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When detergent meets bilayer: Birth and coming of age of lipid bicelles

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1. Introduction

Lipids spontaneously form bilayered structures when brought into an aqueous environment. This is the foundation in the architecture of biological cell membranes. However, lipid bilayers do not lend themselves easily to common biophysical studies; be it of the bilayer itself or of embedded membrane proteins. Detergents, on the other hand, form small aggregates known as micelles that readily solubilize membrane proteins and are well-suited for numerous biophysical methods. However, they are not excellent models of biological membranes as they may denature the structure of a protein and the curvature of the micelle may impose a non-native protein folding. When lipid and detergent meet in an aqueous environment, entities with wholly different properties are formed: lipid bicelles. Bicelles are made of patches of lipid bilayers that are either encircled or perforated by detergent ‘rims’. They combine the advantages of both components alone (micelle and lipid bilayer), namely being good models for a biological membrane and having advantageous properties for biophysical experiments. An additional advantage of certain bicelle preparations is their tendency to macroscopically align when brought into a magnetic field. This fact has been exploited not only in the highresolution structural and dynamics studies of membrane proteins, but also for globular proteins using nuclear magnetic resonance (NMR) experiments.

Fig. 1 gives a graphical introduction to the two types of bicellar phases most commonly employed. At a high detergent concentration and low temperatures, isotropically tumbling disk-like aggregates are formed, the so-called isotropic bicelles (Fig. 1B). At a high lipid concentration and in certain temperature ranges, extended bilayered lamellae are formed that are perforated or delimited by detergent, and have the potential for magnetic alignment (Fig. 1D). Cryo-transmission electron microscopy (TEM) micrographs (A, C) of bicelles taken from the literature [1] are also included in Fig. 1.

Since their first description in 1988, the great potential of bicelles in the study of membrane proteins and proteins in general has been realized. A steady stream of remarkable insights and applications has emerged that is still growing in size. In the present contribution, we will give an introduction to the properties of lipid bicelle phases with an emphasis on NMR

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experimental measurements. In addition, we will discuss some of the most exciting recent applications of bicelles in the structural and dynamic studies of membrane proteins.

2. Different types of model membranes used in NMR studies

2.1. Vesicles

Lipid membranes and membrane proteins have been investigated by NMR spectroscopy for more than 40 years. Numerous types of membrane samples and preparation protocols have been developed. An overview of the most popular ones is depicted in Fig. 2. The choice of a certain type of sample depends on the task in hand. The simplest type of lipid bilayer sample is formed spontaneously when pure lipids are mixed with a buffer. In this case, multilamellar vesicles (MLVs) are formed, which are approximately spherical aggregates up to tens or thousands of μm in diameter where large numbers of lipid bilayers are stacked in the fashion of an onion. Fig. 2A gives a simple schematic idea. By means of sonication, or by extrusion through the pores of suitable membrane filters, MLV samples can be converted into small unilamellar vesicles (SUVs, Fig. 2B) made up of small spheres consisting of only a single lipid bilayer. The size or size distribution of SUVs is governed by the preparation method employed and is usually much more homogeneous when filter extrusion is employed [2–4].

For use in conventional NMR spectroscopy, vesicle samples have a drawback: they do not reorient fast on the NMR time scale, hence the anisotropic NMR interactions (chemical shift anisotropy, dipolar coupling, quadrupolar coupling) dominate the spectra. This is in stark contrast to systems usually investigated in solution-state NMR spectroscopy, where fast molecular reorientation makes anisotropic interactions collapse to an average isotropic value. A situation of fast isotropic tumbling can be recreated in detergent micelles (Fig. 2E) which do not form bilayers and, hence, give unreliable environments for mimicking membrane conditions and may not always preserve the membrane protein structure and function. Alternatively, anisotropic NMR interactions can be suppressed by rapid spinning of a vesicle sample around a certain angle with respect to the external magnetic field. This angle (54°74′) is dubbed as the ‘magic angle’ in solid-state NMR, and magic-angle spinning (MAS) is one of the most commonly used approaches in solid-state NMR today. MAS techniques are also used in the specialized area of membrane proteins in vesicle samples, where MAS has been successfully applied for a long time. MAS at suitable intermediate speeds can also be used to determine the tensorial quantities of anisotropic NMR interactions by analysis of spinning sidebands [5]. Since the tensors of anisotropic NMR interactions observed in peptides or proteins embedded in lipid bilayers are dependent on molecular alignment, information on the global orientation of such molecules can be extracted from these tensors [6,7].

2.2. Mechanically-aligned lipid bilayers

In an alternative experimental approach, anisotropic interactions are not suppressed but are put to good use. By a number of preparation protocols it is possible to generate macroscopically oriented samples of lipid bilayers. In a well-oriented sample, all lipid bilayers are parallel to each other and enclose a single, well-defined angle between their bilayer normal and the external magnetic field of an NMR spectrometer. As a result, proteins or peptides embedded in the lipid bilayers are also partially oriented with respect to the external magnetic field. The result is a dramatically improved spectral resolution, and the possibility to infer geometrical information from the observed values of anisotropic spin interactions [8]. Most commonly, anisotropic interactions are thus used for angle measurements and therefore allow imaging molecules at atomic-level resolution.

The conceptually most straightforward preparation method for macroscopically oriented lipid bilayer samples involves stacks of glass plates. The space between two adjacent glass plates in the stack is filled with the lipid preparation of interest [9]. The lipid preparation is applied to the glass plate in dehydrated form and is then hydrated in an environment of suitable temperature and humidity. Relative humidity is controlled over saturated solutions of a salt [10]. During the hydration process, the lipids form well-hydrated bilayers that orient parallel to the glass plates of the stack. Since thousands of lipid bilayers are typically enclosed between each pair of glass plates, the data measured in NMR spectroscopic experiments are not influenced by the glass plates that provide the mechanical support. A (smaller) number of lipid bilayers oriented between two glass plates is shown schematically in Fig. 2C. Initial studies on a membrane-embedded fd coat protein [11], gramicidin A [12,13], and the retinal of bacteriorhodopsin [14] have established the usefulness of the technique. The Ramamoorthy laboratory has made numerous contributions to the development and application of macroscopically oriented glass plate samples. It was shown that the preparation is feasible over a wide range of temperatures [15] and that the quality of orientation can be decisively improved by including sublimable solids, such as naphthalene or para-dichlorobenzene, in the preparation process [16]. The mechanism of membrane disruption by antimicrobial peptides has been investigated in stacked glass plate samples [17–20], as well as the action of cell signaling peptides [21] and the membrane interaction of myelin basic protein [22].

2.3. Anodic aluminum oxide nanodisks

Anodic aluminum oxide (AAO) is another viable support material for macroscopic alignment. AAO is a porous material that is perforated by highly parallel hollow cylinders ranging in diameter between several nm and several hundred nm [23]. It is commercially available in flat disks of approximately 60 μm height commonly used as a filter material [24], but is equally suitable as an orienting medium for bilayer samples. A fully hydrated liposome preparation can be applied to an AAO disk using an ordinary pipette. Upon contact with the disk material, the lipid immediately covers the surface of the pores, giving macroscopically oriented cylinders or ‘nanotubes’ of lipid bilayers which may contain embedded or attached protein or peptide (Fig. 2D) [25]. The geometrical and dynamic properties of lipid bilayers in AAO have been carefully characterized [26,27]; the latter contribution includes a detailed description of the preparation protocol.

The incorporation of the transmembrane (TM) domain TM-A of the membrane protein acetylenase CREP-1 was a first proof that integral membrane proteins can be studied in AAO-immobilized lipid bilayers [28]. Similarly, a transmembrane domain of the M2 protein from influenza virus was investigated in lipid nanotubes, including the use of two-dimensional ^{1}H-^{15}N-PISEMA spectroscopy [24]. This contribution also discusses the high conductivity of AAO for heat, which may potentially be advantageous in dissipating the heating effects of sophisticated NMR pulse sequences. Rhodopsin, a full-length integral membrane protein with seven transmembrane α-helices, has been successfully aligned in nanotubes [29]. Most remarkably, it was found that rhodopsin retains its binding affinity for G protein in the nanotube environment. In the Ramamoorthy laboratory, immediate alignment in AAO was employed to study membrane damage caused by amyloid-forming peptides [30]. Such a study is not possible using the glass plate samples where the peptide aggregation is much faster than the hydration step necessary in preparing stacked glass plate samples.

Other innovative media examined for the incorporation of membrane proteins include lipoprotein particles [31–34] and amphipols [35].

2.4. Bicelles

Bicelles, the topic of this review article, are a type of lipid sample that combines the advantages of most of the sample preparations introduced so far. Bicelles are formed when bilayer-forming long-chain lipids are mixed with detergent molecules. Under certain conditions, the two components mix to form aggregates, but are spatially separated into a central portion forming an actual lipid bilayer, surrounded or interspersed with ‘rims’ of detergent molecules. Fig. 2F shows the bilayer patch in light gray and the detergent rims in dark gray. At low concentration of a long-chain lipid, the aggregates tumble at a rate that is fast on the NMR time scale, almost comparable to the tumbling rate of detergent micelles (Fig. 2E). In addition, they offer a bilayer environment for embedded membrane proteins, making them a far more realistic membrane mimetic than detergent micelles. The term ‘bicelles’ was coined to denote such bilayer-containing mixed micelle-like aggregates, and the fact that diacylglycerol kinase retains enzymatic activity in bicelles but not in micelles was a proof of their advantageous properties [36].

When the concentration of a long-chain lipid is increased beyond a certain threshold, another advantageous property of bicelles is observed: they assume a well-defined orientation with respect to the magnetic field, see Fig. 2G for a graphic representation. This behavior is rooted in the geometric shape and the anisotropic magnetic susceptibility of bicelles. Hence, macroscopically ordered bicelle samples can be prepared with a degree of alignment that is typically much higher than what is possible in glass plate samples (Fig. 2C) and anodic aluminum oxide material (Fig. 2D). Magnetically-aligned bicelles are often used in solid-state NMR experiments, but can easily be converted into the isotropic bicelles introduced above, which are more amenable for solution-state NMR experiments. Hence, lipid bicelles form an important bridge linking solid- and solution-state NMR approaches. Today, lipid bicelles are a frequently utilized tool in NMR studies of protein structure in both soluble and membrane-bound proteins [8,150,172,236].

It needs to be mentioned that a suitable choice of lipid environment is equally important in crystallization assays of membrane proteins [37,38], and that bicellar lipid samples have also found application in this field. In particular, crystallization of bacteriorhodopsin (bR) from bicelle preparations at 37 °C yielded a novel monomeric structure for bR [39]. A similar result was later obtained at room temperature using different bicelle formulations [40]. For bR, crystallized from bicelle formulations, characteristic properties in optical spectroscopy were reported [41]. A mutant of bR was crystallized from bicelles to investigate structural differences of the proton pump bR and the homologous phototaxis receptor sensory rhodopsin II [42]. LeuT, a neurotransmitter sodium symporter, was crystallized in different crystal forms from bicelles in the presence of a substrate molecule [43]. Membrane protein crystallization using bicelles has recently received an exclusive review that reports a list of protein structures solved by bicelle crystallization [44]. A step-by-step protocol for using bicelles in high-throughput crystallization assays is available in video form [45].

Bicelle formation and morphology can be rationalized by the overall geometry of the involved molecules, depicted in Fig. 3. All involved molecules have hydrophilic head groups and hydrophobic acyl chains. In detergent molecules, on the one hand, the hydrophobic portion tends to be small when compared to the headgroup, giving the detergent molecule a conical overall shape (Fig. 3A). This geometry explains the tendency for detergents to form micelles in an aqueous environment (Fig. 2E). In lipid molecules, on the other hand, the number and size of the acyl chains tend to be larger giving the molecule a cylindrical overall shape (Fig. 3B), and resulting in the formation of bilayered structures in aqueous environments (Fig. 2A). The difference in overall shape results in a low miscibility of lipid and detergent. Hence, lipid and detergent phase-separate into bilayer and rim portions, which causes the formation of flat bilayer patches, i.e. bicelles.

