

Bicelles as Model Membranes for Solid- and Solution-State NMR Studies of Membrane Peptides and Proteins

ISABELLE MARCOTTE,¹ MICHÈLE AUGER²

¹Physical Chemistry, ETH-Hönggerberg, CH-8093, Zürich, Switzerland

²Département de Chimie, Centre de Recherche en Sciences et Ingénierie des Macromolécules, Université Laval, Québec, Québec, Canada G1K 7P4

ABSTRACT: Bicelles are an attractive membrane mimetic system because of their planar surface and lipid composition, which resemble biological membranes. In addition, their orientation and morphologic properties make them amenable to solid- and solution-state NMR. This article reviews the physical properties of bicelles, such as magnetic alignment and viscosity as well as the different models proposed in the literature to explain the bicelle morphology. The utility of bicelles for studying the interaction and structure of membrane peptides and proteins by solid- and solution-state NMR is also presented, along with the advantages and limitations of bicelles. © 2005 Wiley Periodicals, Inc. Concepts Magn Reson Part A 24A: 17–37, 2005

KEY WORDS: bicelles; interactions; structure determination; physical characteristics; solid-state NMR; solution-state NMR

INTRODUCTION

One of today's greatest scientific challenges is to determine the structure of membrane proteins, which constitute about one third of all the proteins encoded

Received 12 May 2004; revised 10 September 2004; accepted 13 September 2004

Correspondence to: M. Auger; E-mail: michele.auger@chm.ulaval.ca

Concepts in Magnetic Resonance Part A, Vol. 24A(1) 17–37 (2005)

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/cm.a.20025

© 2005 Wiley Periodicals, Inc.

in the human genome (1). Of great interest is also the interaction between extrinsic or transmembrane peptides and proteins and cell membranes. Several membrane protein structures have been determined by X-ray crystallography (1), but the difficult purification and crystallization of membrane proteins are a limitation of this technique (2, 3). Most importantly, the function of membrane proteins is strongly dependent on their environment; therefore the protein structure investigation should be done in a membrane milieu (4). This can be performed by nuclear magnetic resonance (NMR), which can render the membrane invisible by appropriate isotopic labeling of either the protein or the lipids. However, because biological

membranes are highly complex mixtures, this task requires the elaboration of membrane model systems in which proteins can be reconstituted. As discussed by Mouritsen and Jorgensen (5), a good model system has to match the extrinsic and transmembrane protein requirement in terms of surface properties and membrane thickness, respectively.

The quest for a suitable membrane model has begun with the use of unilamellar phospholipid vesicles, whose morphology is similar to that of biomembranes. However, their tedious preparation and lack of stability (6) has favored the use of liposomes (or multilamellar vesicles, MLVs), which are more easily made. Unfortunately, both uni- or multilamellar phospholipid vesicles have shown to be inadequate for high-resolution NMR studies of membrane-associated peptides and proteins as their reorientation time is too long on the NMR timescale, giving rise to large bandwidths (7). Therefore, the rapidly reorienting phospholipid micelles were more suitable for high-resolution NMR studies (1). However, micelles are not ideal membrane mimicking systems as they have a monolayer structure with a high curvature, a loose packing of the lipid headgroups, and tighter chain entanglement in the center. These features therefore differ significantly from the biological membrane morphology. In addition, the activity of proteins and enzymes embedded in micelles is not readily preserved (8–10), and the reconstitution of transmembrane proteins in these systems can be complicated as they might require a bilayer in order to adopt a conformation similar to that of the native state (11, 12).

It was thus of great interest to conceive bilayered model membranes that could easily form in aqueous solution. This was first performed by Gabriel and Roberts (13) who showed, in 1984, that unilamellar vesicles (ULVs) could form spontaneously by mixing aqueous suspensions of long-chain lecithins (≥ 14 carbons) with small quantities of synthetic short-chain lecithins (acyl chain length of 6–8 carbon atoms), which have the propensity to form micelles. This novel lipid arrangement was called short-chain/long-chain unilamellar vesicles, or SLUVs. Later, electronic microscopy images published in 1986 (14) showed that SLUVs had a rather ellipsoidal shape, and these structures are, somehow, the bicelle ancestors.

On the basis of X-ray diffraction studies suggesting that certain mixtures of bile salts and phosphatidylcholines could form discoid bilayers of rather uniform size, Prestegard's research group investigated this type of system (15–17). They were the first to show the orientation, in the magnetic field, of discoid micelles made of phospholipids and detergents. These

micelles were composed of the bile salt 3-(cholamidopropyl) dimethylammonio-2-hydroxyl-1-propane sulfate (CHAPSO) and dimyristoylphosphatidylcholine (DMPC), which has two acyl chains with 14 carbon atoms. The use of CHAPSO was motivated by its zwitterionic nature and ability to solubilize proteins without denaturation (16).

The development of SLUVs and of the DMPC/CHAPSO model was followed by the design of a system composed of DMPC and dihexanoyl- (or dicaproyl-) phosphatidylcholine (DHPC or DCPC), which is a short-chain phospholipid (acyl chain length of six carbon atoms). These "binary bilayered mixed micelles" or so-called bicelles were developed by Sanders et al. (18, 19) and were thought to have a discoid shape. The DMPC/DHPC bicelles have quickly replaced the DMPC/CHAPSO system in the study of peptides and proteins because, unlike phosphatidylcholines, CHAPSO is not a natural constituent of biological membranes. Although these bicelles are the most commonly used, other research groups have proposed new bicellar systems composed of phosphatidylcholines with longer acyl chains, such as palmitoylstearyl-, 1-palmitoyl-2-oleoyl-, dilauroyl- and dipalmitoylphosphatidylcholine (20–24) in order to accommodate the hydrophobic length of transmembrane proteins. It is important to point out that in this article, unless specified, the term "bicelle" refers to the DMPC/DHPC system.

With the aim of better mimicking the diversity of biological membranes, the bicelle composition was also modified by incorporating phospholipids with different polar headgroups. Vold's and Macdonald's research groups (25–27) have shown that negatively charged bicelles could be formed by substituting up to 25 mol% of DMPC by dimyristoylphosphatidylglycerol (DMPG) or dimyristoylphosphatidylserine (DMPS). PS is found in the membrane of eukaryote cells, whereas PG exists only in those of prokaryotes (28). It is also possible to replace up to 10 mol% of DMPC by dimyristoylphosphatidylethanolamine (DMPE) (29) to imitate myelin and erythrocyte membranes, which naturally contain 15–18% of PE (28). Cardiolipin-doped bicelles have also been prepared to better resemble mitochondrial membranes (30). Dimyristoylphosphatidic acid (DMPA) can also be incorporated into bicelles (11, 31), as can cholesterol (32–34), the effects of which have been described extensively (33, 34). Positively charged bicelles can also be made with the addition of 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP) (26). Finally, the bicelle composition can also be changed to improve their stability. This effect was observed with the pegylation of the bicelles (35) and the addition of

charged amphiphiles (36, 37). The use of phospholipids containing ether links has also shown a greater stability of these model membranes over a wider range of pH (38).

Bicelles have also been used extensively in the past few years as an alignment medium for proteins in solution with the aim of measuring chemical shift anisotropy and, more importantly, residual dipolar couplings, which provide a measure of the angle, thus orientation, between two atoms. This idea was first introduced by Tjandra and Bax in 1997 (39) who used diluted solutions of aligned bicelles (volume fraction of $\sim 5\%$) to measure the residual dipolar interaction and obtain additional information for structure determination. The use of residual dipolar couplings in the study of protein structure has been recently reviewed by Brunner (40) and Bax (41) and therefore, a detailed description of this technique, which has been mainly applied to the study of proteins in solution, is beyond the scope of this review.

This article describes the physical properties of bicelles, such as magnetic alignment and viscosity and discusses the different models proposed in the literature to explain bicelle morphology. The main focus of this review, however, concerns the utility of bicelles for studying the interaction and structure of membrane peptides and proteins by solid- and solution-state NMR. The advantages and limitations of bicelles are discussed in the conclusion, along with their current and potential applications to other techniques.

PART 1: PHYSICAL CHARACTERISTICS OF BICELLES

Bicelles can display different orientation properties and morphologies depending on the sample preparation and experimental conditions (Fig. 1), which make them amenable to solid- and solution-state NMR. This is an important advantage of bicelles as not only can they orient in the magnetic field but (isotropic), but fast-tumbling bicelles can also be prepared. This section describes the physical properties of bicelle samples, such as magnetic orientation and viscosity, and the different bicelle morphologies.

Orientation and Viscosity

Bicelles spontaneously orient in magnetic fields (B_0) greater than 1 Tesla (42) in a nematic (threadlike) order with the bilayer normal perpendicular to the B_0 direction. This orientation occurs above the DMPC gel-to-fluid transition temperature (43), typically be-

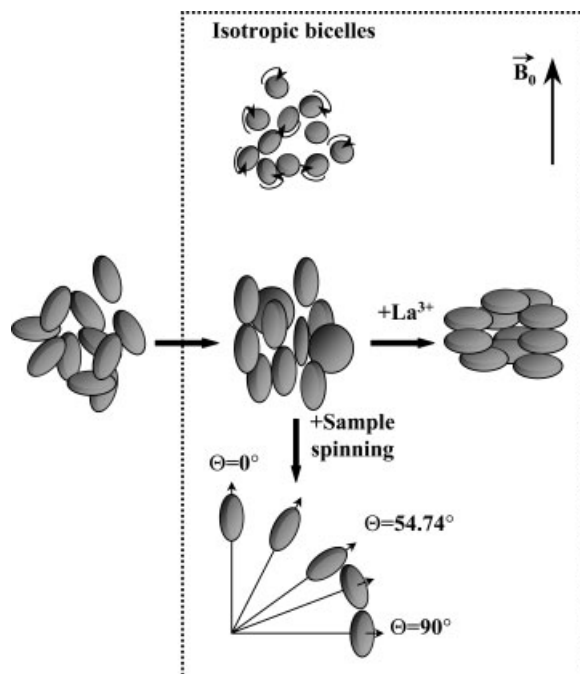


Figure 1 Applications of bicelles in nuclear magnetic resonance, assuming a discoid model. Isotropic bicelles can be used for the structure determination of membrane-associated proteins by solution-state NMR. Oriented bicelles provide information on protein interaction and conformation by solid-state NMR. Dilute oriented bicelles are used as an alignment medium for protein structure determination (solution-state NMR). The addition of lanthanides flips the bicelle orientation and allows the investigation of the interaction and structure of membrane-bound proteins. Slow-spinning bicelles at various angles are also useful to obtain structural information on membrane-associated peptides (58).

tween 30° and 50°C for DMPC/DHPC molar ratios (often referred to as q) above 2.3:1 and lipid concentrations of 3–40% w/v in aqueous solution (17, 38, 44, 45). At small q ratios, isotropic bicelles are formed with a small diameter and rapidly tumble in solution. Typically, bicelles have a thickness of ~ 40 Å (46), which corresponds to that of a bilayer. These model membranes are easily prepared and stable over a wide range of ionic strengths and pH values (18, 43). However, this is not the case for acidic bicelles, which orient on shorter intervals of pH, ionic strength, and temperature, and which are more sensitive to hydrolysis (25, 27). The temperature range of stability for Bic/PE is also reduced (29). Interestingly, the presence of salts such as KCl, NaCl, $CaCl_2$, and $MgCl_2$, in a concentration range of 50–200 mM, has been shown to improve the magnetic orientation (45, 47). Bicelles are also stabilized by dehydration, which

induces a tighter packing of these systems and thus, a greater cooperative alignment (*vide infra*) (47).

The orientation of bicelles in a magnetic field is explained by the anisotropy of the magnetic susceptibility tensor, χ , of the phospholipids acyl chains (48, 49) and, to a lesser extent, the contribution of the ester groups (48, 50). The anisotropy of the magnetic susceptibility is the difference between the magnetic susceptibilities parallel (χ_{\parallel}) and perpendicular (χ_{\perp}) to the axis of a molecule:

$$\Delta\chi = \chi_{\parallel} - \chi_{\perp} \quad [1]$$

If $\Delta\chi < 0$, there is an energetically favorable orientation of the molecules perpendicular to the direction of B_0 , and this is the case for phospholipids and egg lecithins (48, 49, 51). Values of $-0.28 \times 10^{-8} \text{ erg} \cdot \text{cm}^{-3} \cdot \text{G}^{-2}$ (23°C) and $-9 \times 10^{-8} \text{ erg} \cdot \text{cm}^{-3} \cdot \text{G}^{-2}$ (22°C) are found in the literature for egg lecithins and dipalmitoylphosphatidylcholine (DPPC), respectively (49, 51, 52).

