Bicelles and Nanodiscs for Biophysical Chemistry*

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***A tribute to Prof Michèle Auger, University Laval, Canada**

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Highlights

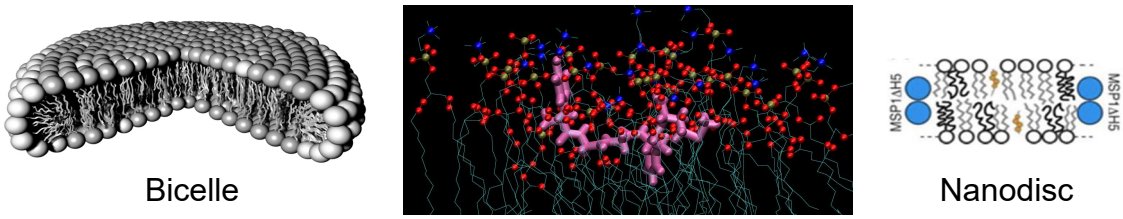
- Bicelles are pure synthetic lipidic discoidal particles of 20-50 nm diameter that may orient parallel or perpendicularly to magnetic fields. They are called “molecular goniometers” and mimic small pieces of biomembranes.
- Bicelles are stable on narrow ranges of composition, temperature and hydration, can embed charged lipids, cholesterol, peptides and membrane proteins and are used as platforms to gain information on protein structure and dynamics by ssNMR.
- Bicelle remain aligned for several hours/days outside magnetic fields and relax according to nematic or smectic properties
- Nanodiscs are smaller particles (10-20 nm) that may be built with a wide variety of lipids and apolipoproteins (MSP) making the rim of the disc.
- As for Bicelles, nanodiscs can embed a wide variety of membrane proteins and can be used as “molecular bricks”.
- The constraints exerted by the MSP proteins forbid any temperature-driven phase transition, providing a membrane liquid-ordered state on large temperature domains.

Abstract

Membrane nanoobjects are very important tools to study biomembrane properties. Two types are described herein: Bicelles and Nanodiscs. Bicelles are obtained by thorough water mixing of long chain and short chain lipids and may take the form of membranous discs of 10-50 nm. Temperature-composition-hydration diagrams have been established for Phosphatidylcholines and show limited domains of existence. Bicelles can be doped with charged lipids, surfactants or with cholesterol and offer a wide variety of membranous platforms for structural biology. Internal dynamics as measured by solid-state NMR is very similar to that of liposomes in their fluid phase. Because of the magnetic susceptibility anisotropy of the lipid chains, discs may be aligned along or perpendicular to the magnetic field. They may serve as weak orienting media to provide distance information in determining the 3D structure of soluble proteins. In different conditions they show strong orienting properties which may be used to study the 3D structure, topology and dynamics of membrane proteins. Lipid Bicelles with biphenyl chains or doped with lanthanides show long

lasting remnant orientation after removing the magnetic field due to smectic-like properties. An alternative to pure lipid Bicelles is provided by nanodiscs where the half torus composed by short chain lipids is replaced by proteins. This renders the nano-objects less fragile as they can be used to stabilize membrane protein assemblies to be studied by electron microscopy. Internal dynamics is again similar to liposomes except that the phase transition is abolished, possibly due to lateral constrain imposed by the toroidal proteins limiting the disc size. Advantages and drawbacks of both nanoplatforms are discussed.

Graphical abstract



Keywords: Bicelles, nanodiscs, NMR, membrane peptides & proteins, lipids, membrane dynamics

Abbreviations: DCPC, 1,2-hexanoyl-1-sn-glycero-3-phosphocholine; DHPC, 1,2-heptanoyl-1-sn-glycero-3-phosphocholine; DMPC, 1,2-myristoyl-1-sn-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol); DMPE, 1,2-myristoyl-1-sn-glycero-3-phosphoethanolamine; DMPS, 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; MAS, magic-angle sample spinning; MLV, multilamellar vesicle; MSP, membrane scaffold proteins; NMR, nuclear magnetic resonance; TOCSY, Total Correlation Spectroscopy; NOESY, nuclear overhauser effect spectroscopy; NOE, nuclear overhauser effect, PISA Wheel, Image of Membrane Protein Helical Wheels; SLF, separated local field spectroscopy; PISEMA, polarization inversion spin exchange at magic angle; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; SUV, small unilamellar vesicle; TBBPC, 1-tetradecanoyl-2-(4-(4-biphenyl)butanoyl)-sn-glycero-3-phosphocholine; DNA, Deoxyribonucleic acid.

1. Introduction

Biological membranes are very important in life as they provide the "barrier" that delimitates the outside from the inside of a cell, often called the plasma membrane. For eukaryotic organisms, cells have in addition many organelles, such as the nucleus made with a double membrane surrounding the aggregated DNA, the endoplasmic reticulum with a quite intricated membrane structure and the mitochondrion with specific in and out membranes. All these "barriers" not only delineate the organelles but provide them with specific functions of signaling, transport and fusion. These membranes are made of a large variety of lipids, sterols and membrane proteins. Both lipids and proteins give to specific membranes their intrinsic dynamic and structural properties. Although it is possible to purify natural membranes and obtain some dynamical information on their components [1, 2], there is a lot of work being done to simplify systems, by reducing the number of components, for instance by isolating a given membrane protein and reinserting it inside relevant, but simpler, membrane lipid matrices. Structural and dynamic information can then be obtained by a wide variety of spectroscopic techniques such as Atomic Force Microscopy, Fluorescence, Electron Microscopy, X-rays and Neutrons scattering or solid-state Nuclear Magnetic Resonance (ssNMR) [3-7].

Lipid membranes are amphiphilic assemblies that can be easily handled and are well known: liposomes, giant vesicles, micelles. It is however interesting having a platform that can be handled almost at will to orient samples and providing an additional information on the topology of the different partners within the membrane. This helps a lot in deciphering membrane-associated biological mechanisms. Lipids deposited on glass plates, as bilayers, have been used for years, for X-Rays, Neutron scattering and solid-state NMR [8-10]. Among these systems Bilayer Micelles and nanodiscs have shown an increase of interest in the Biophysical Chemistry community. Bicelles are made of short chain and long chain lipids and were first proposed from the pioneering work of Gabriel and Roberts using detergents to form "vesicles" of different sizes [11]. They indeed identified Bicelles without naming them. The word "Bicelle" for bilayer micelle was proposed by several scientists such as, Sanders, Vold and Prosser [12, 13]. Although different morphologies have been proposed for Bicelles, the disc topology is the most popular and has been clearly detected by Electron Microscopy [14, 15].

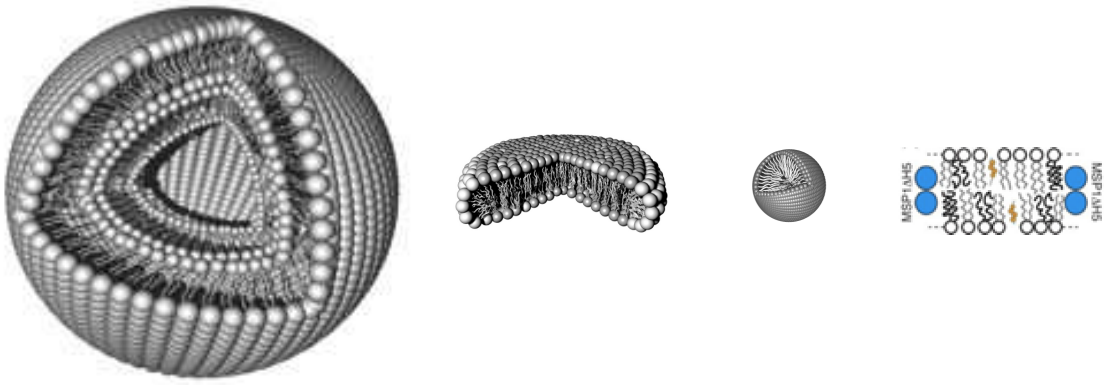


Fig.1 Schematic representation of Multilamellar vesicles (Liposomes), Bicelles (Bilayer Micelles), Micelles and Nanodiscs stabilized by MSP proteins (blue circles). Adapted from [16, 17]

One must mention that perforated lamellae have also been proposed for some conditions of concentration and temperature [18]. As the disc topology with a diameter of a few tenths of nanometers is generally used to describe Bicelles (Fig. 1) we will refer herein to this morphology [19]. These pure lipid discs have very interesting magnetic properties that turn to be very useful for NMR structural determinations. They are indeed aligned by magnetic fields because lipids possess a weak positive or negative magnetic susceptibility anisotropy, $\Delta\chi$, which turns the disc plane perpendicular or parallel to the magnetic field. A change of orientation can also be obtained by using lanthanide ions. Such a property is very useful for structural studies and Bicelles have been termed as "molecular goniometers". In the following, examples of Bicelles use for understanding membrane dynamical properties and structures of peptides and proteins embedded in membranes will be given. However, Bicelles are made of synthetic lipids of specific chain length, the temperature-composition-hydration diagrams where they can be stabilized is restrained and their use may be limited [20, 21]. As a consequence other platforms have been developed by Sligar and coworkers [22, 23]. They consist of smaller discs of nanometer size stabilized by membrane scaffold proteins (MSP) that play the role of short chain lipids in Bicelles : they constitute the rim of the disc. Examples will also be given of the use of such nanodiscs (Fig. 1). For clarity we will use the term nanodisc for MSP-stabilized lipid discs of nanometer size and Bicelle for pure lipids discs of similar sizes. A comparison of advantages and drawbacks of such platforms will be given at the end of the paper. There are many articles, which the reader may consult, that discuss Bicelles and nanodiscs [24, 25], but this review puts emphasis on ssNMR viewpoint that is particularly adapted to compare these nanoplatforms.