A similar conical molecular geometry results when a polyethyleneglycol (PEG) strand is attached to the head groups of a lipid (Fig. 3C). PEGylated lipids insert readily into lipid bilayers. Mixed micelles of lipid and PEGylated lipid are formed at very high concentration of PEGylated lipids. In between both regimes, the formation of flat bilayer disks similar to isotropic bicelles was observed in mixtures of cholesterol, lipids and PEGylated lipids [46]. These discoidal aggregates do not show a propensity for magnetic- alignment and are not referred to as bicelles; no specific name has been introduced for them. Discoidal aggregates can be prepared without cholesterol [47,48]. Their phase behavior has been characterized [49,50]. The influence of the preparation path [51] and the effect of phospholipid hydrolysis [52] on aggregate structure have been studied. Special fluorophores were developed to study the highly curved regions of disks formed by dipalmitoylphosphatidylcholine (DPPC) and PEGylated distearoylposphatidylethanolamine (DSPE-PEG2000) disks by fluorescence resonance energy transfer [53]. Discoidal assemblies of PEGylated lipids have been investigated in a coarse-grained molecular dynamics simulation [54]. Biotin-derivatized discoidal aggregates can be used as model membrane biosensors in surface plasmon resonance [55]. A potential pharmaceutical application has been demonstrated for PEGylated discoidal aggregates, since tight binding of comparably large quantities of melittin to the rim of the stable and well-defined PEG-stabilized disks may be exploited for drug delivery purposes [56]. Similar assemblies were observed when PEGylated lipids were replaced by the more common detergents octaethylene glycol monododecyl ether ($C_{12}E_8$), hexadecyltrimethylammonium bromide (CTAB), and sodium dodecyl sulfate (SDS) [57]. In a similar approach, the lipid dimyristoylphosphatidylethanolamine (DMPE) extended by a diethylenetriaminepentaacetate (DTPA) group chelating a paramagnetic Tm^{3+} or La^{3+} lanthanide ion was used instead of PEGylated lipids. Formation of flat discoidal aggregates was observed, which in the case of Tm^{3+}-doping were slightly orientable in a field of 8 T [58]. The presence of 16 mol% cholesterol in discoidal aggregates of DMPC, DMPE-DTPA, and Tm^{3+} increases the disk size and the propensity for magnetic-alignment [318]. Linear peptide copolymers with hydrophilic and hydrophobic portions of different length have been designed that assemble into vesicle-like structures-dubbed as ‘polymersomes’-and bicelle-like flat discoidal structures [59].

3. What are bicelles?

3.1. General description

Bicelles are formed when long-chain lipids are brought in contact with detergent molecules. Long-chain lipids alone form lipid bilayers, while detergent molecules on their own form detergent micelles. When they are mixed, lyotropic mesophases are observed that combine the properties of both bilayers and micelles. In the simplest case, such a bicellar phase is made up of disk-like aggregates where a central bilayer patch is enclosed by a ‘rim’ of detergent molecules. However, this simple picture does not apply to all bicellar phases, see Section 5 on bicelle morphology. We want to stress three general traits of the bicelle preparations that are in general use today. First, they contain lipid bilayers with very similar properties as found in biological membranes. Second, these bilayers form flat patches rather than having a more or less pronounced curvature found in vesicles. Third, they can potentially be macroscopically aligned by an external magnetic field. This last trait is especially relevant for NMR studies.

3.2. Some landmarks in the development of bicelles

There are numerous systems that can be seen as bicelle precursors. For example, mixtures of sodium decyl sulfate, decanol, sodium sulfate, and water, form disk-like aggregates that orient in an external magnetic field [60]. Numerous other mixtures of hydrophobic, hydrophilic, and amphipathic molecules show similar behavior that has been extensively

investigated in the context of lyotropic liquid crystals [61,62]. However, these systems do not contain the lipids that are the constituents of biological membranes. At high water content of at least 80 wt.%, lipid vesicles can be macroscopically ordered by a strong magnetic field [63]. However, being vesicles, they do not contain flat bilayers. The same objection is true for small unilamellar vesicles that form spontaneously when long- and short-chain phospholipids are mixed in certain conditions. (Obviously, these conditions are different from the conditions favoring the formation of bicelles.) Spontaneously formed short-chain long-chain unilamellar vesicles (SLUVs) have been introduced by Gabriel and Roberts [64] and have non-spherical shapes [65]. SLUVs are still of considerable interest today, and recent contributions have investigated the relationship between SLUVs and bicelles [67,68]. SLUV and bicellar phases can be inter-converted, and the interconversion can be used to generate small unilamellar vesicles of path-dependent size distribution and characteristic shape [68]. Kinetically trapped unilamellar vesicles of uniform size were prepared by passing through a metastable bicellar phase at 10 °C [69].

Coexistence of spherical and disk-like aggregates has been found in mixtures of bile salt and phospholipids [70], and in 1988 magnetic field induced order was reported for such mixtures [71]. This result by the Prestegard laboratory was the first to meet all three criteria for bicelles that were postulated in the preceding section. A bile salt analog, 3-(cholamidopropyl)dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPSO), forms similar bicellar phases with lipids and gives advantageous experimental properties [72]. Lipid/CHAPSO complexes were structurally characterized [73] and used to study the conformation of glyco- and sulfo- lipids and their interaction with membrane binding proteins [74–76]. In 1992, it was demonstrated by the Sanders laboratory that the short-chain phospholipid dihexanoylphosphatidylcholine (DHPC) can be used to replace CHAPSO, eliminating the need for a potentially disadvantageous non-lipid detergent [77]. In the same contribution it was shown that long-chain phospholipids and DHPC in bicelles are spatially separated, presumably into bilayered patches and detergent rims. A first comprehensive review on bicelles, especially with regard to their potential in the study of membrane proteins, was published in 1994 [78] and has since been cited by most studies employing bicelles. Remarkably, the term “bicelles” was not introduced until a year later [36] to denote “binary, bilayered, mixed micelles bearing a resemblance to the classical model for bile-salt phosphatidylcholine aggregates (see Müller 1981)”, citing the article that is reference [70] in the current contribution.

3.3. Bicelle preparation

Most preparation protocols for bicelle samples are straightforward, highly reproducible, and do not require much time or effort. Protocols usually start with mixing of long-chain and short-chain lipids. The most popular choice is dimyristoyl-phosphatidylcholine (DMPC) as a long-chain lipid and dihexanoyl-phosphatidylcholine (DHPC) as a short-chain lipid (or detergent) component. Section 3.4 below gives an overview of other choices. Care must be taken to completely remove any residual organic solvent from the ingredients. The dry lipid-detergent is hydrated by adding a suitable amount of buffer, and after several cycles of cooling/heating (or freeze/thawing, if possible) the bicelle sample should be ready. It is also possible, but less convenient and common, to add detergent to pre-formed vesicular samples, giving the option for a titration with a short-chain lipid component. Preparation protocols have been reviewed [79,4], and Mäler and Gräslund [4] include a comparison to protocols to prepare MLVs and SUVs.

At least two parameters are necessary to describe the composition of bicelles and are needed to establish phase diagrams. The first one is the molar ratio of lipid molecules over detergent molecules. This ratio is usually denoted with the letter q. In the most common case of DMPC as a lipid component and DHPC as a detergent, it is

$$q=[\mathrm{DMPC}]/[\mathrm{DHPC}].$$

It has to be noted that q does not reflect the microscopic ratio in bicellar aggregates with full accuracy. That is because detergents typically have a noticeable solubility in buffer, better known as the critical micelle concentration (CMC). The CMC of DHPC is around 7 mM. Since the presence of 'free' detergent in micelles may reduce the amount of detergent available for bicelle formation, an 'effective-q'-ratio was suggested to take this effect into account [80]. Similarly, a corrected ratio q^* was used to describe the effect of non-negligible amounts of short-chain phospholipid invading the bilayer fraction [81]. The second important parameter is the level of hydration. It is usually given as the ratio between the added lipid and detergent weight with respect to the total weight (or volume) of the sample.

If the ratio of lipid over detergent is raised above a certain threshold, macroscopic alignment of the bilayer patches in a strong external magnetic field can be observed (Fig. 4). Most commonly, the normal of the bilayer patches aligns perpendicularly with respect to the external magnetic field. See Section 3.4 for modifications of this behavior of bicelles. Magnetic field-induced macroscopic alignment is observed for a magnetic field strength above about 1 T [82], which is well below the field strength in modern NMR spectrometers. It has been demonstrated that alignment can be achieved in a weaker field of 0.63 T as used in X-band EPR spectroscopy [83]. Alignment is possible even without an external magnetic field, as demonstrated by shear forces in a Couette flow cell, enabling linear dichroism experiments on embedded molecules [84]. It was shown that the presence of protein, namely the antimicrobial peptide gramicidin A, changes the alignment as a function of q, probably because the embedded protein increases the area of the bilayer patch [85]. Magnetic-alignment can remain present for days after the removal of the external magnetic field [86]. Bicelles with a high concentration of a long-chain lipid or a low hydration level tend to be very viscous. However, they become much more fluid at lower temperatures, which can be used for easy handling using common pipettes.

3.4. Popular bicelle modifications

Numerous other constituents have been used to make bicelles different from the most common choice, DMPC and DHPC. Most bicelle preparations that show magnetic field-induced alignment are oriented with the bilayer normal perpendicular to the applied magnetic field (Figs. 4B and 5B). In a number of cases, a parallel alignment would be better since it can give increased spectral resolution in NMR spectra. Paramagnetic lanthanide ions, especially ytterbium ions, Yb^{3+}, were found to bind to lipid bilayers and reverse the sign of the anisotropy in their magnetic susceptibility. This results in bicelles with parallel magnetic-alignment, often called "flipped" bicelles (Fig. 5A) [87,88]. Lipid-bound chelating agents can sequester the lanthanide ions and protect embedded proteins from possible disadvantageous effects [89]. By means of the chelating agent DTPA attached to DMPE lipids, it was possible to dope bicelles with Cu^{2+} ions [90]. The paramagnetic ions greatly increase spin–lattice relaxation, thus reducing T_1 by a factor of 10 and speeding up NMR experiments accordingly. On the other hand, no significant change in the line width was observed suggesting that the change in the spin–spin relaxation is negligible. Using relaxation enhancement by Cu^{2+} ions, it was possible to record SO-FAST- HMQC spectra within 1 h from a bicelle-embedded antimicrobial peptide that had no isotopic labeling [91]. A special lipid was synthesised that carries a biphenyl group in one of its acyl chains. It forms bicellar phases over a wide range of temperatures, but only for a fairly limited range of q-values. Because the biphenyl group causes the anisotropy of magnetic susceptibility to change its sign, these bicelles have their bilayer normals oriented in parallel to the magnetic field without the need for added lanthanide ions [92,93].

Bicellar phases change with the presence of ions and their concentration, with cations having the stronger effect [94]. Lipids with more stable ether-linkages between their acyl chains and the glycerol backbone have been used to increase the long-term stability of bicelles [95]. Phase diagrams for such ether-linked lipid bicelles have been established with respect to *q*-ratio, hydration level, and temperature [96]. Ether-lipid bicelles have also been applied to residual dipolar coupling (RDC) studies of globular proteins [97]. However, the structure of the antimicrobial peptide novicidin was found to be altered in ether-lipid bicelles when compared to DMPC/DHPC bicelles [98]. Small amounts of added PEGylated lipids are another choice to make bicelle samples more stable [99], but their use is not as widespread as that of ether-lipids. Rather, PEGylated lipids have been extensively used in the study of translational lipid diffusion (see Section 6). Doping of dilute oriented bicelle preparations with charged amphiphiles improves the stability and the degree of alignment [100]. The influences of lipid unsaturation and chain length on bicelle stability have been studied [101]. The addition of cholesterol and especially cholesterol sulfate was reported to stabilize bicelles thermally, with magnetic-alignment possible in an extended temperature range [102]. The influence of divalent cations, which are required by many classes of biomolecules for optimal activity, on the formation and alignment of DMPC/DHPC bicelles has been studied [319]. It was found that higher concentrations of Zn^{2+} and Cd^{2+} disrupt the magneticallyaligned phase, while Ca^{2+} and Mg^{2+} result in more strongly oriented phases.

A number of bicelle modifications have been designed to make bicelles resemble biological membranes more closely. One important characteristic of biological membranes is their bilayer thickness, which affects embedded proteins by means of hydrophobic mismatch [103,21]. Bicelles with different bilayer thickness have successfully been prepared [103]. The effect of varying chain-length in both lipid and detergent components has been investigated systematically [101]. Bicelles formed by DHPC and the phospholipid 1-palmitoyl-2-stearoyl-phosphatidylcholine (PSPC), that is 16:0–18:0-PC, have been investigated [104]. Another important characteristic of biological membranes is their composition with respect to head group charge and cholesterol content. Acidic bicelles with the addition of the charged phospholipid dimyristoylphosphatidylglycerol (DMPG) have been prepared and their stability has been investigated [105,106]. Other researchers have investigated the effect of cholesterol [107–110], of the unsaturated phospholipid 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) [100], and of polyunsaturated phosphatidylcholine [110] added to bicelles. It was shown that sphingomyelin, a common sphingo-lipid in mammalian membranes, and DHPC can form isotropic as well as aligned bicelles [111]. Morphological effects caused by the addition of ceramide to DMPC/DHPC bicelles were studied [112]. Cyclofos-6 as detergent formed bicelles with DMPC; since the critical micelle concentration of Cyclofos-6 is very low (and lower than that of DHPC), bicelle formation is observed down to and below 0.5% total lipid weight per volume [113]. The T-shaped molecule A6/6, which forms closed vesicles in water, was shown to mix with DPPC to form bicelles [114]. In studies of membrane proteins, it is practical that the detergent which is present in the end products of expression, purification, and refolding protocols may in some cases also be used as the short-chain or detergent component in aligned bicelles, as demonstrated for the detergents Triton X-100 [115] and dodecylphosphocholine (DPC) [116] in combination with DMPC.