As described previously (31, 51), the orientation-dependent component of the average Helmholtz free energy of the membrane is given by:

$$F(\beta_m) = \frac{-1}{2}N\Delta\chi(\vec{n} \cdot \vec{B})^2 = \frac{-1}{2}N\Delta\chi\cos^2\beta_m \quad [2]$$

where β_m is the angle between the molecular axis director (normal, \vec{n}) and the magnetic field direction \vec{B} , and N is the number of molecules per unit of volume. In cooperative systems such as bicelles, in which an important number of acyl chains are packed parallel in an ordered structure, the energy $F(\beta_m)$ is sufficient to overcome the thermal energy responsible for Brownian motion (53), which occurs inside or outside a magnetic field. Therefore, the bicelle normal orients perpendicular to the B_0 direction. However, the system should be mobile enough to allow the alignment. Viscosity is thus an important parameter.

There are few reports on the viscosity of bicellar systems in water. A temperature dependence of the viscosity has been published by Struppe et al. (54) for bicelles with $q = 2.9$ at different lipid concentrations ranging from 3% to 15% w/v. An increase in the viscosity by up to three orders of magnitude is observed when the temperature increases. Once the solutions are macroscopically aligned, the viscosity drops monotonically. The viscosity maximum is found to decrease with decreasing lipid concentration. More recently, Hwang and Oweimreen (55) studied the viscosity of bicelles at five different q ratios and temperatures ranging from 20°C to 60°C, and their

results corroborate those of Struppe et al. (54). The temperature dependence of the viscosity is explained by the formation of larger lipid aggregates (55, 56).

It is noteworthy that the orientation of fluid bicelles can be modified under slow sample spinning speed. As demonstrated by Zandomenighi et al. (57, 58), the bicelle director can be changed to perpendicular or parallel to the rotation axis if the spinning angle is respectively smaller or larger than the magic angle, as illustrated in Fig. 1. Interestingly, the nematic order is preserved. This tunable alignment of bicelles is of great interest in the structural study of membrane peptides and proteins because it allows, for example, the scaling of dipolar interactions (59).

Parallel-Oriented Bicelles

As shown above, the negative sign of the magnetic susceptibility anisotropy of phospholipid bilayers dictates that the bicelles align themselves with the bilayer normal perpendicular to the magnetic field. For bilayer constituents undergoing rapid axially symmetric reorientation with respect to the bilayer normal, which is the case for small peptides and drug molecules, as well as the phospholipid molecules themselves, the only disadvantage of the orientation of bicelles is that the anisotropic shifts and the dipolar and quadrupolar splittings are scaled by a factor of $-1/2$ (60) since these interactions are proportional to $3(\cos^2\theta - 1)/2$, where θ is the angle between the axis of motional averaging (the bilayer normal in this case) and the magnetic field direction (see also sections 2 and 3). However, the spectra of larger and slowly reorienting membrane proteins result in cylindrical powder patterns due to the absence of rapid uniaxial averaging of the anisotropic interactions (60, 61).

The bicelle alignment can be flipped from perpendicular to parallel with respect to the magnetic field when sufficient proportions of compounds with positive anisotropy are added to the sample. This was first observed when an aromatic amphiphile, 1-naphtol, was added to DMPC/CHAPSO bicelles (53). High concentration of the ion channel gramicidin A was also found to flip the bicelle alignment (62, 63). Moreover, since the magnetic susceptibility anisotropy of phospholipids and α -helical protein segments have opposite signs, the addition of proteins containing membrane-spanning α -helices may cancel the tendency for the bilayers to align (64).

In 1996 Prosser et al. (60) discovered that the addition of certain trivalent paramagnetic ions, namely Eu^{3+} , Er^{3+} , Tm^{3+} , and Yb^{3+} , to a bicellar system results in a stable liquid crystalline phase with the bilayer normal aligned along the magnetic field

direction. These ions, which are known to bind preferentially to the lipid phosphodiester groups, confer a large positive $\Delta\chi$ to the membrane. For Tm^{3+} , which has the largest $\Delta\chi$ amongst the lanthanides mentioned above, alignment can be obtained with only one Tm^{3+} ion per 155 DMPC lipid molecules (31, 64). Such parallel-aligned bicelles were developed as an alternative to mechanically aligned bilayers on glass plates, for which preparation is tedious, sample volume low, and control of hydration difficult (65, 66). These systems could be particularly useful to determine the orientation and structure of ^{15}N -labeled proteins in lipid bilayers (65, 67, 68).

The electrostatic interaction between Eu^{3+} and bicelles has been investigated by Crowell and Macdonald (69) using solid-state deuterium NMR of choline-deuterated DMPC. Their results indicate that by manipulating the initial membrane surface charge, it is possible to establish conditions under which both perpendicular-aligned and parallel-aligned bicelles exist. On the other hand, solid-state deuterium NMR has been used to study the structural and dynamical properties of perdeuterated stearic acid in bicelles aligned with Yb^{3+} in the absence and presence of cholesterol (70). The results clearly demonstrate that well-resolved ^2H NMR spectra can be obtained for stearic acid in the magnetically aligned bilayers.

Another type of bicelles with a positive anisotropy of the magnetic susceptibility has been described by Cho et al. (71) using a phospholipid containing a biphenyl group. The ^{31}P NMR spectra obtained for this system indicate that parallel alignment was achieved but that the conformation of the polar headgroup in this type of bicelles is different than that obtained in DMPC/DHPC bicelles.

Morphology

It was long accepted that the phospholipids composing isotropic and oriented bicelles were organized into a discoid bilayer (15, 44, 72), as illustrated in Fig. 2(a). The presence of two well-defined resonances in ^{31}P NMR spectra, ^2H NMR experiments on deuterated DMPC and DHPC, and the absence of NOEs between DMPC and DHPC in two-dimensional ^1H NMR spectra (18, 46, 62, 72, 73), suggest that DMPC molecules compose the planar surface of the disk while the short-chain lipids reside on the edges. Because both phospholipids have the same polar headgroup, the ratio between the areas of the planar surface and the rim would be equivalent to that of the long- and short-chain lipid proportion. Therefore, in this discoid model (62, 72), the bicelle size is directly proportional to the molar ratio (q) of DMPC to

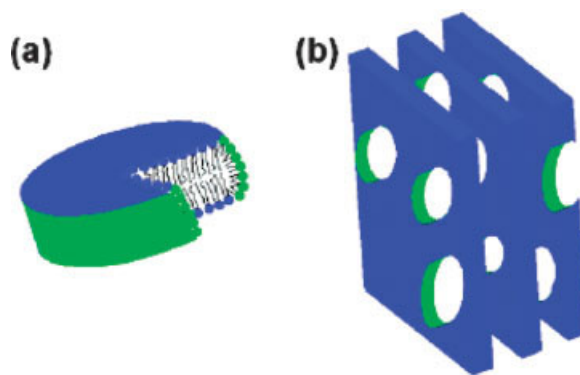


Figure 2 (a) Schematic drawing of a bilayered micelle or so-called bicelle composed of DMPC and DHPC lipid molecules. The short-chain DHPC lipid molecules (in green) occupy the rim of the bicelle, while the long-chain DMPC and DMPG lipids (in blue) are found on the major faces of the micelle. When the bicelles coalesce, the resultant structures are perforated extended lamellae (b) with DHPC molecules coating the pores. Reproduced from Nieh et al. (80) with permission. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

DHPC. Electron microscopy images (47) also support this bicelle model, along with geometrical models and spectral NMR simulations (9, 47, 62).

However, the disk-shape model has been questioned by many research groups. As summarized by Rowe and Neal (74), this model is not consistent with the mechanism of bicelle alignment as a function of temperature in the magnetic field. A disk fusion would be necessary to reach an appropriate size for cooperative alignment (43). Moreover, this model does not reconcile with the increase of viscosity (*vide supra*) occurring at the temperature at which orientation begins and which suggests the formation of large aggregates (74). According to the results published by several research groups using diverse techniques such as small angle X-ray scattering (SAXS), ^{31}P (73, 75), ^2H (73, 76), and ^{23}Na NMR (77), small-angle neutron scattering (SANS) (56, 78–80), and fluorescence probe and resonance transfer (FRET) (74), it appears that different oriented and isotropic lipid organizations exist depending on the temperature, lipid concentration, and DMPC/DHPC (q) ratio. In addition, FRET (74) and SANS experiments (56, 78), along with tracer diffusion measurements performed by NMR (37), have suggested that oriented bicelles would not adopt a discoidal shape, but undergo edge-to-edge contacts, and this is equivalent to DMPC lamellae perforated by DHPC-stabilized pores (37). This model, also known as the Swiss cheese model (31), is presented in Fig. 2(b).

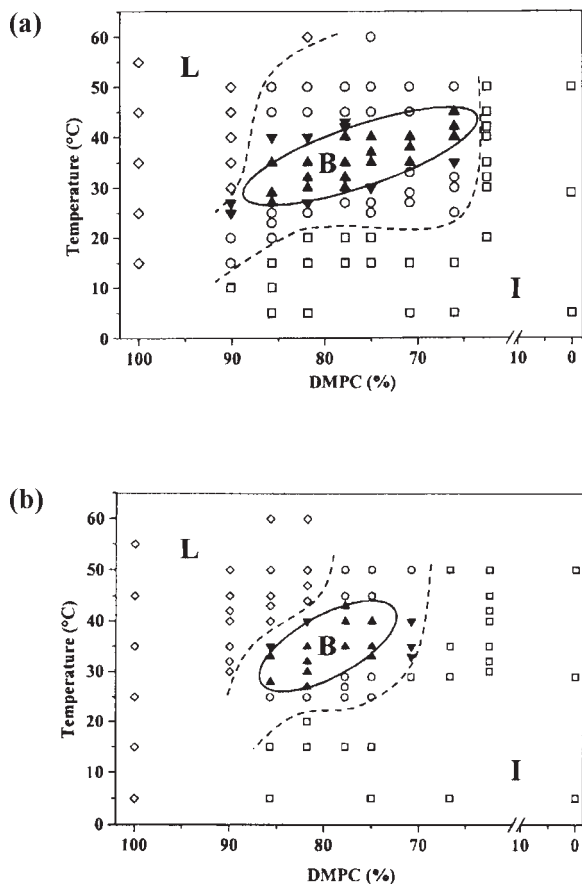


Figure 3 Temperature-composition diagrams of DMPC/DHPC in (a) 80% (w/w) D_2O , 100 mM KCl and (b) 80% w/w D_2O , as determined by ^{31}P NMR: (▲) single phase of bicelles self-orienting into the magnetic field; (▼) self-orienting bicelles coexisting with isotropic or unoriented phases; (○) unoriented plus isotropic phases; (□) isotropic phase (micelles); (◇) lamellar phase. Mole fractions $X = [DMPC] / ([DMPC] + [DHPC])$ are expressed in percent. One-phase regions are denoted I, B, and L for isotropic, bicellar, and lamellar phases. Solid and dashed lines are tentatively drawn to help viewing phase boundaries. Adapted from Raffard et al. (45) and reproduced with permission.

Structural diagrams have been proposed in the literature to illustrate the dependence of the bicelle morphology on the sample conditions. The diagrams proposed by Raffard et al. (45) based on ^{31}P NMR spectra are presented in Fig. 3. They show the changes in the DMPC/DHPC organization as a function of temperature and DMPC/DHPC ratio and at a fixed lipid concentration of 20% w/w in the presence of 100 mM KCl [see Fig. 3(a)] and in the absence of salts [see Fig. 3(b)]. These diagrams define the domain of existence of isotropic and oriented bicelles that can be used in solution- and solid-state NMR studies of

membrane polypeptides, respectively, at a commonly used hydration level of 80%. They lack, however, details in the structures adopted by the lipids, and oriented bicelles are assumed as discoid objects in this work. Yet Fig. 3(a) and 3(b) clearly demonstrate that the presence of salt increases the zone in which bicelles orient. The lipid orientation is observed for proportions of DMPC greater than 70% of total lipids ($q = 2.3$), which compares well with a study performed by Sanders and Schwonek (18) who observed that alignment started near $q = 2.5$ for similar hydration conditions.

