The Magic of Bicelles Lights Up Membrane Protein Structure

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1. INTRODUCTION

1.1. Why Study Membranes and Membrane Proteins?

Biological membranes and membrane proteins, responsible for numerous exciting biological processes, present one of the paramount challenges in biophysics today. Membranes are present in great number and variety in all organisms. They form the boundary between the inside and outside for any bacterium or cell, and they delimit the host of organelles that make up their inner subunits. Each biological membrane is made up of dozens of different types of lipids and sterols, and any particular type of membrane has a characteristic content of these different constituents. As a very basic example, we mention that prokaryotic membranes contain a notable component of negatively charged lipids but almost no cholesterol, while eukaryotic membranes are mostly zwitterionic but have a significant amount of cholesterol. Since the driving biophysical principles of membrane formation are very simple—they lie in the amphipathic properties of any lipid molecule—a single lipid type is sufficient to form membrane-like bilayers in an aqueous environment. Such model membranes are used extensively to study biophysical properties that are representative for most membrane systems. A particularly interesting effect is observed when detergent molecules are added to lipid bilayer samples: the detergents solubilize the bilayers, and in certain regimes so-called bilayered mixed micelles or “bicelles” are formed. In the simplest case, they can be described as microscopic disks where a bilayer patch is encircled by a “rim” of detergent molecules. Bicelles represent a new instance of lipid morphology and are extensively applicable to structural studies of lipid membranes and protein structure.1

Membranes delimit any cell and all of its compartments. They form natural borders for metabolic substances and signaling molecules. Membrane proteins are the porters and gatekeepers that make sure that only proper molecules or signals make it across the membrane. Since membrane proteins perform numerous key functions in cell metabolism and signaling, they contribute over 30% of the genes in typical eukaryotic genomes,2 and they form the targets for over 50% of
drugs in use today.\textsuperscript{3} The number of elucidated structures of membrane proteins has grown exponentially after the first structure was published in 1985, thus equaling the rate at which structure determination of soluble proteins emerged early on.\textsuperscript{4} Still, the number of available high-resolution structures of membrane proteins is limited. There are Internet sites that keep track of newly published structures of membrane proteins. The crystallography-oriented Web site of Dr. Stephen White [http://blanco.biomol.uci.edu/mpstruc] has recently been joined by another site maintained by Dr. Dror Warschawski that is dedicated to structures of membrane proteins elucidated by nuclear magnetic resonance (NMR) spectroscopy [www.drorlist.com/nmr/MPNMR.html]. Another equally important site of Dr. Hartmut Michel [www.mpibp-frankfurt.mpg.de/michel/public/memprotsruct.html] with an emphasis on crystallization conditions is no longer updated, but states that access is still enabled.

In this review article, we aim to give a general overview of lipid bicelles as employed in the study of protein structure. Recent advances in the field of protein structural biology that have been made possible by exploiting the unique properties of lipid bicelles, in both solution and solid-state NMR spectroscopy, will be discussed. During the last five years, review contributions have presented bicelles either within the far more general context of reconstitution media for solution NMR studies (see section 1.4) or have focused on macroscopically aligned bicelles as used for solid-state NMR studies.\textsuperscript{5,6} One very recent contribution has tackled the formidable task of reviewing all membrane mimetics employed in both solution and solid-state NMR studies.\textsuperscript{7} As mentioned above, we will limit the contents of this review article to applications of lipid bicelles, but will cover both the isotropic and the aligned bicelles as used in NMR studies. Some parts of this article can be viewed as an update on the review articles of Opella and Marassi,\textsuperscript{8} Marcotte and Auger,\textsuperscript{9} and Prosser et al.\textsuperscript{10} In addition, some of our recent research involving bicelles is presented in detail.

1.2. Understanding Atomistic-Level Structures Is Important

The intrinsic properties of a cell membrane originate from interactions among molecules like amphiphatic lipids, polysaccharides, cholesterol, proteins, and water. Since the chemical and physical properties of these molecules differ considerably, the minimum free energy of mixing corresponds to a heterogeneous cell membrane. Domains rich in protein, cholesterol and anionic lipids, and rafts have been reported to play important roles in biological activities of cells which have direct implications in viral infection, bacterial infection, amyloid toxicity related to aging diseases, and cancer.\textsuperscript{11–13} For example, the presence of charged lipids in bacterial cell membranes and their absence in mammalian cell membranes are one of the key factors in the selectivity of antimicrobial peptides. Likewise, cholesterol present in mammalian and absent in bacterial cell membranes has been shown to have a similar influence on the selectivity of antimicrobial peptides.\textsuperscript{14} In addition, the process of folding, misfolding or refolding, and aggregation of amyloidogenic proteins in cell membranes is different from that in solution, and also depends on the composition of the cell membrane.\textsuperscript{15} Needless to mention that the secondary and tertiary structures of proteins can be different when they associate with the cell membrane. Therefore, high-resolution structure of individual molecules and their orientation in a membrane environment could reveal the factors that drive the molecular association and their function in this heterogeneous membrane environment. While solving the atomic-level structure of a membrane protein still remains a big challenge for most biophysical techniques, the increasing number of structures determined by X-ray and NMR studies continue to shed light on the functional aspects of membrane proteins. For example, the reported high-resolution structure of the potassium channel forming membrane protein\textsuperscript{16–18} has provided insights into the geometry of the channel, ion selectivity, interactions between lipids and the protein, and the role of individual amino acids in the transportation of potassium ions.

1.3. NMR Is an Ideal Technique to Measure Structure and Dynamics

NMR spectroscopy has played a pivotal role in the structure determination of a host of biomacromolecules, ranging from proteins to nucleic acids. Importantly, NMR spectroscopy has provided scientists with detailed structural and dynamical information that is inaccessible through other biophysical means. First and foremost, X-ray crystallography has elucidated a tremendous number of protein structures in high resolution. The environment of a protein crystal, however, is far from physiological and may shadow important aspects, especially of protein dynamics. In this respect, NMR spectroscopy is both an alternative as well as a complement to X-ray crystallography. The branch of NMR spectroscopy that deals with molecules in solution is known as solution-state NMR spectroscopy. It offers varied, well-tested, and sophisticated tools\textsuperscript{19–25} to routinely deal with any soluble protein that does not exceed a certain molecular weight. The upper limit for molecular weight is currently around 100 kDa\textsuperscript{26} and is continually pushed higher. Lipid membranes are typically not amenable to be studied by solution-state NMR spectroscopy, since they are well above the molecular weight limit. It is often possible, though, to study the structure of membrane proteins when they are solubilized by properly chosen detergents.\textsuperscript{27} Membrane proteins are notoriously hard to study since their highly hydrophobic nature routinely causes misfolding and aggregation, making it very hard to crystallize them in sufficient quality for X-ray diffraction.\textsuperscript{28} In addition, their slow reorientation in a membrane environment prohibits the use of well-established solution-state NMR methodology. The branch of solid-state NMR spectroscopy is rapidly evolving to deal with membrane proteins that are beyond the size limit for solution-state NMR spectroscopy.

Since the NMR observables chemical shift anisotropy and dipolar coupling are sensitive to both the chemical environment and molecular motions, they can be used to probe molecular structure and dynamics associated with biological processes such as ligand binding, conformational exchange and protein–protein interactions. One of the unique advantages of NMR spectroscopy is its ability to interrogate molecular dynamics over a wide range of time scales. Through NMR, motions from nanosecond to microsecond time scales can be probed via measuring different NMR parameters such as spin–lattice relaxation (\(T_1\)), spin–spin relaxation (\(T_2\)), relaxation in the rotating frame (\(T_1\rho\)), residual dipolar couplings, and quadrupolar coupling (for nuclei with spin > 1/2). Thus, NMR spectroscopy is able to paint a very detailed picture of a system, where structure and dynamics as well as function can be correlated. Membrane proteins exhibit a broad time scale of dynamics and these motions highly influence the function of the protein: The residues in transmembrane segments generally
undergo restricted motion on a fast time scale (picosecond-nanosecond), while soluble domains show large amplitude motions with slower correlation times. Loop regions move with intermediate amplitudes on intermediate time scales since they are anchored at transmembrane segments. The entirety of domains may perform collective motions like conformational changes at very slow time scales (microsecond). Typical dynamic properties of different regions were quantified on bacteriorhodopsin by extensive $^{13}$C NMR studies. Therefore, NMR techniques are well suited to study the dynamical structures of membrane proteins. Another unique advantage of NMR spectroscopy is that it can determine the orientation of a membrane protein relative to the lipid bilayer.

In the context of NMR studies of membrane proteins, lipid bicelles have opened completely new ways of preparing samples for NMR studies. This is mostly because the size of lipid bicelles can be custom-tailored for specific tasks. An additional unique property of certain bicelle preparations is their propensity to macroscopically align when brought into an external magnetic field. As a consequence, bicelles disobey the traditional classification of NMR experiments and notoriously cross the border between solution-state and solid-state NMR spectroscopy. Figure 1 gives a graphic overview of the position of lipid bicelles in NMR studies of membrane proteins. Care must be taken to prepare a well-behaved sample for successful structural studies using NMR spectroscopy. As is the case in the study of any membrane protein, the protein needs to be supplied in sufficient amount and purity, needs to have a specific isotope labeling scheme, and needs to be properly folded and reconstituted. Only then can it be taken into formulations that are suitable for NMR spectroscopy. Typically, those have been detergent micelles for solution NMR studies, and multilamellar vesicles (MLVs) of lipid for solid-state NMR studies. Lipid bicelles open a middle ground between these two model membranes, namely, micelles and MLVs. Since their size can be chosen to be small enough to tumble quickly on the NMR time scale, small bicelles (also known as isotropic bicelles) can be investigated using solution NMR experiments. Larger bicelles, especially when aligned macroscopically, are amenable to static solid-state NMR spectroscopy. In addition, magic angle spinning (MAS) NMR experiments can be applied to lipid bicelles.