2. Bicelle Temperature-composition-hydration diagrams

Finding the conditions in which discoidal Bicelles can be stabilized and can be used in structural studies is a compulsory step before undertaking spectroscopic measurements. There is a wide variety of techniques to build "phase" diagrams. X-rays, neutron scattering, [26, 27], electron paramagnetic resonance (EPR) [28], crystallography [29], Circular Dichroism [17, 30, 31] and NMR [15, 18, 32-35] have been mainly used to delineate the boundaries. Interestingly molecular dynamics including coarse grain approaches have been also used [36]. One must however mention that the wording "phase" is not meant in the thermodynamic meaning, because no transition enthalpy has been measured in tentative reporting of Bicelle diagrams. The system DMPC/DCPC (*i.e.*, C14/C6 PC lipids) has been widely studied. The Fig. 2 shows how ssNMR can be used to build temperature-composition-hydration diagrams. Slow tumbling large liposomes give powder NMR spectra, isotropic micellar phases to NMR isotropic lines and bicelar discs, oriented spectra with one or 2 lines depending on the choice of the reporter nucleus (spin ½ or 1). The great advantage of NMR is the capability to distinguish Bicelles that are oriented with their plane parallel or perpendicular to the magnetic field due to the fact that spectra scale according to the $(3\cos^2\beta-1)$ law, β being the angle between the normal to the membrane plane and the magnetic field direction. As can be seen on Fig. 2, Bicelles containing saturated lipid chains only (C14/C6) have a large compositional range but a small temperature span (maximum 10°C), they are oriented perpendicular to the field and are stable even with up to 95% hydration. Conversely, Bicelles made with biphenyl moieties in the long chain component (C14BP/C6) display a reduced composition span but exist in a 60°C temperature range. They are oriented with the normal to the bilayer parallel to the field direction and can be

hydrated as much as their saturated counterparts. There are also some examples of diagrams on C16/C7 PC lipids [17, 36, 37], the temperature span is even smaller (few degrees).

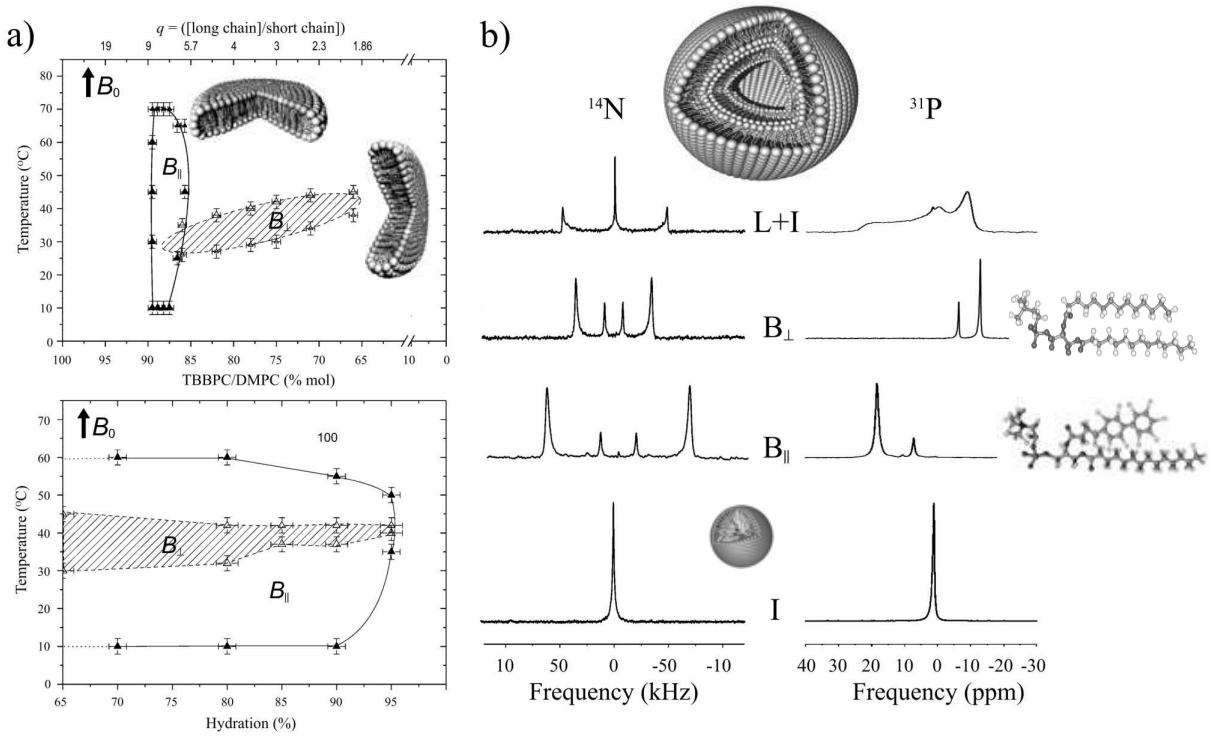
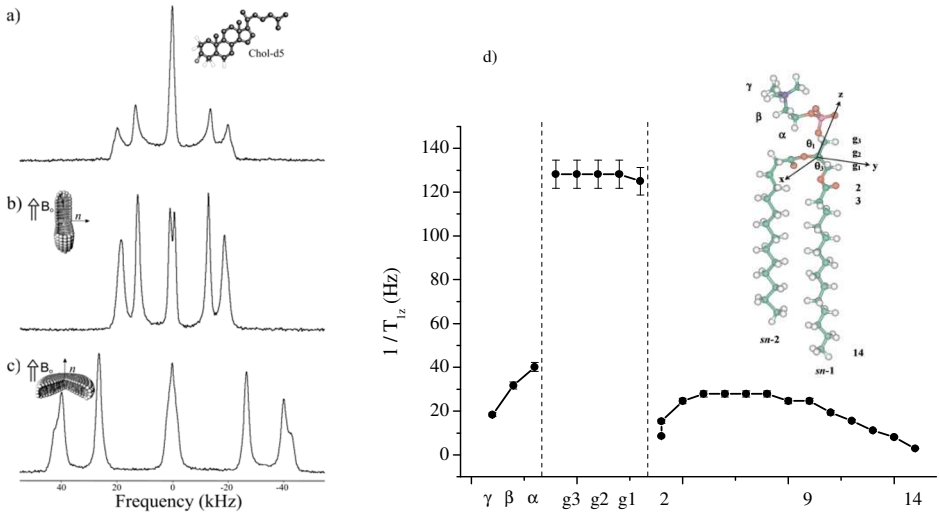


Fig. 2. Diagrams with variable composition, temperature and hydration as determined by solid-state wide-line NMR of TBBPC/DCPC or DMPC/DCPC mixtures. (a) Temperature-composition and temperature hydration diagrams of Bicelle systems being macroscopically oriented by magnetic fields (B_0), either with their bilayer normal, n, perpendicular ($B_\perp$) to the field (DMPC/DCPC) or parallel ($B_{//}$, TBBPC/DCPC). Mole fractions X are expressed in percent. q represents the long chain-to-short chain molar ratio. Lines are drawn as eye guides to delineate domains. (b) Phosphorus-31 and nitrogen-14 solid-sate wide-line NMR spectra characteristic of phosphocholine lipids embedded in different structures (unoriented lamellar (slow tumbling liposomes), L, isotropic, I, Bicelles with normal oriented parallel ($B_{//}$) or perpendicular ($B_\perp$) to the field. On the right, molecular structures of DMPC and TBPPC are shown. Adapted from [17, 38].

Interestingly Bicelles made of PCs with ether-linked hydrophobic chains have also been investigated and show little difference in "phase diagrams" with their ester-linked counterparts [15, 32, 39, 40]. The clear advantage is their hydrolysis-resistant character. Most of Bicelle work has been performed with saturated chains lipids such as DMPC, DPPC, DCPC or DHPC. It is however possible to use unsaturated lipids and obtain stable discoidal micelles [41-43]. Isotropic Bicelles, *i.e.*, fast tumbling and non-magnetically orientable Bicelles have also been reported with native E Coli lipids [44, 45]. For these systems, unfortunately no phase diagram is reported.