From SANS results, Nieh et al. (78) have proposed structural phase diagrams for DMPC/DHPC bicelles ($q = 3.2$), bicelles with 6 mol% of DMPG (Bic/PG, $q = 3.4$, with q representing the molar ratio of both long-chain phospholipids to that of DHPC) and Bic/PG6%/Tm $^{3+}$ [see Fig. 4(a–c)] at different lipid concentrations and as a function of temperature. However, this work was done at only one q ratio. The different structures adopted by the long- and short-chain lipid mixtures are well illustrated. For the DMPC/DHPC mixture [see Fig. 4(a)], randomly oriented disks are observed at high lipid concentration and low temperature, whereas oriented lamellae are formed between 25–45°C and high lipid concentration. It is noteworthy that for the same q ratio (3.2) and lipid concentration (20% w/v), oriented DMPC/DHPC “aggregates” are found in the same temperature range in both Nieh’s and Raffard’s diagrams. The formation of MLVs is observed at low lipid concentration or elevated temperature. This structural diagram is consistent with previous studies performed at similar q ratios and sample conditions (37, 56, 77). Figure 4(b) highlights the effect of negatively charged DMPG in bicelles. In particular, it suggests that high charge density induces a more regular lamellae repeat spacing as a result of interlamellar repulsion. It also shows the presence of only two morphologies, although the Bic/PG system at temperatures above 45°C was not investigated. Interestingly, the addition of Tm $^{3+}$ (~1 mol%) to the Bic/PG system shows a diagram similar to that of the system without lanthanide ions, except for the formation of ULVs, as seen in Fig. 4(c). The bicelle-to-lamellae transition has been investigated (79, 80) using SANS and Bic/DMPG at $q \sim 5$. It is explained by a coalescence of the disks into perforated stacks. A metastable phase in which discoid bicelles or small lamellae are trapped into pores is observed at this high q ratio (80).

Because the bicelle morphology has been investigated at various sample conditions, it is thus currently not possible to draw a definitive phase diagram covering a wide range of temperature, q ratios, and lipid

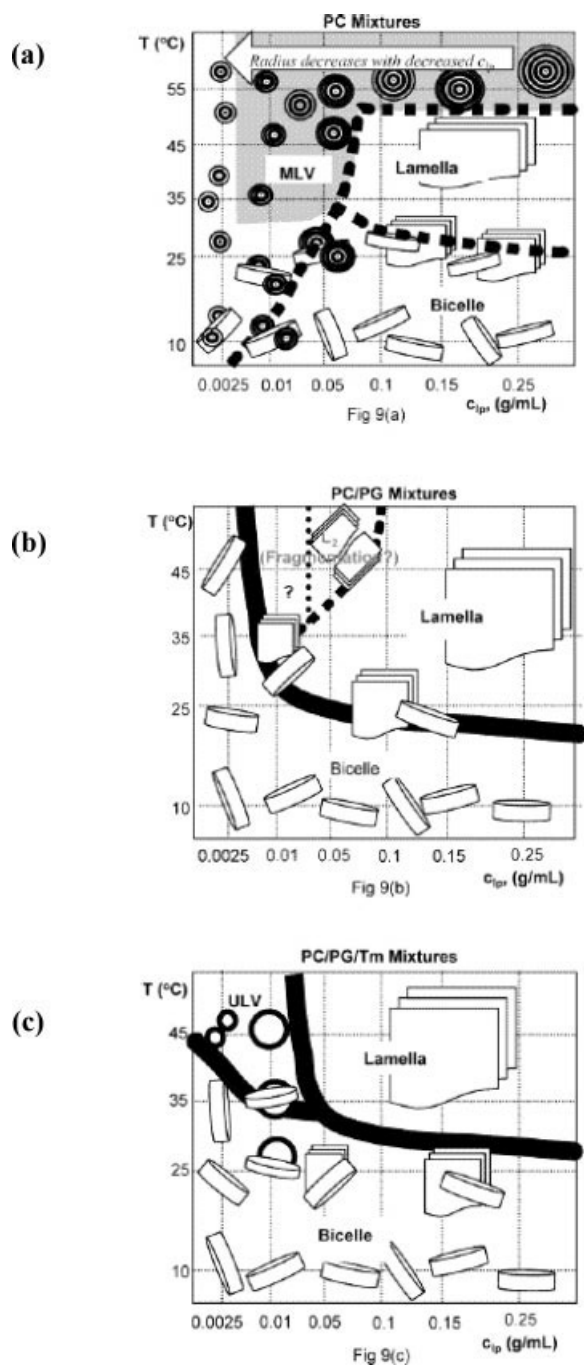


Figure 4 Temperature-lipid concentration diagrams in D₂O for (a) DMPC/DHPC, $q = 3.2$, (b) Bic/PG6%, $q = 3.4$, and (c) Bic/PG6%/Tm³⁺ (~1%), $q = 3.4$. The ratio q is expressed as the molar ratio of the long-chain lipids to that of the short-chain DHPC such as $q = [DMPC + DMPG]/[DHPC]$. The grey regions indicate where some degree of macroscopic phase separation was visually observed. Adapted from Nieh et al. (78) and reproduced with permission.

concentrations. Recently, a bicelle model based on a cubic phase has been considered by Rowe and Neal (74) from their FRET results. These authors have pointed out that such a model has not been thought of in the interpretation of experimental results and ³¹P NMR spectral simulations. Complementary studies are therefore necessary to complete our current knowledge of the bicelle orientation and phase diagrams as a function of all the parameters listed above and explore the different morphology models in simulating the ³¹P and ²H NMR spectra.

There seems to be, nevertheless, a general agreement on the discoid morphology for fast-tumbling bicelles, which are characterized by a low q ratio (typically between 1 and 0.5). SANS experiments and ¹H NMR measurements on fast-tumbling bicelles in the presence of paramagnetic agents (46) have validated the disk model in which DHPC is primarily sequestered to the bicelle rims. ³¹P NMR has also been used to further confirm that DMPC and DHPC are highly segregated over a wide range of DMPC/DHPC ratios and temperatures (81). Additional results obtained by dynamic light scattering support the hypothesis that the isotropic bicellar phospholipid aggregates are disk shaped (81). In the case of dilute bicelles, which are generally used to align proteins in solution, results obtained by Gaemers and Bax (37) are consistent with a disk-like structure below the DMPC phase transition temperature (25°C), and a perforated bilayer morphology at higher temperatures.

Although the term “bicelles” originates from the belief of disk-shaped micelle formation, it now generally designates in the literature the model membranes composed of long- and short-chain phosphatidylcholines, regardless of their morphology. The term “bicelles” will thus be used in the current review. For the bicelles organized as disks or perforated lamellae, the planar DMPC section composing these structures is interesting for the study of peptides and proteins as it is similar to that of the biomembranes. Importantly, it has been shown that the biological activity of the enzyme diacylglycerolkinase (DAGK) is preserved in bicelles (11, 21, 82). In addition, these model membranes orient at physiological temperatures and, just like biomembranes, are in the fluid phase and contain phosphatidylcholines (28).

PART 2: BICELLES IN THE SOLID-STATE NMR STUDY OF MEMBRANE PEPTIDES AND PROTEINS

One of the most important advantages of bicelles is their possible use in solid- and solution-state NMR

investigations of membrane peptides and proteins to obtain information on their conformation, location, and interaction with a unique membrane model system. As evidenced by the temperature-composition diagrams presented in Figs. 3 and 4, this can be achieved by selecting the appropriate long-to-short chain lipid ratio and/or lipid concentration to prepare either oriented or isotropic fast-tumbling bicelles. This section will focus on the solid-state NMR study of bicelle-bound polypeptides.

Sanders and Landis (44) were the first to demonstrate the utility of bicelles for this purpose by publishing well-resolved ^{13}C NMR spectra of cytochrome C and the pentapeptide leucine-enkephalin. Some proteins or peptides are not easily reconstituted into bicelles without disrupting the bicelle orientation and/or organization, as reported by Sanders and Landis (11). As discussed previously, the presence of proteins with positive magnetic anisotropy can alter the bicelle orientation. Phase changes or separation and bicelle fusion can also occur. These perturbations can often be detected by a loss of the sample optical clarity and are also observed on the ^{31}P and ^2H NMR spectra (*vide infra*).

Investigation of Protein- or Peptide-Membrane Interactions

In general, the study of polypeptide-membrane interactions is performed by observing the effect of the peptide or protein on the phospholipids using oriented bicelles. Because of the presence of a phosphorus spin in the phospholipid headgroups, ^{31}P NMR can be used to probe the effects occurring in the bilayer polar region. The hydrophobic portion of lipid bilayers can be studied by ^2H NMR using deuterated phospholipids.

^{31}P NMR

Phosphorus-31 has a spin $\frac{1}{2}$ and a 100% natural abundance, and, with its good sensitivity, is thus extremely useful to investigate the effects of proteins on the polar region of bicelles. The chemical shift anisotropy (CSA) and phosphorus-proton dipolar interactions are dominant in ^{31}P NMR, but the latter are generally removed by high-power irradiation of the protons (decoupling). As described by Seelig (83), the resonance frequency (ω) of the ^{31}P spin depends on the orientation (θ) of the principal axis of motion, \vec{n} , with respect to the magnetic field such as:

$$\omega = \frac{2}{3}\Delta\sigma \frac{(3\cos^2\theta - 1)}{2} + \delta_{\text{iso}} \quad [3]$$

where δ_{iso} is the isotropic chemical shift and $\Delta\sigma$ is the chemical shift anisotropy. In the case of bilayers in the fluid state, like the commonly used bicelles, the phospholipids rapidly rotate along their longitudinal (z) axis (the bilayer normal), and this axially symmetric motion averages out the x and y contributions of the CSA tensor. Therefore, $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$, the difference between the chemical shifts obtained when the magnetic field direction is, respectively, parallel and perpendicular to the lipid long axis. The “powder” spectrum represents the proportion of lipids at each possible orientation with respect to B_0 , from parallel (0°) to perpendicular (90°).

In the case of bicelles aligned with their normal perpendicular to B_0 direction, the long axis of most of the lipids is oriented at 90° . This, therefore, does not lead to a powder pattern but to a characteristic spectrum showing two well-resolved resonances, as displayed in Fig. 5(a). As determined in previous studies (18, 62, 78), the high field resonance corresponds to the DMPC molecules localized on the planar surface of the disk (or perforated lamellae) whereas the second peak is attributed to DHPC constituting the bicelle torus (or lamellae pores). The nearly isotropic position of the DHPC resonance can be explained by the toroidal (porelike) distribution of the lipids partially averaged by their lateral diffusion (18, 62). In the case of bicelles doped with another phospholipid, such as DMPE, DMPG, DMPS, or cardiolipin (26, 29, 30), a third resonance is seen on the spectrum whose position, relative to the DMPC resonance, is consistent with that of their 90° orientation observed on the lipid powder spectra.