1.4. Need for Excellent Model Membranes

The overall architecture of membrane proteins shows little variation: integral membrane proteins transverse the lipid bilayer of the cell membrane either as a single $\alpha$-helix, or as a bundle of $\alpha$-helices, or they form $\beta$-barrels. Since the differences in membrane protein architecture responsible for a specific function are often subtle, excellent model membrane systems are needed. In addition, the secondary and tertiary structures, folding, aggregation, dynamics, stability, orientation, and function of a membrane protein highly depend on the nature of the membrane environment. This is true even if membrane proteins are intrinsically tolerant to changes in the composition of the surrounding membrane. For example, the choice of a good detergent system was found crucial in studies of the enzyme PagP, an integral membrane protein forming a $\beta$-barrel. The detergent used initially was found to deactivate the enzyme because its structure is too similar to the substrate. Only with a more distinct detergent could an active enzyme be studied. Likewise, specific polyunsaturated side chains are present at high molar ratios in the lipids of rod outer segment disk membranes and accumulate near rhodopsin, an integral $\alpha$-helical membrane protein. In the case of the antimicrobial peptide gramicidin A, suitable conditions had to be established to distinguish the physiologically relevant conformation from other conformations. The general awareness of the distinction between physiologically relevant and other conformations has obviously faded recently and had to be called back to mind.

Different types of model membranes have been used for NMR studies. The use of TFE/water mixtures is no longer considered to be a good model membrane. Detergent micelles and lipid vesicles have commonly been used in solution and solid-state NMR applications, respectively. While the use of micelles enables the applications of well-established solution NMR techniques, the potential impact of the curvature of micelles on the structural folding remains a concern. Therefore, a planar lipid bilayer is considered to be a better model membrane than a micelle. As mentioned earlier, bicelles that are devoid of acute curvature like a micelle are considered to be a more suitable model membrane for NMR studies. Nevertheless, micelles have been found to be useful in trapping transiently lived helical structures of amyloid proteins that otherwise rapidly convert into $\beta$-sheet structures in a lipid bilayer.

The importance of detergents in the study of solubilized membrane proteins has been reviewed, at times under imaginative titles referring to detergents as “French swimwear” or denying that they are part of a soap opera. In view of the advantageous properties of bicelles over detergent micelles, another review title states that “small is beautiful, but sometimes bigger is better.” Two other review contributions have reported on bicelles in the context of membrane mimetics and solubilizing agents for solution NMR spectroscopy. These reviews cover micelle-forming detergents as well as innovative solubilizing approaches other than bicelles, such as in situ NMR, amphipols, or nanodisks which are not within the scope of the current review. A comparison of NMR spectra acquired on different membrane proteins in bicelles and nanodisks, both isotropic and aligned, has been performed. Bicelles were investigated as novel surfactants in the context of

Figure 1. A schematic overview of the use of lipid bicelles in the study of structure and dynamics of membrane proteins using NMR spectroscopy.
cell-free expression of membrane proteins. Cell-free production of integral membrane proteins in bicelles was compared to production in lipid protein nanodisks as well as micelles and liposomes. Subunits a and c of ATP-synthase have been produced by cell-free synthesis in the presence of bicelles; subunit a was shown to have a similar fold to native protein extracted from bacterial cell walls.

2. WHAT ARE BICELLES?

When detergent molecules were combined with phospholipids, phases with completely new morphology were found. The microscopic details of these morphologies have been researched extensively, and phase diagrams have been established. With the help of small-angle neutron scattering, detailed descriptions have been given for all morphologies. It was demonstrated that short-chain phospholipids can be used as detergent, giving formulations that consist purely of phospholipids. To date, the combination of dimyristoylphosphatidylcholine (DMPC) as long-chain, bilayer-forming component with dihexanoylphosphatidylcholine (DHP) as detergent component has remained the most popular choice for bicelle formulations. The most important descriptor of bicelle preparations is the molar ratio of long-chain to short-chain component. It is usually denoted as \( q \), and in the most common case, it is \( q = [\text{DMPC}] / [\text{DHP}] \).

The specific values of \( q \), hydration level, temperature, ionic strength, etc. determine the microscopic morphology. Figure 2 gives schematic models for important morphologies. The long-chain lipid component alone can form MLVs (Figure 2A). An addition of a detergent results in defects within the MLVs (Figure 2B) since miscibility between lipid and detergent is low. When increasing the detergent content, the vesicles break up and extended lamellae (Figure 2C) or chiral nematic ribbons (Figure 2D) are formed. Both have the propensity for magnetic alignment, usually with the membrane normal directed perpendicular to the external magnetic field direction of an NMR spectrometer (which would be the vertical z-axis in Figure 2). At even higher concentrations of the detergent, aggregates are formed that have a flat, disk-like geometry and tumble isotropically (Figure 2E). It is important to note that the long-chain component in this geometry is still separated from the detergent and forms a lipid bilayer. Pure detergent will form isotropically tumbling detergent micelles (Figure 2F).

Numerous modifications of bicelles have been developed to make them more closely resemble native biological membranes. The influence of \( q \), hydration level, and temperature on bilayer properties of bicelles has been studied. For spectroscopic purposes, it is advantageous to flip magnetically aligned bicelles to make their membrane normal line up with the external magnetic field axis. This can be achieved by adding lanthanide ions or by using lipids with a biphenyl group in one of their acyl chains. Two very recent studies demonstrated that incorporation of \( \text{Cu}^{2+} \) in a lipid by means of the chelating agent 14:0-PE-DTPA can shorten the \( T_1 \) relaxation and therefore faster data acquisition is feasible; this approach is attractive as an NMR experiment can be completed faster and therefore a sensitive membrane protein can be preserved from RF-pulse induced sample heating. The magnetic-alignment of bicelles can be influenced by the embedded protein, as was shown for gramicidin A, which causes alignment when embedded in small bicelles that would tumble isotropically in the absence of the protein. Ether-lipids can be used to increase sample stability, but have recently been found to alter the structure of an antimicrobial peptide nisin. Hybrid bicelles covered with a siloxane ceramic layer were recently shown to increase the stability. Domain formation could be modeled in bicelle formulations containing unsaturated lipids and cholesterol.

Considerable effort has been invested to establish bicelles as a membrane mimic for studies using electron paramagnetic resonance (EPR) spectroscopy. Alignment can be achieved even at a weak magnetic field strength used in X-band EPR measurements. Structural and dynamic properties of the necessary nitroxide spin labels were investigated and a systematic comparison with NMR results was performed. The conformation of a nitroxide spin label in the homodimeric protein \( \text{Cy}^2 \) was compared in NMR, EPR, and X-ray crystallographic conditions.

Other innovative applications of bicelles outside of NMR spectroscopy include the crystallization of membrane proteins from bicelle formulations, the use of bicelles as delivery vehicles for membrane proteins to oocyte membranes, and the use of bicelles as templates for the synthesis of platinum nanowheels. Today, the term “bicelle” has become so popular that it was even applied to flat, disk-like aggregates formed by linear peptide copolymers with different length hydrophobic and hydrophilic portions. The potential pharmaceutical application of bicellar formulations to the skin has been investigated in detail and has been reviewed. A study involving the drug diclofenac has investigated bicelles as drug carriers in dermal applications.

3. AN OVERVIEW OF THE USE OF BICELLES IN THE STRUCTURAL STUDIES OF PROTEINS

Bicelles are used in a number of ways in the study of proteins, be it a globular or a membrane protein. Figure 3 gives a cartoon overview of the different approaches. In general, both isotropically tumbling as well as magnetically aligned bicelles are valuable tools to study proteins. Membrane proteins can be embedded in both isotropic (Figure 3C) and aligned bicelles.
proteins can be studied in isotropically tumbling bicelles (C). The combination of isotropic bicelles and globular proteins can be used to give a residual preferential orientation to globular proteins (B). Membrane proteins can be studied in isotropically tumbling bicelles (A). Magnetically aligned bicelles can also impose a weak alignment and made the measurement of RDCs possible. Magic angle spinning (MAS) was used to compare these values to isotropic values. RDCs could even be measured on a peptide embedded in isotropic bicelles (Figure 3B). The aligned bicelles impose a weak orientational preference onto the proteins which can be detected in suitable solution-state NMR spectra. Furthermore, membrane interaction of soluble proteins can be studied in the presence of isotropic bicelles (Figure 3D).

The following sections will review each approach. Section 4 and section 5 deal with soluble proteins in the presence of aligned or isotropic bicelles, respectively. Section 6 introduces the special situation of integral membrane proteins in bicelles in general terms and section 7 describes the technical preparation of such samples. Section 8 presents results obtained on integral membrane proteins in isotropic bicelles, section 9 the same in aligned or isotropic bicelles, respectively. Section 6 introduces the special situation of integral membrane proteins in bicelles in general terms and section 7 describes the technical preparation of such samples. Section 8 presents results obtained on integral membrane proteins in isotropic bicelles, section 9 the same in aligned or isotropic bicelles, respectively. Section 6 introduces the special situation of integral membrane proteins in bicelles in general terms and section 7 describes the technical preparation of such samples. Section 8 presents results obtained on integral membrane proteins in isotropic bicelles, section 9 the same in aligned or isotropic bicelles, respectively. Section 6 introduces the special situation of integral membrane proteins in bicelles in general terms and section 7 describes the technical preparation of such samples. Section 8 presents results obtained on integral membrane proteins in isotropic bicelles, section 9 the same in aligned or isotropic bicelles, respectively. Section 6 introduces the special situation of integral membrane proteins in bicelles in general terms and section 7 describes the technical preparation of such samples. Section 8 presents results obtained on integral membrane proteins in isotropic bicelles, section 9 the same in aligned or isotropic bicelles, respectively.

