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NMR Studies of Membrane Proteins

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1.Introduction

Not only do membrane proteins represent a substantial fraction of the information in a genome, but also they are responsible for many essential biological functions, some of which are unique (e.g., as membrane transporters). Consequently, mutations in genes that code for membrane proteins can cause human diseases. Thus, there are many reasons that the structural biology of membrane proteins is of considerable interest in biomedical research. However, because membrane proteins are not soluble in aqueous solution, only a few examples have been amendable to experimental structural analysis with X-ray crystallography and solution nuclear magnetic resonance (NMR) spectroscopy. The relatively few structures of helical membrane proteins that have been determined provide an initial view of the design principles involved in the use of helices as structural and functional elements, setting the stage for the interpretation of new structures of membrane transporters and other membrane proteins in terms of their functions. NMR spectroscopy is an extraordinarily powerful method for describing the structure and dynamics of proteins and other biopolymers, and has the potential to be applied to membrane proteins. Furthermore, Structure-Activity Relationship (SAR) studies by NMR have become a useful tool for the discovery and design of drugs that interact with proteins, and also has the potential to be applied to membrane proteins

2. NMR Spectroscopy

The overall rotational correlation time of a protein, the extent of alignment of the protein molecules in a sample, and strategy for assignment of the resonances to sites in the protein are the principal factors that affect NMR structural studies of proteins. For relatively small globular proteins, the sample conditions, instrumentation, experiments and calculations that lead to structure determination are well established(1). The chief requirement for the structure determination of globular proteins is that samples can be prepared of isotopically labeled polypeptides that are folded in their native conformation and reorient relatively rapidly in solution. Such samples have been prepared for many hundreds of proteins and it is likely that this can be done for thousands more of the polypeptide sequences found in genomes(2). In contrast, each of these factors needs to be revised or developed for applications of NMR to structure determination of membrane proteins(3).

The rotational correlation time problem is paramount. If the protein reorients rapidly enough in solution, as is the case for small membrane proteins in optimized micelles, then the standard methods of NMR can be applied and the structure determined. However, it is not possible to obtain NMR spectra of slowly orienting or immobile proteins such as membrane proteins in phospholipid bilayers, using conventional solution NMR methods and instruments. From the start, NMR studies of membrane proteins require crucial choices about samples, instrumentation and experimental methods. It is feasible to apply both solution NMR and solid-state NMR to helical membrane proteins, depending primarily on the choice of lipid assemblies as well as other sample conditions.

3. Proteins and Lipids

Membrane proteins require the presence of lipids to maintain their native conformations and functions. Figure 1 illustrates the types of lipid assemblies used in NMR structural studies of membrane proteins. The proteins are associated with lipids or mixtures of lipids that self-assemble into micelles, bicelles, or bilayers in aqueous solution. The properties of the lipid-protein complex determine the rotational correlation time of the protein molecules. Micelles can be prepared so that they contain a single polypeptide and reorient rapidly enough for solution NMR spectroscopy. Micelles are thought to be spherical aggregates of lipids with the hydrophobic chains on the interior. Samples for solution NMR spectroscopy require the use of relatively high temperatures and high concentration of lipids that form small micelles(4), most commonly sodium dodecyl sulfate (SDS), dodecyl phosphocholine (DPC), or dihexanoyl phosphatidylcholine (DHPC). Bicelles are made from a mixture of long and short–chain phospholipids that self-assemble into long-chain bilayers that are “capped” by the short-chain lipids(5). The molar ratios of long-chain and short-chain lipids determine the size of the bicelles. Small isotropic bicelles can reorient rapidly without a preferred alignment while larger bicelles form an aligned liquid crystalline phase in the presence of a strong magnetic field with the bilayer normal perpendicular to the applied magnetic field. Bilayers are infinitely large in two dimensions, and two molecules thick in the third. In bilayers, nearly all of the residues in the polypeptide are immobile on time-scales longer that milliseconds, and the relevant dipolar coupling and chemical shift interactions in individual backbone sites are not motionally averaged. As a result, bilayer samples of membrane proteins give very broad and poorly resolved spectra when instruments and methods appropriate for solution NMR are used. On the other hand, bilayer samples are suitable for the application of solid-state NMR methods where radio-frequency irradiations replace molecular reorientation as the principal mechanism of averaging the dipolar couplings responsible for the line broadening. The solid-state NMR approach that uses stationary, uniaxially aligned samples(6) is particularly well suited for membrane proteins in lipid bilayers because the proteins are immobilized and can be highly aligned(7). Spectroscopically, it is the combination of decoupling and heteronuclear dipolar interactions and sample alignment that results in narrow, single-line resonances and well-resolved, orientationally dependent spectra that provide the input for structure determination. There is a direct mapping of protein structure onto the solid-state NMR spectra of aligned samples, and the determination of complete three-dimensional structures from the spectra is feasible when multiple orientationally dependent frequencies are measured for nuclei at each residue.