In the study of membrane-bound peptides, the shape of the ^{31}P NMR spectra of bicelles can be used, as a first step, as a diagnostic of the orientation quality before and after the addition of peptides, lipids, or membrane-binding molecules (11, 17, 24, 26, 29, 30, 32, 34, 45, 47, 54, 64, 67, 73, 84–87). A method has been proposed by Picard et al. (62) to determine the degree of alignment of bicelles from their ^{31}P NMR spectra. This method is based on the determination of the first spectral moment from which an order parameter S_1 (defined below) allows a quantitative analysis of partially oriented spectra.

$$S_1 = \frac{M_1 - \delta_{\text{iso}}}{\delta} \quad [4]$$

In this equation, M_1 is the first spectral moment, δ_{iso} is the isotropic chemical shift, and δ can be defined as:

$$\delta = \delta_{\parallel} - \delta_{\text{iso}} = -2\delta_{\perp} - \delta_{\text{iso}} \quad [5]$$

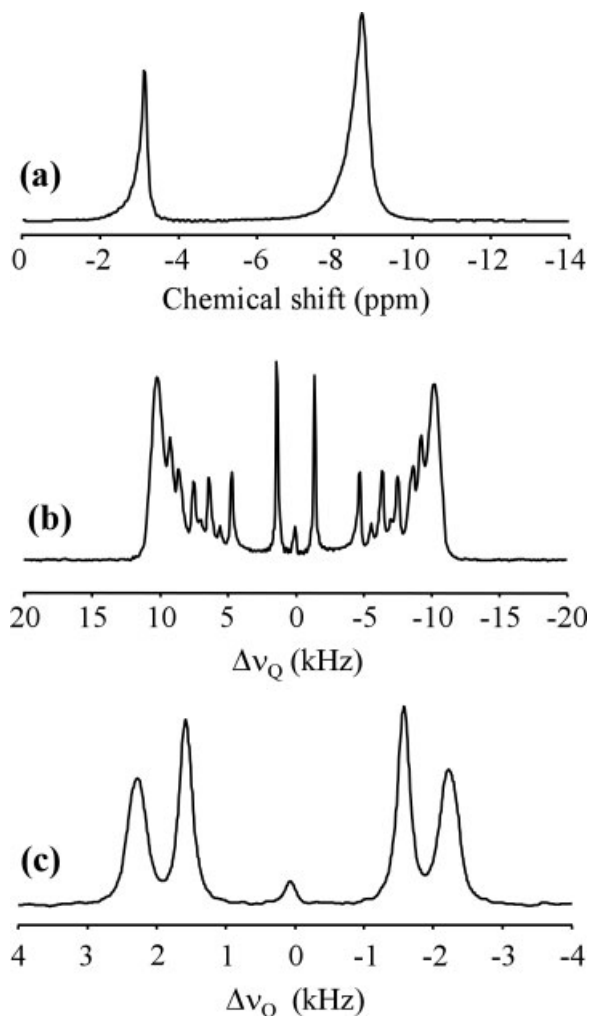


Figure 5 Characteristic spectra of bicelles, 20% w/w, 37°C. (a) ^{31}P NMR spectrum, $q = 2.70$. (b) ^2H NMR spectrum, DMPC- d_{27} , $q = 3.55$. (c) ^2H NMR spectrum, DMPC- d_4 , $q = 3.55$.

where δ_{\parallel} and δ_{\perp} are respectively the chemical shifts measured for the phospholipids oriented parallel and perpendicular to the magnetic field direction. This order parameter S_1 is a measure of the orientation level in the studied system. A system with a value of S_1 close to 1 indicates that the main axis of all the molecules are along the magnetic field direction, whereas a system with a value of S_1 close to -0.5 indicates that all the molecules are oriented with their main axis perpendicular to B_0 . Arnold et al. (47) have expressed the degree of alignment of bicelles in terms of the mosaicity (mosaic spread) of the Gaussian distribution of orientation. The quality of the bicelle orientation, as determined by ^{31}P NMR, can be used to define, by titration, the appropriate amount of peptide to be added to the sample. The appearance of an

isotropic resonance also brings valuable information about the bicelle organization. It is generally indicative of membrane disruption and subsequent formation of fast-tumbling lipid structures. This is often observed with lytic peptides such as antibiotics (67) and melittin (32, 88).

Due to the characteristic ^{31}P NMR spectra of bicelles, the kinetics of enzymatic degradation of phospholipids can be followed. This was demonstrated by Whiles et al. (89) who calculated the rate of lipid degradation by phospholipase A2 in bicelles and DPPC and DMPC bicelles. Resonances of hydrolyzed lipids gradually appeared on the spectra, and the area ratio between the lipid peaks and those of their hydrolyzed counterparts were monitored as a function of time.

Finally, as the ^{31}P chemical shift anisotropy is affected by lipid dynamics (83), it is possible to detect perturbations in the phospholipid headgroup motion induced by the presence of a membrane protein or peptide by observing changes in the chemical shift. For example, shifts in the lipid resonances were observed by Marcotte et al. (29) upon binding of Met-enkephalin to zwitterionic and anionic bicelles, and were correlated to changes in the lipid headgroup motions (29). Similar results were obtained for antibiotic frog peptides in bicelles (67). However, it is possible that shifts can be attributed to deshielding peptide moieties such as aromatic residues in the vicinity of the phosphorus atom.

Another way to determine the effect of a protein on the chemical shift anisotropy is to evaluate a relative order parameter S_2 of the form (18, 62):

$$S_2 = \frac{\delta}{\delta_{\text{ref}}} \quad [6]$$

where δ_{ref} is the chemical shift anisotropy of a reference system. This order parameter is governed by the dynamics of the lipids in the system and is therefore related to the averaging of the chemical shift anisotropy.

^2H NMR

Deuterium NMR is of great interest in the study of polypeptide-membrane interactions as it allows the determination of effects at different parts of the membrane by using lipids that are deuterated on the acyl chains or the headgroup. This atomic substitution does not affect the lipid organization (90), and the weak natural abundance of deuterium (0.016%) does not contribute to the spectra. However, the gel-to-fluid

phase transition of DMPC is slightly decreased ($\sim 2^\circ\text{C}$) by the deuteration of the molecule (85).

Deuterium is a spin-1 nucleus with a quadrupole moment that interacts with the electric field gradient at the nucleus, giving rise to the quadrupolar interaction. Two spin transitions are possible, and a doublet of resonances is observed on a ^2H NMR spectrum, separated by the quadrupolar splitting $\Delta\nu_Q$ (90). For a system with axially symmetric motions, the quadrupolar splitting is given by:

$$\Delta\nu_Q = (3/4)(e^2qQ/h)(3\cos^2\theta - 1)S_{CD} \quad [7]$$

where (e^2qQ/h) is the quadrupole coupling constant (~ 170 kHz for aliphatic C–D) (91), θ is the angle between the bilayer normal and \mathbf{B}_0 , and S_{CD} is the order parameter of a deuterium bond vector. As described extensively (31, 72, 85), this order parameter is the product of several contributions, including bicelle wobbling, intramolecular motions such as trans-gauche isomerizations, and anisotropic reorientation of the whole phospholipid molecules. Hence it is possible to determine variations in lipid chain order by monitoring changes in $\Delta\nu_Q$ values. The description of the different order parameters is however not in the scope of this review and the reader should refer to the references mentioned above for more details.

Because bicelles are oriented with the lipid bilayer normal at 90° with respect to \mathbf{B}_0 , a doublet can be attributed to most of the deuteron positions on the lipid chain. A characteristic spectrum is shown in Fig. 5(b), where the innermost doublet is attributed to the CD_3 at the end of the lipid chain, and the largest quadrupolar splitting is that of the CD_2 attached to the glycerol backbone at the polar-apolar interface. Hence, as in ^{31}P NMR, the quality of the sample orientation can also be evaluated from the ^2H NMR spectrum (17, 25–27, 72, 92), in addition to variations in the dynamics at different parts of the acyl chains.

It is most likely that bicelles in aqueous samples will undergo a wobbling motion, the dynamical effect of which can reduce the measured quadrupolar splitting values. As previously described, it is possible to assess the bicelle wobbling (S_{bw}) by comparing the quadrupolar splitting of a phospholipid deuteron in bicelles (Δ_{Bic}^L) to that in vesicles (Δ_{MLV}^L) (31, 86) such as:

$$S_{bw} = \Delta_{Bic}^L / \Delta_{MLV}^L \quad [8]$$

For bicelles with $q = 3.5$, an order parameter value of 0.84 has been calculated, indicating a high ordering

that compares well to DMPC bilayers aligned on glass plates (86). Bicelle wobbling has been shown to be important at small q values ($q < \sim 3.0$), therefore it is preferable to investigate dynamical effects in ^2H NMR spectra of bicelles at higher q values (29, 72, 85).

^2H NMR has been used extensively to investigate the binding and penetration depth of diverse peptides to bicelles and/or anionic bicelles such as the myristoylated N-terminal 14-residue peptides of cat-14 (catalytic subunit of protein kinase A) (25) and pp60^{v-src} (27), a series of antibiotic peptides extracted from frog skin (67), and the 35-residue fragment of a tyrosine kinase receptor Neu_{TM35} (93). By using either deuterated DMPC or DMPS in bicelles and Bic/PS, Whiles et al. (86) found a preferential interaction of mastoparan X with the negatively charged lipids at the planar region. This was also done to compare the position and verify the preferential interaction of Met-enkephalin in zwitterionic and negatively charged bicelles (29). The insertion of the helicoidal transmembrane peptide P16 was also investigated using ^2H NMR (23).

As mentioned previously, it is also possible to determine the effects of peptides or protein at the bicelle surface using phospholipids with deuterated cholines. In this case, the spectrum [as shown in Fig. 5(c)] is composed of two doublets attributed to the α - and β -methylene close to the phosphate (outer doublet) and trimethylamine groups (inner doublet), respectively. The headgroup average orientation and order can thus be probed. More specifically, the effect of charges on the choline tilt angle can be assessed as the α - and β -splittings change inversely as a function of the negative and positive charges present (24, 26, 94). It is noteworthy that recently the effects of bilayer surface charge on the phosphatidylcholine headgroups in bicelles was investigated using a torsion angle analysis of quadrupolar splittings (^2H NMR) and dipolar couplings (^{13}C NMR) (24).

The combined use of choline- and chain-deuterated DMPC is useful when investigating the interaction of peptides (or drugs) with bicelles as information is obtained at the polar and apolar regions. Such work was done by Parker et al. (30) who found an interaction of doxorubicin with surface and buried sites of negatively charged cardiolipin-doped bicelles. Similarly, Marcotte et al. (29) showed that the penetration of the neuropeptide Met-enkephalin into zwitterionic bicelles and negatively charged bicelles was modulated by the balance between the hydrophobic and electrostatic interactions.

Determination of the Structure and Orientation of Membrane-Bound Peptides

Sanders and Landis (44) were the first to show the possibility of scaling the dipolar couplings for use in structure determination by tuning the bicelle orientation via changes in the q ratio. This method allows the assignment of the ^{13}C resonances by following peaks to their isotropic position, and the calculation of the CSA (11, 53).

The use of oriented bicelles and INADEQUATE experiments have allowed the ^{13}C resonance attribution of the peptide Myr15, the N-terminal fragment of human ADP-ribosylation factor 1 (95). In addition, the orientation of several amide planes in the peptide was determined from the study of residual dipolar couplings and chemical shift anisotropy effects. It is also possible to calculate the helix tilt angle (86, 92, 93) of the deuterated moieties of peptides inserted into oriented bicelles by recording the ^2H NMR spectrum. In the case of ^2H -myristoylated peptides, it was possible to verify if the myristoylated segment was fully inserted by monitoring the degree of ordering along the chain and assess the presence of kinks (25, 27). Similarly, Dave et al. (70) have studied the dynamics of deuterated stearic acid in bicelles and cholesterol-doped bicelles.

The orientation of peptide planes can also be studied by ^{15}N NMR because the ^{15}N chemical shift can identify ^{15}N -H bonds of residues that are perpendicular or parallel to the magnetic field direction, as was shown with the labeled fd coat protein reconstituted in bicelles (65). However, the use of perpendicularly aligned bicelles can complicate the interpretation of the data, in particular for extrinsic peptides that bind to the membrane surface. In such a case, these peptides can have a random distribution on the bilayer with respect to the magnetic field direction, and lead to a powder spectrum spanning σ_{11} to σ_{33} (96). Rapidly reorienting peptides, such as Met-enkephalin, can also lead to an isotropic resonance (97). Therefore the study of transmembrane peptides should sometimes be paralleled by other techniques, such as circular dichroism (CD)—for example, in the case of membrane-spanning peptides, which can have a chemical shift close to the isotropic value when inserted with an orientation close to the magic angle. This was observed by Marcotte et al. (67) with the antibiotic aurein 1.2 for which CD experiments confirmed such an orientation rather than a rapid reorientation of the peptide.