3. Doping Bicelles with sterols, charged lipids or counterions.

When studying the structure and topology of peptides and proteins in interaction or embedded in membranes it is important to have a model membrane that is representative enough of natural membranes, the best being nonetheless to work on natural membranes [1, 2, 46]. As seen in Fig. 3, cholesterol may be inserted in saturated chain PC Bicelles or in Biphenyl PC Bicelles. The sterol displays the same properties as in liposomes : same orientation parallel to the bilayer normal and same dynamics [47]. Moreover it clearly partitions in the flat bilayer part of the disc. The well-known promotion of domain formation (liquid-ordered phases) in liposomes is also detected in Bicelles, provided the disc size in large enough [43]. Unsaturated lipids can also be added to a cholesterol containing Bicelle [42, 48]. An important work has been developed by Michèle Auger and coworkers to make Bicelles with charged entities such as negatively charged lipids [49-51]. They have shown



that depending on the nature of lipid and charge at the interface, membranous peptides insert with different depths in the membrane [51, 52]. It has also been shown that doping the Bicelle with cardiolipin or positively charged lipids such as DOTAP, allows stabilization at the Bicelle surface of anticancer Doxorubicin or DNA, respectively, for drug targeting issues [53, 54]

Fig. 3. Cholesterol-containing Bicelles and Bicelle internal dynamics*. Solid-state deuterium NMR spectra of 10 mol% deuterium-labeled cholesterol (schematic structure) embedded in DMPC liposomes (a), DMPC/DCPC Bicelles (b), and TBBPC/DCPC Bicelles (c), at 35 °C. Bicelle sketches show the orientation of discs with respect to magnetic field. d) Deuterium spin-lattice relaxation rates 1/T1Z, as a function of labeled carbon position, for entirely perdeuterated DMPC incorporated in Bicelles, at 35°C: the higher the rate, the less mobile the carbon deuterium bond. On the right hand side a DMPC molecule is sketched showing the carbon numbering. Adapted from [47, 55]*

Charges at the interface are very important for Bicelle morphology and magnetic field orientation issues. It has been shown that increasing the concentration of cations such as K^+, Na^+, Ca^{2+} and Mg^{2+} leads to an increase of disc size and to better nematic-like alignment in magnetic fields [14]. The optimal salt concentration for optimal alignment ranges in between 50-200 mM. The most striking effect of charges at the interface is obtained with paramagnetic lanthanides ions with large positive $\Delta\chi$ (Eu^{3+}, Er^{3+}, Yb^{3+}, Tm^{3+}, Dy^{3+})[56-58].

This results in tilting the disc plane by 90° in a way very similar to that produced by the biphenyl rings of TBBPC (*vide supra*). Use of paramagnetic lanthanides allows therefore changing the Bicelle orientation with respect to the magnetic field direction.

4. Bicelles internal dynamics

Membrane dynamics describes complex motional processes occurring at very different time and distance scales : from picoseconds and atomic bonds to seconds and micron-millimeter dimensions [3, 55, 59]. ssNMR has been shown to be the best technique to probe all of these motions by combining several relaxation time measurements and line shape analysis. For Bicelles, the local motions that occur at pico-nanosecond timescales are globally conserved compared to liposome data. This is reflected by an order parameter profile for the long chain lipids that partition on the disc plane very similar to that measured in liposomes : very weak ordering near the center of the bilayer and quite rigid near the lipid glycerol backbone region. This is well reflected in Fig. 3d where the rate of spin lattice relaxation ($1/T_{1Z}$) was measured on a fully perdeuterated DMPC molecule : two very dynamic regions (low rate of relaxation) for chains and head groups are separated by a very rigid zone (the glycerol backbone). The variance, compared with liposomes, is the slightly more dynamic character of Bicelle long chain lipids, understandable by the presence of the small chain lipids known to promote disorder [55]. The other variance by comparing with liposomes or large unilamellar vesicles is the absence of collective motions, *i.e.*, membrane undulations linked to hydrodynamic modes of bilayer deformation occurring at microsecond to second time scales [3, 59]. This results for instance in a higher spectral resolution when detecting NMR wide-line spectra. Because Bicelle internal dynamics are very similar to that of liposomes they offer a proper mimic of natural membranes.

5. Weak orienting media: structure of soluble proteins by liquid-state NMR

One of the first uses of saturated Bicelles was to provide a weak orienting medium for structural biology studies by liquid state NMR. Thanks to the pioneering work of Sanders [60], Bax and coworkers [39, 61-64] one could obtain additional distance constraints using diluted Bicelles (3% w/v): indeed at this low concentration the sample takes the form of a *nematic*-like medium where discs orient with their plane parallel to the magnetic field direction and are spaced from each other by 30 nm in average. They have a negligible effect on the free diffusion of soluble proteins or DNA molecules. Very interestingly proteins/DNA exhibits a residual degree of alignment that is proportional to the Bicelle concentration. Residual dipolar couplings, D, can be measured, in addition to the indirect spin-spin couplings J. As the dipolar interaction, also named direct spin-spin coupling depends on nucleus distances through space and orientation with respect to magnetic field, spatial constraints can be obtained in addition to classical measurement of NOE effects.

6. Peptide 3D ssNMR structure in Bicelles

Conversely to soluble proteins or DNA molecules, amphipathic peptides or membrane proteins may strongly interact or partition entirely into the membrane. The use of liquid-state NMR no longer leads to resolved spectra due to the slow rotational diffusion of the 30-50 nm-sized discs. If, in addition, Bicelles are concentrated, *i.e.*, below 95% hydration, the proton spectrum becomes very broad (Fig. 4a) and forbids the use of solution-state NMR techniques. Wide-line ssNMR can then be used to obtain topology information [49-51] (*vide*

infra) or the Bicelle sample can be moderately spun at the magic angle (54.7°) to resolve individual isotropic chemical shifts [65] in proton NMR (Fig. 4b). By comparison with liposomes, which could also be submitted to magic angle sample spinning, the Bicelle structure allows attaining sharper lines due to slightly greater membrane dynamics (*vide supra*) that provide pre-averaging of the strong ^{1}H-^{1}H interactions in the solid state. The MAS rate can therefore be moderate (10 kHz). It is interesting mentioning here that high-resolution proton NMR spectra can be obtained on hydrated membrane proteins in the form of aggregates or with Alzheimer's fibrils but with spinning the sample at very high speeds such as 110 kHz [4, 66]. As an example of a collaboration with prof Michèle Auger, moderate speed-magic angle sample spinning of DMPS-doped Bicelles containing the Methionin Enkephalin neuropeptide (Menk) allowed obtaining a well-resolved ^{1}H-NMR spectrum of the lipid and the peptide (Fig. 4b). The use of classical multidimensional liquid state NMR sequences (TOCSY, NOESY) combined with molecular modeling, allowed finding both the 3D structure of Menk and its localization at the membrane interface (Fig. 4c). This structure confirms earlier works that determined the structure of a parent enkephalin, Leucine Enkephalin, using transferred NOE experiments, which are much more difficult to set up [67].

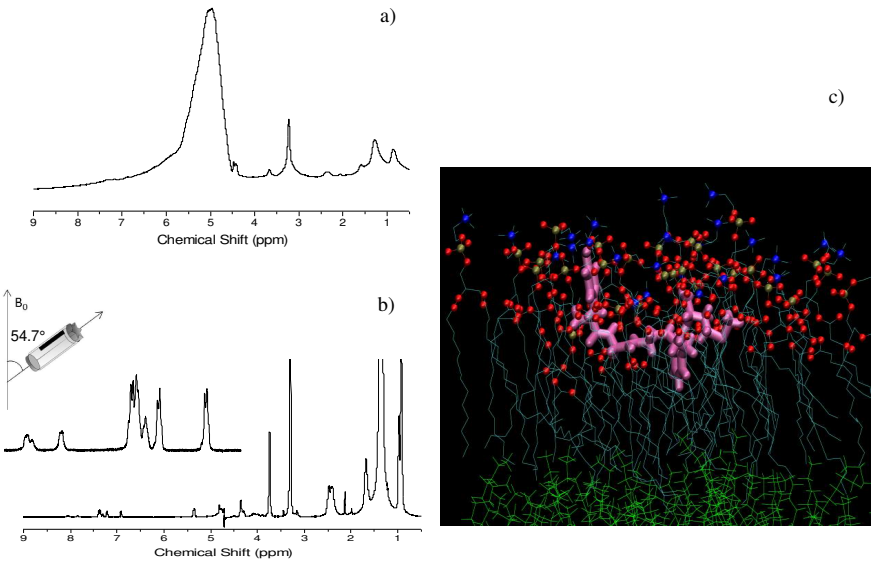


Fig. 4. Peptide 3D structure embedded in Bicelles*. (a) Static ^{1}H-NMR spectrum of DMPC/DCPC Bicelles containing the neuropeptide Menk (lipid-to-peptide molar ratio of 25), at 25 °C. (b) Same as (a) with a magic angle sample spinning speed of 10 kHz. A sketch of the MAS set up together with an expansion of the amide resonances are also shown in (b). (c) Position of Menk at the bilayer interface as reconstructed from 2D-NMR constraints and using molecular dynamics. Color code: peptide in pink; lipids: oxygen atoms of fatty acid chains in red, phosphorus in gold, nitrogen in blue, carbon skeleton in light blue, water molecules are not represented for clarity. Adapted from [17]*

