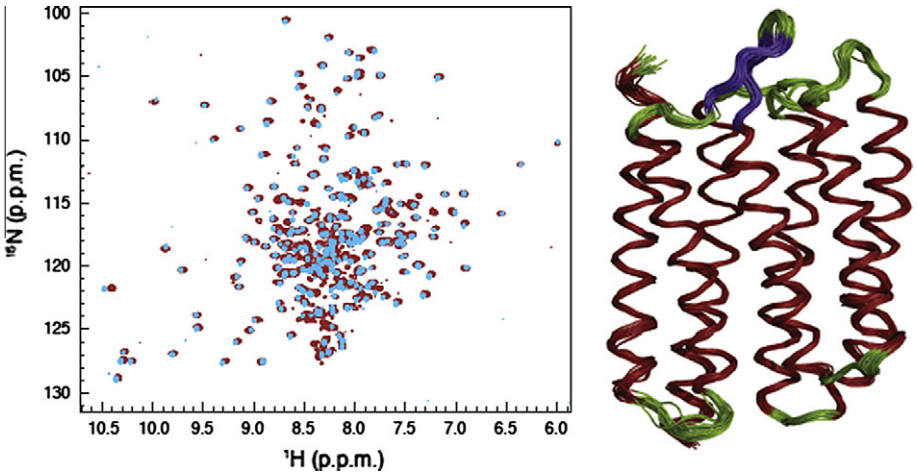

Fig. 4. Left panel: Overlay of ^{1}H–^{15}N TROSY-HSQC spectra of pSRII in DHPC micelles (cyan) and DMPC/c6-DHPC (q = 0.3) small bicelles (red). Right panel: An ensemble of 30 low-energy structures of this 7TM protein derived from NMR restraints. The backbone RMSD for residues 1–221 is 0.48 Å. Loop and strand regions are indicated in green and purple, respectively. The 20 unstructured C-terminal residues are omitted from this figure (adapted from [28]).

tural evidence of a two-step mechanism for SDF-1–CXCR4 interaction based on transferred cross-saturation experiments using ^{2}H, ^{13}C methyl labeled proteins. This method is also useful to study various GPRC-ligand complexes, such as the binding of AMT3100 to CXCR4. They also showed data for CCR5 bound to apoA-1 nanodiscs.

6. Hybrid structural methods

Small/Wide Angle X-ray Scattering (SAXS/WAXS), paramagnetic relaxation enhancement (PRE), residual dipolar couplings (RDCs), pseudocontact shifts (PCS), and various computational methods (*e.g.* docking, simulated annealing) are widely used as complementary methods to NMR spectroscopy. In a case of large and/or multicomponent systems, the combination of several methods is often the only way to answer questions about global structural and dynamics in bigger systems.

Clore [17] presented the use of dipolar coupling constants and SAXS/WAXS data coupled with simulated annealing to determine structures of the 128 kDa E1 dimer and 146 kDa EI–HPr enzyme complexes from the bacterial phosphotransferase system. Later, he demonstrated use of PRE titration measurement for examination of protein–protein encounter complex heterogeneity and differential relaxation measurements to examine the exchange between monomeric and large fibrillar structures. This approach could be very useful for investigation of large protein and protein complexes.

NMR and Docking hybrid methods were presented by Moura [18]. The molecular modeling software Chemera 3.0 with the BiGGER (Bimolecular complex Generation with Global Evaluation and Ranking) protein docking algorithm was used for constrained docking approach (http://centria.fct.unl.pt/~ludi/chemera/index.html), which allows for the inclusion of ambiguous experimental data in the calculations.

A combination of crystallography, molecular biology, and SAXS data with NMR methodology such as intermolecular NOEs, long-range and intermolecular paramagnetic relaxation enhancements, pseudocontact shifts, and residual dipolar couplings was used by Byrd [19]. He applied this methodology to understand multicomponent protein:protein complexes associated with E2:E3 recognition and ubiquitin transfer to substrate.

Lila Gierasch used chemical shift perturbation to sample the energy landscape of the large Hsp70 molecular chaperone system, and showed how dynamic rearrangement of subdomains creates pathways that enable the Hsp70 chaperone machine to respond to allosteric signals [9].

7. The use of paramagnetic spins and the interplay between NMR and EPR

Paramagnetic spins offer the possibility of intermediate and long-range distance restraints from NMR paramagnetic relaxation enhancement (PRE) and EPR techniques, which can be implemented in structure calculations along with NOE restraints.