**Figure 3.** Proteins can be studied in numerous ways employing bicelles. Membrane proteins can be macroscopically oriented using magnetically aligned bicelles (A). Magnetically aligned bicelles can also be used to give a residual preferential orientation to globular proteins (B). Membrane proteins can be studied in isotropically tumbling bicelles (C). The combination of isotropic bicelles and globular proteins can be used to study membrane binding (D).

Globular proteins in solution tumble isotropically, at a rate that is usually fast on the NMR time scale. Hence, the anisotropic nuclear spin interactions, namely, dipolar coupling, chemical shift anisotropy, and quadrupolar interaction, are usually not observable for soluble proteins. An average value is observed instead, which is zero in the cases of dipolar and quadrupolar interactions, and gives the isotropic chemical shift in the case of chemical shift anisotropy. Partly reintroducing an anisotropic interaction, most often dipolar coupling, is a popular and fruitful approach to gain structural information on biomolecules. Dipolar coupling can be partly reintroduced by a large variety of anisotropic ordering media. Magnetically aligned bicelles are often used as an ordering medium. Figure 3B gives a schematic idea of such a sample. The bicelles show macroscopic order that is induced by the magnetic field of the NMR spectrometer. A soluble biomolecule is restricted in its mobility by the presence of oriented bicelles, which basically form “walls” that hinder the reorientation of the investigated molecule. In some cases, a globular protein can have a partial interaction with the head groups of lipids and detergents of bicelles. Other alignment media may also show electrostatic interaction with the molecule under investigation. As a consequence, the investigated molecule is not fully free in its reorientation, but shows a weak preference for a certain induced orientation. The described weak alignment results in a weak dipolar splitting on the order of Hz to several tens of Hz. Since the full magnitude of the dipolar coupling is far larger—for example its value in an amide $^{15}$N–$^{1}$H bond is around 15 kHz,$^{85,86}$—these weak induced dipolar couplings are termed “residual dipolar couplings” (RDCs). RDCs are invaluable parameters in biomolecular structure determination, since they contain information on global molecular structure as well as dynamic information.

RDC measurement was successfully utilized in the study of protein structure using solution NMR experiments.$^{87}$ It was soon demonstrated that RDCs can be used to determine the relative orientation of domains in multidomain proteins.$^{88}$ Since then, this field has expanded, and the utilization of RDC data has been applied widely. An especially intriguing application of RDC studies is in the study of dynamics of biomolecules such as proteins and RNA.$^{89–91}$ A comprehensive overview of pulse sequences used to measure RDCs can be found in the literature.$^{92}$ RDCs are most commonly recorded for amide protons of folded proteins, but can also be determined and utilized for methyl and methylene groups$^{93}$ and in unfolded proteins.$^{94}$ A software dedicated to the analysis of RDC data in structural terms is available.$^{95,96}$ Prediction of the alignment that a given molecular structure will experience in a particular ordering medium has been achieved for purely steric interaction$^{97}$ as well as steric and electrostatic interactions with the ordering medium.$^{98}$ A dedicated software for prediction of alignment from structure (PALES) has been developed.$^{99}$

Some studies have reported specific effects when bicelles were used to collect RDC values. It was found that the presence of two transmembrane domains of the human glycine receptor GlyR in low- $q$ bicelles—which in the absence of protein would tumble isotropically—impose a weak alignment and made the measurement of RDCs possible. Magic angle spinning (MAS) was used to compare these values to isotropic values.$^{100}$ RDCs could even be measured on a peptide embedded in isotropic bicelles that were aligned in stretched polyacrylamide gels.$^{101}$ However, exposing some proteins to bicelles as alignment medium may have adverse effects. For example, in SDF-1/CXCL12, a cardioprotective chemokine, the presence of aligned bicelles for RDC collection was found to favor the presumably inactive dimeric state of the protein.$^{102}$

Magnetically aligned bicelles represent one of the many alignment media used in the study of RDCs. Other orienting media include bacterial phases, stretched polyacrylamide gels, C$_6$F$_{14}$/n-hexanol mixtures, and liquid crystals. Covalently attached paramagnetic tags provide another option to weakly align molecules. Tabulated overviews of alignment media are given in Prestegard et al.$^{92}$ and Tolman and Ruan.$^{89}$ The parallel use of different alignment media can give additional insight, allowing for the resolution of ambiguities and the determination of generalized order parameters. Using 18
different independent ordering media, recognition dynamics on time scales up to μs could be observed in ubiquitin. Since biciples do not play a distinctive role of their own in RDC studies, we do not intend to treat them isolated in the context of the current review article. Instead, we refer the reader to a wide variety of review articles that continue to be published on the topic of RDC studies in structural biology. Excellent review articles can be found on RDC studies in general, on proteins, and on RNA and DNA. The potential of RDC methods in high-throughput studies for structural genomics has also been pointed out.

5. INTERACTION OF SOLUBLE PROTEINS WITH ISOTROPIC BICELLES

The combination of soluble globular proteins with isotropically tumbling biciples (Figure 3D) has been used repeatedly to study protein–membrane interaction. For example, solutions of isotropic biciples modulate the amyloid formation of full-length prion protein. This study did not use NMR spectroscopy, but it is in line with the increasing evidence that lipid membranes play an important role in the formation of amyloid fibrils. In an NMR study of an enzyme, cobra venom phospholipase A₂, isotropic biciples were used as substrate to monitor enzyme function. Binding of the cytosolic domain of rhomboid protease to isotropic biciples has been studied. IFABP, a soluble shuttle protein that transfers hydrophobic ligands to and from membranes, was investigated in the presence of isotropic biciples, and the potential to map out the membrane binding region was reported. For Arf1, ADP-ribosylation factor 1, measurements of effective rotational correlation time were used to characterize the binding of myristoylated and nonmyristoylated Arf1 to biciples with q ranging from 0.75 to 3.5. The structure of the N-terminal activation domain of Formin C was determined (pdb-id 2L1A). The structure of this regulatory domain was found to change significantly in the presence of DPC micelles containing negatively charged phosphoinositides, but not in the presence of phosphatidylycholine micelles and isotropic biciples. For each HAMP signaling domain of four different proteins, two α-helical segments were structurally characterized in the presence of biciples and strong differences were found in α-helical propensities, hinting at possible regulatory dimerization mechanisms. BclXL, extra-large apoptotic repressor protein, was investigated in q = 0.5 biciples to study dimerization in the presence of ligand and lipid bilayers. Membrane binding and phosphatidic acid interaction of the FRB domain of human TOR was probed in different neutral and negatively charged membrane mimetics, including biciples.

6. ARE BICELLES SUITABLE TO STUDY MEMBRANE PROTEINS?

Today, almost 20 years after the first description of bilayered oriented aggregates consisting purely of phospholipids in 1992 and the introduction of the term “bicelle” in 1995, this question has to be seen as purely rhetorical. In a large number of instances, bicelle environments have been found superior to micelle preparations.

It was realized very early on that the enzyme diacylglycerol kinase (DAGK) is active in biciples, while activity is lost in micelles. DAGK activity was quantified under a large number of conditions. HIV envelope peptide was studied in bicle and micelle samples, which were both weakly aligned in a strained gel. In this study, structure determination using RDCs revealed that micelles induce a curvature in the peptide that is not present in a more natural biclele environment. Similarly, structural differences between micelles and isotropic biciples were found for BtuB, a 22-stranded β-barrel protein, by site-directed spin labeling and EPR spectroscopy. It was shown here that oriented biciples preserve structure even better than isotropic biciples. The protein Smr, staphylococcal multidrug resistance pump, binds substrate in isotropic biciples, but shows only reduced or unspecified binding in a number of detergent systems. High quality solution-state NMR spectra were recorded and unambiguous assignments of 55% of the amide and Cα positions were possible. The authors point out the importance of a functional assay to unambiguously identify the functional state of a protein. In their study, Smr was shown to be functional in biciples. Then, protein spectra recorded in biciples were taken as a point of reference to identify detergent systems that support function. In the case of MerF, a bacterial mercury transporter, where no assay for protein function is easily available, the similarity of micelle spectra to biclele spectra was taken as a criterion for proper refolding.

Bicelles where found to be superior to micelles in another respect: For lipolytic enzymes, in this case cobra venom phospholipase A₂, the phospholipids in the biclele can act as substrate and give insight in enzymatic mechanism. Here, short-and long-chain phospholipids were found to be hydrolyzed with similar efficiency. This application and the examples presented previously clearly prove that bicleles have established their place in the structural study of membrane proteins and are regularly found to give superior results.

7. PROTOCOLS FOR RECONSTITUTION OF PROTEINS INTO LIPID BICELLES

It has to be noted that the quality of the achievable results depends primarily on the quality of the biclele sample that is to be investigated. Especially in the case of membrane proteins, this may prove to be very difficult. Whenever a membrane protein is to be embedded in bicleles, there are multiple ways in which these bicleles can be prepared.