4. Bicelles in electron paramagnetic resonance (EPR) spectroscopy

A large body of work, mainly by the Lorigan laboratory, has been dedicated to establish aligned bicelles as a membrane mimetic for electron paramagnetic resonance (EPR) spectroscopy studies. The first successful preparations of aligned bicelles were reported on bicellar samples doped with paramagnetic lanthanide ions [117,118]. As described above and shown in Fig. 5A, bicelles doped in this way orient with their normal parallel to the

external magnetic field. This effect is caused by the large positive anisotropies in the magnetic susceptibilities caused by suitable paramagnetic lanthanide ions (Eu^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}) in the lipid bilayer, which itself has a negative anisotropy in magnetic susceptibility. In the comparably weak magnetic field of 0.63 T used in X-band EPR, no spontaneous alignment of undoped bicelles was observed. However, using Dy^{3+} lanthanide ions which enhance the negative anisotropic magnetic susceptibility of the bilayer, it was possible to achieve alignment of bicelles with their normals perpendicular to the magnetic field (Fig. 5B) [119,83]. EPR studies require the addition of a spin label to the investigated bicelles to detect their orientation; doxyl-labeled cholestane and doxyl-labeled stearic acid were used for this purpose in the studies mentioned so far. Doxyl spin labels attached to different positions along the acyl chain of stearic acid have been used as a probe to study the dynamic properties of the bilayer portions of bicelles and have shown that they agree well with other biological and model membrane systems [120]. Bilayer dynamics and the effect of temperature were studied in more detail using spin-labeled phosphocholines [121]. In addition to the mentioned X-band studies at 9 GHz EPR frequency, bicelles were also introduced for the higher field strength used in Q-band [122] and W-band [123] spectroscopy at 35 and 94 GHz EPR frequency, respectively. At these higher magnetic field strengths of 1.25 T and 3.4 T, respectively, reduced concentrations of lanthanide ions were needed to achieve magneticalignment of bicelles. The magnetic-alignment of bicelles at high q was investigated by EPR and NMR spectroscopy [124].

EPR spectroscopy was applied to study the effect of cholesterol on bicelle model membranes using phosphocholine [125] and cholestane [126] with doxyl spin labels. At Q-band, higher order was observed in cholesterol bicelles than at X-band [126]. Another study on cholesterol in bicelles compared results from EPR and NMR spectroscopy [108]. Nitroxide spin labels can be introduced into proteins to study their properties by EPR spectroscopy, but labeling can potentially perturb protein properties. The sidechain conformation of a nitroxide spin label has been studied in the homodimeric protein CylR2 by comparing results from X-ray crystallography, EPR and NMR spectroscopy [127]. Structural and dynamic properties of the transmembrane protein phospholamban were determined by EPR of aligned bicelles [128]. The helical tilt of the M2δ transmembrane peptide of the nicotinic acetylcholine receptor in aligned bicelles was determined [129]. The analysis method, which is similar to the dipolar waves [130] used in solid-state NMR, is described in detail elsewhere [131]. Similar results could be obtained in unoriented samples [132]. The quenching of EPR spin labels by water-soluble reducing agents can be monitored in real-time to determine details of membrane immersion, as demonstrated for the M2δ peptide [133]. It was shown by EPR that α-synuclein in bicelles forms an extended α-helix rather than a helix-turn-helix structure [134].

5. Phase diagrams and morphology of bicelles

The morphology of bicelles is most often described as microscopic disks of lipid bilayers where the detergent covers the ‘rims’ (see Fig. 2F,G for schematic representations). This picture holds true only in a limited range of conditions, especially for low q-ratios and fast-tumbling bicelles, as seen in small-angle neutron scattering (SANS) [135] and electron microscopy (EM) [94]. In other conditions, more complex models are necessary. To derive unambiguous conclusions and models of complex morphologies, usually complementary techniques such as SANS or EM are needed in combination with NMR spectroscopy.

The influence of the ratio q between DMPC and DHPC on the formation of bicellar phases is shown in Fig. 6. At low q, small isotropically tumbling aggregates are observed, and no magnetically orientable phase is formed. Only in a limited range of q values between ~2.5 and 7.5 is it possible to obtain magnetic alignment. Above a q of 7.5, morphology similar to

multilamellar vesicles is present, where the detergent gathers in nanopore defects [136]. At higher temperatures, annealing of the nanopore defects is observed [137]. ^{31}P- and ^{2}H NMR are the methods of choice to study the structure and dynamics of lipid phases and to quickly map out phase diagrams [138–140]. Partial magnetic-orientation can be determined by the first spectral moments of ^{31}P NMR spectra [141]. The deconvolution of global orientational distributions of lipid bilayers and local order parameters of embedded molecular reporters has been demonstrated [142].

The phase behavior of bicelles, including phase diagrams, has been the objective of numerous studies. The limited miscibility of long- and short-chain lipid components dictates the separation into bilayer and ‘rim’ proportions and is thus the key to the phase behavior of bicelles [143,144]. It was possible to predict phase diagrams from miscibility properties [144]. Based on this insight, lipid mixtures forming bicellar phases in specified concentration and temperature ranges can be rationally designed [101]. As a general rule, it is observed that oriented bicellar phases are formed at temperatures above the main lipid phase transition between the gel state and the liquid–crystalline phase of the long-chain lipid component [145].

Phase diagrams have been established for pure phospholipids [146] as well as for lanthanide-doped bicelle mixtures [147]. Phase diagrams for DMPC/DHPC bicelles at various q-values have been reported [148]. Phase diagrams for ether-lipid bicelles have been established [96]. A review article dedicated to morphology and phase diagrams of bicellar phases is available [149]. A very broad general review on bicelles [150] offers a good focus on morphology. The feasibility of magnetic-alignment of dilute bicellar solutions has been investigated [151].

The model of flat, microscopic disks for bicelle morphology (Fig. 2F and G) is based on the fact that DHPC and DMPC are phase separated into bilayer and ‘rim’ regions in oriented bicelles [77]. A first geometrical model was developed for ideal disk-shaped bicelles [152]. This study also found a new proof in addition to the ones reported [77] that DMPC and DHPC are indeed spatially separated in oriented bicelles, and mentions the need for an experimental proof in the case of isotropic bicelles. This need was again stressed in a work on Mastoparan X in isotropic bicelles [153]. The flat disk model was proven to apply to isotropic bicelles by ^{31}P NMR, dynamic light scattering, and electron microscopy [154]. An independent proof was given by small angle neutron scattering and Eu^{3+}-doping [135]. Further support comes from a recent study of a protein embedded in isotropic lipid bicelles [155].

It has to be noted that from the beginning, the flat disk model has been seen as only tentative [77]. The existence of DHPC pores in DMPC lamellae (Fig. 7C) was clearly seen as an alternative structural model for aligned bicelles, and was termed the “Swiss cheese model” [88]. The high diffusion rate measured for tetramethylsilane (TMS) in oriented bicelles would require extensive transient edge-to-edge contacts in the disk model [156]. Measurements of high viscosity in orientable bicelles [157,158] contradict the theory of individual disks and suggest some entangling. The use of optical microscopy and SANS found yet another bicelle morphology, described as “wormlike micelles” and depicted in Fig. 7B [159]. The use of SANS experiments has in all cases been able to identify the morphology that is present in the bicellar phase [149]. It was possible to obtain very clear cryo-TEM micrographs for isotropic as well as oriented bicellar phases [1,160,161]. The kinetic pathway of the phase transition from bilayered micelles to perforated lamellae has been characterized [162]. Stimulated echo-pulsed field gradient (STE-PFG) experiments were used to measure water diffusion in bicelles [81]. In this study, the fraction of rim-

located and bilayer-embedded DHPC was determined as a function of q and T. As an additional parameter, q^*, the fraction between edge and planar phospholipid, was introduced.

A single contribution has claimed the observation of tight stacks of disk-like bicelles at very high hydration levels [163], similar to the elongated stacks reported for disk-like high-density lipoprotein (HDL) particles [31]. However, the stacking effect claimed for dilute bicelles has not been reported for other systems.

Molecular dynamics (MD) simulations have in a few instances been carried out on bicelle systems. Most studies to date have used coarse-grained models. Structures resembling bicelles were found in the spontaneous aggregation of DPPC into small unilamellar vesicles [164]. A special coarse-grained force field was developed to model zwitterionic lipid assemblies; in coarse-grained MD runs, bicelle-like invaginations referred to as 'buds' were observed to form from DPPC monolayers [165]. A coarse-grained force field called MARTINI was used to simulate discoidal aggregates of PEGylated lipids [54]. A coarse-grained simulation showed that functionalized carbon nanotubes and lipids form bicellar assemblies [166]. The influence of line tension on length and shape of bilayer edges was investigated by course-grained MD of lipid bilayer ribbons of different tail lengths [167]. Only recently, two atomistic simulations on bilayer ribbons were reported which provide insight into lipid behavior in bicelles: the effect of bilayer edge and curvature on the partitioning of lipids by tail lengths was investigated [168]. A two-step semi-grand-canonical mixed Monte Carlo/molecular dynamics approach found a possible mechanism for attraction and merging of DHPC pores [169]. The three-dimensional structure of glycolipids embedded in bilayers starts to be investigated by combined use of isotropic bicelle NMR experiments and molecular dynamics simulations [320].

6. Diffusion studies on bicelles

Molecular diffusion, particularly translational diffusion, is the most fundamental transport process in nature. Importantly, Brownian motion in lipid bilayers governs a variety of important biological processes that ranges from signal transduction to the transport of nutrients across cell membranes such that a significant body of literature is devoted to this subject matter. However, Brownian motion in lipid membranes can be extremely complex due to the heterogeneity of most biological systems; only a single elegant coefficient, namely the lateral diffusion coefficient, i.e. the component of the diffusion tensor that is perpendicular to the bilayer normal, is required to describe this complicated process. The elegance of this coefficient lies in the depth of information it holds, particularly the relationship between the diffusant and its environment. Lateral diffusion coefficients in a cell membrane vary by orders of magnitude: from the rapidly diffusing phospholipids to the slowly moving multi-helix membrane proteins. For species whose sizes are comparable to that of a lipid, their diffusion coefficients seem to follow the free volume model whereas larger species diffuse according to the hydrodynamic model. Currently, most diffusion coefficients are obtained via fluorescence recovery after photobleaching (FRAP) while single molecule tracking is increasingly used to study diffusion of molecules *in situ*. These optical techniques are valuable in providing detailed information on molecular diffusion; yet, these techniques only work if the molecules are inherently fluorescent. Therefore, an alternative approach is required for molecules that lack such an optical property and where the introduction of a fluorescent tag is not an option.

NMR spectroscopy provides an alternative means of measuring diffusion coefficients in a model membrane system. In particular, the pulsed field gradient (PFG) NMR technique introduced by Stejskal and Tanner [66] has evolved into a powerful tool capable of determining the diffusion coefficients for a wide range of macro-molecular systems. PFG

NMR allows for the rapid and simultaneous determination of multiple diffusion coefficients from different species, provided that their resonances are resolvable. The use of the stimulated echo (STE) was subsequently found to be advantageous for the measurement of diffusion coefficients [170]. The seminal publications [66,170] provide the basic theoretical framework for PFG NMR diffusion measurements. Therefore, only a brief description will be given here. The diffusion coefficient of a species, D, under isotropic condition, can be extracted from a STE-PFG NMR experiment by measuring the signal attenuation as a function of gradient duration (δ), gradient amplitude (g) and diffusion time (Δ) as indicated in the pulse sequence of Fig. 9. The observed NMR intensity is attenuated according to

$$\frac{I}{I_0}=\exp\left(-\gamma^2 D\delta^2\left(\Delta-\frac{\delta}{3}\right)g^2\right) \quad (1)$$

where γ is the gyromagnetic ratio of the observed nucleus and I and I_0 are the observed and the initial signal intensity, respectively [66,170].

