NMR Studies of Biomolecular Systems ☆

The late Alex Pines once remarked that Nuclear Magnetic Resonance (NMR) spectroscopy is like an "infinite toy-store", providing a rich platform by which to learn about a wide variety of molecular systems through manipulating their nuclear spins in a non-invasive manner with radio-frequency irradiation (https://www.youtube.com/watch?v=yvhNCFEIP8M). Indeed, the ability of a particular interaction or set of interactions to be selected and manipulated to dissect out the information of interest is one of the greatest strengths of NMR. No where is this more evident than in studies of biomolecular systems, where complex molecules are examined to learn about their structural, motional, and, ultimately, functional properties. In this regard the evolution of bio-NMR spectroscopy has been spectacular, because advances in hardware, software, NMR experiments, and sample production have converged to produce a powerful methodology to study the molecules of life. Yet despite reasons for tremendous optimism regarding the future of NMR in studies of biological systems, one of us (LEK) was somewhat hesitant when approached by the Editor-in-Chief, Mike Summers, to oversee a volume of JMB dedicated to biomolecular NMR. LEK's major concern focused on whether there would be sufficient interest from the NMR community for such an effort and whether enough articles could be collected to make a reasonable issue. These worries were immediately curtailed, however, when close to all the invited participants responded affirmatively when asked to contribute to this venture. In the pages that follow a sampling of the utility of NMR in biological and biophysical studies is presented with a series of 21 papers that captures some of the excitement of biomolecular NMR research.

The great majority of the articles presented focus on the critical role of dynamics in biomolecular function. Although it has long been recognized that NMR spectroscopy is a powerful tool for atomic resolution studies of biomolecular dynamics – in no small part because of its sensitivity to motions spanning from ps to h – it was not so long ago that a major goal was to produce a static structure of the molecule of interest. Although such pictures are aesthetically pleasing, and very useful, this is no longer the primary purpose of most studies, as is evident from the subject matter of many of the articles collected here. Indeed, the development of machine learning tools culminating in AlphaFold [1], has recalibrated the goals of NMR studies to include a large dynamics component, as it is now possible in many cases to generate accurate biomolecular structures computationally in a manner of seconds.

The important roles of dynamics in G protein-coupled receptor (GPCR) function, including recognition of cognate G proteins, are described by Rößler, Gossert and coworkers, who also highlight the importance of studies of human GPCR variants and describe how stabilizing versions of human β1 adrenergic receptors can be constructed [2]. Membrane proteins, such as GPCRs, present challenges for NMR studies related to sensitivity and the need to characterize a plethora of different important interconverting functional states. Prosser and coworkers describe using ^{19}F probes for quantifying dynamics in these systems, and, additionally, present an overview of ^{19}Freporters for studies of drug protein interactions [3]. Shaw and coworkers illustrate an additional compelling case for the use of ^{19}F in studies of the 52 kDa Parkin E3 ubiquitin ligase, both unbound and in complex with phospho-ubiquitin, that shed light on its activation and catalytic cycle [4].

The importance of dynamics extends to molecular chaperones that are critical for protein homeostasis in the cell. Kalodimos, Huang, and coworkers present a cogent account of how dynamics enable selectivity and adaptability in the interactions of chaperones with a variety of different substrate targets [5]. Dynamics play critical roles in molecular interactions that include protein kinases, as described by Veglia and coworkers [6]. The authors present a spin relaxation study showing how a folded substrate of protein kinase A (PKA) interconverts between compact and open conformations; the open state enables kinase binding and subsequent phosphorylation of an other-

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wise occluded site in the substrate. PKA activation is controlled through the binding of cAMP to the regulatory domain of the kinase. Mutations in this regulatory domain, including a 14-residue C-terminal disease-causing truncation, lead to hypoactivation of the kinase. Melacini and coworkers present an NMR study showing that this effect arises not only by impeding activation but also by enhancing a deactivation pathway through perturbations to an allosteric network that controls this process [7].

Molecular dynamics also play significant roles in regulating protein-DNA interactions. van Ingen and collaborators describe studies of the chromatin remodeler ISWI that moves DNA over the histone octamer surface of a nucleosome core particle (NCP) without the need for its disassembly [8]. By studying different variants of ISWI in the absence and presence of NCPs the authors show that histone plasticity is critical for facilitating DNA translocation. Latham and coworkers describe DNA binding studies of Xrs2, a protein involved in the DNA repair process, showing that certain time-scale dynamics are altered upon formation of the binary complex, while others remain unchanged [9]. Their studies provide a framework for understanding how Xrs2 recognizes both DNA and phosphoprotein interactors during double strand break repair. The impact of dynamics on function is further emphasized in a report by Carlomagno and coworkers focused on understanding how non-ribosomal peptide synthetases (NRPSs) mediate formation of peptide bonds between amino acid-like building blocks [10]. This study provides insight into how motion facilitates communication between substrate binding regions in the condensation domain of an NRPS to ensure efficient synthesis of chains of non-proteinaceous peptides.

NMR is also a very powerful technique for elucidating the kinetics and thermodynamics involved in the assembly of large complexes. Tugarinov, Clore, and coworkers present an overview of how NMR can be used to follow protein aggregation in real time and discuss the kinetic models that can be generated to account for the data, focusing on a pathogenic huntingtin protein and Aβ42 [11]. High molecular weight complexes comprised of multiple copies of peptides can be used to template the formation of silica particles, as these complexes can recruit silicates and silicic acid from which silica is condensed on the surface of these large structures. Such structures have potential applications in drug delivery and enzyme encapsulation. Kurzbach and coworkers describe solution NMR approaches that can be used to characterize pathways for silica coating by recording time-dependent data on the peptide scaffolds during silica condensation, establishing a pair of pathways that lead to different sizes of particle [12].