7. Structural topology of membrane proteins in oriented Bicelles

The determination of membrane protein structure and topology in a membrane environment, is very difficult. Most NMR experiments of insoluble membrane proteins have been performed by dissolving the membrane in detergent systems that places membrane proteins in an isotropic environment and allows the use of liquid-state NMR

multidimensional approaches. As mentioned earlier, use of ultra-high speed magic angle sample spinning allows resolving the structure of insoluble membrane proteins under the form of hydrated aggregates. The topology with respect to the membrane is however inaccessible due to the lack of membrane. On the other hand and although convenient from the experimental viewpoint, the main drawback of the detergent approach resides in possible protein misfolding and the inability to control biological function in such media. Membrane protein topology and structure in lipid membranes have been addressed by many people [68, 69] but one must remark the pioneering work of the Opella and Bechinger groups, [5, 70-72] who developed sample alignment experiments that became standards for topology studies of proteins in membranes. Such experiments with macroscopically oriented membrane samples in between glass plates that were placed at defined orientations in the magnetic field offered access to orientational constraints for the calculation of atomic-resolution protein structures and topologies with respect to the membrane plane. For instance it is now very easy to determine whether a protein helix, ^{15}N-labelled in the NH backbone, is perpendicular or parallel to the membrane: ^{15}N-NMR chemical shifts around 200 ppm reflect a perpendicular orientation whereas resonances at ca. 70 ppm stand for helices lying flat on the membrane [73].

In recent years the versatility of Bicelles and the fact that they could be aligned by the magnetic field itself without the need of glass plates has afforded great progresses in the field. Fig. 5 synopsizes this approach by showing 1D and 2D solid-state NMR experiments of uniformly ^{15}N-labelled Pf1 coat protein embedded in biphenyl lipid bilayers Bicelles that are magnetically oriented with their plane normal to the field direction. The 1D spectrum displays very nicely resolved lines compared to a similar sample sandwiched in-between glass plates or even with a sample made of saturated DMPC/DCPC Bicelles that were flipped by 90° after addition of ytterbium(III) chloride, $YbCl_3$ [74]. The ^{15}N chemical shifts are detected in between 160 and 220 ppm a range characteristic of tilted trans-membranous helices. In the 2D separated local field (SLF) experiment shown in Fig. 5 (bottom), almost complete spectral resolution is achieved: each amide site leads to a correlation characterized by a unique spot at the intersection of the ^{1}H–^{15}N dipolar coupling and the ^{15}N chemical shift frequencies. The PISA wheel patterns (dotted ellipse) corresponding to helical wheel projections are formed by the resonances from residues in the transmembrane helix part of the protein. From the breadth of the ellipses a ca. 20–30° tilt can be inferred.

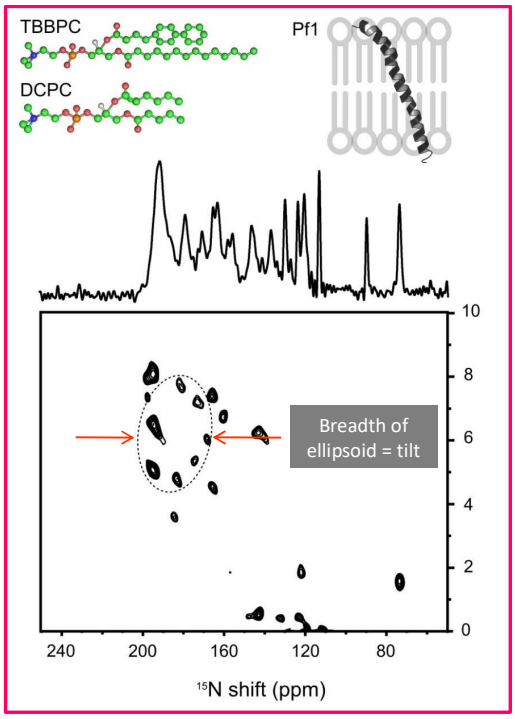


Fig. 5. Topology of membrane proteins in oriented Bicelles*. One-dimensional ^{15}N chemical shift spectrum (middle) and two-dimensional ^{15}N chemical shift/^{1}H–^{15}N dipolar coupling separated local field (SLF) solid-state NMR map (bottom), at 40°C. The Pf1 coat protein is uniformly ^{15}N-labelled and embedded in Bicelle membranes aligned with their bilayer normal parallel to the magnetic field (17.6 T) direction. Bicelles are made with TBBPC/DCPC (sketched on top, left), the molar ratio of lipid bilayers to protein (L/P) is 50. The ideal PISA wheel is superimposed on the 2D-map and leads to the orientation of the transmembrane helix as sketched on top (right). Adapted from [74, 75].*

8. Persistence of Bicelle magnetic orientation

The versatility of the Bicelle sample for NMR measurements has been shown above. Peptides and proteins can be embedded in the bilayer part of the disc and the entire system may be aligned parallel or perpendicular to the magnetic field. This has led us to call this system a "molecular goniometer", which is a slight misuse of language as intermediate orientations cannot yet be reached. Although very interesting such aligned samples thus appear to be restricted to NMR experiments: the magnetic field orients the sample and is used for NMR measurements. The liquid crystal nature of such samples, *nematic* or *smectic*, calls for more investigations outside magnetic fields. As shown in Fig. 6 such systems show a persistence of their orientation outside the magnetic field after having been magnetized. Small Angle X-Rays Scattering has been performed on DMPC/DCPC (Fig.6A), TBBPC/DCPC (Fig.6B) and DMPC/DCPC/Eu3+ (Fig.6C) Bicelles after being magnetized for 15 minutes in a 11.7 T magnetic field (z-axis) [76]. The z-axis of the X-rays set-up correspond to the z-axis of the magnetic field. On the Bicelle system made with saturated lipids, 2 spots are detected along the x-direction indicating a macroscopic orientation of the Bicelle plane perpendicular to the field, *i.e.*, *nematic* orientation. The macroscopic alignment of Bicelle discs is lost after *ca.* 1 hour as shown by a circular distribution of the scattering pattern. The biphenyl Bicelles system show two spots aligned along the z-axis, *i.e.*, with the disc normal parallel to the magnetic field (*smectic* order); orientation is such conditions lasts for several days. An

isotropic distribution, *i.e.*, a complete return to random orientation, is only obtained after 10 days outside the field. When doping the DMPC Bicelle with Europium ions, the Bicelles are flipped by 90° as seen with two spots along the z-axis (*smectic* order). They remain well aligned for several hours. When following their orientation/relaxation kinetics one realizes that the orientation mechanism is governed by the disc size: the smaller discs, DMPC/DCPC Bicelles, or DMPC/DCPC/Eu Bicelles, have similar and fast orientation kinetics: it takes about 5 min to align them (Fig. 6 rigth).

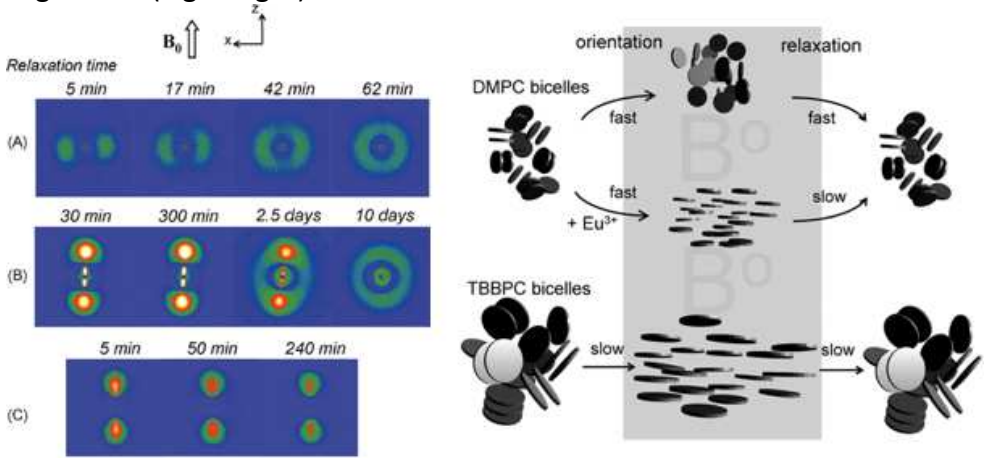


Fig. 6. Remnant orientation of Bicelles*. Left panel: SAXS pattern of (A) DMPC/DCPC Bicelles, (B) TBBPC/DCPC Bicelles, and (C) DMPC/DCPC-Eu Bicelles, which were subjected to an 11.7 T magnetic field (B0) during different durations. The graphs show the variation of the integrated scattered intensity as a function of the azimuthal angle θ. Right panel: Schematic representation of kinetics of orientation and relaxation as a function of Bicelles size and ordering. Adapted from [76]*

