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Preface

Special issue on "Membrane Protein Dynamics: Correlating Structure to Function"

Molecular properties determine the macroscopic behaviors of matter, and biological systems are no exception. For this reason, high-resolution molecular images of biomacromolecules an atomic level have been in high demand. NMR and X-ray crystallography have been the preferred techniques used to generate such images. However, there is a growing sentiment that an album of *still* images alone is inadequate to fully describe biological function. As a result, the former *structure–function* relationship evolved into a new *structure–dynamics–function* paradigm. Nevertheless, the generation of molecular movies showing macromolecules in dynamic motion at an atomic-level resolution is challenging for all spectroscopic techniques. Fortunately, the current developments in NMR methods allow us to film atomic-resolution dynamic action of molecules. We are honored to bring out this special issue that reports the dynamical aspects of a special class of membrane-associated macromolecules, which exhibit a broad range (picoseconds to seconds) of dynamics.

For this topical issue on membrane protein dynamics, we selected 27 papers encompassing the myriad of ways that membrane proteins interact with lipid membranes in several important biological systems. The membrane proteins featured in these papers range from small antimicrobial peptides to membrane-anchored functional peptides and proteins, to integral membrane proteins and channels. The diversity of these proteins and the membranes they interact with require *ad hoc* sample preparations as well as an array of spectroscopic approaches. In addition to the variety of experimental approaches, the most salient aspects of this special issue are the versatility and creativity that the researchers have applied to characterize the structure and dynamics of these biomolecules and to correlate these features to the biological function. From a spectroscopic view point, two major NMR approaches are represented: solution and solid-state NMR. Solution NMR methods for membrane proteins are traditionally applied to membrane proteins reconstituted in detergent micelles. However, a new method reviewed here encapsulates membrane proteins in reverse micelles, improving the overall rotational correlation time with a substantial gain in sensitivity and resolution. On the other hand, solid-state NMR does not suffer as a result of the slow tumbling of the molecular complexes, and can be used either for randomly oriented samples or for magnetically or mechanically aligned lipid membranes. The papers reported in this issue show that irrespective of the approach used, information on the dynamics of these polypeptides can be correlated to their biological function.

Tamm and co-workers [REF] used solution NMR to analyze the dynamics of the detergent solubilized OmpA. This Gram-negative bacterial protein is comprised of a β-barrel transmembrane domain that may function as an ion channel. The detailed analysis of the dynamics using nuclear spin relaxation measurements reveals a collective view of the motions reminiscent of sea anemone. This complex motion, the anisotropic shape of the protein/lipid complex, and the interactions between the protein and the detergent molecules all complicated the analysis of the relaxation data, revealing the limitations of the classical Lipari-Szabo approach and opening the possibility that membrane protein dynamics might be more complicated than globular proteins.

Traaseth and Veglia [REF] used the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence to monitor the conformational equilibrium dynamics of phospholamban in DPC micelles, estimating the activation energy of the transition between the T-state (resting) and R-state (excited). The promotion from the T- to the R-state has been shown to be the key to regulation of the Ca^{2+}-ATPase. Understanding the energy balance in this equilibrium will show us how to tip the equilibrium toward the excited state using site directed mutagenesis, and represents a possible avenue to design new therapeutic approaches to treat heart failure.

Markley and co-workers [REF] review their recent approach to directly probe binding of several sweet agonists and antagonists to the human taste receptor in membranes prepared from human embryonic kidney cells. In their review, they show how NMR made it possible to measure binding where previous approaches failed. Other activity assays performed were unable to distinguish between mutations that altered ligand-binding sites from those that altered signal transduction downstream of the binding site.

Mascioni et al. [REF] used solution NMR approaches to characterize the dynamics of the meningococcal lipidated outer-membrane protein LP2086. Using nuclear spin relaxation measurements, these researchers found that the folded domains of the antigen are neither in a direct contact with the micelle nor with the membrane. Therefore, both N- and C-domains of the protein are exposed to the extra-cellular space and accessible to the host immune system. The structural and dynamic model that emerged from these studies correlates with binding data using monoclonal antibodies, and provides a framework to explain the efficacy of this class of proteins as a vaccine candidate against meningitis.

Mutagenesis and nuclear spin relaxation studies have been combined by Rainey and co-workers [REF] to analyze the helical propensities and dynamics of the transmembrane segment VII of Na^+/H^+ exchanger 1.

Three other papers show the application of NMR dynamics analysis to study the conformational dynamics of antimicrobial peptides in interaction with micelles. Defensin Psd1 [REF], LL37 [REF], aurein 1.2 [REF], and mellitin [REF] were studied using either isotopically labeled samples or by using ^{13}C natural abundance, offering insight into the restriction of motion induced by the presence of a membrane-mimicking environment.