Samples can be prepared with no, weak or complete alignment of the protein molecules. Complete alignment is possible only with immobile proteins. Rapidly reorienting proteins can have either no or weak alignment. The vast majority of NMR studies have been performed on samples without molecular alignment. This is the case for globular proteins, which undergo effectively isotropic reorientation in solution. It is also the case for membrane proteins in micelles and isotropic bicelles. For these samples, structure determination is based on measurements that reflect internal molecular parameters, such as nuclear Overhauser enhancements (NOEs) that give short-range distance measurements and variations in isotropic chemical shifts associated with secondary structure(8). In the past few years, there has been a great deal of interest in weakly aligned samples of proteins,. Because the proteins are reorienting rapidly, solution NMR methods are used for the measurement of residual dipolar couplings (RDCs). It is possible to weakly align membrane proteins in micelles and small bicelles through lanthanide ion(9, 10) and gel methods(11).

Unoriented, immobile samples, including membrane proteins in bilayers, can be studied using magic-angle, sample spinning (MAS) solid-state NMR spectroscopy(12), where distances and torsion angles are measured through spectroscopic parameters affected by both

homonuclear and heteronuclear dipolar interactions. Immobile bilayer samples can be completely aligned to a degree rivaling that observed in single crystals of small peptides. Membrane proteins in bilayers can be aligned mechnically between glass plates, while membrane proteins in large bicelles can be aligned magnetically and then optionally "flipped" 90° so that the bilayer normal is parallel to the applied magnetic field through the addition of lanthanide ions(13).

The traditional approach to protein structure determination is based on the same overall principles, whether solution NMR or solid-state NMR methods are used and whether the sample is aligned. This involves the resolution of resonances through the use of isotopic labels and multidimensional NMR experiments, the measurement of spectral parameters associated with individual resonances (e.g., NOEs, J couplings, dipolar couplings, or chemical shift frequencies), the assignment of all resonances to specific sites in the protein and then the calculation of structures. There are examples of the application of this approach to membrane proteins in micelles(8), bicelles(14) and bilayers(15). The availability of orientational information associated with individual resonances means that it is now possible to make effective use of limited amounts of assignment information (i.e. some reidue-type assignments or a few sequential assignments). It may also be feasible to implement an "assignment-free" approach(16). The use of either limited or no assignment information prior to calculating structures greatly speeds the process of structure determination by NMR spectroscopy, especially in the case of membrane proteins, for which assignments are difficult to make in nearly all situations because of overlap of resonances and unfavorable relaxation parameters.

4. The Local Field

"The interaction between two nuclear spins depends on the magnitude and orientation of their magnetic moments and also the length and orientation of the vector describing their relative positions"(17). The local field, which results from the interaction of two or more proximate nuclei, provides a particularly direct source of structural information. Other nuclear spin interactions, especially the chemical shift, can also provide essential structural constraints, but their interpretation is generally more complicated and less firmly grounded. Taken together, the restrictions resulting from measurements of spectral parameters from several spin interactions, where at least one of them is a local field, are sufficient to determine the three-dimensional structures of proteins.

It is possible to study unoriented "powder" samples, including membrane proteins with the use of magic-angle spinning. However, there is a second way to obtain high-resolution chemical shift spectra of solid-samples, and that entails the use of single crystals, where depending on the space group, the molecules have only one or a few orientations relative to each other and the applied magnetic field. Fortunately, it is not necessary to have a single-crystal sample. One direction of molecular orientation is sufficient, as long as it is parallel to the magnetic field or the molecules undergo rotational diffusion about a single axis, to give all of the spectroscopic benefits of sample orientation. It is generally easier to prepare uniaxially oriented samples, each site on one molecule can be transformed into the identical site on another molecule through a combination of translation, inversion and rotation operations about an axis parallel to the direction of the applied magnetic field. As is the case for single crystals, the spectra of oriented protein samples are characterized by narrow single-line resonances rather than powder patterns. The resonance frequencies reflect the orientations of the individual groups and, as a result, the solid-state NMR spectra of oriented samples are a rich source of structural information.