Figure 4 gives a schematic overview of preparation protocols. (A) Detergent may be added to preformed proteoliposomes, containing the reconstituted membrane protein of interest. This may be done gradually, resulting in a “q-titration” and subsequently investigating aligned and isotropic bicleles. (B) Detergent-solubilized protein may be brought in contact with lipid vesicles, disrupting the vesicles and at the same time inserting the membrane protein. (C) In cases were protein can be solubilized without a detergent, pure protein may spontaneously insert into preformed bicleles. (D) For membrane proteins that have an extraordinarily stable fold, it may be possible to prepare a lyophilized mixture of protein, lipid, and detergent, which forms a biclele sample upon addition of buffer. A comparison of different preparation protocols and optimization of all parameters may critically improve the quality of the resulting NMR sample. Optimization of q-ratio in isotropically tumbling bicleles can differentiate between mobile and structured residues in embedded proteins, as demonstrated.
for seven membrane proteins consisting of one to seven α-helices.132 Protocols for the production and reconstitution of G protein-coupled receptors for structural biology studies have recently been reviewed.133,134

8. SOLUTION NMR STUDIES OF MEMBRANE-ASSOCIATED PEPTIDES AND PROTEINS IN NEAR-ISOTROPIC BICELLES

Solution-state NMR spectroscopy is a well-established experimental technique and offers a tremendous wealth of proven tools to answer almost any question on the structure and dynamics of small soluble proteins.20–23 Solution-state NMR spectra are characterized by nuclear resonances of very small line width resulting in highly resolved spectra with the option of resolving site-specific properties. The antimicrobial peptide mastoparan X was the first biological system to be studied by solution-state NMR in isotropic bicelles.135 More and more systems have been studied since then, with amazing results. In the following, a comprehensive overview of membrane proteins, protein fragments, and peptides that have been investigated in isotropically tumbling lipid bicelles is given (Figure 3C). The use of isotropic bicelles is a necessary criterion and is not reiterated in each case. Comprehensive galleries of high resolution structures of membrane proteins solved by solution-state NMR are presented in two recent review publications.24,25

8.1. Proteins and Protein Fragments

The structure of myristoylated Arf1, ADP ribosylation factor 1, was solved in q = 0.25 bicelles (pdb-id 2KSQ).136 It makes for a fruitful comparison to an earlier structure determination with neither bilayer nor detergent present.121 A novel experiment to measure one- and two-bond N−C couplings that are complementary to more common RDCs was developed and introduced in the study of Arf1.137 A myristoylated N-terminal fragment of the protein has been studied earlier in isotropic138 and aligned bicelles.139

The structure of OmpX, a bacterial outer membrane porin that forms an eight-stranded β-barrel, has been solved, and NOE crosspeaks that report on contacts between the protein and the DMPC and DHPC molecules in the bicelle have been observed.140 For BtuB, a 22-stranded β-barrel protein, an EPR study found that isotropic bicelles with q = 2.0 do not stabilize the native fold, but q = 4.0 bicelles do.127 Other EPR studies have shown that α-synuclein in bicelles forms a single extended α-helix rather than a helix−turn−helix structure141 and picked up typical periodicity for an α-helical segment of the M2δ subunit of the nicotinic acetylcholine receptor (AChR).142 The kinetics of nitroxide spin label reduction by ascorbic acid has been used to characterize membrane immersion of the M2δ-peptide of AChR in EPR experiments.143

For the seven-helix transmembrane receptor sensory rhodopsin II, a solution-state NMR structure was determined in micelles (pdb-id 2KSY). Measurements in bicelles were taken as an indication that the micelle structure is similar to the structure in lipid bilayers.144 Sensory rhodopsin and its apoprotein, opsin, were investigated in bicelles of very different q-values. In addition, phospholipids with varying chain length were used with either DHPC or CHAPS as solubilizing agents to find optimal conditions to keep the proteins stable.145 Opsin was tested in q = 1.0 bicelles for stability against urea unfolding in DMPC/DHPC and DMPC/CHAPS formulations. Higher stability was observed in DMPC/CHAPS bicelles by tryptophan fluorescence and far-UV circular dichroism.146 The coupling efficiency of rhodopsin and transducin was investigated in q = 2.8 bicelles by absorbance measurements and was found to be dramatically stabilized in bicelles containing 30% anionic lipids.147 An activated rhodopsin/transducin complex used as a constitutively active mutant of rhodopsin was prepared in q = 0.65 bicelles.148

It has been shown that both DMPC/CHAPS and DMPC/DHPC bicelles can be used to refold bacteriorhodopsin from a denatured state. Increased DMPC content was found to slow down the formation of a partially folded intermediate, which is ascribed to increased bending rigidity of the bilayer portion.149 The kinetic mechanism of SDS-denatured bacteriorhodopsin being refolded by stopped-flow mixing with bicelles was studied by pulsed oxidative labeling and optical spectroscopy.150 The successful stabilization of the seven-helix transmembrane opioid receptor ORL-1 in sterol/detergent micelles was attributed to the bicelle-like geometry of these mixed micelles.151

Smr, the small multidrug-resistance pump from S. aureus, is functional as a homodimer of four transmembrane α-helices each. Lipid bicelles were found to stabilize this functional form.128 A major review on solution NMR of membrane proteins has presented Smr as a prototypical example.43 Recently, a backbone assignment of the functional form was reported.152 EmrE, a small multidrug resistance transporter from E. coli known to be highly sensitive to its environment, was reconstituted into isotropic bicelles with improved sample stability and expanded lipid composition profile.153 It forms asymmetric antiparallel homodimers that were found functional in isotropic bicelles; global conformational exchange between identical inward- and outward-facing states was found and exchange rates were measured quantitatively.154 The protein MerF from the bacterial mercury detoxification system was investigated in isotropic bicelles and different detergent micelles. Similarity of micelle to bicelle spectra was used to find a micelle system supporting native protein fold. SDS micelles were chosen to solve the structure, which contains two parallel transmembrane helices.159 Similarly, for LR11/SorLA, a binding partner of the human amyloid precursor protein, a 1H−15N-TROSY spectrum recorded in isotropic bicelles was used as a ‘gold standard’ in screening for a suitable detergent system.155 Conformational equilibria of phospholamban, a single-pass transmembrane protein, were studied in neutral and negatively charged isotropic bicelles.156 This is part of a broader
A structure determination of the integrin αβ transmembrane (TM) domain was performed in both detergent micelles and isotropic bicelles. A kinked α-helical structure was found that was very similar in bicelles containing long-chain phospholipids varying in length and also in charged bicelles, but had distinct deviations from the structure determined from micelles. Similarly, a structure was determined for the integrin αIIbβ3 TM domain. Both integrin TM domains formed a stable heterodimeric complex whose solution NMR structure gives insight into integrin TM signaling. Methods for efficient construction of covalent TM complexes and high-throughput selection of membrane mimics were established using integrin αIIbβ3 as a model system; bicelles were identified as the best membrane mimic.

For the TM α-helix of BNip3, a prominent representative of apoptotic Bcl-2 proteins, a homodimeric structure was determined. The structures of several TM segments of receptor tyrosine kinases have also been elucidated. For the TM region of growth factor receptor ErbB2, a homotypic right-handed α-helical bundle was found. The monomers interact via an N-terminal double GG4-like motif. The TM regions of ErbB1 and ErbB2 form similar heterodimeric right-handed α-helical bundles by association of N-terminal GG4-like and glycine zipper motifs. The energetics and kinetics of the weak dimerization of the ErbB4 transmembrane domain has been investigated in isotropic bicelles with different protein to lipid ratios. For the TM domain of EphA1, the ephrin receptor tyrosine kinase, a dimeric right-handed α-helical bundle was found. A pH-dependent change in conformation was observed. The TM domain of EphA2 dimerizes in a left-handed α-helical bundle, interacting through an extended heptad repeat motif, indicating diversity in helix packing among receptor tyrosine kinases of the same family. A recent review article provides more details about studies on bitopic membrane proteins.

A 25-residue peptide from MARCKS-ED, the effector domain of the myristoylated alanine-rich C-kinase substrate, was synthesized. This segment, which reversibly binds the full-domain of the myristoylated alanine-rich C-kinase substrate (TM) domain was performed in both detergent micelles and isotropic bicelles. The bound proportion of DMPG triggers daptomycin oligomerization. The structures of three C-terminal analogues of the human AMP mastoparan X have since been refined and extended to solid-state NMR methods. The structure of the AMP alamethicin was solved and compared to results from a molecular dynamics simulation on a DMPC bilayer. The peptide was found in a transmembrane configuration, and its high degree of dynamics and heterogeneity could not be described by a single conformational model. Membrane binding of the magainin-derived AMP MSI-78 has been studied by 19F-NMR on a variety of fluorine-labeled analogues of MSI-78. For arenicin-2, an AMP from a marine polychaete, a bent β-hairpin structure was found in solution, which assembles into flat dimers in DPC micelles and retains this structure in DPC/DMPG bicelles. The structures of three C-terminal analogues of the human AMP β-defensin-3 showed that dimer formation and aconformation of well-defined structures upon interaction with lipid membranes contributes to compactization of positive charges within peptide oligomers and antimicrobial activity. Bicelles with a high bilayer content, \( q = 3 \), were used at low temperature to avoid magnetic alignment and rather observe solution NMR spectra in isotropically tumbling bicelles. The relevance and implications of solution NMR structures for the mode of a peptide’s action has been critically reviewed for amphibian AMPs, with a special focus on the synergy of different AMPs. Another review focuses on the role of membrane lipids in the action of AMPs as well as pore-forming peptides and proteins in general. Excimer fluorescence spectroscopy on an analogue of the lipo-AMP daptomycin in a q-titrated bicelle experiment showed that stochiometric binding of DMPG triggers daptomycin oligomerization.

