COMMENTARY

Membrane proteins, magic-angle spinning, and in-cell NMR

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In PNAS, Renault et al. (1) describe an advance that will help reveal the atomic details of membrane proteins in living cells. Membrane proteins comprise one-third of all cellular proteins, including biologically crucial molecules such as ion channels, G protein-coupled receptors (GPCRs), and transporters. The GPCR superfamily alone comprises nearly half of our current drug targets. Despite their importance and prevalence, relatively few membrane protein structures are known. Of the more than 20,000 unique high-resolution protein structures in the Protein Data Bank (www.pdb.org) (2), less than 0.5% are membrane proteins. The paucity of membrane protein structures reflects difficulties in preparing sufficient quantities of protein for structural studies as well as technical challenges faced by both X-ray crystallography and NMR spectroscopy.

The ability to obtain a crystal structure depends on the regular 3D spacing of protein molecules in a crystal lattice. It could be considered a stroke of luck that proteins even form crystals, because there is no selective pressure favoring crystallization. Moreover, membrane proteins are often especially difficult to crystallize because at least a part of every membrane protein lies within the hydrophobic environment of the bilayer. This hydrophobic component often resists ordering, leaving the job of stabilizing the lattice to intermolecular interactions between solvent-exposed regions of the protein.

Solution-State NMR

NMR can give atomic-level information about proteins without the need for crystals (3). In essence, a structure is obtained by analyzing a list of atom identities and distances between them. In NMR, this distance information resides in the dipolar coupling between nuclei. In solution NMR, where the sample is dissolved in an isotropic medium (e.g., aqueous buffer), the fast random tumbling of proteins averages the dipolar coupling to zero. This averaging gives rise to sharp signals, which allows the assignment of individual signals to specific atoms. Importantly, even though the dipolar coupling is averaged to zero, the distance information is not lost. It can be retrieved from changes in signal intensity by using experiments that measure the nuclear Overhauser effect. Unfortunately, proteins in real membranes and artificial liposomes do not tumble fast enough to average out the dipolar coupling. The resulting broad signals make assignment impossible, obviating efforts to derive a structure.

Solid-State NMR

Enter magic-angle-spinning solid-state NMR (MASssNMR), the technique that Renault et al. (1) use. Whereas solution-state NMR requires tumbling, MASssNMR requires the opposite—solid samples with limited motion. When most of the residual motion is eliminated (e.g., by freezing) and the sample is rotated rapidly about an axis tipped at the so-called “magic angle” (54.7°) with respect to the magnetic field, the dipolar coupling “disappears” and narrow signals emerge (3). These beautiful spectra, however, come with a price. The distance information is lost. Efforts by many spectroscopists over many years have yielded methods to reintroduce enough dipolar coupling to yield assignments and distances but not enough to broaden the signals into the baseline (4). With magic-angle spinning, the solution NMR requirement for rapid tumbling no longer applies; therefore, the size of the protein and its requisite membrane component is much less of a problem. Thus, MASssNMR makes structural analysis of membrane proteins an achievable goal.

Native membrane environments do not inherently contribute to the broadness of signals.

Previous Studies

Independent of whether crystallography or NMR is used, however, nearly all attempts to obtain structural information about membrane proteins ignore the intricacy of their natural environment. Biological membranes contain a diverse set of lipids, small molecules, and proteins, and the function of the membrane proteins can depend on subtle combinations of these components (5). Nevertheless, almost all structural efforts undertaken so far involve removing the protein from its native membrane, replacing the natural lipids with detergents to facilitate protein purification, and then reconstituting the purified protein into a simplified membrane or membrane-like environment.

Clearly, it would be preferable to study membrane proteins in natural membranes. Given the difficulty of obtaining crystals of membrane proteins even under simplified conditions, however, the goal of using native membranes in crystallography is incredibly challenging. NMR would appear to be better suited to provide structural information under physiologically relevant conditions. Until recently, however, bacteriorhodopsin was the only protein that had been structurally characterized in natural membranes with MASssNMR (6–10). Last year, we showed the feasibility of analyzing a small recombinant transmembrane peptide in native *Escherichia coli* (*E. coli*) membranes (11). These studies were performed ex vivo, that is, on membrane samples that had been removed from cells.

Current Studies

Renault et al. (1) go an important step further by improving the conditions for ex vivo measurements and, most importantly, by examining membrane proteins in living *E. coli* cells. A major challenge to studies using native membranes is the presence of background signals from other membrane components. A partial solution is to incorporate NMR-active nuclei into the protein. Renault et al. (1) use this strategy and report two additional ones that should pave the way for future in-cell studies. First, they used an *E. coli* strain lacking the genes for two abundant membrane proteins. The second strategy was to use an ex vivo preparation of the outer membrane, eliminating the background from inner membrane components. These efforts allowed detection of signals mainly from the targeted molecules and facilitated signal

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assignment and conformational analysis. Their observation that native membrane environments do not inherently contribute to the broadness of signals is an important one because resolution is a major limiting factor for in-cell NMR.

Despite the increased complexity of the MASssNMR data from the *E. coli* membranes, a large number of signals overlap with those from studies conducted using artificial membranes. This overlap not only allowed assignment data to be transferred from the artificial to the native membranes but strongly suggested that the protein's backbone structure is similar in both environments. It is important to note, however, that most of the assigned residues are located either in an extracellular loop or at the membrane-water interface. It is uncertain whether such structural similarities will hold for residues deeply embedded in the membrane, where interactions with lipids and other proteins are expected. Furthermore, Renault et al. (1) observe some differences at the level of the amino acid side chains. This is important because the details of protein function often depend on the conformation of side chains. Despite these caveats, the results raise our optimism that it will soon be possible to study the structures of membrane proteins routinely in their natural environments.

The Future

Where does the field go from here? Further improvements in sensitivity, resolution, and background suppression will extend applicability. Dynamic nuclear polarization should improve sensitivity, and its application to native membrane systems is feasible (12, 13). New expression systems offer even more powerful ways to suppress background signals (14, 15), and membrane fractionation is a tried and true method for improving sample homogeneity and reducing background (16, 17). Finally, site-specific labeling with unnatural amino acids provides residue-specific information for protein in real membranes (18).

What challenges remain? Overexpressing the protein of interest is a common approach to overcoming the sensitivity limitations of NMR, but this may adversely affect the biological relevance of the results. A key control will be to show that overexpression does not perturb protein function. In addition, a thorough characterization of lipid composition is needed. Under most conditions used for membrane protein overexpression, *E. coli* increases lipid synthesis to maintain a constant lipid-to-protein ratio (19). The proliferation of intracellular membrane and the synthesis of excess non–bilayer-prone lipids on overexpression have been reported (20–22). Perhaps the biggest obstacle is moving from bacterial cells to higher eukaryotic cells, where cell viability is harder to maintain and overexpression is more difficult. Nevertheless, the work of Renault et al. (1) opens the way to understanding membrane protein structure under the most biologically relevant of conditions—in living cells.

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