The SAMMY pulse sequence(18) is a high-resolution version of separated local field spectroscopy(19). It utilizes on-resonance “magic sandwich” pulses to accomplish CP transfer between two spins (i.e. ^{15}N and ^{1}H) while effectively decoupling the abundant spins (^{1}H) during the t1 evolution period of the pulse sequence. Very high-resolution two dimension heteronuclear dipolar spectra of solid samples can be obtained. The combination of narrow lines and favorable scaling factor has such a dramatic effect on the appearance of the spectra that it is now feasible to formulate solid-state NMR experiments where heteronuclear dipolar coupling frequencies provide a mechanism for resolution among similar chemical sites. In addition, dipolar coupling and chemical shift frequencies can be used in a number of complementary ways to enhance resolution in reduced and maximum dimensional experiments and to provide qualitative and quantitative indications of molecular structure. Two-dimensional $^{1}H/^{15}N$ SAMMY spectra have narrow linewidths in both the heteronuclear dipolar coupling and chemical shift frequency dimensions, enabling dipolar couplings to complement chemical shifts for resolution among sites as well as the measurement of readily interpretable orientationally dependent frequencies. SAMMY experiments are being used on a variety of systems, including membrane proteins.

5. Wheels and Waves

The secondary structure and topology of membrane proteins can be determined from the two dimensional $^{1}H/^{15}N$ SAMMY spectra of uniformly ^{15}N-labeled samples in oriented bilayers. The characteristic “wheel-like” patterns of resonances observed in these spectra reflect helical wheel projections of residues in both transmembrane and in-plane helices and, hence, provide direct indices of secondary structure and topology of membrane proteins in phospholipid bilayers. We refer to these patterns as PISA (polarity index slant angle) wheels(20, 21).

The resonance frequencies in both the $^{1}H/^{15}N$ heteronuclear dipolar and ^{15}N chemical shift dimensions in SAMMY spectra of aligned samples of membrane proteins depend on helix orientation, as well as on backbone dihedral angles, the magnitudes and orientations of the principal elements of the amide ^{15}N chemical shift tensor, and the N-H bond length. Consquently, it is possible to calculate spectra for any protein structure(22). The principles involved in the PISA wheel analysis of helices(23) are illustrated in Fig. 2. In Fig. 2A the projection down the axis of a helical wheel shows that the 3.6 residues per turn periodicity characteristic of an a-helix results in an arc of 100° between adjacent residues. The illustration of a peptide plane in Fig. 2B shows the orientations of the principal axes of the three operative spin interactions of the 15N labeled amide site. The 17° difference between the N-H bond axis and the $\sigma 33$ principal element of the amide ^{15}N chemical shift tensor is of a PISA wheel. The striking “wheel-like” pattern of the resonances calculated from a two-dimensional SAMMY spectrum of the ideal helix is shown in Fig. 2C. A PISA wheel reflects the slant angle (tilt) of the helix and the assignment of the resonances reflects the polarity index (rotation) of the helix.

When the helix axis is parallel to the bilayer normal, all of the amide sites have an identical orientation relative to the direction of the applied magnetic field and, therefore, all of the resonances overlap with the same dipolar coupling and chemical shift frequencies. Tilting the helix away from the membrane normal results in variations in the orientations of the amide N-H bond vectors relative to the field. This is seen in the spectra as dispersions among both the heteronuclear dipolar couplings and chemical shift frequencies. The result is a “wheel-like pattern” in the SAMMY spectrum like the one illustrated in 2C. Essentially all trans-membrane helices in membrane proteins are tilted with respect to the bilayer normal, and it is the combination of the tilt and the 17° difference between the tensor orientations in

the molecular frame that make it possible to resolve many resonances from residues in otherwise uniform helices.

Dipolar waves can serve as maps of protein structure in NMR spectra of both weakly and completely aligned samples(23). The periodicity inherit in secondary structure elements is key to the use of both PISA wheels and dipolar waves as indices of secondary structure and topology in membrane proteins. Figure 2C displays a classical two-dimensional PISA wheel of an a-helix. Figure 2D illustrates the periodic-wavelike variations of the magnitudes of the static heteronuclear dipolar couplings as a function of residue number. Similar patterns are observed in RDCs measured for weakly aligned proteins. Simulations like those shown in Figure 3 of helices with tilt angles of 0, 10, 20, 30 and 40° can be used in conjunction with experimental spectra to determine the orientation of the helices without making a single resonance assignment.