The membrane-induced structure of a bee venom peptide melittin was found to be correlated with lipid fluidity. Melittin was also studied in discoidal aggregates formed when pegylated lipids are added to bilayers. These aggregates have a disk-like morphology similar to isotropic bicelles, and it has...
been argued that they are a superior membrane mimic in partitioning studies. Melittin was bound tightly in comparably large quantities to the rim of the stable and well-defined PEG-stabilized disks, which might be exploited for drug delivery purposes.\textsuperscript{198}

A structural study on a cell-penetrating peptide (CPP) transportan bound to neutral bicelles is reported (pdb-id 1SMZ).\textsuperscript{201} Transportan was further studied in neutral and partly charged isotropic bicelles.\textsuperscript{202} Penetratin, a cell-penetrating fragment of the Antennapedia homeodomain protein of Drosophila, was studied in two different bilayer mimetics.\textsuperscript{203} In addition, penetratin's dynamics and diffusion were studied using \textsuperscript{15}N relaxation and PFG NMR experiments.\textsuperscript{204} Membrane interactions of CPPs have been reviewed in a recent review article.\textsuperscript{205}

For two model transmembrane peptides, KALP-21 and KALP-23, changes in lipid dynamics were observed in bicelles with different bilayer thickness due to different long-chain lipid components, namely, DLPC, DMPC, and DPPC.\textsuperscript{206} The 22-residue model peptide P16 assumes a transmembrane orientation as determined by amide–water chemical exchange and lipid NOEs.\textsuperscript{207} (A parallel solid-state investigation on P16\textsuperscript{208} is described below.)

## 9. SOLID-STATE NMR STUDIES ON MAGNETICALLY ALIGNED BICELLES

Dramatic recent developments in pulse sequences, instrumentation, and sample preparations enabled high-resolution structural studies of biological solids using solid-state NMR spectroscopy. Solid-state NMR spectra are characterized by nuclear resonances of considerable line width due to anisotropic interaction which often make site-specific information hard to observe. However, numerous experimental strategies are available to overcome these obstacles. Solid-state NMR has been applied successfully to study a variety of membrane proteins and peptides in a large number of instances, and today it is fully established as a standard tool in the study of membrane protein structure, dynamics, and orientation.\textsuperscript{206} Since native membrane proteins are restricted in their isotropic reorientation by the lipid bilayer, they naturally display strong anisotropic nuclear spin interactions, making solid-state NMR the natural approach to study their properties. Two strategies have been developed to deal with strong anisotropic interactions. One of the approaches is magic angle spinning (MAS), which suppresses anisotropic interactions to render "solution-like" high-resolution spectra of solids. This approach enjoys the benefits from the use of ultrafast spinning, multidimensional pulse sequences, recoupling techniques to selectively measure an anisotropic interaction, homogeneous sample preparation, and low temperature capabilities. MAS techniques have been applied to proteins embedded in MLVs but only rarely utilized to study proteins incorporated in bicelles, as described in section 10. The second strategy involves the application of static solid-state NMR experiments on macroscopically aligned samples. Here, aligned bicelles are obviously a very helpful tool to achieve high-quality macroscopic alignment (Figure 3A). This section will first give a quick overview of solid-state NMR techniques that are designed especially for the study of proteins or peptides aligned in lipid bilayers. In the following, successful studies of membrane proteins or peptides embedded in aligned bicelles will be reviewed. Again, the aim of this section is to give a comprehensive overview of membrane proteins, protein fragments, and peptides studied in magnetically aligned bicelles. The use of aligned bicelles is a presupposition for each mentioned study and not stated explicitly each time.

### 9.1. Aligned Molecules Enable High-Resolution Molecular Imaging

**Use of Unaligned Lipids.** Solid-state NMR studies commonly utilize unaligned MLVs and aligned lipids under static conditions. Unaligned lipid bilayers are traditionally characterized using one-dimensional \textsuperscript{31}P chemical shift spectral lines as they can distinguish different phases (gel, lamellar, hexagonal, cubic) of lipids and can measure the changes in the dynamics and conformation of lipid headgroup. Therefore, \textsuperscript{31}P NMR experiments on unaligned MLVs have been well utilized to study lipid–lipid, lipid–protein/peptide, and lipid-drug interactions. In addition to \textsuperscript{31}P NMR, quadrupole coupling parameters measured from \textsuperscript{14}N (only from choline-containing lipids)\textsuperscript{210} and \textsuperscript{2}H (only from deuterated lipids)\textsuperscript{211} NMR spectra have been useful in probing the electrostatic interactions and dynamics associated with the lipid headgroup. \textsuperscript{2}H NMR has also been used to measure the order/disorder of C–D bonds in different regions of a lipid in MLVs.\textsuperscript{212} While unaligned MLVs continue to be used in solid-state NMR applications, the use of aligned samples can provide more site-specific information on lipids and also from embedded peptides/proteins.

**Approaches to Prepare Aligned Lipid Bilayers.** Macroscopically aligned lipid bilayer samples can be prepared using three different approaches. The first approach uses the mechanical alignment of lipids between glass plates.\textsuperscript{213,214} This approach has been used in the structural studies of membrane proteins and peptides. The main advantage of this type of sample is that various combinations of lipids can be incorporated. But the main disadvantages are (i) it takes more than a day to prepare samples in spite of using the recently developed naphthalene procedure\textsuperscript{214} to speed up the hydration process. (ii) The filling factor in the NMR sample coil is poor as the glass plates occupy most of the space. (iii) A flat-coil probe is needed to accommodate the glass-plate sandwich sample.

The second approach uses aluminum oxide nanodiscs to mechanically align lipid bilayers.\textsuperscript{215–217} While this approach renders a quick way to prepare aligned samples, the extent of alignment is small for high-resolution structural studies on membrane proteins. Nevertheless, this approach has been well utilized in various applications.\textsuperscript{218} The third approach is to use magnetically aligned bicelles as explained earlier. Some of the main advantages are as follows: (i) It is easy to prepare well-hydrated bicelles. (ii) Bicelles of varying sizes can be prepared. (iii) It is devoid of glass plates and therefore the filling factor is very high. (iv) The presence of bulk water can enable native-like folding of membrane proteins particularly those containing large water-soluble domains. As mentioned above, bicelles are increasingly applied because of these advantages.

**Examining the Quality of Aligned Lipid Bilayers.** The quality of alignment of lipids is commonly examined using a \textsuperscript{31}P chemical shift spectrum. A well-aligned lipid bilayer sample exhibits a narrow spectral line revealing the direction of alignment relative to the external magnetic field. One-dimensional chemical shift spectra of \textsuperscript{1}H, \textsuperscript{31}P, and \textsuperscript{13}C nuclei from aligned samples are easy to obtain and therefore commonly used to study lipids and their interactions with other embedded molecules. Quadrupole coupling spectra of \textsuperscript{14}N and \textsuperscript{2}H nuclei from aligned samples are also useful to study
lipid bilayers as mentioned above. Spectra of peptides or proteins labeled with $^{15}$N, $^{13}$C, $^2$H or $^{19}$F embedded in aligned lipid bilayers are useful in determining their orientation relative to the lipid bilayer surface or normal.

9.2. Custom-Tailored NMR Experiments

One-dimensional static solid-state NMR experiments on aligned lipid bilayers containing peptides or proteins labeled with $^{15}$N, $^{13}$C, $^2$H, or $^{19}$F have been well utilized in various instances. For example, it has become very common to determine the overall orientation of an antimicrobial peptide in a lipid bilayer in order to understand its mode of action. However, the spectral resolution rendered by a 1D spectrum is insufficient to resolve spectral lines arising from a uniformly labeled membrane protein. On the other hand, a well-equipped arsenal of solid-state NMR experiments is ready for the study of macroscopically aligned proteins and peptides. A central role is taken by two-dimensional separated-local-field (SLF) experiments that correlate $^{15}$N chemical shift and $^2$H--$^{19}$F dipolar coupling, thus reporting on the geometry and alignment of peptide groups. The prototype of such experiments is the polarization inversion by spin exchange at the magic angle (PISEMA) experiment. PISEMA experiments display characteristic patterns that report on a molecule’s orientation with respect to the lipid bilayer. Most notably, $\alpha$-helices give circular spectral patterns known as polarity index slant angle (PISA) wheels which can be used to infer a peptide’s tilt within the bilayer.

The analysis of PISA wheels requires detailed knowledge of the chemical shift anisotropy tensor within the geometry of an amide bond. PISEMA can be improved by using different mixing schemes in the indirect dimension, e.g., BB-PISEMA, HIMSELF (heteronuclear isotropic mixing spin exchange via local field) or HERSELF (heteronuclear rotating-frame spin exchange via local field) or SAMMY. Methods to enhance sensitivity in SLF experiments, in heteronuclear correlation spectroscopy, and in proton evolved local field experiments using Hadamard encoding, all in oriented systems, have been reported. Cross-polarization can be made more efficient by performing multiple repetitive contacts. Specific strategies for backbone assignment in oriented samples have been described, utilizing controlled reintroduction of proton spin diffusion, or mismatched Hartmann–Hahn magnetization transfer, or a previously assigned isotropic chemical shift spectrum. De novo sequential assignment was demonstrated for 26 residues out of the 31-residue membrane protein sarcolipin in uniformly $^{15}$N-labeled form. Influence of orientational and motional narrowing of lineshapes in PISEMA-type experiments has been investigated theoretically and experimentally and can potentially yield an additional angular constraint in structure calculations.

Aligned bicelles have been established for EPR spectroscopy and can give similar information on the tilt of transmembrane domains. The structural and dynamic properties of the necessary spin label have been characterized. For a transmembrane $\alpha$-helical segment of the M2 subunit of the nicotinic acetylcholine receptor, a helical tilt of 14° with respect to the bilayer normal was determined by EPR. It was established experimentally on the M2$\alpha$ peptide as well as theoretically that unoriented bicelles can be used for the same purpose. The helical tilt of phospholamban, a regulatory single-pass transmembrane protein, and its segmental mobility were probed by EPR in oriented bicelles.