Lateral diffusion in a lipid bilayer environment is anisotropic such that the diffusion coefficient is represented as a diffusion tensor according to

$$\begin{pmatrix} D_\perp & 0 & 0 \\ 0 & D_\perp & 0 \\ 0 & 0 & D_\parallel \end{pmatrix}. \quad (2)$$

There is uniaxial symmetry about the bilayer normal and so only two principal components are required to represent the system. These two components correspond to molecular diffusion along perpendicular ($D_\perp$) and parallel ($D_\parallel$) directions to the bilayer normal as illustrated in Fig. 8.

If the gradient is applied along the laboratory z-axis, chosen along the direction of the external magnetic field B_0 (see Fig. 8), then only the D_{zz} component of the diffusion tensor in the laboratory frame is measured. The relationship between D_{zz} and the principal components in the molecular frame, which align parallel and perpendicular with respect to the bilayer normal as described above, is given by

$$D_{zz}=D_\parallel\cos^2\theta+D_\perp\sin^2\theta \quad (3)$$

If the molecules forming the phase have a low critical micelle concentration (CMC), as is typical for lipids in general, then there is no detectable diffusion parallel to the normal of the bilayer, $D_{//}=0$. Thus, only the term $D_{zz}=D_\perp\sin^2\theta$ remains in Eq. (3). Interestingly, for the case where the bilayer normal is perpendicular to the applied gradient, the observed diffusion coefficient in the laboratory frame (D_{zz}) is equal to that of the lateral diffusion ($D_\perp$) in the lipid bilayer. Therefore, in order to measure the diffusion coefficient of a molecule in a lipid bilayer system, an aligned sample is required and bicelles provide a suitable medium for the purpose. Recently, Soong and Macdonald [171] demonstrated the feasibility of measuring diffusion of a polymer-grafted lipid, namely DMPEPEG2000, in magnetically-aligned bicelles using the STE-PFG NMR technique; the pulse sequence is shown in Fig. 9.

The diffusion coefficient is measured by monitoring the decay of the ^{1}H signal intensity of polymer-grafted lipids as a function of the gradient duration. The rapid internal motion of PEG yields a narrow ^{1}H resonance, which made the measurement feasible. The measured

diffusion coefficient was found to be comparable in magnitude to the FRAP measurements of DMPC in the liquid crystalline phase [171]. Therefore, this illustrates the viability of bicelles as a medium for the lateral diffusion studies of membrane-associated amphiphiles in a bilayer environment. The results also demonstrate the possibility of extracting the diffusion coefficient of a membrane protein via STE-PFG NMR, which is important to our understanding of protein trafficking in lipid bilayers. Therefore, this illustrates that bicelles are more than just a mere reconstitution medium for membrane proteins; in fact, they can be used as a platform for lateral diffusion studies via NMR. An interesting result regarding bicelle morphology was also obtained in these diffusion measurements: the molecular constituents exhibit free diffusion over micron distances. This observation is consistent with the perforated lamellae morphology of bicelles [172] and also corroborates with SANS data [159] and tetramethylsilane diffusion studies in dilute bicelle solutions [156].

Diffusion studies can also be done on bicelles with low q ratios ($0.5 \leq q \leq 1$). Due to their small size, these bicelles tumble isotropically, hence are suitable for high-resolution studies of membrane proteins. Interestingly, bicelles with a low q ratio exist as disk-like aggregates and are relatively monodisperse in their diameter and thickness. At $q = 0.5$, their estimated diameter is about 8 nm, double the assumed bilayer thickness [152]. The diameter of these fast-tumbling bicelles changes in the presence of membrane associated peptides. Recently, these bicelles have been used for the measurement of lateral diffusion coefficients of membrane binding peptides and the results are comparable to the literature values. While these experiments demonstrate the feasibility of using bicelles for diffusion studies, caution needs to be taken since the lateral diffusion coefficient is measured in a relative sense as all the components in the sample diffuse at different rates. In particular, the lipid bicelle as a whole does not provide a stationary reference frame, since it itself undergoes substantial lateral diffusion. Nevertheless, these bicelles are excellent model membrane systems for the investigation of binding kinetics of membrane-associated peptides and amphiphiles.

Diffusion measurements by PFG NMR were used to investigate the morphology of three media that are commonly used in the study of residual dipolar couplings, namely oriented lipid bicelles, cetylpyridinium bromide, and a mixture of PEG and *n*-hexanol [156]. The hydrodynamic radius of micelles and isotropic bicelles has been measured by bipolar pulsed gradients [173]. In these measurements, lysozyme was used as a reference compound to correct for the differences in hydrodynamic volume between dry and hydrated protein, bicelle, or micelle. The motion of constituent lipids in isotropic bicelles was studied by ^{13}C relaxation, PFG NMR, and EPR [174]. Local mobility was found to depend much more strongly on the detergent used than on bicelle size. Different peptides were found to have an impact on apparent dynamics. In a later study, diffusion measurements were used to study the size and shape of bicelles of low q ratio which tumble isotropically [175]. Three different long-chain lipid components, namely dilauroylphosphatidylcholine (DLPC), DMPC, and DPPC, were used to study lipid dynamics as a function of bilayer thickness [176].

Rotational diffusion concerns a molecule’s rotational degrees of freedom in contrast to translational motion discussed so far. Lipids as well as proteins in a bilayer sample experience rotational diffusion, which occurs most freely around the bilayer normal. It is usually fast on the NMR time scale, as long as proteins and peptides of moderate molecular size are studied. In NMR spectra, rotational diffusion becomes evident from the observed NMR lineshape as the averaging of anisotropic spin interactions along the axis of rotation alters the spectral lineshape and the magnitude of the interaction. As a consequence, multilamellar vesicles (MLVs) can potentially give the same information as macroscopically oriented samples [6,7,177,178], and the choice is mostly determined by ease of preparation and sensitivity issues. However, it was reported that structural measurements for the

antimicrobial peptide PGLa may be influenced by different hydration levels present in MLVs compared with data obtained from macroscopically oriented samples made of stacks of glass plates [7]. For disk-like bicelles, rotational diffusion about the bilayer normal was found to be fast enough to average the cylindrical distribution about that axis, even in the rare cases where the embedded protein itself does not undergo rotational diffusion fast enough for the required averaging [179]. As a result, even very large proteins and protein complexes are amenable to studies in unflipped bicelles.

7. Separated local field (SLF) NMR studies on bicelles

7.1. Separated local field spectroscopy

Though one-dimensional ^{31}P, ^{1}H and ^{13}C NMR experiments are commonly used to characterize magnetically-aligned bicelles [180], sophisticated two-dimensional (2D) experiments are essential to probe the order/disorder of lipid and detergent molecules in bicelles as well as to measure the interaction of ligand or peptide with hydrophilic and hydrophobic domains of bicelles. Experiments correlating short-range heteronuclear dipolar couplings with the chemical shift of a given nucleus, referred to as separated local field (SLF) experiments, are powerful in providing insights into the atomic-level structure of bicelles. Magnetically-aligned bicelles were found to be readily accessible objects in 2D SLF studies [181–183]. Fig. 10 demonstrates how 2D SLF gives piercing insights into bicelle properties. The chemical structure of a DMPC lipid molecule is shown in Fig. 10A. The commonly used labeling scheme is indicated: C_α, C_β, C_γ denote the carbon positions in the choline headgroup, C_{g1}, C_{g2}, C_{g3} make up the glycerol backbone, and C_1 to C_{14} the myristoyl chains. The very high concentration of DMPC molecules in typical bicelle preparations means that natural abundance ^{13}C NMR of DMPC carbons is sufficient for one- and two-dimensional NMR experiments.

The one-dimensional ^{13}C chemical shift spectrum in Fig. 10B shows well-resolved resonances for almost all positions; assignments are available for all resolved resonances and are given in Fig. 10B. In an SLF experiment, an incrementable time delay is added to evolve the transverse magnetization under heteronuclear dipolar couplings. The heteronuclear dipolar couplings (also known as the local field) associated with each chemically distinct carbon nucleus in the indirect dimension of the 2D spectrum are separated by the ^{13}C-chemical shift frequency; hence the name ‘separated local field’. The SLF spectrum in Fig. 10C shows well-resolved dipolar multiplets for each carbon group. A large splitting corresponds to a large molecular order parameter of the respective ^{1}H–^{13}C bond, whereas a small splitting indicates a small order parameter. The magnitude of the dipolar splitting can be plotted versus the carbon position in the lipid molecule, yielding an order parameter profile over the whole lipid bilayer. In this fashion the dynamics of the whole lipid molecule, from the headgoup to the acyl chains, can be thoroughly mapped out via one single 2D experiment. This is similar to order parameter profiles acquired by ^{2}H NMR spectroscopy [138,139], but does not need deuterated lipids and gives unambiguous site-specific information. Static and/or macroscopically oriented samples are not a necessary prerogative for SLF experiments; SLF is equally possible under MAS conditions by re-introducing dipolar interactions [184,185].

Spectroscopically, SLF experiments can be divided into two categories: laboratory-frame and rotating-frame experiments. In a rotating-frame SLF, the heteronuclear dipolar couplings are evolved under the influence of a spin-lock matched at the appropriate Hartmann–Hahn condition. Prominent examples include polarization inversion spin exchange at the magic angle (PISEMA) [186], *h*eteronuclear *i*sotropic *m*ixing *s*pin *e*xchange via *l*ocal *f*ield (HIMSELF) or *he*teronuclear *r*otating-frame *s*pin *e*xchange via *l*ocal *f*ield (HERSELF) [181], and magic-sandwich PISEMA (SAMMY) [187]. The PISEMA

experiment and its improved derivatives are commonly used to investigate the structure and dynamics of membrane proteins in aligned lipid bilayers as they provide ultra-high spectral resolution; since those are not the main focus of this article, they will not be discussed further. Readers can consult a review article on PISEMA spectroscopy [188].

In the second type of SLF experiment, laboratory-frame SLF, the heteronuclear dipolar couplings are evolved through the protons and are subsequently transferred, by cross polarization, to the dilute spin such as ^{13}C or ^{15}N for detection. An example is proton evolved local field spectroscopy (PELF) [321]. Rotating-frame SLF is preferable particularly when large dipolar couplings are measured by efficiently suppressing large homonuclear proton–proton dipolar couplings, as in a single crystal or in transmembrane proteins. Laboratory-frame SLF, on the other hand, is suitable when motionally averaged dipolar couplings are present. The latter case is found in magnetically-aligned bicelles. Consequently, laboratory- frame SLF experiments have recently been successfully applied to a number of biological problems.

7.2. Application of SLF to study bicelle properties

As a proof of feasibility, our laboratory has recorded 2D ^{1}H-^{13}C-PELF spectra on oriented q = 3.5 DMPC/DHPC-bicelles [189]. In addition, a 2D correlation spectrum of ^{13}C chemical shift and ^{13}C–^{31}P dipolar couplings was acquired, which reports on the conformation of the phosphocholine headgroup region of DMPC. Similar results were obtained with a solution-state NMR spectrometer using a SAMMY experiment [182]. In a subsequent study, experimental parameters were optimized to maximize sensitivity and resolution [180]. Ramped cross-polarization [190] and the FLOP-SY- 8 [191] scheme were found to be the best choices for polarization transfer and heteronuclear decoupling, respectively. By carefully calibrating the sample temperature and minimizing sample heating, spectral quality could be further improved. A direct experimental comparison of 2D SLF, PELF, and rotating-frame SLF schemes proved the superior quality of the PELF approach [183]. The method was further developed by reintroducing proton-spin diffusion to aid in resonance assignment of embedded molecules [192].

^{1}H–^{13}C-PELF spectra were used to characterize bilayer properties in bicelles for a wide range of conditions [193]. For different q-values, temperatures, and hydration levels, ^{1}H-^{13}C-PELF spectra were recorded. The extracted order parameter profiles are reproduced in Fig. 11. The order parameter profiles show that at higher q values (higher DMPC content, Fig. 11B), the bilayered regions become more rigid. The same is true for lower temperatures (Fig. 11C). More surprising is the fact that mobility seems to be fairly constant over a large range of hydration levels (Fig. 11D).