6. Application of NMR to an Ion Channel

The three-dimensional structures of a number of functional membrane proteins has been determined by solution(8, 24–32) and solid-state NMR(15, 33–37). In these cases, relatively large quantities of isotopically labeled protein required for NMR spectroscopy were prepared by expression of recombinant protein in Esherichia coli. The incorporation of purified proteins into lipid bilayers has been shown to yield functional channels as demonstrated by single-channel current recordings under voltage-clamp conditions.

The experimental two-dimensional SAMMY spectrum in Figure 4 of selectively ^{15}N-leucine labeled Hepatitis C Virus p7 protein in aligned bicelles has reasonable resolution, with each amide resonance characterized by ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling frequencies. The experimental SAMMY spectrum in Fig. 4 is similar to that of the PISA wheel in Fig. 2C and, based on simulations, has at least one helix with a tilt angle of about 10°. Differences between an experimental spectrum and a calculated spectrum are a result of deviations between the experimentally determined backbone dihedral angles and those of an ideal a-helix and differences resulting from variations of the chemical shift tensors among the various amide sites.

The polarity of the resonances observed in the “wheel-like” pattern of a SAMMY spectrum provides a direct measure of the angle of the helix rotation about its long axis within the membrane. In principle, one well-resolved two-dimensional SAMMY spectrum of an aligned sample of a uniformly ^{15}N-labeled protein provides sufficient information for complete structure determination. The orientationally dependent frequencies associated with each resonance depends on the magnitudes and orientations of the principal elements of the spin-interaction tensors in the molecule and on the orientation of the molecular site with respect to the direction of the applied magnetic field. Because the orientation of the bilayer is fixed by the method of sample preparation and the properties of the nuclear spin-interaction tensors are generally well characterized, each frequency reflects the orientation of a specific site in the protein with respect to the bilayer. The backbone structure of a protein is defined by the planes formed by the individual rigid peptide bonds and their directly bonded atoms. This is equivalent to the conventional description by vectors representing bonds between non-hydrogen atoms. The standard planar peptide geometry serves as the building block for the structure assembly process. The orientation of a peptide plane consists of the NMR frequencies measured from a SAMMY spectrum, the magnitudes and orientations of the principal elements of the amide ^{15}N and ^{1}H chemical shift tensors, and the N-H bond length. Once the orientations of all the peptide planes in the protein are determined from the experimental data, neighboring planes of fixed orientation are connected through their common a-carbon atom, with the only constraint of a fixed

tetrahedral angle of 110°. Another program calculates the ϕ and χ dihedral angles for the two contiguous peptide plane combinations that satisfy tetrahedral angle geometry at the a-carbon.

The advantage of this direct mathematical analysis is that it is possible to determine the standard deviations in ϕ and μ based on uncertainties in the experimentally determined angles. Another important advantage of the method is that it results in the "piecewise" assembly of structures. Different parts of the protein structure can be determined independently. This is not possible in methods for which distance constraints between different regions of the protein must be established for three-dimensional structure determination. Because the orientation of individual peptide planes is determined relative to a unique external reference, any errors are not cumulative.

7. Drug Discovery and Development by NMR

The emergence of Structure Activity Relationship (SAR) studies by NMR(38) has shown great promise for the identification of lead compounds for drug development (39–45). NMR can be used to analyze the structural and chemical details of intermolecular interactions between proteins and small molecules. . In solution NMR a simple correlation spectrum (e.g., HSQC) can display changes in chemical shifts, an indication of an effect on the local chemical environment. The use of solid-state NMR, although slightly more laborious in sample preparation can indicate local as well as global changes in protein structure as a result of drug interaction. The magnetically aligned bilayer samples can provide information as to the orientation of the protein before and after the addition of drugs. Such samples avoid the time correlation problem associated with solution NMR, thus providing the opportunity to look at larger protein systems in environments similar to the ones used to characterize their channel activity. Molecules that bind specifically, albeit weakly or in mixtures, to the protein cause changes in the chemical shift and/or dipolar coupling frequencies of resonances associated with the residues in the binding site.

An appealing feature of SAR by NMR comes from its ability to identify compounds that bind to different parts of the protein. With only a basic outline of the protein architecture, it is possible to link two binding fragments to make a drug candidate that binds with high affinity and specificity. In solid-state NMR experiments, spectral resolution results from the orientation dependence of the frequencies in aligned samples; consequently, the resonances in the spectra are segregated by topology, and residues in loops can be readily differentiated from those in helices. The trans-membrane helices of tranporter proteins are the likely binding sites for channel blockers, and amphipathic cytoplasmic domains and basic inter-helical loops may provide separate binding sites.