9.3. Proteins and Protein Fragments

Solid-state NMR methodology is routinely applied to membrane proteins and protein fragments embedded in magnetically aligned bicelles. Among the most challenging targets for structure elucidation are G-protein coupled receptors (GPCR) that consist of seven transmembrane $\alpha$-helices. A high-resolution structure determination was recently achieved for a GPCR by solution-state NMR (ref 144 and section 8.1), and solid-state NMR investigations of GPCR are becoming increasingly common. The chemokine receptor CXCR1, another GPCR, was successfully incorporated in aligned bicelles and studied in solid-state NMR. Solution-state NMR assignment experiments could identify only a limited number of resonances in CXCR1. A combination of solution- and solid-state NMR experiments was used to characterize local and global dynamics of this protein and binding to its ligand interleukin-8. The C-terminal domain of human cannabinoid 1 GPCR was found to modulate the structure of its membrane environment. A reconstitution protocol for Y$_2$, a human GPCR, into lipid bicelle environment has been described. Reviews are available on the expression, solubilization, and reconstitution of GPCR in membrane mimetic environments including bicelles.

The second transmembrane domain (TM2) of the $\alpha_4$ subunit of the neuronal $\alpha_4/\beta_2$ nicotinic acetylcholine receptor (nAChR) was prepared as a selectively $^{15}$N-Leu labeled peptide. In the presence of unlabeled TM2 from the $\beta_2$ subunit, it forms functional $(\alpha_4)_{4}(\beta_2)_{5}$ pentamers, for which the tilt and azimuthal rotation of the $\alpha_2$-TM2 subunit could be determined. Structural changes were observed in the presence of anesthetic drug molecules.

The membrane protein p7 from hepatitis C virus could be expressed as a fusion protein to give sufficient yield for NMR samples; incorporation in aligned bicelles was successfully achieved. PISA wheels corresponding to two $\alpha$-helical transmembrane segments could be identified with the help of a truncated construct corresponding to the second transmembrane $\alpha$-helix. Further experiments including a “titration” gave RDC and isotropic NMR data and helped refine the structural model to define seven distinct structural regions within the 63-residue protein. The three-dimensional structure of the membrane-spanning domain of Vpu from HIV-1, consisting of a single $\alpha$-helix, was solved, and changes were investigated in the presence of channel-blocking drugs. A review comparing both viral proteins, p7 and Vpu, is available. The major coat protein of bacteriophage PF1 was investigated in diphenyl bicelles that orient with their lipid bilayer normal parallel to the applied magnetic field. A combination of solution- and solid-state NMR yielded a full structure of the protein in lipid bilayer environment, which consists of a tilted transmembrane $\alpha$-helix and a second, orthogonal $\alpha$-helix.

MerFt, a truncated construct of the bacterial mercury transport protein MerFt, was found to consist of two membrane-spanning $\alpha$-helices and a short loop region. An $\alpha$-helical transmembrane segment from the pore forming component TatA of the twin-arginine translocase was found to have a tilt of 17° with respect to the bilayer normal. Further studies on larger fragments revealed that a second adjacent $\alpha$-helix is immersed in the phospholipid headgroup region.

For TOMPa, the transmembrane portion of bacterial outer membrane porin A which spans the membrane as an eight-stranded $\beta$-barrel, successful reconstitution in aligned bicelles...
was reported. For OmpX, another eight-stranded β-barrel, orientational constraints from solid-state NMR were combined with atomic coordinates from X-ray crystallography to give the protein’s overall orientation within the bilayer.

Structural propensities of an exceptionally long linker region from the human voltage-gated K⁺ channel hERG were found to be dependent on bicelle composition, as determined by solution and solid-state NMR experiments. In addition, this study used isotropic bicelles to characterize membrane binding affinity of hERG. The interaction of two different Arg-rich paddle domains of voltage gated K⁺ channels with the lipid bilayer have been characterized in oriented bicelles. The myristoylated 14-residue peptide Cat14 from the catalytic unit of cAMP-dependent protein kinase A was incorporated in q = 3.5 bicelles to study interaction with lipids by 2H NMR. A myristoylated N-terminal 14-residue peptide from pp60c-src was studied in neutral and acidic bicelles.

9.4. Peptides

The effect of bound Menk, the neuropeptide methionine enkephalin, on different types of lipid bilayers was investigated using oriented bicelles. Binding and arrangement of aromatic pharmacophores were investigated for the δ-opiate DPDPE. The neurotoxin pardaxin permeabilizes vesicles more efficiently by pore formation than by disruption. It assumes a transmembrane orientation in neutral bicelles, while it is restricted to headgroup contacts in DMPC-doped bicelles.

The binding of two fragments of rat islet amyloid polypeptide (rIAPP, also known as rat amylin) to aligned bicelles was investigated. The nontoxic rIAPP(1−37) binds to the bilayered regions of low curvature, while the toxic rIAPP(1−19) binds to detergent-rich regions of high curvature. Neither peptide caused membrane fragmentation.

The consequences of hydrophobic mismatch and peptide sequence were investigated in the transmembrane model peptide P16. A 21-mer cytotoxic model peptide modified with crown ethers stabilized bicelle structure and orientation and perturbed the lipid polar headgroup conformation. For a similar 14-mer peptide modified with crown ethers, no significant change in the morphology and orientation of bicelles was found.

The antimicrobial peptide (AMP) mastoparan X was found to orient perpendicular or parallel to the membrane normal of the bilayer patch depending on bilayer charge. Various AMPs found in the skin of Australian amphibians were characterized in aligned bicelles and compared to results obtained in mechanically aligned DMPC bilayers. For the AMP novicidin, significant structural and conformational differences were observed between ordinary DMPC/DHPC bicelles and bicelles with analogous ether-lipids. This result has a considerable impact, since ether-lipids are regularly employed to increase sample stability and lifetime. For lactophorin I and II, two AMP found in bovine milk, tertiary structure and membrane orientation were determined. The bee venom peptide melittin disrupts aligned q = 1.8 bicelles, unless they are protected by embedded cholesterol.

9.5. Cytochrome b₅

The Ramamoorthy laboratory is currently investigating cytochrome b₅, a membrane-anchored protein that is mostly found in the endoplasmic reticulum of liver cells and plays a supportive role in the biodegradation of a large number of toxic and drug molecules. We have given a comprehensive overview of the structure and function of cytochrome b₅ and its physiological interaction partners, especially with respect to NMR spectroscopic investigations. Briefly, cytochrome b₅ supports members of the cytochrome P450 family of enzymes to oxidize their substrates, which are typically drug or toxic molecules that need to be prepared for excretion. Cytochrome b₅ accelerates the oxidation process for numerous cytochrome P450 isozymes, most probably by transferring an electron.

The function of cytochrome b₅ is intimately linked to its topology, which is represented as a cartoon in Figure 5. A globular domain contains a heme prosthetic group which carries electrons that are to be transferred to cytochrome P450. However, this transfer is not possible unless the globular domain is attached to the membrane of the endoplasmic reticulum by a membrane anchor. The membrane anchoring part of cytochrome b₅ consists of a putatively α-helical transmembrane domain and a flexible linker region. It was shown that the length, but not the actual amino acid composition of the linker domain is critical for electron transfer to cytochrome P450. The globular domain of cytochrome b₅ truncated from the holo-protein has been the subject of extensive structural investigations. Very few structural studies, however, have been conducted on cytochrome b₅ in its holoform of 16.7 kDa molecular weight. This is particularly unsatisfactory as cytochrome b₅ function is critically dependent on the presence of its membrane anchor. For this reason, we decided to investigate holo-cytochrome b₅ in lipid bicelle environment.

Bicelle samples made from DMPC and DHPC at a q ratio of 3.5 were used to incorporate full-length rabbit cytochrome b₅ into a bilayer environment. The quality of the bicellar phase in terms of orientation and mosaic spread was monitored by 31P NMR. Figure 6A shows the 31P NMR spectrum of a pure q = 3.5 DMPC/DHPC bicelle preparation. Two well-separated resonances originate from the phosphocholine headgroups of DMPC and DHPC and report on their orientation. The very narrow width of the lines reflects the high quality of alignment reached in this case. Upon addition of 1 cytochrome b₅ molecule per 86 DMPC molecules, there are still two distinct 31P NMR resonances, shown in Figure 6B. However, the width and overall shape of the lines indicates that only a very limited

Figure 5. Schematic of the topology of full-length cytochrome b₅ (cyt b₅, yellow). The protein consists of an α-helical transmembrane domain, a highly flexible linker region, and a globular domain that carries a prosthetic heme molecule. Also shown is the bicellar environment used to macroscopically align cyt b₅ with respect to the external magnetic field, B₀.

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amount of macroscopic orientation is reached. In samples with a lower ratio of protein per lipid, macroscopic orientation can be recovered. Figure 6C shows the $^{31}$P NMR spectrum of a sample with 170 DMPC molecules per cytochrome $b_5$. Macroscopic orientation is recovered, but the quality of alignment is still poor. A ratio of 212 DMPC molecules per cytochrome $b_5$ is necessary to reach a quality of alignment that is comparable to pure DMPC/DHPC bicelle samples (see Figure 6D).