It was mentioned above that the order parameter profiles of lipid bilayers can be—and traditionally have been—recorded using ^{2}H NMR. A tremendous number of results has been achieved from ^{2}H NMR studies of lipid vesicles [138,139]. The determination of order parameters of lipid C–C-bonds from ^{2}H quadrupolar splittings has been thoroughly investigated, and a number of conformational details could be determined [194]. It has been shown that structural and dynamical details of DMPC in bicelles can be studied by deuteration [195]. However, there are serious drawbacks to this technique: a study [195] found that progressive deuteration has an effect on thermotropic behavior. The fact that partially deuterated molecules were employed, rather than single-site deuteration, makes their assignments ambiguous. Earlier results on DMPC conformation in crystals [196] or MLVs [177] were confirmed and refined for the bicelle environment. As described, the ^{1}H–^{13}C-PELF method gives equivalent results and does not require isotope labeling and does not suffer from the sensitivity and assignment problems imposed by ^{2}H-labeled

phospholipids. A promising extension would be the use of order parameters as orientational angular constraints in molecular dynamics simulations [197].

The DMPC choline head group in DMPC/DHPC bicelles has found particular interest. It was found that a deuterated head group can be employed as molecular voltmeter, since its conformation reacts almost linearly to the presence of negatively charged DMPG and positively charged 1,2-dimyristoyl-3-trimethylammoniumpropane (DMTAP) [198,199]. POPC with site-specific ^{2}H- and ^{13}C-labels was used to record ^{2}H quadrupolar as well as ^{13}C–^{31}P dipolar splittings, which can be used to provide torsional constraints on conformation [200]. Again, carbon dipolar couplings with ^{1}H and ^{31}P nuclei can be detected in 2D HIMSELF or HERSELF experiments without the need for isotopic labeling. In addition, quadrupolar splittings of the naturally most abundant nitrogen isotope, ^{14}N, can be employed to report on head group conformation [180,201]. The immersion depth and orientation of the headgroup of ganglioside GM1, a glycosphingolipid, was investigated in isotropic DMPC/CHAPSO bicelles by paramagnetic relaxation enhancement experiments [202].

The application of ^{1}H–^{13}C-PELF to membrane-associated peptides and small molecules has already yielded interesting results. The antimicrobial peptide MSI-78 (also known as pexiganan) was found to reduce the order parameter profile smoothly in each position, indicating that MSI-78 fragments the bicelles into smaller, more dynamic segments [189]. The antidepressant desipramine was found to be localized in the glycerol backbone and head group regions in DMPC/DHPC bicelles [183]. A ^{1}H–^{13}C-PELF study on the interaction between curcumin and membranes shows an ordering effect of the lipid acyl chain at low curcumin concentrations but at a high concentration of curcumin, a disordering of the acyl chains was observed [203]. The latter two studies dealt with the interaction of small molecules with lipid bicelles. This topic has also found interest in other types of studies, and will be further discussed in Section 9.

8. Bicelles under magic-angle spinning (MAS)

Magic-angle spinning (MAS) experiments on macroscopically aligned samples have been established which used solid supports, such as stacks of round glass plates [204], or a polymer film wrapped into a cylinder [205]. Similarly, lipid bicelles have been investigated under magic-angle spinning. It was found by variable- angle sample spinning (VASS) that at spinning angles smaller than the magic-angle the bilayer normal will align perpendicular to the rotation axis [206]. It was demonstrated that this fact can be used to determine the relative signs of dipolar and scalar (or *J*-) couplings. Perpendicular alignment at spinning angles smaller than the magic-angle was confirmed by ^{2}H NMR [207]. For spinning angles larger than 54.7°, the same study found parallel alignment with respect to the spinning axis. At the magic-angle itself, no preferential orientation is present, and the bilayer normals are distributed isotropically. In addition, the study described metastable phases with different alignment behavior whose presence depends on spinning speed and can be manipulated by switched-angle spinning to yield arbitrary angles between bilayer normal and applied magnetic field. The width of the distribution of the bilayer normal around its average value, known as mosaic spread, was the subject of another study [208]. In this study, mosaic spread in spinning bicelles was investigated by ^{31}P NMR and found to be small at spinning angles far away from the magic-angle. As the spinning axis approaches the magic-angle, mosaic spread becomes larger until an isotropic distribution is reached at the magic-angle.

It was shown that studies of peptides and proteins under MAS can benefit from using bicelles rather than MLVs [209]. Both isotropic and aligned spectra for the study of RDCs in soluble proteins can be collected from the same sample using bicelles and MAS [210].

Precise values of chemical shift anisotropy (CSA) were determined for a transmembrane segment of an acetylcholine receptor in bicelles under MAS [211]. Two-dimensional MAS experiments on bicelles containing a membrane-associated cytochrome b_5 have been reported and the results suggest that bicelles form better model membranes than MLVs or SUVs [212].

9. Interaction of small molecules with bicelles

Bicelles have in a few cases been used to study the interaction of small organic molecules with lipid bilayers. Both isotropically tumbling and magnetically-aligned bicelles were used. Tea catechins interact with isotropic bicelles according to the partition coefficient and their amphiphilic properties, with attachment mostly to the lipid headgroup region [213]. Erythromycin A, a macrolide antibiotic, showed shallow insertion into isotropic bicelles in a paramagnetic relaxation enhancement (PRE) study [214]. Salinomycin, an ionophore antibiotic, showed different conformation in presence and absence of sodium ions in isotropic bicelles [215]. The conformation found for the salinomycin-sodium complex is significantly different from the structure found in solution or in a crystal. The conformation and a model of insertion were established for amphidinol 3, a potent antifungal agent, in isotropic bicelles [216]. The study of small molecules in isotropic bicelles has been reviewed [217]. The polyene antibiotic amphotericin B was investigated in aggregates formed with dioctadecyl dimethylammonium bromide that are presumed to have discoidal geometry similar to bicelles [218].

Magnetically-aligned bicelles have also been used to study small molecules. RDCs were measured for ethanol [219]. The orientation and motion of ethanol in its membrane bound state could be inferred from these measurements. About 4% of ethanol is bound to the membrane; the lifetime of ethanol association is on the order of a few nanoseconds. Structural and dynamic properties of stearic acid-d_{35} in bicelles containing cholesterol have been studied by ^{2}H NMR [220] and later compared to EPR results [221]. Two cannabinoids were investigated in the presence of oriented bicelles [222]. In this study, two deuterium labeled sites yielded orientational constraints from spectra obtained on a solution-state NMR spectrometer, and were analyzed in terms of structure and dynamics. In an earlier parallel study, the same two cannabinoids were investigated in isotropic bicelles of high DMPC content, $q = 2.0$, which was necessary in order to observe a bilayer-bound conformation [223]. Another cannabinoid was investigated by ^{2}H NMR in bicelles of different lipid composition [224]. Glycosidic torsional motions were determined in a bicelle-associated disaccharide using RDCs [225]. Single-site deuterium labeled epigallocatechin gallate was found to interact with aligned bicelles [226]. Three fullerene derivatives in magnetically-alignable bicelles were investigated by EPR spectroscopy and were found to reside as single molecules just below the hydrophilic/hydrophobic interface with a preferential orientation [227]. Our own studies on membrane distortion by the antidepressant desipramine [183] are presented in Section 7.

In biopartitioning chromatography (BPC), a biomembrane-mimetic (e.g. liposomes, phospholipid monolayers, micelles, or bicelles) is introduced into a chromatographic system to study drug-membrane interactions [228]. Pure and highly stable phases of PEG-stabilized bilayer disks (described in Section 2.4) were developed as model membranes for drug partition studies, and have the potential to give more accurate results than liposomes [229]. Formulations designed to model porcine brush border membrane [230] and plant tissue [231] in drug partition studies were described. Discoidal aggregates of PEGylated lipids and lipids were used as pseudostationary phases in capillary electrophoresis to study drug partitioning [232,233] and the results verified by using a quartz crystal microbalance [233].

Numerous studies on peptides in bicelles have been conducted, for example on antimicrobial peptides [234] and neuropeptides [235]. A recent review [236] gives a comprehensive overview of proteins and peptides studied in bicelles and therefore we do not report on peptide studies here.

10. Magic touch added to studies of protein structure

The most exciting and fruitful area of bicelle application is without doubt found in structural biology of membrane proteins. Bicelles can be used in four fundamentally different ways to study membrane protein structure, as illustrated in Fig. 12. The difference is found in the proteins studied and in the type of bicelle chosen for their study. Integral membrane proteins (Fig. 12A and B), soluble proteins (Fig. 12C), and membrane-interacting proteins (Fig. 12D) have all been studied in bicelles. Both aligned (Fig. 12A and C) and isotropically tumbling (Fig. 12B and D) bicelles are in common use. In addition, a variation in the lipid:detergent ratio (the so called *q* titration) converting one type into the other can be performed and may be useful for differentiating between structured and mobile residues of membrane proteins [237]. In this section, a very short and highly subjective overview of the most exciting results on proteins is presented. For a comprehensive presentation, the reader is referred to our recent review article [236]. Several other, excellent review contributions on proteins studied in bicelles are available [8,150,172].

The propensity of bicelles for magnetic-alignment opened a completely new route for the preparation of macroscopically oriented membrane samples. Typically, proteins embedded in magnetically- aligned bicelles (Figs. 2G and 12A) reach a much higher quality of alignment than is possible for example in stacks of glass plates [15,16] or anodic aluminum disks (see Section 2 and Fig. 2C and D). Solid-state NMR of macroscopically aligned, static samples is a well-established and very productive branch of solid-state NMR spectroscopy [238]. It offers a wide array of well-tested custom-tailored pulse sequences, such as the separated local field experiments that were described in Section 7.1. Magneticallyaligned bicelles have given fresh impetus to this field, especially in the study of incorporated membrane proteins.

The largest integral membrane protein studied in this fashion to date is CXCR1, a human chemokine receptor that consists of seven transmembrane α-helices. CXCR1 was successfully incorporated in aligned bicelles [239]. Local and global dynamics of CXCR1 were studied by a combination of solid- and solution-state NMR experiments [240]. The interaction of CXCR1 with its ligand interleukin- 8 was characterized in aligned as well as isotropic bicelles [241]. For OmpX, outer membrane porin X which forms an 8-stranded β-barrel, the absolute orientation with respect to the surrounding bicelle bilayer was determined [242]. Functional $(\alpha 4)_2(\beta 2)_3$ pentamers of transmembrane α-helices from the α4β2 neuronal nicotinic acetylcholine receptor were prepared in aligned bicelles and the effect of anesthetics was investigated by observing a selectively ^{15}N-Leu labeled α4 peptide [243]. Results on the viral proteins p7 and Vpu in aligned bicelles have recently been reviewed [244]. Although both proteins belong to the same family of viroporins, p7 of HCV passes the membrane in two α-helical stretches, while Vpu of HIV-1 consists of only one transmembrane α-helix plus two α-helices that are attached peripherally to the membrane. The structure of MerFt was determined, a truncated version of a bacterial mercury transporter which has two transmembrane α-helices [245]. Several fragments of TatA, twin-arginine translocase consisting of a transmembrane α-helix and two membrane-associated α-helices, were investigated in aligned bicelles [246,247]. Bacteriophage Pf1 coat protein has a single transmembrane α-helix and was studied in bicelles with biphenyl lipid side-chains that align with their bilayer normal parallel to the external magnetic field [248] (Section 3.4). The feasibility of *de novo* sequential assignments in oriented bicelles has been

demonstrated on sarcolipin, where 26 out of 31 amide ^{15}N-resonances could be unambiguously assigned [249].

The Ramamoorthy laboratory has studied the integral membrane protein cytochrome b_5 in aligned bicelles [250]. Cytochrome b_5 consists of a transmembrane α-helix that serves as a membrane anchor, and a water soluble, globular domain. It was found to give high quality of orientation in aligned bicelles, which resulted in solid-state NMR spectra of very high resolution [251]. This result suggests that bicelles with their high degree of hydration are especially useful in the common case where membrane proteins have large globular domains in addition to their transmembrane portions. It was possible to reconstitute a complex of cytochrome b_5 with its binding partner, the 56 kDa enzyme cytochrome P450 [250]. Innovative pulse sequences were developed on cytochrome b_5 in bicelles that used proton-evolved local-field experiments to distinguish transmembrane and soluble domains [252], and used insensitive nuclei enhancement by polarization transfer (INEPT)-type magnetization transfer to study side-chain dynamics [253]. When cytochrome b_5 in aligned bicelles was studied under magic-angle spinning conditions, where macroscopic orientation vanishes, spectra of very high quality could be observed, confirming the favorable conditions that bicelles offer for membrane proteins [212].

