New and Notable

A Solid View of Membrane Proteins In Situ

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Studying biomolecules at atomic resolution in their native environment is a central goal of structural biology. In the context of membrane proteins and their complexes, such efforts are complicated by the presence of a dynamic and heterogeneous cell membrane often containing a plethora of proteins and other biomolecules. Indeed, capturing such supermolecules at atomic resolution seems a formidable task (1). Yet, the field of solid-state NMR has seen remarkable progress in the last few years on this subject, and the article by Ward et al. (2) published in this issue of the *Biophysical Journal* discusses another milestone in this direction.

For a long time, solid-state NMR has been used to study membranes and membrane proteins (see, e.g., Fleischer et al. (3)) at atomic scale. In the last decade, progress in methodology, spectroscopic sensitivity, and sample preparation has significantly expanded the use of this method to study the structure and dynamics of membrane proteins, and comprehensive studies of protein structure in relation to the surrounding bilayer have become possible (see, e.g., Weingarth et al. (4)). Importantly, such studies are usually not restricted by the choice of the lipid bilayer, which allows for a wide range of synthetic and native membrane lipids to be used. Such flexibility provides unique opportunities to dissect the structural interplay between protein and membrane in close reference to membrane protein function. A logical question that arises next is: Can we perform cellular solid-state NMR experiments to directly study the protein of interest in the cellular membrane?

Because solubilization and reconstitution procedures can be abandoned, this idea also seems very appealing in terms of sample preparation. However, such studies are confronted with other technical challenges: the membrane protein of interest must be detected in a complex biomolecular background and at sufficient spectroscopic sensitivity and resolution. Some of these issues can be addressed by using promotor-driven overexpression protocols leading to the properly timed production of a specific isotope-labeled (membrane) protein such as described by Ward et al. (2). The situation is further simplified in Gram-negative bacteria by separating the inner membrane. Indeed, several studies (e.g., Fu et al. (5), Miao et al. (6), and Jacso et al. (7)) have focused on membrane proteins residing in the *Escherichia coli* inner membrane where individual protein residues as well as specific amino-acid stretches could be studied by solid-state NMR.

For the first time, the work of Ward et al. (2) reports a comprehensive view of a seven- transmembrane helical microbial photosensor *Anabaena* sensory rhodopsin (ASR) in such a membrane environment and compares findings to earlier studies using x-ray and solid-state NMR on DMPC/DMPA liposomes. For evaluating functionality and the oligomeric state of ASR, Ward et al. (2) elegantly combined their solid-state NMR work with absorption spectroscopy, circular dichroism spectroscopy in the visible range, and small-angle x-ray scattering studies. From the NMR experiments, Ward et al. (2) were able to obtain solid-state NMR resonance assignments for 40% of all ASR residues. With these parameters, key functional ASR regions such as the retinal binding pocket, the trimer interface, and the extracellular B-C loop could be studied, suggesting their structural conservation in synthetic and *E. coli* inner membrane lipid bilayers. On the other hand, local mobilities seen in the peripheral helix E′ and variations in overall protein packing may be the result of changes in the local lipid environment, particularly in regard to the hydrophobic thickness.

The observed structural conservation is remarkable. In part, it may be related to the multimeric nature of ASR seen in crystals and bilayers and the intrinsic chemical balance of the *E. coli* inner membrane bilayer in reference to the cytoplasm and the cellular envelope. Such a notion would be in line with studies on the M2 channel (6) and recent work in our group on the bacterial KcsA channel. For both tetrameric proteins, solid-state NMR correlations of channel residues critical for function seem to be unaltered when comparing synthetic and *E.coli* inner membrane bilayers.

It will be interesting to extend such studies to cellular membranes and proteins that lack such symmetries and for which synthetic in vitro models are difficult to establish. For example, we have conducted cellular solid-state NMR studies focusing on the outer-membrane protein PagL (8). In this case, the use of special *E. coli* deletion strains that lack naturally highly abundant outer-membrane proteins, i.e., OmpA and OmpF, further suppressed the influence of endogenous protein signals. Our initial studies suggested that protein regions potentially exposed to the highly asymmetric outer membrane comprising both phospholipids and lipid A together with lipopolysaccharides may change structure and dynamics compared to (symmetric) in vitro lipid bilayer preparations.

Further work along the lines demonstrated by Ward et al. (2) will be needed to examine the interplay between the

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cellular membrane and their associated proteins in comprehensive detail. Clearly, such studies will not be restricted to bacterial cells. For example, Wang et al. (9) reported elegant studies in plant cell walls and Kulminskaya et al. (10) examined chlorosomes. Ongoing work in our laboratory suggests that cellular solid-state NMR studies are also possible for protein complexes that span several molecular compartments and in eukaryotic organelles. In such experiments, further technical advancements ranging from a tighter control of endogenous protein expression relative to the target protein of interest, to the use of sensitivity-optimized NMR approaches, will be useful.

With the information to be obtained, a powerful spectroscopic basis may be established to track cellular membranes and their proteins under variable cellular conditions or in the presence of additional molecular players that modulate cellular organization and function. After all, in situ views of membrane proteins may provide novel structural and dynamic insights that cannot be obtained in vitro.

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