Important and unique insights into protein dynamics can be obtained from solid state NMR studies, including on timescales that can be difficult to access via solution-based methods. Schanda, Lichtenecker, and coworkers contribute an article describing the use of magic angle spinning techniques to quantify the dynamics of Arg residues in ubiquitin and the tetrameric enzyme malate dehydrogenase, where the Arg probes are perdeuterated and ^{12}C except at the δ position which is $^{13}CH_2$ labeled [13]. They detect a range of motions, with some Arg sites rigid on timescales extending to many tens of ms, while other Arg residues display rapid, ns dynamics.

Solution NMR spectroscopy provides unprecedented insights into highly dynamic systems, including intrinsically disordered regions of proteins and intrinsically disordered proteins. This is illustrated in the current issue through studies of several proteins that consist of folded domains separated by sequences containing significant regions of disorder. Barbar and coworkers illustrate how a range of NMR techniques, include paramagnetic relaxation enhancement, solvent water exchange, and saturation transfer approaches, can be used to identify novel transient structures and interactions within the intermediate chain of dynein, a MDa protein involved in cargo transport in the cell [14]. Huang and coworkers use multi-dimensional NMR methods, including spin relaxation and chemical shift perturbations, to characterize interactions within the p47 adaptor, a protein that interacts with the p97/VCP ATPase, targeting the ATPase for Golgi reassembly after cell division [15]. Novel contacts between different regions of the molecule are elucidated that may be important functionally. The utility of NMR to explore multivalent, dynamic interactions is further established in a study by Felli and coworkers exploring the nucleocapsid protein N and its binding with heparan sulfate that enables this protein to localize to the surface of cells [16]. A comparative study of different regions of N with heparin oligosaccharides is reported revealing a correlation between ligand size and affinity and establishing unique binding signatures depending on the size of the N variants examined.

The development of new labeling strategies has stimulated novel experiments and applications for advancing NMR studies of biomolecules. An example is found in the work of Schanda, described above, involving synthesis of a novel Arg precursor, but many other compounds have been designed over the years. Lichtenecker and coworkers describe the synthesis of selective ^{15}N and ^{13}C labeled histidine, recognizing the importance of this amino acid in biology [17]. Histidine can act as a base or acid, as a hydrogen bond acceptor or donor, as a metal chelator, and often plays essential roles in enzyme catalysis. The authors give examples with several proteins to illustrate the utility of their precursors in NMR applications. Konrat and coworkers provide an illustration of the synergy between new labeling

approaches and the development of new NMR experiments. An isovaleric acid precursor for generating proteins was synthesized as 2-^{13}C, 3-methyl-^{13}C, 4-^{13}C, 3-^{2}H that leads to the production of Val and Leu residues with ^{13}C labels at C^{γ},C^{α} and C^{δ},C^{β} carbons, respectively, and the addition of ^{13}C-gluose to the growth medium ensured that all backbone carbons are also ^{13}C-labeled [18]. Incorporation of this labeling scheme into proteins facilitated the assignment of methyl groups of Val and Leu residues via three-bond J_{CH} transfers connecting the ^{13}C carbons added by the precursor using a pulse sequence that the authors describe.

In parallel to the development of NMR methods for studies of proteins of increasing size and complexity there have been important advances in approaches to characterize both the structure and the dynamics of nucleic acids. Summers and coworkers present a cogent review of the various strategies, involving both labeling and NMR methodology, to increase the sizes of RNAs that can be studied [19]. Although heteronuclear methods are not as useful for RNA studies as for proteins, the authors describe recently developed impressive labeling approaches that enable structural studies of RNAs greater than 700 nucleotides with molecular weights in the hundreds of kilodaltons. The importance of dynamics and transient conformers in RNA processing is described in an article by Keane and coworkers [20]. Using a combined NMR and SAXS approach these investigators elucidate the structure of pre-miR-20a, an oncogenic microRNA. Their studies identify a flexible loop that can adopt multiple conformations that self-regulate pre-miR-20a's processing, with a single nucleotide bulge important for this process.

Counterions surrounding proteins and nucleic acids are critical for stabilizing their structures, controlling their functions, and maintaining their interactions with targets. In a perspective in this issue, Iwahara describes the use of solution NMR to quantify the dynamics of these ions and their binding to the macromolecule of interest, highlighting challenges and describing recent experimental advances that have resulted in new insights in this field [21].

The articles described briefly above naturally focus on a limited number of topics. A final article by Walters and coworkers reviews the utility of NMR in studies ranging from ligand binding to molecular structure, from in vitro applications to in-cell investigations, and includes sections on metabolic profiling, macromolecular dynamics, NMR in drug discovery, synergies between NMR and other atomic resolution structural modalities, and prospects for using artificial intelligence based computation for analysis of NMR spectra [22].

The papers in this edition make it clear that NMR science is vibrant, playing multiple roles in enhancing our understanding of the fascinating molecules of life. Using past advances as a predictor of the future it is obvious that new methods and instrumentation to tackle today's challenging problems will be developed, limited only by the creativity and ingenuity of NMR scientists. Alex Pines's apt description of 'NMR as a toy-store' serves to remind us of the wonderful developments that have been made to this point. It is exciting to imagine what toys the next generation of NMR scientist will discover to further elucidate the inner workings of biomolecules.

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