8. Summary

Nuclear magnetic resonance structural studies of membrane proteins yield valuable insights into their structure and topology. For example, the tilt angle and rotation of the helices in an ion channel can be determined by solid-state NMR spectroscopy in aligned lipid bilayers. This has important consequences for ion-channel pore geometry and conduction, as it leads to the assembly of a symmetric, oligomeric, and funnel-like pore with its wide opening at the N-terminal side of the membrane. Details about the structure of the protein in aligned phospholipids environments are immediately apparent from inspection of the SAMMY spectrum and the data can be further used for the determination of atomic resolution three-dimensional structures. SAR by NMR is a technique that is well suited for the field of membrane transporter proteins. The experiments on protein/phospholipid samples provide a unique insight into the interaction of drugs and the functional proteins.

The advances required to transform solid-state NMR from a spectroscopic technique to a generally applicable method for determining molecular structures included multiple-pulse sequences, double-resonance methods, and separated local field spectroscopy. It also required improvements in instrumentation, especially the use of high field magnets and efficient probes capable of high-power radio-frequency irradiations at high frequencies. The pace of development is accelerating and the local field is being utilized in an increasing number of ways in spectroscopic investigations of molecular structure and dynamics. Applications to many helical membrane proteins are underway and promise to add to our understanding of membrane proteins in health and disease.

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References

1. Cavanagh, J.; Fairbrother, WJ.; Palmer, AG., III; Skelton, NJ. Protein NMR Spectroscopy. Acedemic; San Diego, CA: 1995.
2. Wuthrich K. The second decade--into the third millenium. Nat Struct Biol. 1998; 5(Suppl):492–495. [PubMed: 9665176]
3. Opella SJ. NMR and membrane proteins. Nat Struct Biol. 1997; 4(Suppl):845–848. [PubMed: 9377156]
4. McDonnell PA, Opella SJ. Effect of detergent concentration on multidimensional solution NMR spectra of membrane proteins in micelles. J Magn Reson. 1993; B102:1205–1225.
5. Sanders CR, Hare BJ, Howard K, Prestegard JH. Magnetically Oriented Phospholipid Micelles as a Tool for the Study of Membrane-Associated Molecules. Progress in NMR Spectroscopy. 1994; 26:421–444.
6. Opella SJ, Stewart PL, Valentine KG. Protein structure by solid-state NMR spectroscopy. Q Rev Biophys. 1987; 19:7–49. [PubMed: 3306759]
7. Marassi FM, Ramamoorthy A, Opella SJ. Complete resolution of the solid-state NMR spectrum of a uniformly 15N-labeled membrane protein in phospholipid bilayers. Proc Natl Acad Sci U S A. 1997; 94:8551–8556. [PubMed: 9238014]
8. Almeida FC, Opella SJ. fd coat protein structure in membrane environments: structural dynamics of the loop between the hydrophobic trans-membrane helix and the amphipathic in-plane helix. J Mol Biol. 1997; 270:481–495. [PubMed: 9237913]
9. Ma C, Opella SJ. Lanthanide ions bind specifically to an added “EF-hand” and orient a membrane protein in micelles for solution NMR spectroscopy. J Magn Reson. 2000; 146:381–384. [PubMed: 11001856]
10. Veglia G, Opella SJ. Lanthanide Ion Binding to Adventitious Sites Aligns Membrane Proteins in Micelles for Solution NMR Spectroscopy. Journal of the American Chemical Society. 2000; 122:11733–11734.
11. Chou JJ, Kaufman JD, Stahl SJ, Wingfield PT, Bax A. Micelle-induced curvature in a water-insoluble HIV-1 Env peptide revealed by NMR dipolar coupling measurement in stretched polyacrylamide gel. J Am Chem Soc. 2002; 124:2450–2451. [PubMed: 11890789]
12. Griffin RG. Dipolar recoupling in MAS spectra of biological solids. Nat Struct Biol. 1998; 5(Suppl):508–512. [PubMed: 9665180]
13. Howard KP, Opella SJ. High-resolution solid-state NMR spectra of integral membrane proteins reconstituted into magnetically oriented phospholipid bilayers. J Magn Reson. 1996; B 112:91–94.
14. Park SH, De Angelis AA, Nevzorov AA, Wu CH, Opella SJ. Three-dimensional structure of the transmembrane domain of Vpu from HIV-1 in aligned phospholipid bicelles. Biophys J. 2006; 91:3032–3042. [PubMed: 16861273]