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Two technical papers complete the series of solution NMR applications. The first one is a review from Sanders and co-workers [REF] that stresses the importance of correct data processing to interpret paramagnetic relaxation data for structure determination. The use of paramagnetic centers for structure determination of membrane proteins is becoming routine to supply long-range distance restraints. This paper describes the nuts and bolts of the effects of processing in the estimation of the inter-proton distances. In the second paper, Wand and co-workers revisit the critical steps for the encapsulation of KcsA ion channel into reverse micelles in low viscosity alkanes. Given the lack of sensitivity of the membrane protein samples in detergent micelles, the use of reverse micelles represents a possible avenue to obtain high resolution spectra to measure dynamics in large membrane proteins.

As for the systems studied by solution NMR spectroscopy, the membrane proteins analyzed by solid-state NMR technique vary in sample preparations as well as in the size of the proteins.

Tang et al. [REF] report the effects of an anesthetic compound (halothane and isoflurane) on the structural dynamics and topology of the second-transmembrane (TM2) domain of the alpha4beta2 nicotinic acetylcholine receptor (nAChR). In addition to the dynamics of a specific site, this study reports on the dynamic and topological changes in the transmembrane helices. Unlike their soluble counterparts, hydrophobic helices tend to be more stable in the hydrocarbon core of the membrane, and the structural changes are often associated with changes in the topology (i.e. relative orientation with respect to the membrane bilayer). Rotational dynamics and tilt of the secondary structure elements are important parameters that define the function of channels; hence they are critically affected by ligand binding.

Saito et al. [REF] presents new insights into the dynamic communication between the helical domains of bacteriorhodopsin (bR) using site directed ^{13}C NMR spectroscopy and analyzing the suppressed or recovered intensities (SRI) of site-directed ^{13}C solid-state NMR spectra of 2D crystalline preparations. Using this approach, these researchers identify at the atomic resolution residues undergoing low-frequency motions and are also able to evaluate their fluctuation frequencies to reconstruct the dynamic mechanism of proton transfer. The main advantages of site-specific ^{2}H spectroscopy are also highlighted in a review by Brown and co-workers [REF], where they offer an excursion of all of their work on the characterization of retinal dynamics during the light activation of rhodopsin.

Two articles by Weliky et al. [REF] and de Groot et al. [REF] describe the NMR approaches used towards understanding membrane fusion. The first article focuses on the HIV fusion peptide (HFP) as a model system to study virus/host cell fusion. Using ^{31}P relaxation studies, these researchers analyzed the dynamic properties of the membrane, showing a correlation between the dynamics and conformational (curvature) changes induced by the HFP due to its fusion catalytic function. In the second paper, de Groot and co-workers demonstrate how to use ^{31}P NMR spectroscopy to analyze the interaction of fusogenic membrane peptides. Specifically, they found that fusogenic peptides recruit phosphatidyl ethanolamine lipid in a non-bilayer phase, probably seeding membrane fusion.

Muscle proteins are extremely dynamic, and the SERCA regulator phospholamban is an excellent example. Lorigan et al. [REF] support and enrich the studies of phospholamban dynamics performed in detergent micelles [REF] with the analysis of both wild-type and mutant phospholamban in lipid membranes. These researchers show how site-specific ^{2}H and ^{15}N spectroscopy can detect the modulation of the dynamics in the connecting loop of phospholamban upon mutation. Given the important role of the loop dynamics in the control of SERCA activity [REF], this paper opens up the possibility of correlating structural dynamics to function and disease.

KL(4) is a 21-residue peptide which has successfully replaced lung surfactant protein B in clinical trials of synthetic lung surfactants for restoration of lung compliance. The structure of this peptide is quite controversial. Long and co-workers [REF] use solid-state NMR techniques to elucidate the partitioning and dynamics of KL(4) in phospholipid bilayers. The ability of this peptide to differentially partition in various bilayers and also to induce different fluid phases suggests that plasticity and dynamics is crucial for the biological action of this peptide on lung cell membranes.

Five articles feature the growing field of NMR to study antimicrobial peptides and toxins. Ramamoorthy and co-workers [REF] report on the effects of dynamics on the channel-forming pardaxin, a versatile 33-residue antimicrobial peptide (AMPs) that adapts its mechanism of action to the membrane composition. The abundant presence of cholesterol in mammalian cell membranes has been thought to be an important factor contributing to the selective antibiotic properties of AMPs. In this study, they have shown that the reduction of the dynamics of this peptide due to cholesterol could be the key to its membrane selectivity of its function. Along these lines, the Cotton group [REF] reports on the *snorkeling* of piscidin on the surface of the lipid bilayers using ^{15}N solid-state NMR. These studies may help us understand the kinetics of the antimicrobial response. Auger and co-workers [REF] present a study on a 21-mer cytotoxic peptide, which forms ion channels. The study is carried out in a combination of membrane mimicking environments, including bicelles and aligned glass plates. The combination of these approaches brought these authors to propose a new mechanism of interaction for this peptide that suggests it may absorb on the surface of the bilayer and perturb the lipid head groups. A similar approach is used by Separovic and co-workers for equinatoxin II, a pore-forming protein from Actinia equina that lyses red blood cells. Ulrich and co-workers [REF] focus on the analysis of NMR parameters (chemical shifts, quadrupolar, and dipolar couplings) obtained from mechanically or magnetically aligned lipid membranes. The authors suggest that a careful choice of labeling schemes is needed for the correct interpretation of the topology and dynamics of oriented polypeptides.