The future prospects in the use of oriented bicelles for gleaned information on membrane-associated peptides appear to lie on the use of sample spinning,

which leads to high-resolution spectra. As suggested by preliminary results published by Sizun et al. (93) on Neu_{TM35}, it would be possible to use magic angle spinning and ^1H NMR to perform the structure determination of peptides bound to oriented deuterated bicelles. This group also showed, using Met-enkephalin, that the localization of peptides into the bilayer could be assessed by monitoring intermolecular peptide-lipid NOEs. In addition, Zandomenighi and Meier (59) extracted the ^1H - ^1H dipole coupling from the observed scalar/dipolar coupling values using slow spinning of oriented bicelles at variable angles in ^1H NMR by fitting the peak splitting as a function of the angle between the rotor axis and B_0 direction. Recently, the amide bond dipolar couplings for Leu-enkephalin were measured using the variable angle spinning technique, offering a measure of the amide bond orientation with respect to the membrane surface (98).

Study of Peptides in Lanthanide-Doped Bicelles

The first attempt to incorporate peptides or proteins in lanthanide-doped bicelles was published in 1996 by Howard and Opella (65). They compared spectra of a uniformly ^{15}N -labeled membrane protein reconstituted into phospholipid bilayers oriented parallel and perpendicular relative to the magnetic field direction. Their results clearly indicate that the ^{15}N NMR spectrum of the protein in the lanthanide-doped bicelles has its effective frequency range increased by a factor of $|2.0 \pm 0.2|$ compared to that obtained for the perpendicular-oriented bicelles. This doubling is indicative of a change in the ordering of the bilayer director from $S_{zz} = -1/2$ to $S_{zz} = 1$. The order parameter S_{zz} is defined as $3(\cos^2 \theta - 1)/2$, where θ is the angle between the bilayer normal and the applied magnetic field. The frequency breadth obtained in the parallel-oriented bicelles is however slightly less than that obtained for the same protein oriented between glass plates due to the wobbling of the magnetically oriented bicelles about their average orientation.

Providing that the orientation of bicelles is good, it is of interest to study the interaction of peptides, proteins, or other molecules (70) with parallel deuterated bicelles because the quadrupolar splittings values are doubled, as can be deduced from Eq. [7] with $\theta = 0^\circ$ (60). It is thus easier to monitor, for example, changes occurring at different positions on the lipid chains. The orientation of the sample can also be better assessed by ^2H NMR as the ^{31}P NMR spectra are complicated by the presence of paramagnetic shift

agents, generally located in the vicinity of the phosphorus atoms (64), as they are known to shift and broaden NMR resonances.

Lanthanides might bind to negatively charged sites in membrane protein or peptide of interest, resulting in a change of conformation of the peptide or protein, and the addition of peptide also often necessitates higher lanthanide concentrations to maintain alignment (*vide infra*). To overcome these limitations, Prosser et al. (99) suggested incorporating into the bicellar system a phospholipid molecule that strongly binds or chelates the lanthanides. This has been shown to significantly reduce paramagnetic broadening and prevent direct association of peptides with the lanthanide ions. More specifically, a phospholipid chelate complexed with ytterbium (DMPE-DTPA: Yb³⁺) has been shown to be readily incorporated into bicelles (99). This has been demonstrated with gramicidin A for which the ²H NMR spectra of ²H exchange-labeled peptide indicate that the chelate sequesters the lanthanides away from the peptide binding sites (99). The use of the DMPE-DTPA chelate in positively aligned Yb³⁺-doped bilayers has also been tested for two membrane-associated peptides (99), and the spectra appeared to be well aligned and stable. A positively ordered smectic (layered) phase has been obtained with as little as 1% DMPE-DTPA:Yb³⁺. Another amphiphilic chelate, DTPA-18, has also been proposed by Prosser et al. (87) and has been shown by deuterium NMR to be useful in aligning bicelles with the average bilayer normal along the magnetic field direction. This study also showed that the conformational change induced by Tm³⁺ in the surface-associated Leu-enkephalin is reversed by the addition of the chelate. The use of lanthanide chelates therefore appears to be a promising avenue in the study of the structure of peptides in parallel-aligned bicelles.

PART 3: BICELLES IN THE SOLUTION-STATE NMR STUDY OF MEMBRANE PEPTIDES AND PROTEINS

Introduction

Detergent micelles, composed of either the anionic surfactant sodium dodecylsulfate or the zwitterionic surfactant dodecylphosphocholine, have been extensively used as a membrane mimetic since the early days of peptide and protein structure determination (100). Recent examples of the structure determination of small α -helical membrane proteins in micelles include the coat proteins from the filamentous bacterio-

phages M13 (50 residues) (101) and fd (50 residues) (102), and the 81-residue human immunodeficiency virus (HIV) membrane-associated protein Vpu (103). In addition, the use of the TROSY (transverse relaxation-optimized spectroscopy) technique, combined with refined isotope labeling strategies, has extended significantly the size of the proteins that can be investigated in micelles (104). Recent examples include the study of the outer membrane proteins OmpX (148 residues) (105, 106), OmpA (177 residues) (105), and PagP (164 residues) (107) from *Escherichia coli* in micelles with size of 50–60 kDa. All of these proteins consist of an eight-stranded antiparallel β -barrel structure, and the TROSY approach has not yet been applied to α -helical transmembrane proteins.

However, the high surface curvature of micelles has been shown to induce strain in proteins interacting with the membrane surface (9, 12). To circumvent this problem, the use of mixed micelles or bicelles has been proposed. Because the bicelle interior consists of a true lipid bilayer, this system presents a more natural environment for membrane proteins. It has been shown that the activity of some proteins and enzymes incorporated into micelles is not readily preserved (8–10) but that the biological activity of the enzyme diacylglycerolkinase (DAGK) is preserved in bicelles (11, 21, 82). In addition, it has been shown that the position of a peptide in a micelle can be significantly different from its position in a phospholipid bilayer (108, 109). Finally, the lipid composition of bicelles can be varied to better mimic biological membranes.

Extending the application of bicelles into the isotropic phase has first been suggested in 1997 by Vold et al. (9). By using a total phospholipid concentration of 15% (w/v) and increasing the content of short-chain phospholipid (DHPC) until a q ratio of 0.5 was reached, isotropic (or so-called fast-tumbling) bicelles have been obtained. A resolution sufficient for high-resolution NMR studies of membrane-associated peptides was achieved with this system, as discussed in the examples presented below. As mentioned previously, fast-tumbling bicelles are believed to have a disk-shape morphology and several experiments have indicated that DHPC is primarily sequestered to the bicelle rims (46, 81).

It is interesting to note that the diameter estimated for DMPC/DHPC bicelles at a $q = 0.5$ is 80–100 Å. As demonstrated by Vold et al. (9), if bicelles were rigid object, their overall correlation time would be on the order of 100 ns, compared to 26 ns for a spherical micelle with a diameter of 50 Å. This would make high-resolution NMR studies impossible, and it has therefore been concluded that the narrow lines observed for peptides associated to fast-tumbling bi-

celles is the consequence of the high internal mobility of membrane constituents (9, 110).

Structure, Dynamics and Association of Peptides in Fast-Tumbling Bicelles

After their introduction in 1997, fast-tumbling bicelles have been used by several research groups to investigate the structure of various peptides, as detailed below. The size of the peptides investigated varies from five amino acids for methionine-enkephalin (111) to 27 amino acids for the peptide transportan (108). Typically, the peptide structures have been determined by the use of two-dimensional ^1H NMR techniques. Resonance assignments for the protons are readily obtained from analysis of COSY and TOCSY data and distance constraints are obtained from two-dimensional NOESY data. Additional torsion angle constraints can also be obtained from coupling constants. Fast-tumbling bicelles have not yet been used to investigate the structure of uniformly $^{15}\text{N}/^{13}\text{C}$ -labeled peptides by multidimensional (3D and 4D) NMR methods.

The resolution obtained for peptides associated to fast-tumbling bicelles was first demonstrated on the tetradecameric peptide mastoparan from *Vespa lewisii* (9). The two-dimensional ^1H NMR spectra of the peptide in fast-tumbling bicelles indicate that the peptide adopts a well-defined conformation in association with the bilayers, in contrast to the random coil structure observed in water. No evidence of positive peptide NOEs was found in the two-dimensional NOESY spectrum at mixing times of 150 ms and 250 ms, indicating that the dominating peptide reorientation times are long. Note the absence of peptide-phospholipid intermolecular NOEs in these systems, due to the fast lipid and/or peptide motion relative to a specific lipid molecule (9).

This study was further extended to the comparison of the structure of mastoparan X in zwitterionic and anionic bicelles (86). The results demonstrate that MPX forms a well-structured amphiphatic helix in these two types of bicelles. The dynamics of MPX was also investigated in these systems, further indicating that this peptide is bound to bicelles as a well-structured helix. An interesting aspect of this study is the demonstration that the introduction of anionic lipids, in proportion of up to 25%, does not alter the resolution obtained with zwitterionic fast-tumbling bicelles. The fact that fast-tumbling bicelles can easily be modified with anionic lipids is particularly interesting considering that several membrane-associated peptides and proteins bear a global positive charge.

Andersson and Mäler (112, 113) recently investigated the structure and location of several peptides in fast-tumbling bicelles, including the 22 amino acid hormone motilin, two variants of the peptide penetratin (109, 114), and transportan (108). More specifically, the structure of motilin has been investigated in zwitterionic and acidic bicelles, in which 30% of DMPC was replaced by DMPG (112). The results of these studies clearly demonstrate that the peptide is less structured and more flexible in zwitterionic bicelles compared with anionic bicelles. The dynamical behavior of the peptide was also probed by the analysis of relaxation data for the Leu10 $^{13}\text{C}^\alpha - ^1\text{H}$ spin pair. A global correlation time of $t_m = 14.2$ ns was obtained with the model-free approach of Lipari and Szabo (115, 116) for motilin in acidic bicelles, indicating that the peptide is attached to a large object. A shorter correlation time (7.0 ns) was obtained for motilin in neutral bicelles, indicating different peptide-bicelle interactions.

The translational diffusion of the motilin peptide in association with acidic and neutral bicelles has been investigated using pulsed field gradient NMR (113). More specifically, if the peptide is in rapid exchange on the NMR timescale between the free and the bound states, it is possible to calculate the amount of bound peptide by measuring the translational diffusion constants for the different molecular species (117). The populations of free and bicelle-bound molecules can be estimated from:

$$D_{\text{complex}} = xD_{\text{bound}} + (1 - x)D_{\text{free}} \left(\frac{D_{\text{H}_2\text{O,complex}}}{D_{\text{H}_2\text{O,free}}} \right) \quad [9]$$

where x is the amount of molecules bound to the bicelles, D_{bound} is the diffusion coefficient of the long chain lipids, D_{free} is the diffusion coefficient for the free molecule (DHPC or motilin in this case), and D_{complex} is the diffusion constant for the molecule in the presence of bicelles. The diffusion coefficients for water, $D_{\text{H}_2\text{O}}$, are introduced to account for differences in viscosity of the different solutions. The results obtained by this technique indicate that about 90% and 84% of motilin is bound to acid and zwitterionic bicelles, respectively. The large difference in dynamics between the peptide bound to acidic and neutral bicelles cannot be entirely explained by the amounts of bicelle-bound peptide but is most likely due to a higher flexibility of the peptide in neutral bicelles (112, 113).

A similar approach has been used to compare the structure, dynamics, and association of two variants of the peptide penetratin in SDS micelles and bicelles

(109, 114). This peptide is the third helix (from residues 43–58) of the Antennapedia homeodomain protein of *Drosophila* and has been shown to penetrate biological membranes. The results indicate that penetratin and its nontranslocating analog (W48F,W56F) have similar structure, with a helical structure present in about 50% of the peptide (Lys46–Met54). In addition, diffusion measurements indicate that penetratin binds almost fully to the bicelles, and relaxation data indicate that the peptide has a more restricted flexibility in negatively charged bicelles compared to neutral bicelles.