15. Opella SJ, Marassi FM, Gesell JJ, Valente AP, Kim Y, Oblatt-Montal M, Montal M. Structures of the M2 channel-lining segments from nicotinic acetylcholine and NMDA receptors by NMR spectroscopy. Nat Struct Biol. 1999; 6:374–379. [PubMed: 10201407]
16. Nevzorov AA, Opella SJ. Structural fitting of PISEMA spectra of aligned proteins. J Magn Reson. 2003; 160:33–39. [PubMed: 12565046]
17. Abragam, A. The Principles of Nuclear Magnetism. Oxford University Press; Oxford, UK: 1961.
18. Nevzorov AA, Opella SJ. A “Magic Sandwich” pulse sequence with reduced offset dependence for high-resolution separated local field spectroscopy. J Magn Reson. 2003; 164:182–186. [PubMed: 12932472]
19. Waugh JS. Uncoupling of local field spectra in nuclear magnetic resonance: determination of atomic positions in solids. Proc Natl Acad Sci U S A. 1976; 73:1394–1397. [PubMed: 1064013]
20. Marassi FM, Opella SJ. A solid-state NMR index of helical membrane protein structure and topology. J Magn Reson. 2000; 144:150–155. [PubMed: 10783285]
21. Wang J, Denny J, Tian C, Kim S, Mo Y, Kovacs F, Song Z, Nishimura K, Gan Z, Fu R, Quine JR, Cross TA. Imaging membrane protein helical wheels. J Magn Reson. 2000; 144:162–167. [PubMed: 10783287]
22. Bak M, Schultz R, Vosegaard T, Nielsen NC. Specification and visualization of anisotropic interaction tensors in polypeptides and numerical simulations in biological solid-state NMR. J Magn Reson. 2002; 154:28–45. [PubMed: 11820824]
23. Mesleh MF, Veglia G, DeSilva TM, Marassi FM, Opella SJ. Dipolar waves as NMR maps of protein structure. J Am Chem Soc. 2002; 124:4206–4207. [PubMed: 11960438]
24. Williams KA, Farrow NA, Deber CM, Kay LE. Structure and dynamics of bacteriophage IKe major coat protein in MPG micelles by solution NMR. Biochemistry. 1996; 35:5145–5157. [PubMed: 8611498]
25. MacKenzie KR, Prestegard JH, Engelman DM. A transmembrane helix dimer: structure and implications. Science. 1997; 276:131–133. [PubMed: 9082985]
26. Papavoine CH, Christiaans BE, Folmer RH, Konings RN, Hilbers CW. Solution structure of the M13 major coat protein in detergent micelles: a basis for a model of phage assembly involving specific residues. J Mol Biol. 1998; 282:401–419. [PubMed: 9735296]
27. Townsley LE, Tucker WA, Sham S, Hinton JF. Structures of gramicidins A, B, and C incorporated into sodium dodecyl sulfate micelles. Biochemistry. 2001; 40:11676–11686. [PubMed: 11570868]
28. Sorgen PL, Cahill SM, Krueger-Koplin RD, Krueger-Koplin ST, Schenck CC, Girvin ME. Structure of the Rhodobacter sphaeroides light-harvesting 1 beta subunit in detergent micelles. Biochemistry. 2002; 41:31–41. [PubMed: 11772000]
29. Zamoon J, Mascioni A, Thomas DD, Veglia G. NMR solution structure and topological orientation of monomeric phospholamban in dodecylphosphocholine micelles. Biophys J. 2003; 85:2589–2598. [PubMed: 14507721]
30. Yushmanov VE, Mandal PK, Liu Z, Tang P, Xu Y. NMR structure and backbone dynamics of the extended second transmembrane domain of the human neuronal glycine receptor alpha1 subunit. Biochemistry. 2003; 42:3989–3995. [PubMed: 12667090]
31. Yushmanov VE, Xu Y, Tang P. NMR structure and dynamics of the second transmembrane domain of the neuronal acetylcholine receptor beta 2 subunit. Biochemistry. 2003; 42:13058–13065. [PubMed: 14596621]
32. Howell SC, Mesleh MF, Opella SJ. NMR structure determination of a membrane protein with two transmembrane helices in micelles: MerF of the bacterial mercury detoxification system. Biochemistry. 2005; 44:5196–5206. [PubMed: 15794657]
33. Ketchem RR, Hu W, Cross TA. High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR. Science. 1993; 261:1457–1460. [PubMed: 7690158]
34. Wang J, Kim S, Kovacs F, Cross TA. Structure of the transmembrane region of the M2 protein H(+) channel. Protein Sci. 2001; 10:2241–2250. [PubMed: 11604531]
35. Valentine KG, Liu SF, Marassi FM, Veglia G, Opella SJ, Ding FX, Wang SH, Arshava B, Becker JM, Naider F. Structure and topology of a peptide segment of the 6th transmembrane domain of the Saccharomyces cerevisae alpha-factor receptor in phospholipid bilayers. Biopolymers. 2001; 59:243–256. [PubMed: 11473349]

