The ideal sample for structure determination of membrane proteins can be envisaged as a mixture of lipids, salts, and water that, upon addition to purified polypeptide, self-assembles into bilayers that both immobilize the protein in its active, native conformation and align it magnetically. This is remarkably close to the description of bicelles;1 however, their use in structural studies of expressed proteins can be envisaged as a mixture of lipids, salts, and water that, upon addition to purified polypeptide, self-assembles into bilayers to align magnetic ions.

Bicelles consist of long-chain phospholipids that form planar bilayers and short-chain lipids that “cap” the rim of the bilayer of bicelles;1 however, their use in structural studies of expressed proteins has been limited by several factors. Previous studies of lipid assemblies aligned perpendicular to the magnetic field2 have been restricted to synthetic peptides where motional averaging was essential to obtain resolution in samples with more than one labeled site. In this paper, we demonstrate that magnetically aligned bicelles can be used for structure determination of uniformly 15N-labeled membrane proteins by solid-state NMR spectroscopy; this is possible because the proteins undergo rotational diffusion about an axis perpendicular to the magnetic field, parallel “flipped” bicelles, and perpendicular to the magnetic field, parallel “unflipped” bicelles, and (C) in “unflipped” bicelles. (D) 15N Trp-labeled protein in “unflipped” bicelles (one residue). (E) Diagram of an “unflipped” bicelle containing a transmembrane helix. (F) Val (six residues) and Leu (one residue) selectively 15N-labeled proteins in “unflipped” bicelles. (H–J) Simulated 15N NMR spectra for a single NH bond in “unflipped” bicelles undergoing axial diffusion about the bilayer normal n on different time scales. The experimental spectra were obtained at 313 K using a Bruker Avance console, a 16.4 T Magnex magnet with an 1H resonance frequency of 700 MHz, and a home-built probe with a 5 mm ID double-tuned solenoid coil. Acquisition parameters were 54 kHz 1H B1 field, 1K scans, 1 ms cross-polarization mixing time, 7 s recycle delay, and 10 ms acquisition time using SPINAL-16 for heteronuclear decoupling.14

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Nonhydrolyzable ether-linked lipids 1,2-O-ditetradeyl-sn-glycero-3-phosphocholine (14-O-PC) and 1,2-O-dihexyl-sn-glycero-3-phosphocholine (6-O-PC), previously shown to form a lyotropic liquid crystalline phase under similar conditions as bicelles consists of the phospholipids 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC),6 contribute to sample stability. The resulting q = 3.2 bicelles have near-perfect uniaxial alignment and a molecular order parameter of 0.8, reflecting a small degree of high-frequency “wobble” of the disks. The 160 μL samples have 2.8 mg of protein and 28% (w/v) lipids (Avanti). “Flipped” bicelles contain 3 mM YbCl3·6H2O (Sigma). Proteins were incorporated in bicelles, as described previously for DMPC/DHPC bicelles.1c

Figure 1. 15N solid-state NMR spectra of the transmembrane domain of Vpu. (A) Uniformly labeled protein in bilayers on glass plates, (B) in “flipped” bicelles, and (C) in “unflipped” bicelles. (D) 15N Trp-labeled protein in “unflipped” bicelles (one residue). (E) Diagram of an “unflipped” bicelle containing a transmembrane helix. (F) Val (six residues) and Leu (one residue) selectively 15N-labeled proteins in “unflipped” bicelles. (H–J) Simulated 15N NMR spectra for a single NH bond in “unflipped” bicelles undergoing axial diffusion about the bilayer normal n on different time scales. The experimental spectra were obtained at 313 K using a Bruker Avance console, a 16.4 T Magnex magnet with an 1H resonance frequency of 700 MHz, and a home-built probe with a 5 mm ID double-tuned solenoid coil. Acquisition parameters were 54 kHz 1H B1 field, 1K scans, 1 ms cross-polarization mixing time, 7 s recycle delay, and 10 ms acquisition time using SPINAL-16 for heteronuclear decoupling.14

The solid-state 15N NMR spectra (Figure 1A–C) of a 36-residue polypeptide7 aligned in bilayers on glass plates with normals parallel to the magnetic field, parallel “flipped” bicelles, and perpendicular “unflipped” bicelles contain narrow single-line resonances. Significantly, there is no evidence of residual “powder pattern” line shapes in any of the spectra. By comparison with simulated spectra (Figure 1H–J), the rotational diffusion coefficient about the bicelle axis of alignment n is $D_\| \geq 1 \times 10^3 \text{ s}^{-1}$. 

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The resonance line widths (1–2 ppm) for the magnetically aligned bicelle samples are narrower than those typically observed for mechanically aligned bilayers or peptide single crystals. The dramatic differences in chemical shift frequencies between “flipped” (Figure 1B) and “unflipped” (Figure 1C) bicelles demonstrate that the orientations of individual peptide planes relative to the magnetic field are reflected in the spectra. PISEMA (polarization inversion spin exchange at the magic angle)\(^9\) yields high-resolution separated local field spectra where each resonance is characterized by orientation-dependent heteronuclear \(^1^H–^{15}^N\) dipolar coupling and \(^{15}^N\) chemical shift frequencies. Helices result in characteristic PISA (polarity index slant angle) wheel patterns of resonances that reflect their tilt and polarity in the bilayers.\(^8\) The magnitudes of the chemical shift and dipolar coupling frequencies measured from these spectra can be plotted as a function of residue number, generating sinusoidal waves with a period of 3.6 for an \(\alpha\)-helix.\(^10\) Furthermore, atomic-resolution structures of the proteins can be calculated\(^1\) or obtained by “structural fitting”\(^2\) to the orientation-dependent frequencies.

Panels A and C of Figure 2 contain experimental PISEMA spectra of uniformly \(^{15}^N\)-labeled membrane proteins in “unflipped” bicelles. Comparison of the wheel-like pattern of resonances in Figure 2A to the simulated PISA wheels in Figure 2D shows that the transmembrane helix of Vpu has a tilt angle of approximately 30°. The ability to obtain spectra like those in Figure 2 means that bicelles can be used as samples for structure determination of membrane proteins. Significantly, there is no reason that this method is limited to membrane proteins with only one or two transmembrane helices. Although the spectra of larger proteins will be more crowded, the resonances will not be broader or weaker, and there are many multidimensional solid-state NMR experiments that can be used to resolve and assign resonances that overlap in two-dimensional spectra.\(^1\) Among the advantages of magnetically aligned bicelles are their ease of preparation, the use of a sealed sample tube that ensures sample stability (>1 year), and the placement of the sample inside a solenoid coil for optimal probe performance; in contrast, the widely used glass plate samples are marginally sealed in plastic films, have a significant fraction of the active volume wasted on glass, and require the use of “flat coil” probes. However, the most important feature of these samples is that the membrane proteins are functional\(^2\) in fully hydrated lipid bilayers under physiological conditions.

Acknowledgment. This research was supported by Grants EB002169, GM064676, and GM066978, the Biomedical Technology Resource for NMR Molecular Imaging of Proteins (EB002031), postdoctoral fellowships GM65833 to A.D. and GM66487 to A.M., and a Training Grant DK54441 position to S.H. all from the National Institutes of Health.

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JA045631Y