After establishing experimental conditions for oriented bicelle samples with very low mosaic spread, we used uniformly $^{15}$N-labeled cytochrome $b_5$ to investigate its molecular structure. Figure 7B shows one-dimensional $^{15}$N NMR chemical shift spectra obtained using different NMR pulse schemes. Shown in the figure are cross-polarization (CP) spectra obtained at contact times of 3.0, 0.8, and 0.1 ms. The CP spectrum at 0.8 ms contact time shows the strongest overall signal intensity. It displays intensity in the spectral range around 125 ppm; this range is typical for isotropic amide $^{15}$N chemical shifts. In addition, signal is observed in a range around 80 ppm, which indicates that the protein is macroscopically oriented and experiences $^{15}$N chemical shift anisotropy. When the CP contact time is lowered to 0.1 ms, the intensity in this spectral region stays visible, while it drops severely in the region around 125 ppm. This is consistent with macroscopically oriented protein components with high molecular order resulting in fast and efficient polarization transfer from protons by strong $^{15}$N−$^1$H dipolar couplings. If, on the other hand, the contact time is increased to 3.0 ms, intensity in the spectral region around 85 ppm is lost because of increased relaxation due to strong $^1$H−$^1$H dipolar couplings. The signal in the isotropic range around 125 ppm is still visible at 3.0 ms contact time, indicating that it arises from highly mobile regions of the molecule. In mobile molecular segments, dipolar coupling is decreased by motional averaging, resulting in a less efficient cross-polarization and slower relaxation losses. Remarkably, the spectral intensity around 125 ppm is observable in refocused-INEPT experiments that are usually applied to soluble proteins in solution-state NMR (see top spectrum in Figure 7B). We conclude that our bicelle samples indeed confer macroscopic orientation to cytochrome $b_5$ and that by different NMR pulse schemes, we can distinguish domains of cytochrome $b_5$ that display different degrees of molecular mobility.

In order to understand how the spectral properties observed in 1D $^{15}$N NMR experiments relate to the domains of cytochrome $b_5$, we conducted two-dimensional NMR experi-

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**Figure 6.** Proton-decoupled $^{31}$P NMR chemical shift spectra of different bicelle preparations used in the study of cytochrome $b_5$. Phosphorus-$^{31}$ NMR spectra report directly on the quality of bicelle alignment. (A) Pure $q = 3.5$ DMPC/DHPC bicelles. Bicelles in the presence of one cytochrome $b_5$ molecule per 86 (B), 170 (C), and 212 (D) molecules of DMPC. (E) Bicelles in the presence of both cytochrome $b_5$ and cytochrome P450. The resonance observed at approximately 0 ppm originates from phosphate buffer.

**Figure 7.** Structural observations on full-length cytochrome $b_5$ using solid-state $^{15}$N NMR spectroscopy. (Top) Molecular model of cytochrome $b_5$ in a lipid bilayer. (Middle) One-dimensional $^{15}$N NMR spectra and (bottom) two-dimensional H Roths spectrum of uniformly $^{15}$N-labeled cytochrome $b_5$ embedded in DMPC/DHPC $q = 3.5$ bicelles.
ments that correlate 15N chemical shift with 2H−15N dipolar coupling. Heteronuclear isotropic mixing by HIMSELF experiments are advantageous compared to the more common PISEMA-type experiments. HIMAGE experiments correlate the 15N chemical shift of amide nitrogens with the 2H−15N dipolar coupling they exhibit with the directly bonded amide proton. Since the 15N-CSA-tensor and the 2H−15N dipolar interaction do not line up perfectly, their correlation gives distinctive spectral shapes that are characteristic for certain types of a secondary structure. Figure 7C shows the HIMSELF spectrum of a uniformly 15N-labeled cytochrome b5 in DMPC/DHPC q = 3.5 bicelles. A circular spectral pattern is observed in the region around 85 ppm of 15N chemical shift, which was found to represent rigid molecular regions. Such a circular spectral pattern is associated with transmembrane α-helices and is referred to as a PISA-wheel. We conclude that the spectral region around 85 ppm represents the membrane anchor of cytochrome b5, which transverses the lipid bilayer as a transmembrane α-helix. This is especially remarkable since in our preparation protocol, cytochrome b5 was added to preformed bicelles. Hence, the transmembrane α-helix of cytochrome b5 is actually able to insert spontaneously into lipid bilayers. It has to be noted that the observed PISA-wheel of Figure 7C is far from perfect. This may be related to the fact that a proline residue is found in the center of the α-helical domain, since proline residues are known to induce kinks in α-helices. A best-fit analysis of the observed PISA-wheel revealed a tilt angle of 15° for the transmembrane domain of cytochrome b5 with respect to the lipid bilayer normal.

In conclusion, we found that magnetically aligned bicelles are a suitable environment to study the structure of cytochrome b5 in the membrane. Figure 7A shows a molecular model that summarizes the results. The membrane anchor was found to span the lipid bilayer as a rigid α-helix. It may be kinked due to a proline residue that is shown in green in the model. The globular domain is highly mobile and is tethered only very loosely to the membrane anchor by the linker region. The linker region is an example of an “entropic chain” or intrinsically disordered region.

Opening ways for further investigations, it was possible to extend the studies to other types of lipid bicelles. These modified bicelles have tunable bilayer thickness and charge, and they subtly influenced cytochrome b5’s structure. Moreover, and most remarkably, it was found that cytochrome b5 can be studied in complex with its most important interaction partner, cytochrome P450, an integral membrane protein of approximately 56 kDa molecular weight. Figure 6E shows the 31P NMR spectra of DMPC/DHPC bicelles harboring the cytochrome b5/cytochrome P450 complex. The quality of alignment was very high and gave HIMSELF spectra of comparable quality as the one shown in Figure 7C. Our bicelle samples also gave interesting spectra under magic-angle spinning.

Recently, new methods have been developed for the study of proteins incorporated into aligned bicelles containing cytochrome b5. By using two-dimensional proton-evolved local-field (PELF) in combination with WIM and COMPOZER-CR pulse sequences, we were able to clearly resolve peaks for both the transmembrane and soluble domains of bicelle-bound cytochrome b5. Furthermore, the helical tilt angle of cytochrome b5’s transmembrane helix was determined to be 13° with respect to the bilayer normal, with 8° of fluctuation. Dipolar enhanced polarization transfer (DREPT) is based on INEPT-type magnetization transfer; it eliminates 2H−2H dipolar interactions, making it highly sensitive and especially useful for the detection of side-chain dynamics in proteins embedded in aligned bicelles. When applied to cytochrome b5, it was found that the immobile transmembrane domain and the mobile soluble domain can be selectively observed by changing the length of the refocusing period, as seen in Figure 8. In addition, by utilizing 2D DREPT it was possible to measure 15N−1H dipolar couplings in histidine, tryptophan, and arginine side chains in cytochrome b5. Further studies using DREPT may provide important insights into the exact orientation of these side chains and the mechanism of cytochrome b5’s function.

10. MAS STUDIES ON BICELLES

The broad nuclear resonances observed in solid samples are dealt with by two different major strategies. In the preceding section, the use of macroscopically aligned samples was described. The alternative approach of magic-angle spinning (MAS) is more common but has found less application to bicellar samples. This section presents the application of MAS to proteins in bicelles.

10.1. Bicelles under MAS

By running a solid-state NMR experiment while spinning the sample at the “magic angle” of 54.7° relative to the external magnetic field, the dominant anisotropic interactions dipolar coupling, chemical shift anisotropy, and quadrupolar coupling can be suppressed. In particular, very narrow lines can be observed since homogeneous line broadening caused by strong dipolar coupling is absent under magic angle spinning (MAS).
Thus, high-resolution spectra (comparable to those of solution NMR) full of dynamic and structural information about bilayer-associated membrane proteins can be obtained. The effect of sample spinning on aligned bicelles has been studied in detail. When aligned bicelles are spun at an angle smaller than the magic angle, their bilayer normal aligns perpendicular with respect to the spinning axis. This alignment was used to determine signed values of residual dipolar coupling for a myristic acid derivative in the bicelles. At angles larger than the magic angle, the bilayer normal aligns parallel with the spinning axis. When the spinning axis approaches the magic angle, mosaic spread increases. Finally, at the magic angle, bicelle alignment vanishes; that is, there is no more preferred orientation for the bilayer normals and they distribute isotropically.

MAS experiments conducted on spinning bicelles have proven useful in the study of peptides and proteins. It has been found that the width of lines observed in spinning bicelles can be reduced by a factor of 3 compared to what is typically observed in lipid vesicles. A direct comparison of line width in bicelles and proteoliposomes under MAS was performed and interpreted with respect to theoretical expectations. This study used the pentapeptide methionine-enkephalin and NeuTm35 for demonstration, a 35-residue transmembrane study used the pentapeptide methionine-enkephalin and cross-polarization (RampCP) for polarization transfer to incorporated into bicelles than into liposomes. By using ramped bilayer normal.297

A tilt of 15° CSA and isotropic chemical shift. The measured values indicate transfer, refocused INEPT (RINEPT) pulse sequence, and spinning spectra yielded precise values for 13C- and 15N-nuclei under MAS, it was possible to observe not only backbone amide-15N resonances, but also arginine and lysine side chain-15N resonances of cyt b5 in bicelles. The study of residual dipolar couplings (RDC) of soluble proteins in the presence of oriented bicelles can also benefit from sample spinning. Because bicelle alignment is suppressed by MAS, the same sample can be used with and without MAS to record isotropic and weakly aligned spectra, respectively. Recording both spectra in the presence of bicelles keeps the influence of protein–bicelle interaction identical for both spectra. This has been demonstrated for ubiquitin, where precise site-specific 15N CSA tensors could be determined. Similarly, the use of variable-angle spinning has improved the observation of scaled RDC in ubiquitin. This was later expanded to include very strong RDC and chemical shift variations of ubiquitin’s 15N resonances. For the second and third transmembrane segment of GlyR, the human glycine receptor, incorporation into low-δ bicelles resulted in weak alignment; MAS and static spectra yielded J-couplings and RDC values with high accuracy.