Dynamic contributions to NMR parameters and conformational equilibria are also the subject of a short review by Bechinger and coworkers [REF]. This paper shows a survey of recent solid-state NMR work highlighting the effects of molecular motions, lateral and rotational diffusion, as well as dynamic oligomerization equilibria within fluid phase lipid bilayers.

The technical article from Baldus and co-workers closes this issue and projects the application of solid-state NMR methodology to increasingly larger systems. In particular, these authors demonstrate that solid-state NMR can monitor residue-specific backbone dynamics in membrane embedded proteins. In contrast to the site-specific labeling schemes used in the majority of solid-state NMR papers, this method will offer a collective view of the protein motions. The elegant application of the method to the potassium channels gives a unique view of the dynamics of the selectivity filter, thus connecting structure, dynamics, and function.

As with all special issues, the space limitations do not allow us to comprehensively view this growing field. We believe that this special issue offers a few “video clips” that will stimulate the readers to try these valuable NMR methods on a variety of exciting biological systems. We take this opportunity to thank all of the contributors, reviewers, and the journal office for bringing out this issue.

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Gianluigi Veglia is an Associate Professor of Chemistry and Biochemistry at the University of Minnesota. He received his M.S. from the University of Rome, La Sapienza, with a thesis on the "Synthesis and NMR Characterization of New β-Carboline Derivatives" under the direction of Professors M.R. Del Giudice and M. Delfini. He received his Ph.D. in physical chemistry from the University of Rome, La Sapienza. His thesis focused on the study of "Macromolecular Interactions by NMR Spectroscopy" carried out in the laboratory of Professor M. Delfini. During this time, he also studied the dynamics of small peptides and molecules using molecular dynamics simulations in collaboration with Professor A. Di Nola. Veglia carried out his postdoctoral studies with Professor S. Opella in the Chemistry Department of the University of Pennsylvania, where he optimized solution NMR methods for the structure determination of membrane proteins. In 2000, he joined the Department of Chemistry at the University of Minnesota as an Assistant Professor, where he began his independent program on the structural and dynamic characterization of soluble and membrane-bound proteins responsible for muscle contractility. He was then promoted to Associate Professor with a joint appointment between the Department of Chemistry and the Department of Biochemistry, Molecular Biology & Biophysics. Dr. Veglia uses an interdisciplinary approach to study the structure, dynamics, and interactions of integral and peripheral membrane proteins. Specifically, he combines solution NMR, solid-state NMR, and computational methods into a hybrid approach to determine the high-resolution structures and dynamic movements of proteins in membranes. More details about Dr. Veglia's research projects can be found at www.chem.umn.edu/groups/veglia/.

Professor Ayyalusamy Ramamoorthy obtained his Ph.D. in Chemistry in 1990 from the Indian Institute of Technology (Kanpur, India) working on the development of NQR spectroscopy. He subsequently moved to the Central Leather Research Institute (a national research laboratory in Madras/Chennai, India) as a Fellow Scientist to develop scalar coupling based NMR methods for structural studies using solution NMR spectroscopy. In 1992, he joined JEOL Ltd (Tokyo, Japan) as a Scientist in the laboratory of Professor Kuniaki Nagayama to develop recoupling techniques (including USEME and *J*-HOHAHA) for magic angle spinning NMR studies on biological solids. He then joined the Stanley Opella group (University of Pennsylvania, Philadelphia) in 1993 to further develop and apply solid-state NMR techniques (including PISEMA, PSEUDO and PISEMAMAT) for structural studies of membrane proteins. In 1996, he joined the University of Michigan in Ann Arbor where he currently holds a joint appointment as Professor in Biophysics and Department of Chemistry. His main research interests are on the development and applications of solid-state NMR spectroscopy to study the structure, dynamics and function of membrane protein complexes, amyloid peptides, antimicrobial peptides, nanomedicine, and bone. His recent research revealed atomic-level mechanisms of membrane permeation/disruption by antimicrobial peptides and amyloid peptides, and reported high-resolution structure of membrane proteins. More details about his current research can be found at www.umich.edu/~ramslab.