Conversely, larger discs, TBBPC/DCPC Bicelles, need to stay 15 min in the magnetic field to be aligned. Relaxation outside the field of Bicelles with *smectic* orientation (DMPC/DCPC/Eu Bicelles or TBBPC/DCPC Bicelles) was very slow, whereas the DMPC/DCPC Bicelles with *nematic* orientation relaxed back to isotropic orientation after about one hour. Such a remnant long-lived orientation of Bicelles outside the magnetic field was attributed to the higher degree of organization of the *smectic* phases with respect to *nematic* ones and was demonstrated by the analysis of anisotropic scattering pattern [76]. Such a long lasting orientation outside the magnetic field, after having been magnetized for a few minutes, is unprecedented and represents an excellent tool for structural studies by X-rays or Neutrons diffraction.

9. Nanodiscs made of lipids and helical proteins

Bicellar discs are very nice systems for structural biology but remain fragile to handle. If one wishes to embed membrane proteins or peptides into Bicelles, the latter may destabilize the discs as they may compete with the small chain lipids that compensate for membrane edge effect (half torus). This is what indeed happened with Apolipoprotein-like protein (which are amphipathic along the helix edge): Bicelles without short chain lipids are stabilized. They are called nanodiscs. Recent works [77] have shown that such 10 nm diameter nanodiscs are good tools to reconstitute membrane proteins in a native environment and solve their 3D structure by spectroscopic techniques. They originated from apolipoprotein A1 (Apo A1), which solubilizes lipids and cholesterol, to deliver them to the liver as high-density lipoprotein (HDL) particles [78]. As sketched in Fig. 7a,b they stabilize a piece of lipid bilayer

containing or not cholesterol and are well imaged by cryo-electron microscopy. The Apo A1 is also called membrane scaffolding protein (MSP) in the nanodisc community and stabilizes the lipid disc by direct van der Walls interaction of its hydrophobic face with lipid hydrophobic chains. The nanodisc is of small size (10 nm), compared to Bicelles but efforts are being made to design longer MSP proteins to form larger discs, with a diameter of ca. 20 nm or more [79].

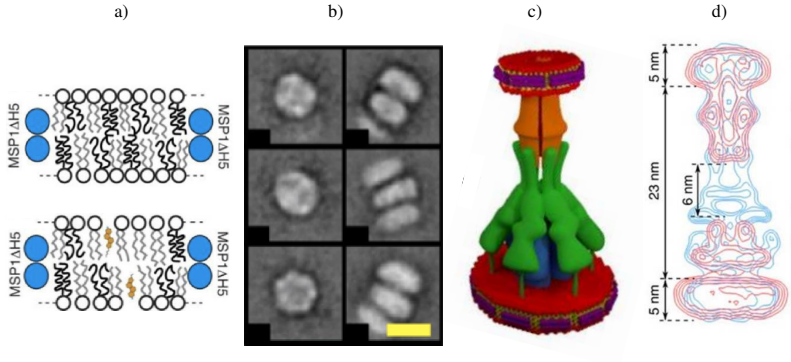


Fig.7. Nanodiscs for structural biology*. a) cartoons representing lipid nanodiscs: the MSP protein helices are represented as blue circles and lipid acyl chains are indicated in black and grey, cholesterol is displayed in orange. b) Electron micrographs of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) MSP nanodiscs (scale bar: 10 nm): the left column shows top views of the nanodiscs, whereas the right column shows side views of nanodiscs. c) cartoon representing the assembly of a multimeric (blue, green and orange) membrane protein complex after insertion and stabilization into nanodiscs: the integral membrane proteins are reconstituted into a small lipid bilayer wrapped by MSPs (purple) forming the nanodisc. Lipids are red/yellow. d) isocontour map obtained from cryo-electron microscopy experiments on the multimeric membrane protein complex stabilized into nanodiscs (c). Adapted from [7, 16].*

A very nice example of use of nanodiscs has been recently published by Lambert and coworkers [7] with the 3-component multimeric multidrug efflux system of Gram-negative bacteria. The intermembrane complex is composed of an inner membrane transporter, an outer membrane channel and a periplasmic adaptor protein. It is hypothesized that they form channels within the periplasm facilitating the exit of the drug through the two bacterial membranes. The inner and outer membrane transporter have been reconstituted in MSP-nanodiscs and single-particle analysis by electron microscopy has demonstrated that the inner and outer membrane protein components are linked together via the periplasmic adaptor protein (Fig. 7c,d). The 2D electron microscopy projection of all three complexes (Fig. 7d) clearly pictures the essential building block role of the periplasmic adaptor protein in extending the channel over 30 nm (periplasmic space between the inner and outer membranes). It is also interesting mentioning that polymer-encased lipid nanodiscs have been proposed by Ramamoorthy and coworkers: the MSP protein is replaced by polymers [80, 81].

10. Nanodiscs internal dynamics

Although there is a general consensus concerning the driving forces in nanodisc assembly with Apo A1/MSP proteins little is known about the internal dynamics of lipids and the possible dynamic interplay between lipids and proteins in relation with membrane protein activity. Incorporation of fluorescent probes into membranes [82] suggested a decrease in

membrane fluidity in nanodiscs, by comparison to that of vesicles,. However, the use of fluorescent probes might disturb the lipid cooperative behavior [83], and direct methods should be preferred. The internal lipid dynamics and their thermotropism has recently been reported by ^{2}H NMR spectroscopy of chain perdeuterated DMPC molecules embedded in nanodiscs. Such a method allows direct measurement of chain ordering and is very sensitive to the lipid phase nature and chain melting during thermal transitions.

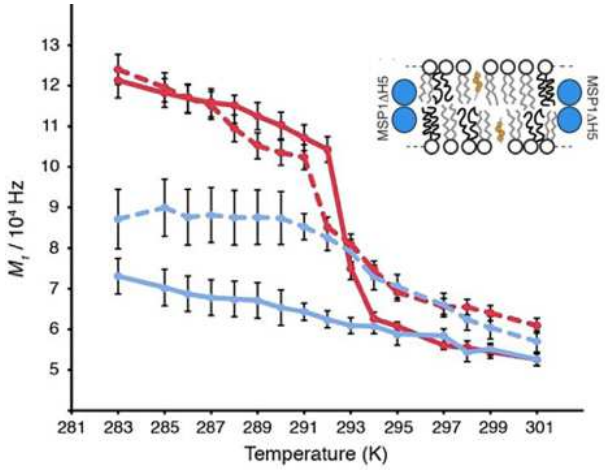


Fig. 8. Nanodisc and liposome thermotropism and internal chain ordering*. Thermal variation of the first spectral moment (M_1) of perdeuterated DMPC, as determined from solid-state ^{2}H NMR spectra for liposomes (red) and nanodiscs (blue) in the presence (dashed lines) and absence (solid line) of 7.5 mol% cholesterol. M_1 values near 140 kHz correspond to a chain order parameter near 1 (chains fully extended, full rigidity) and near 0 to entire disorder as in liquids. M_1=70 kHz represents a chain ordering of 0.5. The insert shows a representation of the cholesterol containing nanodisc as already described in Fig.7. Adapted from [16].*

It has been shown (Fig. 8) that chain ordering in the fluid phase is very similar to that of DMPC in large micrometer-size liposomes, although one observes a 10-15% increase in chain ordering. This has been attributed to the constraining role of MSP proteins that limit lateral and rotational diffusion. The well-known ordering effect of cholesterol in natural and synthetic liposomes [3, 84-86] is also observed in 10 nm diameter nanodiscs. The most striking effect is however the observation of fluid phase spectra at temperatures for which one usually observes gel phases with liposomes (Fig. 8). The lamellar gel-to lamellar fluid ($L_{\beta'}$ - L_{α}) phase transitions, which are well observed by the temperature variation of the first spectral moment in liposomes, are almost completely abolished in the nanodisc. The small size (10 nm) of the nanodisc indicates that there are ca. 70 lipids by leaflet, a value that is very (too) small to define a cooperative unit of lipids and therefore a phase transition. Cooperative units for liposomes are in the range of several thousand lipids and lead to very sharp transitions (1-2 °C). Those observed for small unilamellar vesicles obtained by sonication (20 nm diameter) lead to very broad transitions (10-20 °C) and are composed of cooperative units of a few hundred of lipids [87-89]. It thus appears that nanodiscs at low temperatures are maintained in some kind of fluid state due to the constraints produced by the MSP proteins. Interestingly, this result differs from that obtained with larger nanodiscs (ca. 20 nm) from which phase behavior is observed. This reminds also the observations on

large Bicelles were solid-ordered and liquid-disordered phases have been observed by NMR [43].