For the fourth transmembrane domain of the γ-subunit of the nicotinic acetylcholine receptor in high-δ bicelles, a similar comparison of static and spinning spectra yielded precise values for 13C- and 15N-CSA and isotropic chemical shift. The measured values indicate a tilt of 15° for the transmembrane domain with respect to the bilayer normal.

10.2. Cytochrome b5 in Bicelles under MAS

The Ramamoorthy laboratory has utilized MAS techniques to study cyt b5 (cyt b5) in bicelles. Using full-length rabbit cyt b5 with uniform 15N-labeling and 5 kHz MAS, we found that cyt b5 yielded higher resolution spectra when incorporated into bicelles than into liposomes. By using ramped cross-polarization (RampCP) for polarization transfer to 15N-nuclei under MAS, it was possible to observe not only backbone amide 15N resonances, but also arginine and lysine side chain-15N resonances of cyt b5 in bicelles (Figure 9A). We also studied uniformly 13C,15N-labeled cyt b5 in bicelles under MAS. Experiments using nuclear Overhauser effect (NOE) transfer, refocused INEPT (RINEPT) pulse sequence, and RAMP for polarization transfer to 15C-nuclei were compared, finding that NOE experiments produced particularly strong resonances for the carbonyl carbons of cyt b5, while RINEPT produced strong signals for the acyl carbons of the bicelle lipids, DHPC and DMPC. In addition, two-dimensional CTUC COSY and DARR experiments were conducted on cyt b5 to record 13C−15C correlations. Because of the high resolution achieved in these MAS experiments, it was possible to assign peaks to specific amino acids. It was found that RINEPT and CTUC experiments are best suited for the study of cyt b5’s mobile domain, while RampCP and DARR experiments showed resonances mainly from the immobile, transmembrane domain of the protein (Figure 9B).

We conclude that MAS on bicelles is especially useful in the study of membrane bound proteins with soluble domains, such as cyt b5, where both highly mobile and immobile regions are present. MAS techniques may become invaluable tools in the structure determination of such proteins.

11. LIPID–PROTEIN INTERACTIONS BY SLF-NMR SPECTROSCOPY OF BICELLES

In addition to the study of protein structure and dynamics, bicelles can also be used to determine the effect that a protein has on the surrounding lipid bilayer. Traditionally, the addition of deuterated lipids to liposomes has been used to determine site-specific lipid order parameters in 2H NMR experiments. 2H NMR investigation of deuterated lipid probes has been applied to bicelles; but deuteration of lipids is costly and was found to alter thermostropic behavior while site-specific assignment is ambiguous. As an alternative approach, SLF experiments were employed to study the long-chain lipid molecules in a bicelle. SLF experiments, such as the PISEMA experiment, are described in section 9.2. In the current context, they correlate 1H−13C
dipolar coupling (which gives information on local order parameters) with $^{13}$C chemical shift (which gives unambiguous identification of each $^{13}$C-site in the lipid molecule). Most notably, these experiments do not need isotopic labeling since natural-abundance $^{13}$C-nuclei in the lipid molecules give sufficient intensity to carry out two-dimensional experiments. By using the HIMSELF scheme, $^{229}$ SLF could be successfully applied to magnetically aligned bicelles. $^{303,304}$ Advantages of using laboratory-frame SLF experiments to measure small heteronuclear dipolar couplings from mobile regions of bicelles and also from embedded ligands have also been demonstrated. $^{305}$

By running an SLF experiment on bicelles, $^1$H–$^{13}$C dipolar couplings can be measured for each resolved $^{13}$C-site, yielding an order parameter profile as given in Figure 10. The open symbols in Figure 10 give a $^1$H–$^{13}$C dipolar coupling value determined for each resolved $^{13}$C-site in the DMPC molecule as shown on top of Figure 10. As is known, large dipolar couplings, that is, order parameters, are found in the rigid bilayer region of the glycerol backbone, while order parameters become small toward the mobile end of the choline headgroup and especially toward the very mobile ends of the acyl chains. The SLF experiment was used to characterize order parameter profiles for bicelles in a wide range of temperatures, hydration levels, and $q$-ratios. $^{38}$ It was also used to characterize the bilayer perturbation caused by an antimicrobial peptide MSI-78 $^{306}$ and the ligands desipramine $^{305}$ and curcumin. $^{306}$ By use of the SAMMY pulse sequence, $^{230}$ similar investigations were carried out on bicelles containing the transmembrane segment of phospholamban, an antimicrobial peptide (KIGAKI)$_3$, and cholesterol. $^{307}$ Recent studies have shown that analogous experiments are also possible under magic angle spinning conditions. $^{308–310}$ 2D RPDLF experiments have been used to determine the interaction of dendrimers with lipid bilayers. $^{311}$

As a demonstration of the potential of this application of SLF experiments, we present our unpublished results on myelin basic protein (MBP). MBP is a major component of the myelin sheath in the central nervous system of higher vertebrates and is implicated in multiple sclerosis. MBP is intrinsically unstructured in solution, but binds to bilayers and may assume tertiary structure in membrane environment. $^{312–315}$ Previously, we had used $^{31}$P- and $^1$H NMR to investigate the interaction of MBP with MLVs and mechanically aligned bilayers. $^{316}$ For conducting SLF experiments, we incorporated bovine MBP at 10 wt % into $q = 3.5$ DMPC/DHPC bicelles. The profile of $^1$H–$^{13}$C dipolar couplings acquired on MBP-containing bicelles is shown as filled symbols in Figure 10. When compared to results on identical bicelles without MBP, shown as open symbols, several observations can be made. For the choline headgroup, almost no change in local order is observable. In the glycerol backbone and the acyl chains, the order parameter profile shows an overall decrease in order parameter to about 90%. For the $\text{g}$-carbon of the DMPC glycerol backbone, three different $^1$H–$^{13}$C splitting values were observed. This may indicate a tight and specific interaction of MBP with this particular site of the DMPC molecule in the lipid bilayer.

12. SUMMARY AND OUTLOOK

Lipid bicelles have added yet another facet to the tremendous wealth of lipid morphologies. $^{317}$ The structural and thermodynamic properties of bicellar phases have been understood in detail, and powerful techniques are available to quickly and reliably establish phase diagrams and characterize morphological properties. Properties of bicellar formulations are so well understood and so many specific compositions have been established that today they are routinely used in an ever increasing number of structural studies of membrane proteins.

Some very specific and unique properties of bicelles lie at the core of their success, not only in NMR but in fields as diverse as crystallography, chromatography, and drug formulations. Bicelles are the most versatile model membrane system presently available. Dozens of compositions have been tested and used, and there is most probably potential for more. Bicelles with small $q$ values can be used for high-throughput solution NMR studies, while those with large $q$ values are ideal for solid-state NMR studies. Since bicelles contain bulk water, they enable natural folding of even those membrane-associated proteins that contain large soluble domains and therefore render the feasibility of physiologically relevant structural studies. This property of bicelles is therefore well suited to investigate the structures of single-pass in addition to multipass transmembrane proteins that are unusually difficult to study due to their combination of hydrophobic and water-soluble domains. In fact, very few structures of single pass TM proteins are reported in the PDB. The most serious drawback of bicellar phases is the fact that they are found in restricted regions of the phase diagram that are bound by limiting temperatures and hydration levels that may be restrictive for some application. Continuous effort is put into developing new formulations where the region of bicellar phase is extended. New developments include adding designed lipids with biphenyl-containing acyl chains $^{31,62}$ or stabilizing bicelles by sialylated lipids. $^{68}$

Today, bicelles have perfused most areas where structure and dynamics of membrane proteins are investigated. In fact, the current contribution reads like a cross-section through the entire field of structural biology of membrane proteins. As a result, the scope of this contribution—proteins studied in bicelles—may feel too restricted in the very near future, since the focus of the most interesting studies will be purely on...
structural and functional aspects of membrane proteins. Bicelles as the actual tool used to gain these insights—however powerful they may be—will step into the background.

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Dr. Ulrich H. N. Dürr heads the NMR spectroscopy department at INFAI GmbH in Cologne, Germany. He graduated with a physics degree from Göttingen University (1999) and obtained his doctorate with Prof. Anne S. Ulrich at the University of Karlsruhe (2005), using 19F-NMR to study membrane-active peptides. He conducted postdoctoral studies on membrane protein structure with Prof. Ayyalusamy Ramamoorthy at the University of Michigan (2005–2007) and with Prof. Markus Zweckstetter at the Max-Planck-Institute for biophysical chemistry (2007–2009). At Michigan, most of his research involved bicelles. Currently he is interested in medical applications of NMR spectroscopy.

Melissa Gildenberg is a medical student in the University of Iowa Carver College of Medicine’s Medical Scientist Training Program. She graduated from the University of Michigan with a degree in biochemistry in 2012. There, she worked in Prof. Ramamoorthy’s laboratory where her research focused on the development of low temperature bicelles for high-resolution structural studies of membrane proteins using solid-state NMR spectroscopy.

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. In 1992, he joined JEOL Ltd (Tokyo, Japan) as a Scientist in the laboratory of Professor Kuniaki Nagayama to develop NMR techniques for 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 for atomic-level resolution imaging 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 proteins, antimicrobial peptides, and bone. His recent research revealed the high-resolution structure, dynamics and interaction of membrane-bound cytochrome-P450-cytochrome-b₄, and atomic-level mechanisms of membrane permeation/disruption by amyloid peptides and antimicrobial peptides. More details about his current research can be found at www.umich.edu/~ramslab.

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