The more traditional field of solution-state NMR has also made rapid progress in the study of integral membrane proteins, and bicelles have made a significant contribution. Solution-state NMR offers a wide range of sophisticated tools to study proteins that tumble quickly on the NMR time scale [254–258]. Integral membrane proteins that are natively embedded in membranes typically do not meet the criterion of fast reorientation. For this reason, they are regularly solubilized in detergent micelles that do show fast tumbling. The detergent used for solubilization has been humorously described as “French swimwear” for the protein [259]. Naturally, smaller protein–detergent aggregates will result in faster reorientation and better solution-state NMR spectra. However, it has been found that not all proteins retain their functional form in protein–detergent micelles. Isotropic bicelles are especially attractive as an alternative in this context, as they possess a bilayered portion that offers a native-like environment for the embedded protein (Fig. 12B). For example, the functional form of Smr, staphylococcal small multidrug-resistance pump, is stabilized in bicelles [260]. A review on solubilizing agents presents Smr as prototypical case and stresses the importance of an assay to verify that a protein is present in its active form [261]. This publication states with respect to protein–detergent aggregates that “small is beautiful, but sometimes bigger is better”. A complete backbone assignment for the functional form of Smr in isotropic bicelles has been reported [262]. Several other contributions have reviewed the importance of detergent systems in the study of membrane proteins [263–266]. Two review contributions present comprehensive galleries of integral membrane proteins solved by solution-state NMR spectroscopy [267,268].

The structure determination of sensory rhodopsin II, a seven transmembrane helix receptor, in detergent micelles (pdb-id 2KSY) [269] has shown that these proteins are now within reach of solution-state NMR. The same study used spectra recorded in isotropic bicelles to show that environment-specific effects are minimal when using mild detergents. A complex of rhodopsin and transducin was prepared in isotropic bicelles and displayed dramatically increased stability [270], and it was possible to generate and purify a rhodopsin/transducin complex with constitutively active, recombinant rhodopsin [271]. A bicelle-associated structure was determined for full-length myristoylated ARF1, ADP ribosylation factor 1, which consists of an N-terminal bicelle-associated and a C-terminal catalytic domain connected by a flexible linker (pdb-id 2KSQ) [272]. EmrE, a small multidrug-resistance transporter, forms homodimers where each monomeric unit consists of four transmembrane α-helices. EmrE is highly sensitive to its environment but could successfully be

incorporated into isotropic bicelles [273]. It was then shown that asymmetric antiparallel EmrE exchanges between inward- and outward-facing states that are identical except that they have opposite orientation in the membrane [274]. Homo- and hetero-dimers of single-pass transmembrane α-helices are of special interest since they play central roles in a large class of transmembrane signaling receptors [275]. A structure determination has been reported for the heterodimer of the αIIb and β3 transmembrane helices of integrin (pdb-id 2K9 J) [276]. Similarly, the spatial structure of the transmembrane domain heterodimer of ErbB1 and ErbB2 receptor tyrosine kinases was reported (pdb-id 2KS1) [277]. Phospholamban, a single-pass α-helical transmembrane protein, has found special interest because of its role in regulating SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase [278]. Magic angle spinning of phospholamban in isotropic bicelles was used to probe equilibria between ground and excited states [279]. For OmpX, a bacterial outer membrane porin that forms an 8-stranded β-barrel, it was possible to observe intermolecular NOE contacts to DMPC lipids in the surrounding isotropic bicelle, but not to DHPC detergent, proving that the protein is embedded solely in the bilayered portion of the bicelle [155].

When soluble, globular proteins are introduced into dilute samples of strongly aligned bicelles (Fig. 12C), they experience a weak macroscopic alignment that gives rise to small anisotropic NMR interactions, so-called residual dipolar couplings or RDCs, on the order of several Hz. The transferred partial orientation of the soluble protein is caused by interaction with the aligned medium which forms an obstacle to the free re-orientation that would be observed in solution. Numerous alignment media are used besides bicelles in the study of RDCs; tabulated overviews of alignment media are available [280,281]. The alignment that a molecule will experience in a certain environment can be predicted on the basis of steric interaction alone [282], or additionally taking into account electrostatic interaction [283]; the software PALES is available for this task [284].

RDC measurements were first reported on ubiquitin partially ordered in $q = 2.9$ DMPC/DHPC bicelles [285]. Their role in structure determination of biomolecules has been reviewed repeatedly; we mention a contribution that includes an overview of pulse sequences for recording RDCs [280]. Other review contributions have focused on RDCs as probes of biomolecular dynamics [281], and on RDC studies on proteins [286] and RNA [287]. It has to be noted that the interaction with the alignment medium may change the properties of the studied protein. For example, the putatively inactive dimeric state of the chemokine SDF-1/CXCL12 is favored when bicelles are used as alignment medium [288]. Since RDCs report on global orientation, they are especially useful for determining the overall orientation of protein domains [289]. Three independent sets of RDCs in aligned bicelles of three different conditions were determined for the three N-terminal domains of human factor H and analyzed to yield their relative orientation [290]. For ubiquitin, 18 independent ordering media including bicelles gave sufficient RDC data to observe protein recognition dynamics on a microsecond time scale [291].

An especially intriguing situation occurs when bicelles in RDC studies are not used as alignment medium but are themselves subjected to partial alignment. Isotropic bicelles containing the HIV-1 Env peptide were introduced into a stretched hydrogel and RDCs could be recorded that showed considerable differences when compared to RDCs recorded on the same peptide in detergent micelles [292]. Similarly, DNA tetrads can be used as alignment medium to measure RDCs on molecules embedded in isotropic bicelles [293].

The interaction of soluble, globular proteins with lipid bilayers can be studied in the presence of isotropic bicelles (Fig. 12D). This is different from the separated local field (SLF) studies described in Section 7, where strong binding to aligned bicelles is a prerequisite. In the case of globular protein and isotropic bicelle, neither strong nor partial

alignment of the protein is observed and protein-lipid interactions can be investigated. For example, details of membrane binding of myristoylated and non-myristoylated ARF1, ADP-ribosylation factor 1 (pdb-id 2K5U), was compared in isotropic bicelles [294]. Spontaneous insertion of the apoptotic repressor BclXL into $q = 0.5$ DMPC/DHPC bicelles was found to cause a dramatic conformational change that inhibits binding with its BH3 ligand [295]. Isotropic bicelles were used to supply lipid substrate to cobra venom phospholipase A_2 for kinetic assays [296]. It has also been observed that amyloid formation is modulated in bicellar solutions [297].

11. New and notable

A number of recent publications have presented new bicelle designs and novel bicelle applications that do not fit easily into the categories of this review article, but that feel particularly noteworthy. A new bicelle was designed that contains both cholesterol and unsaturated lipid sidechains and forms separated domains that can potentially be used as models for physiological lipid rafts [298]. Bicelles were stabilized by encompassing them into a layer of siloxane ceramics [299]. Newly designed peptide copolymers form flat discoidal structures similar to bicelles [59]. The use of special detergents extends the possible range of bicelle concentrations to below 0.5% lipid weight per volume [113]. Even lower concentrations can be reached by encapsulating bicelles in vesicles; such formulations have been introduced under the name ‘bicosomes’ [300].

More traditional bicelle formulations have been applied in completely novel ways. The use of bicelles in the crystallization of membrane proteins [38,44] has been discussed in detail in Section 2.4. Bicelles as detergents in the cell-free expression of membrane proteins offer distinct advantages [34,301,302]. The effects of applying bicellar preparations to the skin have received broad attention [303–308] and were recently reviewed [309]. Of special interest seems to be the prospect of delivering drug substances to the skin using bicelles, as demonstrated for diclofenac [310]. The voltage gated potassium channel modulatory membrane protein KCNE3 was delivered into oocytes in functional form using bicelles [311].

In the field of nanotechnology, bicelles were used as a temporary scaffold to synthesize rigid organic nanodisks by polymerization [312] and to generate platinum nanowheels [313,314]. Bicelles were used to build assemblies of multiple highly fluid free lipid bilayers that are tethered together by cholesterol-anchored double-stranded DNA [315]. Supported and suspended lipid bilayers have been generated on silicon chips using bicelles [316].

12. Summary and conclusion

Bicelles have quickly emerged as another amazing possibility in the host of lipid morphologies [317]. The present contribution has focused on the studies that generated today’s comprehensive understanding of bicelle properties. NMR experiments along with scattering and diffusion measurements can give quick and unambiguous characterizations of bicelle morphology and phase diagrams. Bicelles quickly took on an important role in the context of lipid bilayer samples for NMR and EPR spectroscopy. SLF and MAS experiments on bicelles are regularly used to study bilayer properties. The interaction of small molecules with bilayers can also be studied in bicelle samples. The study of membrane protein structure forms the most prominent practical application of bicelles.

The application of bicelles in the structural studies of membrane proteins has a number of unique advantages. Bicelles can embed membrane proteins into truly flat, bilayered lipid patches with lipid compositions that can be chosen from a wide array of options. Bicelles form only in limited ranges within the phase diagram, but these ranges are continuously

expanded and typically coincide with ranges of conditions that are desirable for the study of membrane proteins. By the ratio q of lipid over detergent, the size of bicelles can be freely chosen. Bicelles with small q values can be used for high-throughput solution-state NMR studies while those with large q values are ideal for solid-state NMR studies. Even q-variations ranging from solid-state into solution-state conditions are possible. Since bicelles contain bulk water, they enable natural folding of even those membrane-associated proteins that contain large soluble domains and therefore make physiologically relevant structural studies possible. In summary, bicelles are the most versatile model membrane and as a consequence they are increasingly and routinely used in studies ranging from crystallography to NMR spectroscopy.

Today, the field extends so widely that it is virtually impossible to cover the phenomenon “bicelle” in a single review article. Insights on protein structure gained with the help of bicellar formulations are emerging at an amazing pace. At the same time, research that is focused on bicelles in their own right has elucidated most of their basic structural and dynamic properties. The most exciting activity is found on the fringes of the field, where new imaginative variations and applications keep emerging at a similar pace.

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Glossary of abbreviations

AAO	anodic aluminum oxide
BPC	biopartitioning chromatography
bR	bacteriorhodopsin
$C_{12}E_{18}$	octaethylene glycol monododecyl ether
CHAPSO	3-(cholamidopropyl)dimethylammonio-2-hydroxy-1-propanesulfonate
CMC	critical micelle concentration
CSA	chemical shift anisotropy
CTAB	hexadecyltrimethylammonium bromide
DHPC	dihexanoylphosphatidylcholine
DLPC	dilauroylphosphatidylcholine
DMPC	dimyristoylphosphatidylcholine
DMPE	dimyristoylphosphatidylethanolamine
DMPG	dimyristoylphosphatidylglycerol
DMTAP	1,2-dimyristoyl-3-trimethylammonium-propane
DPC	dodecylphosphocholine
DPPC	dipalmitoylphosphatidylcholine
DSPE	distearoylphosphatidylethanolamine
DTPA	diethylenetriaminepentaacetate
EM	electron microscopy
EPR	electron paramagnetic resonance

FRAP	fluorescence recovery after photobleaching
HDL	high-density lipoprotein
HERSELF	heteronuclear rotating-frame spin exchange via local field
HIMSELF	heteronuclear isotropic mixing spin exchange via local field
INEPT	insensitive nuclei enhancement by polarization transfer
MAS	magic-angle spinning
MD	molecular dynamics
MLV	multilamellar vesicle
NMR	nuclear magnetic resonance
PEG	polyethyleneglycol
PELF	proton evolved local field spectroscopy
PFG	pulsed field gradient
PISEMA	polarization inversion spin exchange at the magic angle
POPC	1-palmitoyl-2-oleoyl-phosphatidylcholine
PRE	paramagnetic relaxation enhancement
PSPC	1-palmitoyl-2-stearoyl-phosphatidylcholine
RDC	residual dipolar coupling
SAMMY	magic-sandwich PISEMA
SANS	small-angle neutron scattering
SDS	sodium dodecyl sulfate
SLF	separated local field
SLUV	short-chain long-chain unilamellar vesicle
STE	stimulated echo
SUV	small unilamellar vesicle
TEM	transmission electron microscopy
TM	transmembrane
TMS	tetramethylsilane
VASS	variable-angle sample spinning

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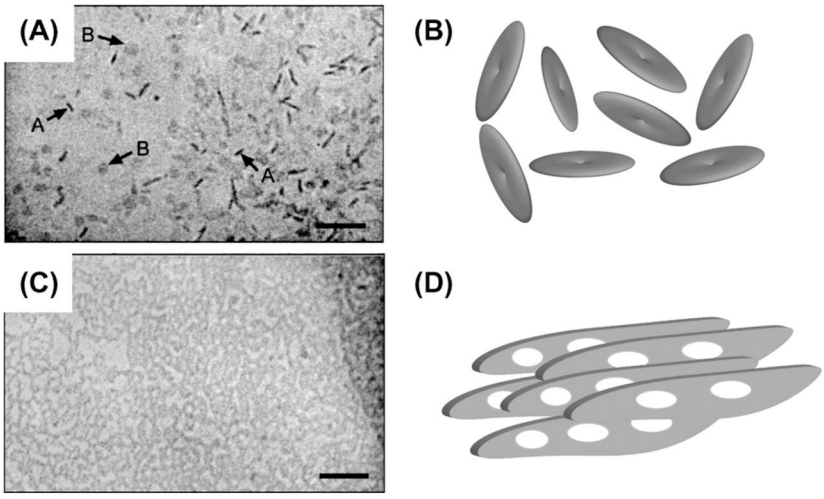

Fig. 1.