11. Conclusion

Bicelles and Nanodiscs turn to be very interesting platforms primarily for the study of membrane peptides and proteins. They are also used for the structural study of soluble proteins and nucleic acids, as well as possible platforms for drug targeting. Whereas Bicelles are membranous discs entirely made of lipids, Nanodiscs require the presence of MSP proteins or polymers for stability. In both cases increasing the size of these nanoplatforms is a clear requirement to have enough lipids in the disc plane to correctly mimic membrane thermotropism and dynamics. The internal dynamics of Bicelles and Nanodiscs resembles that of liposomes with the variance that lipid order slightly decreases in Bicelles and slightly increases in Nanodiscs on comparing with liposomes. Very interestingly, Bicelles can be manipulated with magnetic fields to be oriented parallel of perpendicular to the field direction, offering the access to “molecular goniometers” for experimentalists. In addition, Bicelles show a remnant alignment after having been magnetized and taken out of the field. In some case this alignment persisting outside the magnetic field lasts for days, offering a new tool for crystallography. Such properties are not yet studied with Nanodiscs made with MSP proteins and deserve to be considered.

Although the structure and dynamics of Bicelles and nanodiscs can be compared, much more information on the composition of nanodiscs is urgently needed. Such a lack is not surprising since nanodiscs were first invented by mimicking natural HDLs while Bicelles were discovered by examining lipid-detergent mixtures. Composition-temperature-hydration diagrams for nanodiscs are needed. The ability to accommodate controlled amounts of cholesterol and sphingolipids would be of primary interest: so-called lipid rafts could be isolated.

The question of phase transitions is also open. While MSP proteins seem to prevent temperature-induced gel-fluid transitions in small nanodiscs, such transitions are difficult to observe in Bicelles because temperature variation modifies the morphology of the pure lipid assembly: very small micelles are observed when the temperature is below Tc. Here again, it would be good to stabilize large nanodisks or Bicelles with enough lipids (a few thousand) to have a cooperative unit capable of producing thermodynamic phase changes.

A review of the literature shows that Bicelles have been considered over the last two decades as the ultimate membrane mimetics for the study of the structure and dynamics of biomolecules. However, the situation is now changing due to the relative fragility of Bicelles; nanodiscs made from MSP or polymers surrounding natural lipids appear to be becoming the fashionable new membrane platform that is robust and easy to manipulate.

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References

[1] P. Uzureau, S. Uzureau, L. Lecordier, F. Fontaine, P. Tebabi, F. Homble, A. Grelard, V. Zhendre, D.P. Nolan, L. Lins, J.-M. Crowet, A. Pays, C. Felu, P. Poelvoorde, B. Vanhollebeke, S.K. Moestrup, J. Lyngso, J.S. Pedersen, J.C. Mottram, E.J. Dufourc, D. Perez-Morga, E. Pays, Mechanism of Trypanosoma brucei gambiense resistance to human serum, Nature 501(7467) (2013) 430-+.
[2] R. Dazzoni, A. Grélard, E. Morvan, A. Bouter, C.J. Applebee, A. Loquet, B. Larijani, E.J. Dufourc, The unprecedented membrane deformation of the human nuclear envelope, in a magnetic field, indicates formation of nuclear membrane invaginations. , Scientific Reports in press (2020).
[3] E.J. Dufourc, Sterols and membrane dynamics, J. Chem. Biol. 1 (2008) 63–77.
[4] A. Loquet, N. El Mammeri, J. Stanek, M. Berbon, B. Bardiaux, G. Pintacuda, B. Habenstein, 3D structure determination of amyloid fibrils using solid-state NMR spectroscopy, Methods 138 (2018) 26-38.
[5] B. Bechinger, The structure, dynamics and orientation of antimicrobial peptides in membranes by multidimensional solid-state NMR spectroscopy, Biochim. Biophys. Acta-Biomembr. 1462(1-2) (1999) 157-183.
[6] L. Vigh, P.V. Escriba, A. Sonnleitner, M. Sonnleitner, S. Piotto, B. Maresca, I. Horvath, J.L. Harwood, The significance of lipid composition for membrane activity: New concepts and ways of assessing function, Prog. Lipid Res. 44(5) (2005) 303-344.
[7] L. Daury, F. Orange, J.-C. Taveau, A. Verchere, L. Monlezun, C. Gounou, R.K.R. Marreddy, M. Picard, I. Broutin, K.M. Pos, O. Lambert, Tripartite assembly of RND multidrug efflux pumps, Nature Communications 7 (2016).
[8] B. Bechinger, L.M. Gierasch, M. Montal, M. Zasloff, S.J. Opella, Orientations of helical peptides in membrane bilayers by solid state NMR spectroscopy, Solid State Nucl. Magn. Reson. 7(3) (1996) 185-191.
[9] D.B. Fenske, J.L. Thewalt, M. Bloom, N. Kitson, MODELS OF STRATUM-CORNEUM INTERCELLULAR MEMBRANES - H-2 NMR OF MACROSCOPICALLY ORIENTED MULTILAYERS, Biophys. J. 67(4) (1994) 1562-1573.
[10] A. Ramamoorthy, F.M. Marassi, M. Zasloff, S.J. Opella, 3-DIMENSIONAL SOLID-STATE NMR-SPECTROSCOPY OF A PEPTIDE ORIENTED IN MEMBRANE BILAYERS, Journal of Biomolecular Nmr 6(3) (1995) 329-334.
[11] N.E. Gabriel, M.F. Roberts, Spontaneous formation of stable unilamellar vesicles, Biochemistry 23(18) (1984) 4011-4015.
[12] C.R. Sanders, G.C. Landis, RECONSTITUTION OF MEMBRANE-PROTEINS INTO LIPID-RICH BILAYERED MIXED MICELLES FOR NMR-STUDIES, Biochemistry 34(12) (1995) 4030-4040.
[13] R.R. Vold, R.S. Prosser, Magnetically oriented phospholipid bilayered micelles for structural studies of polypeptides. Does the ideal bicelle exist?, Journal of Magnetic Resonance Series B 113(3) (1996) 267-271.
[14] A. Arnold, T. Labrot, R. Oda, E.J. Dufourc, Cation modulation of bicelle size and magnetic alignment as revealed by solid-state NMR and electron microscopy, Biophys. J. 83(5) (2002) 2667-2680.
[15] F. Aussenac, B. Lavigne, E.J. Dufourc, Toward bicelle stability with ether-linked phospholipids: Temperature, composition, and hydration diagrams by H-2 and P-31 solid-state NMR, Langmuir 21(16) (2005) 7129-7135.