Ubbink [20] showed how paramagnetic relaxation enhancement NMR spectroscopy combined with Monte Carlo methods was used to characterize structure and dynamics of three electron transfer complexes: (a) nitrite reductase with pseudoazurin, (b) adrenodoxin reductase with adrenodoxin and (c) cytochrome c (Cc) with cytochrome c peroxidase (CcP). The data was used to

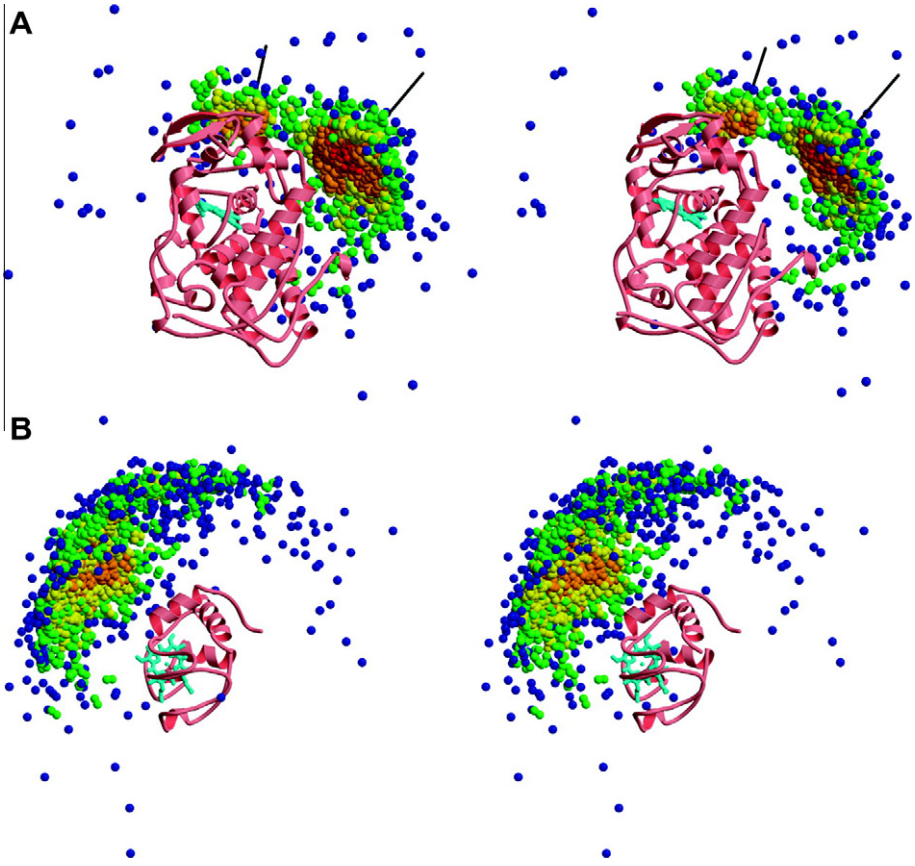

Fig. 5. Simulated encounter complex of the Cc–CcP complex. Stereo representations of the ensemble structures with CcP (A) and Cc (B) superimposed are shown in ribbons with the hemes in cyan. The centers of mass of Cc (A) and CcP (B) are shown as spheres, colored to indicate the density of the distributions, decreasing from red to blue. The highest densities denote the most favorable electrostatic orientations. Densities were determined by counting the number of neighbors within 2 Å. The lines in A indicate the two centers of high density (adapted from [29]).

show the ensemble of encounter states for the later complex, which characterize 30% of the 'bound' state (Fig. 5). Using this example he showed that the initial encounter step is crucial for successful complex formation.

Goldfarb [21] presented Gd^{3+} spin labeling for nanometer range distance measurements in proteins by high field pulse EPR. She and her colleagues used two types of spin labels covalently attached to proteins, nitroxides and Gd^{3+} tags. They showed that Gd^{3+} labeling could be a useful technique for distance measurements at high fields and has the advantage over nitroxide labeling of having the intrinsic absence of orientation dependence.

Otting [22] illustrated new strategies for site-specific protein tagging with paramagnetic lanthanides. He showed use of both close and long-range PREs using the example of elucidation of leucine zipper quaternary structure. He also showed how bipyridylalanine (Bpa) could be site-specifically incorporated into proteins using amber stop codons to create PCSs with Co^{2+} bound to Bpa.