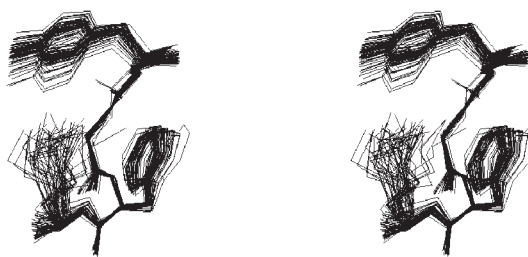
Finally, the NMR solution structure of transportan in neutral bicelles has recently been investigated (108). Transportan is a chimeric cell-penetrating peptide constructed from the peptides galanin and mastoparan and it has the ability to translocate through biological membranes and to carry large hydrophilic load through the membranes. The results of this study demonstrate that the mastoparan part of transportan presents a helical conformation, whereas the galanin moiety also has a tendency, although smaller, to adopt a helical conformation. This is in contrast to studies of transportan in SDS, which have revealed that the galanin section of the peptide is less structured. These results therefore clearly illustrate the possible influence of the model membranes on the structure of peptides.

The results obtained in the studies discussed above have shown a unique structure for all the peptides investigated in the different bicelle environments. Recently however, Marcotte et al. (111) found distinct conformations of the peptide methionine-enkephalin in fast-tumbling bicelles. The effect of the membrane composition on the peptide conformation was explored using zwitterionic and negatively charged bicelles (Bic/PG). Pulse field gradients experiments allowed the determination of the proportion of Met-enkephalin bound to the model membranes. Approximately 60% of the water-soluble enkephalin was found to associate to the bicellar systems. Structure calculations from torsion angle and NOE-based distance constraints suggest the presence of μ - and δ -selective conformers of Met-enkephalin in each system and slightly different conformers in PC bicelles and Bic/PG. In contrast to previous studies of enkephalins in membrane mimetic systems, the results show that these opiate peptides could adopt several conformations in a membrane environment, which is consistent with the flexibility and poor selectivity of enkephalins. An example of the two types of conformation obtained in that study is shown in Fig. 6, which indicates that the structure of methionine-enkephalin in Bic/PG divides into two groups of con-

80 structures



Group A



Group B



Figure 6 Stereoview of the structure of methionine-enkephalin in Bic/PG, divided into two groups of conformers. The structures were generated by the program Molmol (129). Adapted from Marcotte et al. (111) and reproduced with permission.

formers, which could correspond to μ - and δ -selective conformers.

Position of Peptides in Fast-Tumbling Bicelles

There are several methods to investigate the position of a peptide in micelles or in fast-tumbling bicelles, including the use of paramagnetic probes inserted at different positions within the bicelles, the study of amide exchange, and the observation of peptide-phospholipid NOE effects (118). The first approach has

been used to investigate the position of the peptides motilin (113), penetratin (109), and transportan (108), as discussed in the previous section. Several paramagnetic probes have been used to look at different locations in the bicelles, namely Mn^{2+} , to probe the bicelle surface (headgroup region), and phospholipids with a doxyl group at either the fifth or twelfth position in the fatty acid chain, to explore different depths in the bicelle interior. The position of the peptides with respect to the bicelles is investigated by observing the effects on $\text{H}^{\text{N}}-\text{H}^{\alpha}$ cross-peaks in TOCSY spectra of the peptides after the addition of paramagnetic probes.

The results obtained for motilin indicate that the helix resides close to the surface of the bicelle, and the N-terminus part of the peptide inserts into the bicelle interior. For the two variants of penetratin, the results indicate that both peptides are positioned within the bicelle but that subtle differences exist in the positioning of the two peptides. Finally, transportan has been shown to lie parallel to the membrane surface, in the headgroup region. The positions of motilin and penetratin in bicelles have been compared to those obtained in SDS micelles, and that small differences exist between the locations of both peptides in these two environments. These results could therefore help explain how these peptides interact with a real biological membrane. In addition, they illustrate that fast-tumbling bicelles may provide a more realistic membrane-like environment for the study of peptides.

The location of residues in transmembrane peptides incorporated into fast-tumbling bicelles has been investigated by a combination of NOE measurement and amide-water chemical exchange (118). The use of several band-selective 2D-NOESY spectra, acquired in bicellar solutions prepared with three combinations of deuterated and protonated DMPC and DHPC, has been useful to distinguish the NOEs due to amide-peptide, amide-DHPC, and amide-DMPC interactions. The results demonstrate that the peptide P16 has a transmembrane orientation and that it interacts primarily with the long-chain lipids of the bicelles rather than with the rim. This study further confirms the advantage of disk-shaped bicelles over round-shaped micelles in the study of transmembrane peptide, as the bilayer section of the bicelle has several important roles—namely, shielding hydrophobic residues from exposure to solvent molecule and aiding in the formation of secondary and tertiary structures through peptide-lipid contacts.

The location of the myristoylated alanine-rich C-kinase substrate (MARCKS) effector domain has been investigated in negatively charged bicelles by NOE measurements in combination with the study of

the effects of molecular oxygen on the paramagnetic enhancements of nuclear relaxation (119). For the NOE measurements, selective excitation of the amide and aromatic region in ω_2 was used so that cross-peaks between this region and the aliphatic region of the spectrum are not obscured by the intense lipid diagonal peaks (120). The NOE results indicate a close distance between the phenylalanine aromatic protons and the lipid acyl chain methylenes 3–14. The aromatic proton position with respect to the bicelle phospholipid has been determined by examining the effect of O_2 on the ^1H spin-lattice relaxation of the peptide and lipid protons. The results indicate that the average position of the phenylalanine aromatic protons is in the bilayer hydrocarbon region, a few angstroms from the aqueous-hydrocarbon interface.

By combining various techniques, including NOE measurements, paramagnetic probes, amide-water exchange, and the effects of molecular oxygen on the paramagnetic enhancements of nuclear relaxation, a precise location of peptides in fast-tumbling bicelles can be determined. This, in combination with structural, dynamical, and association data, can provide a detailed picture of the interaction between peptides and lipid membranes.

CONCLUSION

The literature of the past 10 years has shown an increasing popularity of bicelles as model membranes for the NMR study of extrinsic and intrinsic peptides and proteins. This can be attributed to their phase, composition, and planar section, which are similar to that of eukaryote and prokaryote membranes, but also to their easy preparation done in an aqueous environment. Moreover, the hydration, pH, and ionic strength of bicelles can be controlled, allowing biologically relevant conditions (93). The possibility of performing solid- and solution-state NMR investigations with a single system by only changing the DMPC/DHPC molar ratio and/or lipid concentration also makes bicelles a unique mimetic system. These model membranes can be used to study the interaction of membrane-associating peptides and proteins, such as their effect on the lipids and their localization in the bilayer, as well as their structure and orientation.

The use of bicelles has, however, some limitations. For example, DMPC/DHPC bicelles can hinder the reconstitution of membrane proteins when the bilayer thickness does not match the hydrophobic domain of proteins. This was reported by Sanders and Landis (11) for the α -helical P24 ($\text{K}_2\text{GL}_{24}\text{K}_2\text{A}$), in which the 36 Å poly-leucine hydrophobic stretch could not be

incorporated into bicelles. Similarly, DAGK was shown to have a higher enzymatic activity in the thicker DPPC bicelles than in those containing DLPC or DMPC (21). However, bicelles can be made using phospholipids with longer acyl chains. It is also possible that the small size of isotropic bicelles does not preserve the native structure of large proteins. Fanucci et al. (121) observed that the conformation of BtuB was conserved in oriented bicelles ($q = 4$), whereas the native fold was not maintained when $q = 0.5$. There are also reports on the instability and precipitation of bicelles (36, 43, 64, 122). This problem can be circumvented, however, by maintaining the bicelle samples at a pH of 6–7 (38) or modifying their composition by adding dialkyl DMPC and DHPC analogues (38), polyethylene glycol (35) or CTAB (36).

The use of bicelles is not limited to NMR spectroscopy. It has also been extended to electron paramagnetic resonance (EPR) to study the effect of a BtuB fragment (121), cholesterol (33), and lanthanide ions (123, 124) on bicelles. These model membranes have also shown a promising application in micellar electrokinetic chromatography as they mimic physiologically relevant conditions. More specifically, the bilayered structure allowed the separation of six beta blockers that were not soluble in micelles (125), and the membrane affinity of peptides to membranes was also studied using this technique (126). Bicelles are also useful in circular dichroism (CD) studies of membrane peptides, which complement well the structural data obtained by NMR. CD was used with a series of antibiotic frog peptides (67), motilin (113), penetratin (109), the N-myristoylated 15 amino acid peptide Myr15 (95), and a membrane-associated helical domain (110–136) of prion protein (92). Bicelles have shown some potential as a starting medium for crystallization of proteins, as was performed for bacteriorhodopsin in DMPC/CHAPSO membranes (127). Finally, bicelles could be suitable biomembrane models for Fourier transform infrared (FTIR) studies of membrane proteins and peptides (128).

Since their introduction a decade ago, bicelles have proven to be a useful and versatile membrane-mimetic system for solution- and solid-state NMR studies of membrane polypeptides. It is therefore expected that the structure and interaction of an increasing number of membrane peptides and proteins will be investigated in fast-tumbling and oriented bicelles. Ongoing efforts in the characterization of the bicelle morphologies and properties should lead to the development of new membrane-mimicking systems that could be used with a variety of spectroscopic techniques.

ACKNOWLEDGMENTS

This work was supported by the Natural Science and Engineering Research Council (NSERC) of Canada, by the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), and by the Centre de Recherche en Sciences et Ingénierie des Macromolécules (CERSIM). I.M. wishes to thank Professor Beat H. Meier for insightful comments, group members for their support, and Dr. Alexandre Arnold for help and discussions. I.M. is also grateful to the NSERC of Canada for the award of a postdoctoral scholarship.

REFERENCES

1. Arora A, Tamm LK. 2001. Biophysical approaches to membrane protein structure determination. *Curr Opin Struct Biol* 11:540–547.
2. Ostermeier C, Michel H. 1997. Crystallization of membrane proteins. *Curr Opin Struct Biol* 7:697–701.
3. Byrne B, Iwata S. 2002. Membrane protein complexes. *Curr Opin Struct Biol* 12:239–243.
4. Gohon Y, Popot J-L. 2003. Membrane protein-surfactant complexes. *Curr Opin Coll Interf Sci* 8:15–22.
5. Mouritsen OG, Jorgensen K. 1998. A new look at lipid-membrane structure in relation to drug research. *Pharm Res* 15:1507–1519.
6. Nieh M-P, Harroun TA, Raghunathan VA, Glinka CJ, Katsaras J. 2004. Spontaneously formed monodisperse biomimetic unilamellar vesicles: the effect of charge, dilution and time. *Biophys J* 86:2615–2629.
7. Watts A, Spooner PJR. 1991. Phospholipid phase transitions as revealed by NMR. *Chem Phys Lipids* 57:195–211.
8. Seelig J, Borle F, Cross TA. 1985. Magnetic ordering of phospholipid membranes. *Biochim Biophys Acta* 814:195–198.
9. Vold RR, Prosser RS, Deese AJ. 1997. Isotropic solutions of phospholipid bicelles: a new membrane mimetic for high-resolution NMR studies of polypeptides. *J Biomol NMR* 9:329–335.
10. Kleinschmidt JH, Wiener MC, Tamm LK. 1999. Outer membrane protein A of *E. coli* folds into detergent micelles, but not in the presence of monomeric detergent. *Protein Sci* 8:2065–2071.
11. Sanders CR, Landis GC. 1995. Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies. *Biochemistry* 34:4030–4040.
12. Chou JJ, Kaufman JD, Stahl SJ, Wingfield PT, Bax A. 2002. Micelle-induced curvature in a water-insoluble HIV-1 Env peptide revealed by NMR dipolar coupling measurement in stretched polyacrylamide gel. *J Am Chem Soc* 124:2450–2451.
13. Gabriel NE, Roberts MF. 1984. Spontaneous forma-