[16] D. Martinez, M. Decossas, J. Kowal, L. Frey, H. Stahlberg, E.J. Dufourc, R. Riek, B. Habenstein, S. Bibow, A. Loquet, Lipid Internal Dynamics Probed in Nanodiscs, ChemPhysChem 18(19) (2017) 2651-2657.
[17] A. Diller, C. Loudet, F. Aussenac, G. Raffard, S. Fournier, M. Laguerre, A. Grelard, S.J. Opella, F.M. Marassi, E.J. Dufourc, Bicelles: A natural 'molecular goniometer' for structural, dynamical and topological studies of molecules in membranes, Biochimie 91(6) (2009) 744-751.
[18] M.N. Triba, D.E. Warschawski, P.F. Devaux, Reinvestigation by phosphorus NMR of lipid distribution in bicelles, Biophys. J. 88(3) (2005) 1887-1901.
[19] U.H.N. Duerr, M. Gildenberg, A. Ramamoorthy, The Magic of Bicelles Lights Up Membrane Protein Structure, Chemical Reviews 112(11) (2012) 6054-6074.
[20] M. Beaugrand, A.A. Arnold, J. Henin, D.E. Warschawski, P.T.F. Williamson, I. Marcotte, Lipid Concentration and Molar Ratio Boundaries for the Use of Isotropic Bicelles, Langmuir 30(21) (2014) 6162-6170.
[21] D.E. Warschawski, A.A. Arnold, M. Beaugrand, A. Gravel, E. Chartrand, I. Marcotte, Choosing membrane mimetics for NMR structural studies of transmembrane proteins, Biochim. Biophys. Acta-Biomembr. 1808(8) (2011) 1957-1974.
[22] T.H. Bayburt, J.W. Carlson, S.G. Sligar, Reconstitution and imaging of a membrane protein in a nanometer-size phospholipid bilayer, J. Struct. Biol. 123(1) (1998) 37-44.
[23] T.H. Bayburt, Y.V. Grinkova, S.G. Sligar, Assembly of single bacteriorhodopsin trimers in bilayer nanodiscs, Archives of Biochemistry and Biophysics 450(2) (2006) 215-222.
[24] K. Klopfer, F. Hagn, Beyond detergent micelles: The advantages and applications of non-micellar and lipid-based membrane mimetics for solution-state NMR, Progress in Nuclear Magnetic Resonance Spectroscopy 114 (2019) 271-283.
[25] R. Puthenveetil, O. Vinogradova, Solution NMR: A powerful tool for structural and functional studies of membrane proteins in reconstituted environments, Journal of Biological Chemistry 294(44) (2019) 15914-15931.
[26] T.A. Harroun, M. Koslowsky, M.P. Nieh, C.F. de Lannoy, V.A. Raghunathan, J. Katsaras, Comprehensive examination of mesophases formed by DMPC and DHPC mixtures, Langmuir 21(12) (2005) 5356-5361.
[27] M.P. Nieh, V.A. Raghunathan, C.J. Glinka, T. Harroun, J. Katsaras, Structural phase behavior of high-concentration, alignable biomimetic bicelle mixtures, Macromolecular Symposia 219 (2004) 135-145.
[28] S.M. Garber, G.A. Lorigan, K.P. Howard, Magnetically Oriented Phospholipid Bilayers for Spin Label EPR Studies, J. Am. Chem. Soc. 121(13) (1999) 3240-3241.
[29] S. Faham, J.U. Bowie, Bicelle crystallization A new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure, Journal of Molecular Biology 316(1) (2002) 1-6.
[30] L. Khemtemourian, S. Buchoux, F. Aussenac, E.J. Dufourc, Dimerization of Neu/Erb2 transmembrane domain is controlled by membrane curvature, European Biophysics Journal with Biophysics Letters 36(2) (2007) 107-112.
[31] I. Marcotte, K.L. Wegener, Y.H. Lam, B.C.S. Chia, M.R.R. de Planque, J.H. Bowie, M. Auger, F. Separovic, Interaction of antimicrobial peptides from Australian amphibians with lipid membranes, Chem. Phys. Lipids 122(1-2) (2003) 107-120.
[32] F. Aussenac, B. Lavigne, O. Dubernet, G. Raffard, E.J. Dufourc, Phase diagrams of acyl chain ester- vs. ether-linked lipid bicelles: Towards the bicelle stability, Biophys. J. 80(1) (2001) 333A-333A.

[33] J. Bian, M.F. Roberts, Phase separation in short-chain lecithin/gel-state long chain lecithin aggregates, Biochemistry 29(34) (1990) 7928-7935.
[34] G. Raffard, S. Steinbruckner, A. Arnold, J.H. Davis, E.J. Dufourc, Temperature-composition diagram of dimyristoylphosphatidylcholine-dicaproylphosphatidylcholine "bicelles" self-orienting in the magnetic field. A solid state H-2 and P-31 NMR study, Langmuir 16(20) (2000) 7655-7662.
[35] E. Sternin, D. Nizza, K. Gawrisch, Temperature dependence of DMPC/DHPC mixing in a bicellar solution and its structural implications, Langmuir 17(9) (2001) 2610-2616.
[36] K. Koshiyama, M. Taneo, T. Shigematsu, S. Wada, Bicelle-to-Vesicle Transition of a Binary Phospholipid Mixture Guided by Controlled Local Lipid Compositions: A Molecular Dynamics Simulation Study, J. Phys. Chem. B 123(14) (2019) 3118-3123.
[37] P.W. Yang, T.L. Lin, Y. Hu, U.S. Jeng, Small-Angle X-ray Scattering Studies on the Structure of Mixed DPPC/diC(7)PC Micelles in Aqueous Solutions, Chin. J. Phys. 50(2) (2012) 349-356.
[38] C. Loudet, S. Manet, S. Gineste, R. Oda, M.F. Achard, E.J. Dufourc, Biphenyl bicelle disks align perpendicular to magnetic fields on large temperature scales: A study combining synthesis, solid-state NMR, TEM, and SAXS, Biophys. J. 92(11) (2007) 3949-3959.
[39] M. Ottiger, A. Bax, Bicelle-based liquid crystals for NMR-measurement of dipolar couplings at acidic and basic pH values, Journal of Biomolecular Nmr 13(2) (1999) 187-191.
[40] S.H. Park, A.A. De Angelis, A.A. Nevzorov, C.H. Wu, S.J. Opella, Three-dimensional structure of the transmembrane domain of Vpu from HIV-1 in aligned phospholipid bicelles, Biophys. J. 91(8) (2006) 3032-3042.
[41] R.E. Minto, P.R. Adhikari, G.A. Lorigan, A H-2 solid-state NMR spectroscopic investigation of biomimetic bicelles containing cholesterol and polyunsaturated phosphatidylcholine, Chem. Phys. Lipids 132(1) (2004) 55-64.
[42] H.S. Cho, J.L. Dominick, M.M. Spence, Lipid Domains in Bicelles Containing Unsaturated Lipids and Cholesterol, J. Phys. Chem. B 114(28) (2010) 9238-9245.
[43] M.L. Schmidt, J.H. Davis, Liquid disordered-liquid ordered phase coexistence in bicelles containing unsaturated lipids and cholesterol, Biochim. Biophys. Acta-Biomembr. 1858(4) (2016) 619-626.
[44] J. Liebau, P. Pettersson, P. Zuber, C. Arioz, L. Maler, Fast-tumbling bicelles constructed from native Escherichia coli lipids, Biochim. Biophys. Acta-Biomembr. 1858(9) (2016) 2097-2105.
[45] A.A. Arnold, I. Marcotte, Studying Natural Structural Protein Fibers By Solid-State Nuclear Magnetic Resonance, Concepts in Magnetic Resonance Part A 34A(1) (2009) 24-47.
[46] A. Grelard, P. Guichard, P. Bonnafous, S. Marco, O. Lambert, C. Manin, F. Ronzon, E.J. Dufourc, Hepatitis B subvirus particles display both a fluid bilayer membrane and a strong resistance to freeze drying: a study by solid-state NMR, light scattering, and cryo-electron microscopy/tomography, Faseb J. 27(10) (2013) 4316-4326.
[47] C. Loudet, A. Diller, A. Grelard, R. Oda, E.J. Dufourc, Biphenyl phosphatidylcholine: A promoter of liposome deformation and bicelle collective orientation by magnetic fields, Prog. Lipid Res. 49(3) (2010) 289-297.
[48] M.N. Triba, P.F. Devaux, D.E. Warschawski, Effects of lipid chain length and unsaturation on bicelles stability. A phosphorus NMR study, Biophys. J. 91(4) (2006) 1357-1367.
[49] M. Ouellet, G. Bernard, N. Voyer, M. Auger, Insights on the interactions of synthetic amphipathic peptides with model membranes as revealed by P-31 and H-2 solid-state NMR and infrared spectroscopies, Biophys. J. 90(11) (2006) 4071-4084.