8. Special mention

In the last scientific talk of the meeting, Kalodimos [23] presented a stunning array of spectra of very large biological systems approaching the MDa size (Fig. 6). These included studies of allostery and dynamics in protein trafficking, cell signaling, gene regulation, protein secretion, and DNA and RNA helicases. These

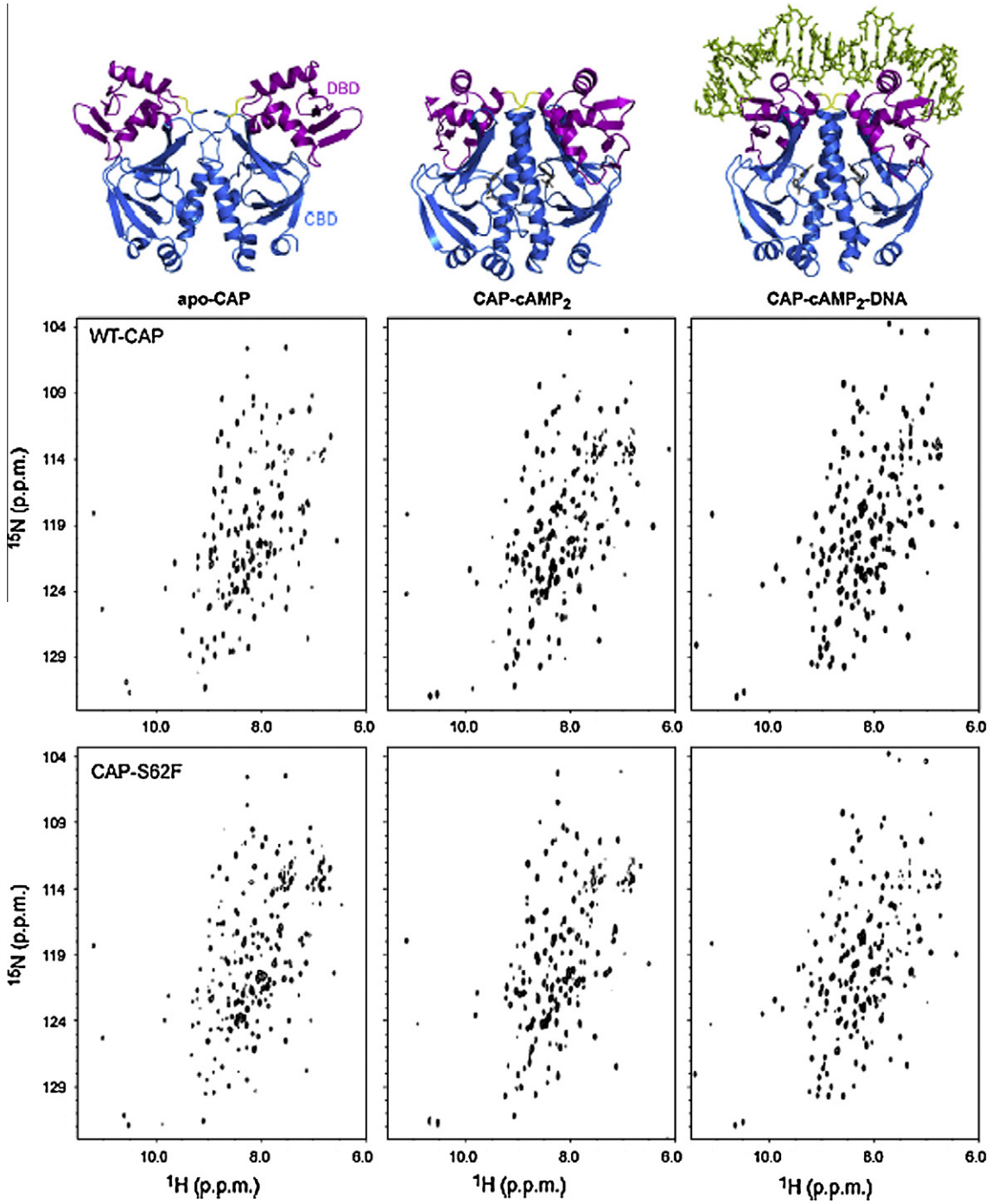

Fig. 6. ^{1}H–^{15}N TROSY-HSQC spectra of WT-CAP and S62F-CAP in several states (left: apo; middle: $cAMP_2$ bound; and right: $cAMP_2$ DNA bound) (adapted from [30]).

demonstrated how far one can go with selective labeling approaches, high field NMR methods, and control over the biological system under study.

9. Summary

Amazingly, progress has not been abated. The data presented was exciting, and challenges one to go further.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2010.09.015.

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