36. Marassi FM, Opella SJ. Simultaneous assignment and structure determination of a membrane protein from NMR orientational restraints. Protein Sci. 2003; 12:403–411. [PubMed: 12592011]

37. Park SH, Mrse AA, Nevzorov AA, Mesleh MF, Oblatt-Montal M, Montal M, Opella SJ. Three-dimensional structure of the channel-forming trans-membrane domain of virus protein “u” (Vpu) from HIV-1. J Mol Biol. 2003; 333:409–424. [PubMed: 14529626]

38. Hajduk PJ, Meadows RP, Fesik SW. Discovering high-affinity ligands for proteins. Science. 1997; 278:497–499. [PubMed: 9381145]

39. Hajduk PJ, Meadows RP, Fesik SW. NMR-based screening in drug discovery. Q. Rev. Biophys. 1999; 32:211–240. [PubMed: 11194565]

40. Lepre CA, Moore JM, Peng JW. Theory and applications of NMR-based screening in pharmaceutical research. Chem. Rev. 2004; 104:3641–3676. [PubMed: 15303832]

41. Huth JR, Sun C, Sauer DR, Hajduk PJ. Utilization of NMR-derived fragment leads in drug design. Methods Enzymol. 2005; 394:549–571. [PubMed: 15808237]

42. Takeuchi K, Wagner G. NMR studies of protein interactions. Curr. Opin. Struct. Biol. 2006; 16:109–117. [PubMed: 16427776]

43. Yu L, Sun C, Song D, Shen J, Xu N, Gunasekera A, Hajduk PJ, Olejniczak ET. Nuclear magnetic resonance structural studies of a potassium channel-charybdotoxin complex. Biochemistry. 2005; 44:15834–15841. [PubMed: 16313186]

44. Watts A. Solid-state NMR in drug design and discovery for membrane-embedded targets. Nat Rev Drug Discov. 2005; 4:555–568. [PubMed: 16052240]

45. Luca S, White JF, Sohal AK, Filippov DV, van Boom JH, Grisshammer R, Baldus M. The conformation of neurotensin bound to its G protein-coupled receptor. Proc Natl Acad Sci U S A. 2003; 100:10706–10711. [PubMed: 12960362]

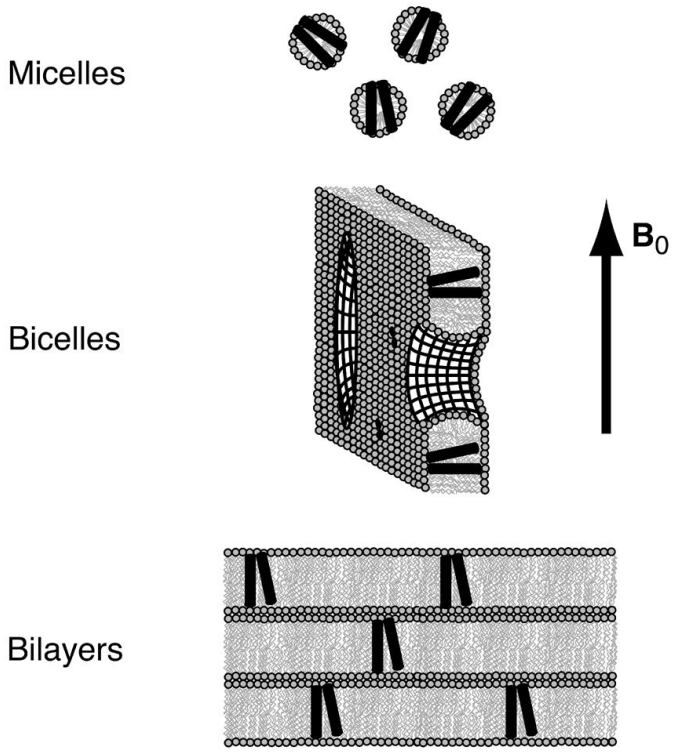

Figure 1.
Representation of proteins in micelles (top), bicelles (middle) and bilayers (bottom).