- tion of stable unilamellar vesicles. *Biochemistry* 23: 4011–4015.
14. Gabriel NE, Roberts MF. 1986. Interaction of short-chain lecithins with long-chain phospholipids: characterization of vesicles that form spontaneously. *Biochemistry* 25:2812–2821.
 15. Ram P, Prestegard JH. 1988. Magnetic field induced ordering of bile salt/phospholipid micelles: new media for NMR structural investigations. *Biochim Biophys Acta* 940:289–294.
 16. Sanders CR, Prestegard JH. 1990. Magnetically orientable phospholipid bilayers containing small amounts of a bile salt analogue, CHAPSO. *Biophys J* 58:447–460.
 17. Sanders CR, Hare BJ, Howard KP, Prestegard JH. 1994. Magnetically-oriented phospholipid micelles as a tool for the study of membrane-associated molecules. *Progr NMR Spectros* 26:421–444.
 18. Sanders CR, Schwonek JP. 1992. Characterization of magnetically orientable bilayers in mixtures of dihexanoylphosphatidylcholine and dimyristoylphosphatidylcholine by solid-state NMR. *Biochemistry* 31:8898–8905.
 19. Sanders CR, Prosser RS. 1998. Bicelles: a model membrane system for all seasons? *Structure* 6:1227–1234.
 20. Hare BJ, Prestegard JH, Engelman BM. 1995. Small angle X-ray scattering studies of magnetically oriented lipid bilayers. *Biophys J* 69:1891–1896.
 21. Czerski L, Sanders CR. 2000. Functionality of a membrane protein in bicelles. *Anal Biochem* 284:327–333.
 22. Tiburu EK, Moton DM, Lorigan GA. 2001. Development of magnetically aligned phospholipid bilayers in mixtures of palmitoylstearylphosphatidylcholine and dihexanoylphosphatidylcholine by solid-state NMR spectroscopy. *Biochim Biophys Acta* 1512:206–214.
 23. Whiles JA, Glover KJ, Vold RR, Komives EA. 2002. Methods for studying transmembrane peptides in bicelles: consequences of hydrophobic mismatch and peptide sequence. *J magn reson* 158:149–156.
 24. Semchyschyn DJ, Macdonald PM. 2004. Conformational response of the phosphatidylcholine headgroup to bilayer surface charge: torsion angle constraints from dipolar and quadrupolar couplings in bicelles. *Magn Reson Chem* 42:89–104.
 25. Struppe J, Komives EA, Taylor SS, Vold RR. 1998. ^2H NMR studies of a myristoylated peptide in neutral and acidic phospholipid bicelles. *Biochemistry* 37: 15523–15527.
 26. Crowell KJ, Macdonald PM. 1999. Surface charge response of the phosphatidylcholine head group in bilayered micelles from phosphorus and deuterium nuclear magnetic resonance. *Biochim Biophys Acta* 1416:21–30.
 27. Struppe J, Whiles JA, Vold RR. 2000. Acidic phospholipid bicelles: a versatile model membrane system. *Biophys J* 78:281–289.
 28. Cullis PR, Fenske DB, Hope MJ. Physical properties and functional roles of lipids in membranes. In: Vance JE, editor. *Biochemistry of lipids, lipoproteins and membranes*. Amsterdam: Elsevier; 1996. p 1–33.
 29. Marcotte I, Dufourc EJ, Ouellet M, Auger M. 2003. Interaction of the neuropeptide met-enkephalin with zwitterionic and negatively charged bicelles as viewed by ^{31}P and ^2H solid-state NMR. *Biophys J* 85:328–339.
 30. Parker MA, King V, Howard KP. 2001. Nuclear magnetic resonance study of doxorubicin binding to cardiolipin containing magnetically oriented phospholipid bilayers. *Biochim Biophys Acta* 1514:206–216.
 31. Prosser RS, Hwang JS, Vold RR. 1998. Magnetically aligned phospholipid bilayers with positive ordering: a new model membrane system. *Biophys J* 74:2405–2418.
 32. Sasaki H, Fukuzawa S, Kikuchi J, Yokoyama S, Hirota H, Tachibana K. 2003. Cholesterol doping induced enhanced stability of bicelles. *Langmuir* 19: 9841–9844.
 33. Lu J-X, Caporini MA, Lorigan GA. 2004. The effects of cholesterol on magnetically aligned phospholipid bilayers: a solid-state NMR and EPR spectroscopy study. *J Magn Reson* 168:18–30.
 34. Tiburu EK, Dave PC, Lorigan GA. 2004. Solid-state ^2H NMR studies of the effects of cholesterol on the acyl chain dynamics of magnetically aligned phospholipid bilayers. *Magn Reson Chem* 42:132–138.
 35. King V, Parker MA, Howard KP. 2000. Pegylation of magnetically oriented lipid bilayers. *J Magn Reson* 142:177–182.
 36. Losonczi JA, Prestegard JH. 1998. Improved dilute bicelle solutions for high-resolution NMR of biological macromolecules. *J Biomol NMR* 12:447–451.
 37. Gaemers S, Bax A. 2001. Morphology of three lyotropic liquid crystalline biological NMR media studied by translational diffusion anisotropy. *J Am Chem Soc* 123:12343–12352.
 38. Ottiger M, Bax A. 1999. Bicelle-based liquid crystals for NMR measurement of dipolar couplings at acidic and basic pH values. *J Biomol NMR* 13:187–191.
 39. Tjandra N, Bax A. 1997. Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium. *Science* 278:1111–1114.
 40. Brunner E. 2001. Residual dipolar couplings in protein NMR. *Concepts Magn Reson* 13:238–259.
 41. Bax A. 2003. Weak alignment offers new NMR opportunities to study protein structure and dynamics. *Protein Sci* 12:1–16.
 42. Katsaras J, Donaberger RL, Swainson IP, Tennant DC, Tun Z, Vold RR, Prosser RS. 1997. Rarely observed phase transitions in a novel lyotropic liquid crystal system. *Phys Rev Lett* 78:899–902.
 43. Ottiger M, Bax A. 1998. Characterization of magnetically oriented phospholipid micelles for measurement of dipolar couplings in macromolecules. *J Biomol NMR* 12:361–372.
 44. Sanders CR, Landis GC. 1994. Facile acquisition and

- assignment of oriented sample NMR spectra for bilayer surface-associated proteins. *J Am Chem Soc* 116:6470–6471.
45. Raffard G, Steinbruckner S, Arnold A, Davis JH, Dufourc EJ. 2000. Temperature-composition diagram of dimyristoylphosphatidylcholine-dicaproyl-phosphatidylcholine “bicelles” self-orienting in the magnetic field. A solid-state ^2H and ^{31}P study. *Langmuir* 16:7655–7662.
 46. Luchette PA, Vetman TN, Prosser RS, Hancock REW, Nieh M-P, Glinka CJ, Krueger S, Katsaras J. 2001. Morphology of fast-tumbling bicelles: a small angle neutron scattering and NMR study. *Biochim Biophys Acta* 1513:83–94.
 47. Arnold A, Labrot T, Oda R, Dufourc EJ. 2002. Cation modulation of bicelle size and magnetic alignment as revealed by solid-state NMR and electron microscopy. *Biophys J* 83:2667–2680.
 48. Sakurai I, Kawamura Y, Ikegami A, Iwayanagi S. 1980. Magneto-orientation of lecithin crystals. *Proc Natl Acad Sci USA* 77:7232–7236.
 49. Scholz F, Boroske E, Helfrich W. 1984. Magnetic anisotropy of lecithin membranes. *Biophys J* 45:589–592.
 50. Lonsdale K. 1939. Diamagnetic anisotropy of organic molecules. *Proc R Soc Lond Ser A* 171:541–568.
 51. Boroske E, Helfrich W. 1978. Magnetic anisotropy of egg lecithin membranes. *Biophys J* 24:863–868.
 52. Kawamura Y, Sakurai I, Ikegami A, Iwayanagi S. 1981. Magneto-orientation of phospholipids. *Mol Cryst Liq Cryst* 67:77–88.
 53. Sanders CR, Schaff JE, Prestegard JH. 1993. Orientational behavior of phosphatidylcholine bilayers in the presence of aromatic amphiphiles and a magnetic field. *Biophys J* 69:1891–1896.
 54. Struppe J, Vold RR. 1998. Dilute bicellar solutions for structural NMR work. *J Magn Reson* 135:541–546.
 55. Hwang JS, Oweimrenn GA. 2003. Anomalous viscosity behavior of a bicelle system with various molar ratios of short- and long-chain phospholipids. *Arab J Sci Eng* 28:45–49.
 56. Nieh M-P, Glinka CJ, Krueger S, Prosser RS, Katsaras J. 2001. SANS study of the structural phases of magnetically alignable lanthanide-doped phospholipid mixtures. *Langmuir* 17:2629–2638.
 57. Zandomenighi G, Tomaselli M, van Beek JD, Meier BH. 2001. Manipulation of the director in bicellar mesophases by sample spinning: a new tool for NMR spectroscopy. *J Am Chem Soc* 123:910–913.
 58. Zandomenighi G, Tomaselli M, Williamson PTF, Meier BH. 2003. NMR of bicelles: orientation and mosaic spread of the liquid-crystal director under sample rotation. *J Biomol NMR* 25:113–123.
 59. Zandomenighi G, Williamson PTF, Hunkeler A, Meier BH. 2003. Switched-angle spinning applied to bicelles containing phospholipid-associated peptides. *J Biomol NMR* 25:125–132.
 60. Prosser RS, Hunt SA, DiNatale JA, Vold RR. 1996. Magnetically aligned membrane model systems with positive order parameter: switching the sign of S_{zz} with paramagnetic ions. *J Am Chem Soc* 118:269–270.
 61. Ulrich AS, Watts A. 1993. Deuterium NMR line-shapes of immobilized uniaxially oriented membrane proteins. *Solid State Nucl Magn Reson* 2:21–36.
 62. Picard F, Paquet M-J, Levesque J, Bélanger A, Auger M. 1999. ^{31}P NMR first spectral moment study of the partial magnetic orientation of phospholipid membranes. *Biophys J* 77:888–902.
 63. Marcotte I, Bélanger A, Auger M. 2004. Manuscript in preparation.
 64. Prosser RS, Volkov VB, Shiyonovskaya IV. 1998. Solid-state NMR studies of magnetically aligned phospholipid membranes: taming lanthanides for membrane protein studies. *Biochem Cell Biol* 76:443–451.
 65. Howard KP, Opella SJ. 1996. High-resolution solid-state NMR spectra of integral membrane proteins reconstituted into magnetically oriented phospholipid bilayers. *J Magn Reson* 112:91–94.
 66. Drechsler A, Separovic F. 2003. Solid-state NMR structure determination. *IUBMB Life* 55:515–523.
 67. Marcotte I, Wegener KL, Lam Y-H, Chia BCS, de Planque MRR, Bowie JH, Auger M, Separovic F. 2003. Interaction of antimicrobial peptides from Australian amphibians with lipid membranes. *Chem Phys Lipids* 122:107–120.
 68. Opella SJ, Marassi FM. 2004. Structure determination of membrane proteins by NMR spectroscopy. *Chem Rev* 104:3587–3606.
 69. Crowell KJ, Macdonald PM. 2001. Europium (III) binding and the reorientation of magnetically aligned bicelles: insights from deuterium NMR spectroscopy. *Biophys J* 81:255–265.
 70. Dave PC, Tiburu EK, Nusair NA, Lorigan GA. 2003. Calculating order parameter profiles utilizing magnetically aligned phospholipid bilayers for ^2H solid-state NMR studies. *Solid State Nucl Magn Reson* 24:137–149.
 71. Cho G, Fung BM, Reddy VB. 2001. Phospholipid bicelles with positive anisotropy of the magnetic susceptibility. *J Am Chem Soc* 123:1537–1538.
 72. Vold RR, Prosser RS. 1996. Magnetically oriented phospholipid bilayered micelles for structural studies of polypeptides. Does the ideal bicelle exist? *J Magn Reson* 113:267–271.
 73. Sternin E, Nizza D, Gawrish K. 2001. Temperature dependence of DMPC/DHPC mixing in a bicellar solution and its structural implications. *Langmuir* 17:2610–2616.
 74. Rowe BA, Neal SL. 2003. Fluorescence probe study of bicelle structure as a function of temperature: developing a practical bicelle structure model. *Langmuir* 19:2039–2048.
 75. Bolze J, Fujisawa T, Nagao T, Norisada K, Saitô H, Naito A. 2000. Small angle X-ray scattering and ^{31}P