Lipid bicelles are supramolecular aggregates that are formed when appropriate amounts of lipids and detergents are mixed in an aqueous environment. The size and phase of bicellar aggregates depend on the [lipid]:[detergent] ratio as well as on the temperature. Two fundamentally different phases of bicellar preparations have proven highly useful in the study of protein structure using NMR spectroscopy: isotropic bicelles rapidly tumble freely and are formed at a high detergent concentration (A and B). At low detergent concentrations extended bilayered lamellae are formed (C and D), that spontaneously align macroscopically in a magnetic field. Cryo-TEM micrographs (A and C) are reproduced from the literature [1]. Micrograph (A) contains arrows marked A and B that point to disk-like bicelles viewed from the side and the top, respectively.

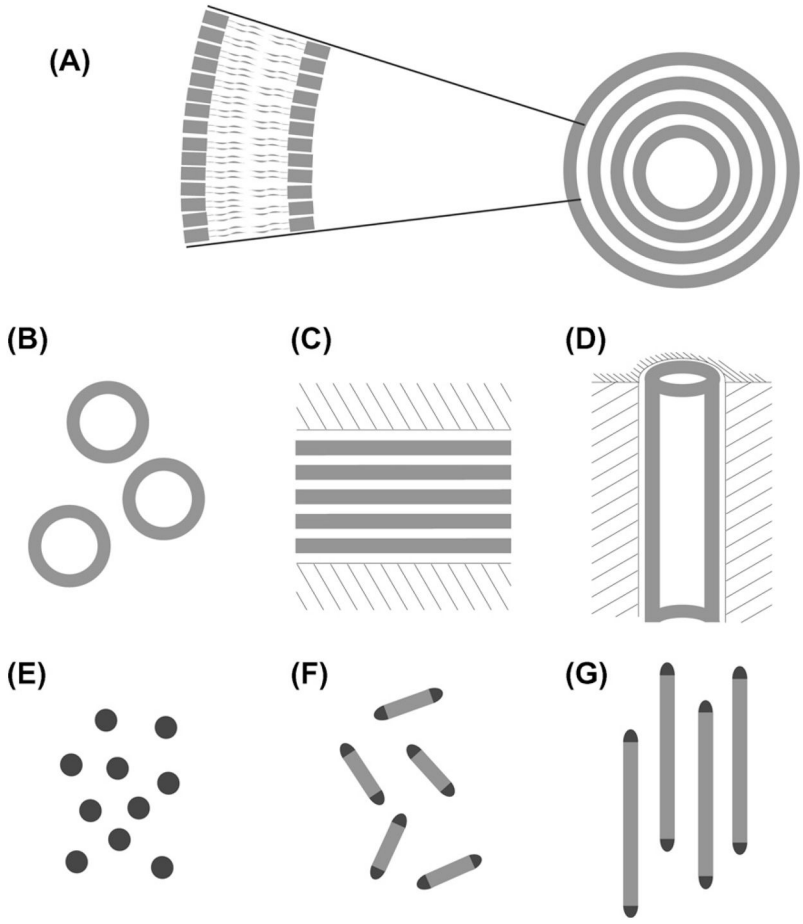

Fig. 2.

Different types of model membrane samples suitable for studying membrane proteins by NMR spectroscopy: (A) lipid bilayers (inset) are present in multilamellar vesicles; (B) small unilamellar vesicles and macroscopically-aligned bilayer samples using either (C) glass plates or (D) cylindrical nanopores in anodic aluminum oxide. (E) Micelles are formed from pure detergents. When lipid and detergent mix, isotropic bicelles (F) and magnetically-aligned bicelles (G) are formed. Light gray color indicates a lipid bilayer, dark gray is detergent, and hatching represents either a glass plate (C) or aluminum oxide (D) as solid supporting material.

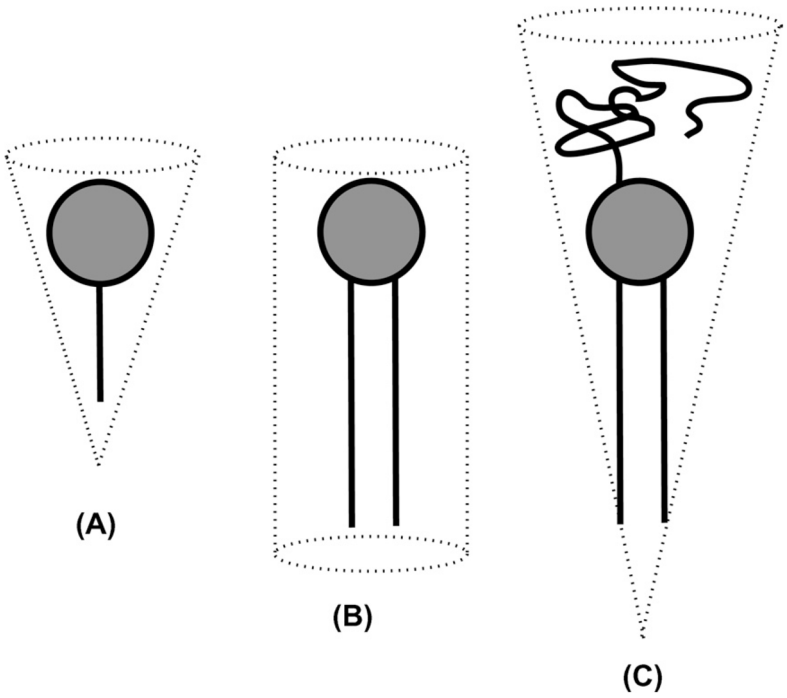

Fig. 3.

The overall geometrical shape of detergents tends to be conical (A), while phospholipid molecules have mostly cylindrical overall geometry (B). The geometry of pegylated phospholipids again tends to be conical (C).

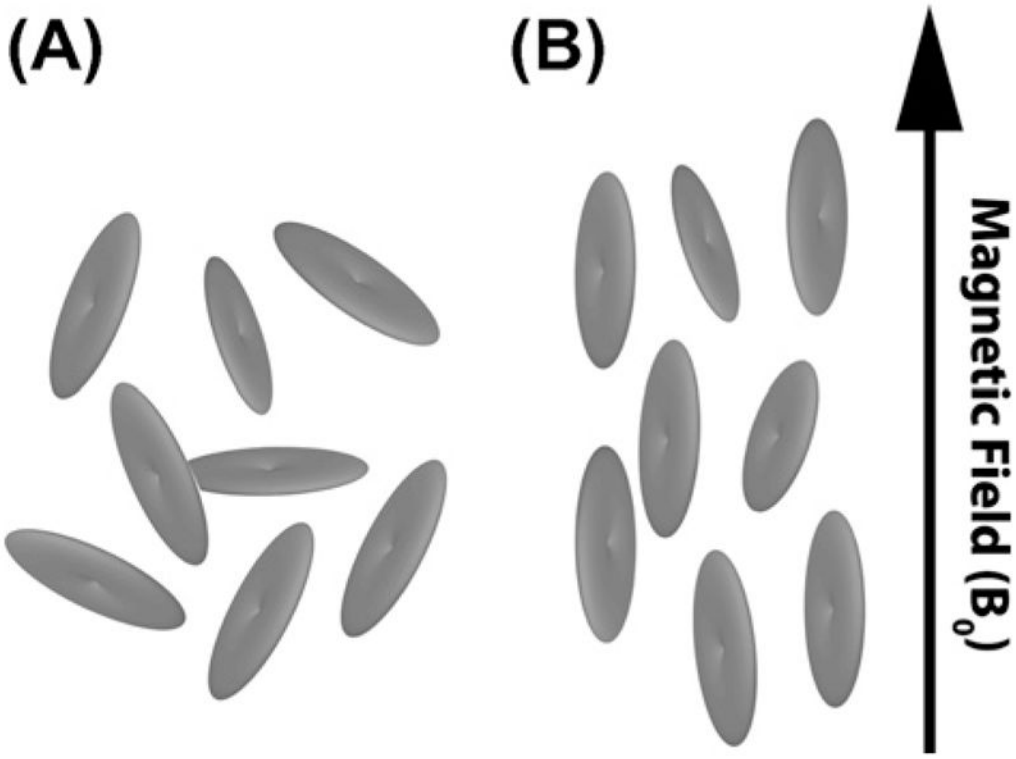

Fig. 4.
(A) In the absence of a magnetic field, bicelles assume random orientations. (B) Anisotropy in magnetic susceptibility causes macroscopic alignment of bicelles when an external magnetic field is applied.

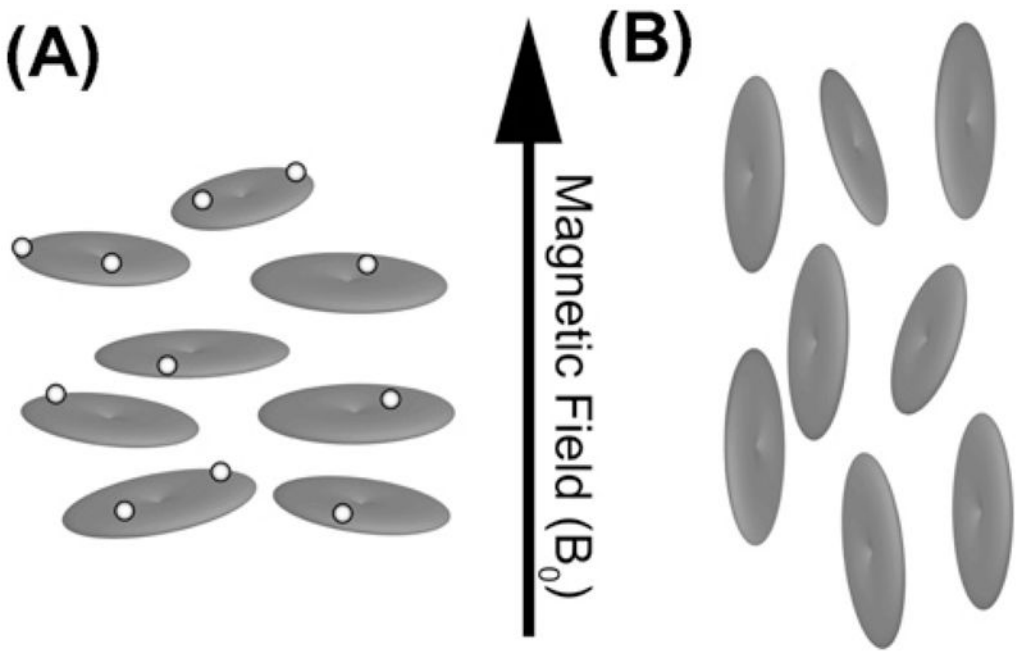

Fig. 5.
In the presence of lanthanide ions (light spheres), lipid bicelles adopt an orientation where the bilayer normal is parallel to the applied magnetic field (A). This is termed as “flipped” bicelle with respect to the perpendicular orientation adopted by undoped bicelles (B).

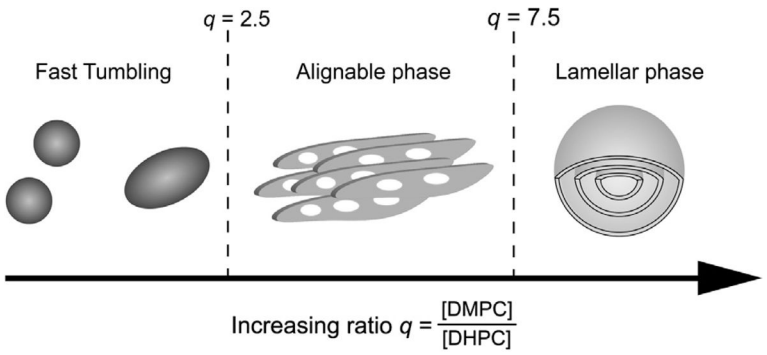

Fig. 6.