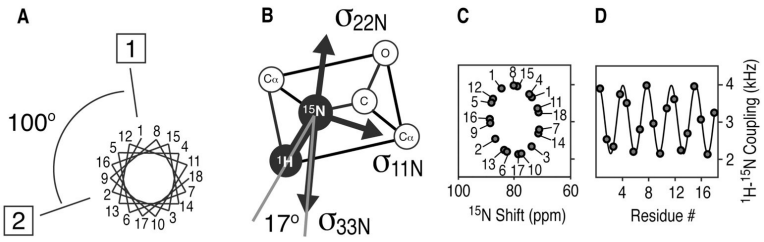

Figure 2.

Principles of PISA wheels. (A) Helical wheel showing the 100° arc between adjacent residues that is a consequence of the periodicity of 3.6 residues per turn in an α-helix; (B) orientations of the principal elements of the spin-interaction tensors associated with ^{15}N in a peptide bond. (C) PISA wheel for an ideal α -helix; (D) dipolar wave for an ideal α-helix.

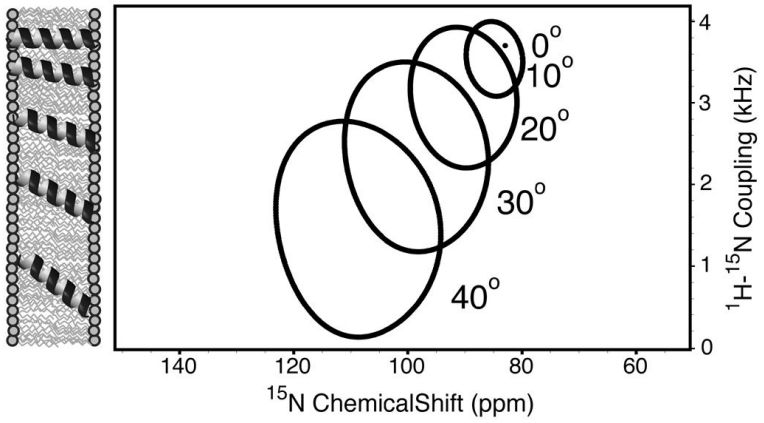

Figure 3.
Simulated PISA wheel patterns in "unflipped" bicelles undergoing rapid rotational diffusion for an α-helix with uniform dihedral angles (ϕ,ψ) = (−61°, −45°) tilted at 0, 10, 20, 30 and 40° with respect to the bilayer normal.

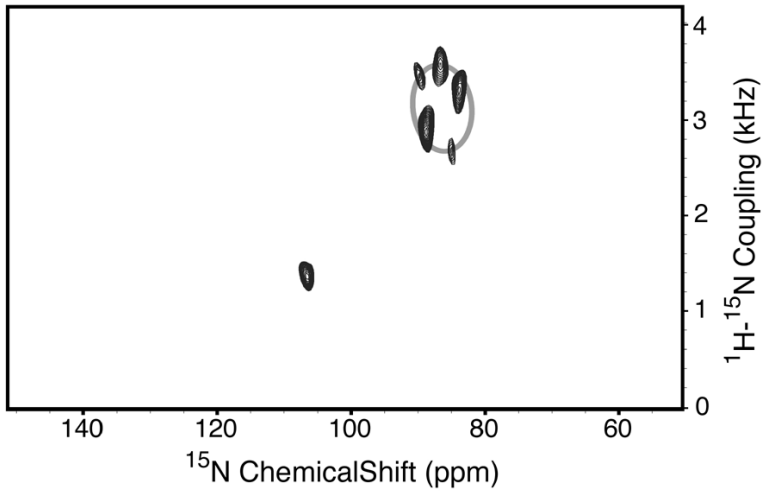

Figure 4.
Two-dimensional ^{1}H-^{15}N dipolar coupling and ^{15}N chemical shift solid-state SAMMY spectrum of selectively labeled ^{15}N leucine HCV p7 in “unflipped” bicelles. A simulated wheel of 10° has been superimposed to show the approximate tilt angle of the transmembrane helix.