- NMR studies on the phase behavior of phospholipid bilayered mixed micelles. *Chem Phys Lett* 329:215–220.
76. Sternin E, Schäfer H, Polozov IV, Gawrish K. 2001. Simultaneous determination of orientational and order parameter distributions from NMR spectra of partially oriented model membranes. *J Magn Reson* 149:110–113.
 77. Vyas S, Weekley AJ, Tenn BK, Flinders JC, Dieckmann T, Augustine MP. 2003. Using sodium cation organization to study the phase behavior of bicelle solutions. *J Phys Chem B* 107:10956–10961.
 78. Nieh M-P, Glinka CJ, Krueger S, Prosser RS, Katsaras J. 2002. SANS study of the effect of lanthanide ions and charged lipids on the morphology of phospholipid mixtures. *Biophys J* 82:2487–2498.
 79. Wang H, Nieh M-P, Hobbie EK, Glinka CJ, Katsaras J. 2003. Kinetic pathways to the bilayered-micelle to perforated-lamellae transition. *Phys Rev E* 67:902.
 80. Nieh M-P, Raghunathan VA, Wang H, Katsaras J. 2003. Highly aligned lamellar lipid domains induced by macroscopic confinement. *Langmuir* 19:6936–6941.
 81. Glover KJ, Whiles JA, Wu G, Yu N-J, Deems R, Struppe JO, Stark RE, Komives EA, Vold RR. 2001. Structural evaluation of phospholipid bicelles for solution-state studies of membrane-associated biomolecules. *Biophys J* 81:2163–2171.
 82. Vinogradova O, Sonnichsen FD, Sanders CR. 1998. On choosing a detergent for solution NMR studies of membrane proteins. *J Biomol NMR* 4:381–386.
 83. Seelig J. 1978. ^{31}P nuclear magnetic resonance and the head group structure of phospholipids in membranes. *Biochim Biophys Acta* 515:105–140.
 84. Guo J, Pavlopoulos S, Tian X, Lu D, Nikas SP, Yang D-P, Makriyannis A. 2003. Conformational study of lipophilic ligands in phospholipid model membrane systems by solution NMR. *J Med Chem* 46:4838–4846.
 85. Aussenac F, Laguerre M, Schmittter J-M, Dufourc EJ. 2003. Detailed structure and dynamics of bicelle phospholipids using selectively deuterated and perdeuterated labels. ^2H NMR and molecular mechanics study. *Langmuir* 19:10468–10479.
 86. Whiles JA, Brasseur R, Glover KJ, Melacini G, Komives EA, Vold RR. 2001. Orientation and effects of mastoparan X on phospholipid bicelles. *Biophys J* 80:280–293.
 87. Prosser RS, Bryant H, Bryant RG, Vold RR. 1999. Lanthanide chelates as bilayer alignment tools in NMR studies of membrane-associated peptides. *J Magn Reson* 14:256–260.
 88. Lam Y-H, Marcotte I, Separovic F. 2001. Unpublished results.
 89. Whiles JA, Deems R, Vold RR, Dennis EA. 2002. Bicelles in structure-function studies of membrane-associated proteins. *Bioorg Chem* 30:431–442.
 90. Seelig J, Seelig A. 1980. Lipid conformation in model membranes and biological membranes. *Q Rev Biophys* 13:19–61.
 91. Seelig J, Seelig A, Tamm L. 1982. Nuclear magnetic resonance and lipid-protein interactions. In: Griffith OH, editor. *Lipid-protein interactions*. Volume 2. New York: John Wiley & Sons. p 127–148.
 92. Glover KJ, Whiles JA, Wood MJ, Melacini G, Komives EA, Vold RR. 2001. Conformational dimorphism and transmembrane orientation of prion protein residues 110–136 in bicelles. *Biochemistry* 40:13137–13142.
 93. Sizun C, Aussenac F, Grelard A, Dufourc EJ. 2004. NMR methods for studying the structure and dynamics of oncogenic and antihistaminic peptides in biomembranes. *Magn Reson Chem* 42:180–186.
 94. Seelig J, Macdonald PM, Scherer PG. 1987. Phospholipid head groups as sensors of electric charge in membranes. *Biochemistry* 26:7535–7541.
 95. Losonczi JA, Prestegard JH. 1998. Nuclear magnetic resonance characterization of the myristoylated, N-terminal fragment of ADP-ribosylation factor 1 in a magnetically oriented membrane array. *Biochemistry* 37:706–716.
 96. Cornell BA, Separovic F, Baldassi AJ, Smith R. 1988. Conformation and orientation of gramicidin A in oriented phospholipid bilayers measured by solid-state carbon-13 NMR. *Biophys J* 53:67–76.
 97. Marcotte I. 2003. Étude de l'Interaction de la Méthionine-Enképhaline et de Peptides Antibiotiques avec des Membranes Modèles par Spectroscopies de RMN et Infrarouge. PhD thesis, Université Laval, Québec.
 98. Zandomenighi G, Meier BH. Adiabatic-passage cross polarization in N-15 NMR spectroscopy of peptides weakly associated to phospholipids: determination of large RDC. *J Biomol NMR*, in press.
 99. Prosser RS, Volkov VB, Shiyanovskaya IV. 1998. Novel chelate-induced magnetic alignment of biological membranes. *Biophys J* 75:2163–2169.
 100. Wuthrich K, Bosch C, Brown LR. 1980. Conformational studies of lipid-bound polypeptides by elucidation of proton-proton cross-relaxation networks. *Biochem Biophys Res Commun* 95:1504–1509.
 101. Papavoine CHM, Christiaans BEC, Folmer RHA, Konings RNH, Hilbers CW. 1998. Solution structure of the M13 major coat protein in detergent micelles: a basis for a model of phage assembly involving specific residues. *J Mol Biol* 282:401–419.
 102. Almeida FCL, Opella SJ. 1997. fd coat protein structure in membrane environments: structural dynamics of the loop between the hydrophobic trans-membrane helix and the amphipathic in-plane helix. *J Mol Biol* 270:481–495.
 103. Park SH, Mrse AA, Nevzorov AA, Mesleh MF, Oblatt-Montal M, Montal M, Opella SJ. 2003. Three-dimensional structure of the channel-forming trans-membrane domain of virus protein “u” (Vpu) from HIV-1. *J Mol Biol* 333:409–424.
 104. Fernandez C, Wuthrich K. 2003. NMR solution struc-

- ture determination of membrane proteins reconstituted in detergent micelles. *FEBS Lett* 555:144–150.
105. Fernández C, Hilty C, Bonjour S, Adeishvili K, Pervushin K, Wüthrich K. 2001. Solution NMR studies of the integral membrane proteins OmpX and OmpA from *Escherichia coli*. *FEBS Lett* 504:173–178.
 106. Fernández C, Hilty C, Wider G, Guntert P, Wüthrich K. 2004. NMR structure of the integral membrane protein OmpX. *J Mol Biol* 336:1211–1221.
 107. Hwang PM, Choy W, Lo EI, Chen L, Forman-Kay JD, Raetz CRH, Privé GC, Bishop RE, Kay LE. 2002. Solution structure and dynamics of the outer membrane enzyme PagP by NMR. *Proc Natl Acad Sci USA* 99:13560–13565.
 108. Barany-Wallje E, Andersson A, Graeslund A, Mäler L. 2004. NMR solution structure and position of transportin in neutral phospholipid bicelles. *FEBS Lett* 567:265–269.
 109. Lindberg M, Biverstahl H, Gräslund A, Mäler L. 2003. Structure and positioning comparison of two variants of penetratin in two different membrane mimicking systems by NMR. *Eur J Biochem* 270:3055–3063.
 110. Chou JJ, Baber JL, Bax A. 2004. Characterization of phospholipid mixed micelles by translational diffusion. *J Biomol NMR* 29:299–308.
 111. Marcotte I, Separovic F, Auger M, Gagné SM. 2004. A multidimensional ^1H NMR investigation of the conformation of methionine-enkephalin in fast-tumbling bicelles. *Biophys J* 86:1587–1600.
 112. Andersson A, Mäler L. 2002. NMR solution structure and dynamics of motilin in isotropic phospholipid bicellar solution. *J Biomol NMR* 24:103–112.
 113. Andersson A, Mäler L. 2003. Motilin-bicelle interactions: membrane position and translational diffusion. *FEBS Lett* 545:139–143.
 114. Andersson A, Almqvist J, Hagn F, Mäler L. 2004. Diffusion and dynamics of penetratin in different membrane mimicking media. *Biochim Biophys Acta* 1661:18–25.
 115. Lipari G, Szabo A. 1982. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity. *J Am Chem Soc* 104:4546–4559.
 116. Lipari G, Szabo A. 1982. Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 2. Analysis of experimental results. *J Am Chem Soc* 104:4559–4570.
 117. Lindman B, Puyal MC, Kamenka N, Rymden R, Stilbs P. 1984. Micelle formation of anionic and cationic surfactants from Fourier transform proton and lithium-7 nuclear magnetic resonance and tracer self-diffusion studies. *J Phys Chem* 88:5048–5057.
 118. Glover KJ, Whiles JA, Vold RR, Melacini G. 2002. Position of residues in transmembrane peptides with respect to the lipid bilayer: A combined lipid NOEs and water chemical exchange approach in phospholipid bicelles. *J Biomol NMR* 22:57–64.
 119. Ellena JF, Burnitz MC, Cafiso DS. 2003. Location of the myristoylated alanine-rich C-kinase substrate (MARCKS) effector domain in negatively charged phospholipid bicelles. *Biophys J* 85:2442–2448.
 120. Seigneuret M, Levy D. 1995. A high-resolution ^1H NMR approach for structure determination of membrane peptides and proteins in non-deuterated detergent: application to mastoparan X solubilized in n-octylglucoside. *J Biomol NMR* 5:345–352.
 121. Fanucci GE, Lee JY, Cafiso DS. 2003. Membrane mimetic environments alter the conformation of the outer membrane protein BtuB. *J Am Chem Soc* 125:13932–13933.
 122. Clore GM, Starich MR, Gronenborn AM. 1998. Measurement of dipolar residual couplings of macromolecules aligned in the nematic phase of a colloidal suspension of rod-shaped viruses. *J Am Chem Soc* 120:10571–10572.
 123. Caporini MA, Padmanabhan A, Cardon TB, Lorigan GA. 2003. Investigating magnetically aligned phospholipid bilayers with various lanthanide ions for X-band spin-label EPR studies. *Biochim Biophys Acta* 1612:52–58.
 124. Dave PC, Inbaraj JJ, Lorigan GA. 2004. Electron paramagnetic resonance studies of magnetically aligned phospholipid bilayers utilizing a phospholipid spin label. *Langmuir* 20:5801–5808.
 125. Holland LA, Leigh AM. 2003. Bilayered phospholipid micelles and capillary electrophoresis: a new additive for electrokinetic chromatography. *Electrophoresis* 24:2935–2939.
 126. Mills JO, Holland LA. 2004. Membrane-mediated capillary electrophoresis: interaction of cationic peptides with bicelles. *Electrophoresis* 25:1237–1242.
 127. Faham S, Bowie JU. 2002. Bicelle crystallization: a new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure. *J Mol Biol* 316:1–6.
 128. Marcotte I, Ouellet M, Auger M. 2002. Unpublished results.
 129. Koradi R, Billeter M, Wüthrich K. 1996. MOLMOL: a program for display and analysis of macromolecular structures. *J Mol Graph* 14:51–55.

BIOGRAPHIES



Isabelle Marcotte, BSc, MSc (chemistry), received her PhD at Université Laval under the supervision of Professor Michele Auger, where she studied membrane-peptide interactions by NMR and FTIR spectroscopy. During her undergraduate and postgraduate studies, she was awarded scholarships from the NSERC of Canada and the FQRNT of Québec. She is currently a postdoctoral fellow at the ETH in Zürich with Professor Beat H. Meier where she studies the relationship between the structure and mechanical properties of spider silk. Her scientific interests include the study of the interaction of bioactive compounds with membranes and the investigation of biological fibers by NMR.



Michèle Auger, BSc, PhD, is Professor of Chemistry at Université Laval in Québec City, Canada. As a Natural Sciences and Engineering Research Council (NSERC) of Canada 1967 awardee, she earned her PhD in chemistry under the direction of Ian C.P. Smith at the University of Ottawa and the National Research Council. She did her postdoctoral work in the laboratory of Robert G. Griffin at the Massachusetts Institute of Technology in Cambridge, where she studied amyloid and membrane protein structure by novel solid-state NMR techniques. Her current research efforts are focused on the solid-state NMR study of protein structure, protein-lipid interactions and domain formation in model membranes.