The morphology of bicellar preparations is dependent on the ratio q between a long-chain lipid and a detergent. Fast tumbling bicelles exist when $q < 2.5$ (left panel), macroscopically aligned bicelles exist between q ratios of 2.5 and 7.5 (middle), and at high $q > 7.5$, multilamellar vesicles are formed (right).

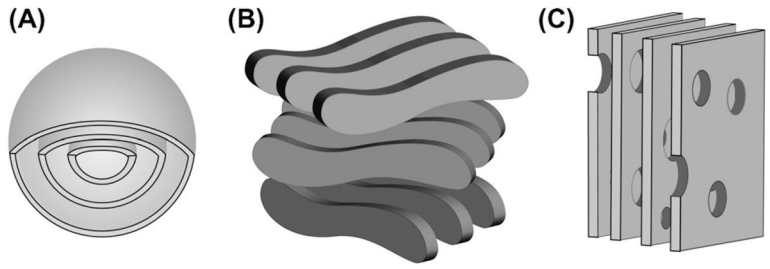

Fig. 7.
Bicelles exhibit a wide range of morphologies. Beside the simple nanodisk morphology, multilamellar vesicles (A), chiral nematic ribbons (B), and perforated lamellae (C) are found under different sample conditions.

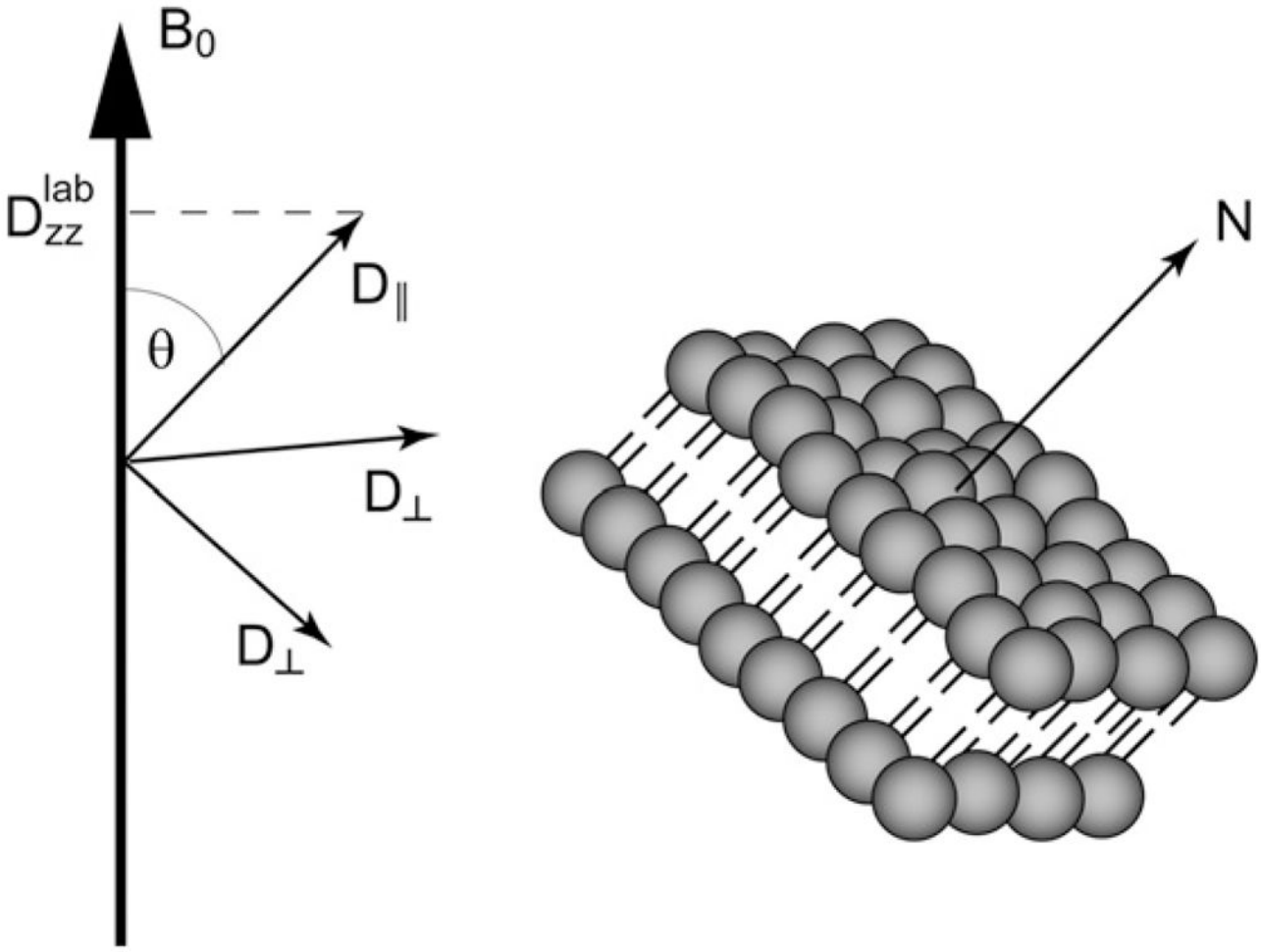

Fig. 8.

In a lipid bilayer system, only two components are needed to describe the diffusion tensor. The relative orientation of the bilayer normal and hence the principal components of the diffusion with respect to the external magnetic field is described by the angle θ. It determines the component D_{zz}^{lab} of the diffusion tensor that lines up with the magnetic field and can be measured.

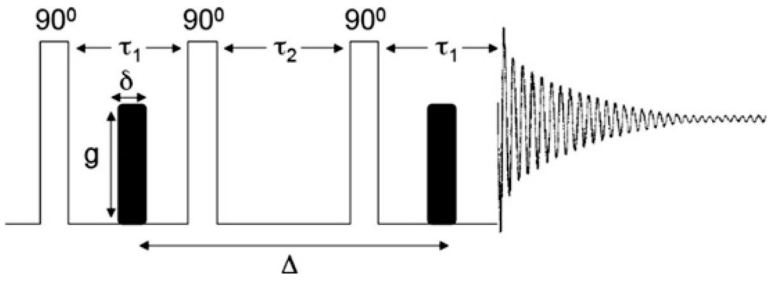

Fig. 9.

STE-PFG NMR pulse sequence composed of three 90° radio frequency pulses and two gradient pulses of identical amplitude and duration. The STE-PFG NMR experiment is arranged such that the decay of the echo intensity, as a function of δ, g or Δ, is proportional to the diffusion coefficient of the species of interest.

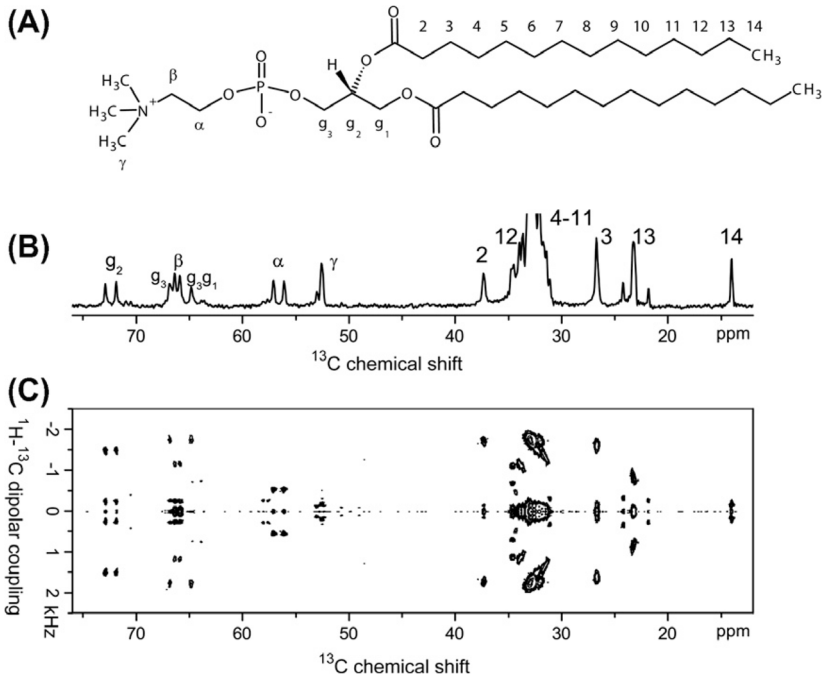

Fig. 10.

^{13}C NMR spectroscopy of magnetically-aligned DMPC/DHPC bicelles, $q = 3.5$. At this ratio of long- to short-chain component, resonances from the long-chain component dominate the spectra. (A) Molecular structure of DMPC, including the commonly employed nomenclature. (B) The natural-abundance ^{13}C NMR chemical shift spectrum on a 400 MHz NMR spectrometer shows clearly resolved resonances for most carbon sites in DMPC. (C) A 400 MHz 2D SLF-spectrum correlates ^{13}C chemical shift to ^{1}H–^{13}C dipolar coupling of carbon nuclei to adjacent hydrogen nuclei and yields information on the local structure and mobility of lipid molecules in bicelles.

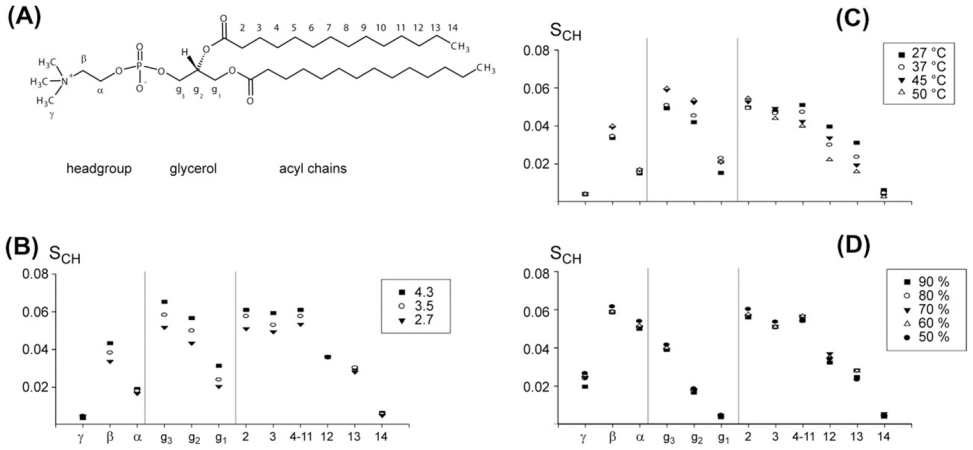

Fig. 11.

Order parameter profiles determined from 2D ^{1}H–^{13}C-PELF NMR spectra of magnetically-aligned DMPC/DHPC bicelles [193]. (A) DMPC structure with nomenclature and indication of distinct molecular regions. Order parameter profiles were determined for different values of (B) composition *q*, (C) temperature and (D) hydration level. These results demonstrate that experimentally measured ^{13}C–^{1}H dipolar couplings can be utilized in measuring the changes in the order/disorder associated with various regions of the lipid and detergent in bicelles without the need for isotopic enrichment. Such measurements have been shown to provide insights into the mechanism of membrane disruption by antimicrobial peptides and amyloid peptides/proteins.

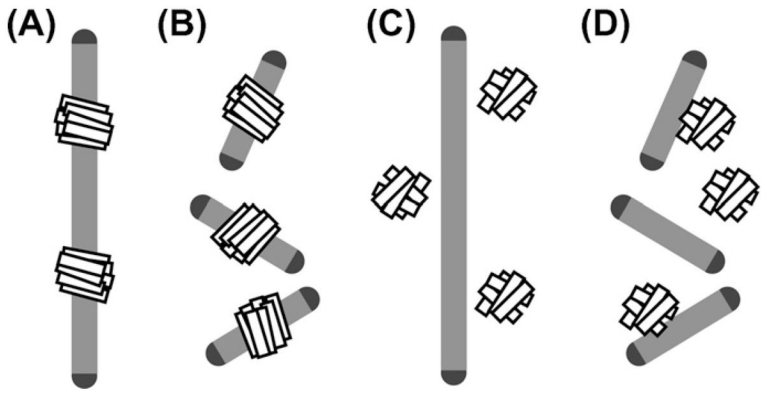

Fig. 12.

Graphic representation of the four fundamentally different ways in which bicelles are used in protein structure and protein–membrane interaction studies. Integral membrane proteins can be studied in aligned (A) as well as isotropically tumbling bicelles (B). Aligned bicelles can be used to impose a residual orientation on soluble proteins for RDC measurements (C). Isotropic as well as aligned bicelles can be used to study membrane interaction of soluble proteins (D).