[50] I. Marcotte, M. Auger, Bicelles as model membranes for solid- and solution-state NMR studies of membrane peptides and proteins, Concepts in Magnetic Resonance Part A 24A(1) (2005) 17-37.
[51] I. Marcotte, E.J. Dufourc, M. Ouellet, M. Auger, Interaction of the neuropeptide Met-enkephalin with zwitterionic and negatively charged bicelles as viewed by P-31 and H-2 solid-state NMR, Biophys. J. 85(1) (2003) 328-339.
[52] S.B. Baek, S.C. Lim, H.J. Lee, H.C. Lee, C. Kim, An NMR Study on the Conformation of Substance P in Acidic Bicelles, Bulletin of the Korean Chemical Society 32(10) (2011) 3702-3706.
[53] M.A. Parker, V. King, K.P. Howard, Nuclear magnetic resonance study of doxorubicin binding to cardiolipin containing magnetically oriented phospholipid bilayers, Biochim. Biophys. Acta-Biomembr. 1514(2) (2001) 206-216.
[54] P.W. Yang, T.L. Lin, T.Y. Lin, C.H. Yang, Y. Hu, U.S. Jeng, Packing DNA with disc-shaped bicelles, Soft Matter 9(48) (2013) 11542-11548.
[55] F. Aussenac, M. Laguerre, J.M. Schmitter, E.J. Dufourc, Detailed structure and dynamics of bicelle phospholipids using selectively deuterated and perdeuterated labels. 2-H NMR and molecular mechanics study, Langmuir 19(25) (2003) 10468-10479.
[56] E.K. Tiburu, D.M. Moton, G.A. Lorigan, Development of magnetically aligned phospholipid bilayers in mixtures of palmitoylstearoylphosphatidylcholine and dihexanoylphosphatidylcholine by solid-state NMR spectroscopy, Biochim. Biophys. Acta-Biomembr. 1512(2) (2001) 206-214.
[57] C. Ma, S.J. Opella, Lanthanide ions bind specifically to an added "EF-hand" and orient a membrane protein in micelles for solution NMR spectroscopy, J. Magn. Reson. 146(2) (2000) 381-384.
[58] R.S. Prosser, S.A. Hunt, J.A. DiNatale, R.R. Vold, Magnetically Aligned Membrane Model Systems with Positive Order Parameter: Switching the Sign of Szz with Paramagnetic Ions, J. Am. Chem. Soc. 118(1) (1996) 269-270.
[59] E.J. Dufourc, C. Mayer, J. Stohrer, G. Althoff, G. Kothe, Dynamics of phosphate head-groups in biomembranes - Comprehensive analysis using P-31 Nuclear-Magnetic-Resonance lineshape and relaxation time measurements., Biophys. J. 61(1) (1992) 42-57.
[60] C.R. Sanders, J.P. Schwonek, Characterization of magnetically orientable bilayers in mixtures of dihexanoylphosphatidylcholine and dimyristoylphosphatidylcholine by solid-state NMR, Biochemistry 31(37) (1992) 8898-8905.
[61] J. Lorieau, L.S. Yao, A. Bax, Liquid crystalline phase of G-tetrad DNA for NMR study of detergent-solubilized proteins, J. Am. Chem. Soc. 130(24) (2008) 7536-+.
[62] Z.G. Wu, F. Delaglio, N. Tjandra, V.B. Zhurkin, A. Bax, Overall structure and sugar dynamics of a DNA dodecamer from homoand heteronuclear dipolar couplings and P-31 chemical shift anisotropy, Journal of Biomolecular Nmr 26(4) (2003) 297-315.
[63] N. Tjandra, S. Tate, A. Ono, M. Kainosho, A. Bax, The NMR structure of a DNA dodecamer in an aqueous dilute liquid crystalline phase, J. Am. Chem. Soc. 122(26) (2000) 6190-6200.
[64] N. Tjandra, A. Bax, Direct Measurement of Distances and Angles in Biomolecules by NMR in a Dilute Liquid Crystalline Medium, Science 278(5340) (1997) 1111-1114.
[65] C. Carlotti, F. Aussenac, E.J. Dufourc, Towards high-resolution 1H-NMR in biological membranes: magic angle spinning of bicelles, Biochim Biophys Acta 1564(1) (2002) 156-164.
[66] J. Stanek, L.B. Andreas, K. Jaudzems, D. Cala, D. Lalli, A. Bertarello, T. Schubeis, I. Akopjana, S. Kotelovica, K. Tars, A. Pica, S. Leone, D. Picone, Z.Q. Xu, N.E. Dixon, D. Martinez,

M. Berbon, N. El Mammeri, A. Noubhani, S. Saupe, B. Habenstein, A. Loquet, G. Pintacuda, NMR Spectroscopic Assignment of Backbone and Side-Chain Protons in Fully Protonated Proteins: Microcrystals, Sedimented Assemblies, and Amyloid Fibrils, Angew. Chem.-Int. Edit. 55(50) (2016) 15503-15509.
[67] A. Milon, T. Miyazawa, T. Higashijima, Transferred Nuclear Overhauser Effect Analyses of Membrane-bound Enkephalin Analogs by H-1 Nuclear Magnetic Resonance - Correlation between Activities and Membrane-bound conformations, Biochemistry 29(1) (1990) 65-75.
[68] T.A. Cross, S.J. Opella, SOLID-STATE NMR STRUCTURAL STUDIES OF PEPTIDES AND PROTEINS IN MEMBRANES, Current Opinion in Structural Biology 4(4) (1994) 574-581.
[69] F. Creuzet, A. McDermott, R. Gebhard, K. Vanderhoef, M.B. Spijkerassink, J. Herzfeld, J. Lugtenburg, M.H. Levitt, R.G. Griffin, DETERMINATION OF MEMBRANE-PROTEIN STRUCTURE BY ROTATIONAL RESONANCE NMR - BACTERIORHODOPSIN, Science 251(4995) (1991) 783-786.
[70] S.J. Opella, NMR and membrane proteins, Nat. Struct. Biol. 4 (1997) 845-848.
[71] B. Bechinger, M. Zasloff, S.J. Opella, STRUCTURE AND ORIENTATION OF THE ANTIBIOTIC PEPTIDE MAGAININ IN MEMBRANES BY SOLID-STATE NUCLEAR-MAGNETIC-RESONANCE SPECTROSCOPY, Protein Sci. 2(12) (1993) 2077-2084.
[72] K.J. Shon, Y.G. Kim, L.A. Colnago, S.J. Opella, NMR-STUDIES OF THE STRUCTURE AND DYNAMICS OF MEMBRANE-BOUND BACTERIOPHAGE-PFL COAT PROTEIN, Science 252(5010) (1991) 1303-1304.
[73] F.M. Marassi, C. Ma, H. Gratkowski, S.K. Straus, K. Strebel, M. Oblatt-Montal, M. Montal, S.J. Opella, Correlation of the structural and functional domains in the membrane protein Vpu from HIV-1, Proc. Natl. Acad. Sci. U. S. A. 96(25) (1999) 14336-14341.
[74] S.H. Park, C. Loudet, F.M. Marassi, E.J. Dufourc, S.J. Opella, Solid-state NMR spectroscopy of a membrane protein in biphenyl phospholipid bicelles with the bilayer normal parallel to the magnetic field, J. Magn. Reson. 193(1) (2008) 133-138.
[75] E.J. Dufourc, S. Buchoux, J. Toupe, M.-A. Sani, F. Jean-Francois, L. Khemtemourian, A. Grelard, C. Loudet-Courreges, M. Laguerre, J. Elezgaray, B. Desbat, B. Odaert, Membrane Interacting Peptides: From Killers to Helpers, Current Protein & Peptide Science 13(7) (2012) 620-631.
[76] C. Loudet-Courreges, F. Nallet, E.J. Dufourc, R. Oda, Unprecedented Observation of Days-Long Remnant Orientation of Phospholipid Bicelles: A Small-Angle X-ray Scattering and Theoretical Study, Langmuir 27(15) (2011) 9122-9130.
[77] F. Hagn, M. Etzkorn, T. Raschle, G. Wagner, Optimized Phospholipid Bilayer Nanodiscs Facilitate High-Resolution Structure Determination of Membrane Proteins, J. Am. Chem. Soc. 135(5) (2013) 1919-1925.
[78] P.T. Duong, H.L. Collins, M. Nickel, S. Lund-Katz, G.H. Rothblat, M.C. Phillips, Characterization of nascent HDL particles and microparticles formed by ABCA1-mediated efflux of cellular lipids to apoA-I, J. Lipid Res. 47(4) (2006) 832-843.
[79] M.A. McLean, M.C. Gregory, S.G. Sligar, Nanodiscs: A Controlled Bilayer Surface for the Study of Membrane Proteins, in: K.A. Dill (Ed.), Annual Review of Biophysics, Vol 472018, pp. 107-124.
[80] T.J. Knowles, R. Finka, C. Smith, Y.P. Lin, T. Dafforn, M. Overduin, Membrane Proteins Solubilized Intact in Lipid Containing Nanoparticles Bounded by Styrene Maleic Acid Copolymer, J. Am. Chem. Soc. 131(22) (2009) 7484-+.
[81] T. Ravula, J. Kim, D.K. Lee, A. Ramamoorthy, Magnetic Alignment of Polymer Nanodiscs Probed by Solid-State NMR Spectroscopy, Langmuir 36(5) (2020) 1258-1265.

[82] M. Nakano, M. Fukuda, T. Kudo, M. Miyazaki, Y. Wada, N. Matsuzaki, H. Endo, T. Handa, Static and Dynamic Properties of Phospholipid Bilayer Nanodiscs, J. Am. Chem. Soc. 131(23) (2009) 8308-8312.
[83] S.S.W. Leung, J. Thewalt, Link between Fluorescent Probe Partitioning and Molecular Order of Liquid Ordered-Liquid Disordered Membranes, J. Phys. Chem. B 121(6) (2017) 1176-1185.
[84] J.G. Beck, D. Mathieu, C. Loudet, S. Buchoux, E.J. Dufourc, Plant sterols in "rafts": a better way to regulate membrane thermal shocks, Faseb J. 21(8) (2007) 1714-1723.
[85] F. Aussenac, M. Tavares, E.J. Dufourc, Cholesterol dynamics in membranes of raft composition: A molecular point of view from H-2 and P-31 solid-state NMR, Biochemistry 42(6) (2003) 1383-1390.
[86] E.J. Dufourc, E.J. Parish, S. Chitrakorn, I.C.P. Smith, Structural and dynamical details of cholesterol lipid intearaction as revealed by deurerium NMR., Biochemistry 23(25) (1984) 6062-6071.
[87] I.P. Sugar, COOPERATIVITY AND CLASSIFICATION OF PHASE-TRANSITIONS - APPLICATION TO ONE-COMPONENT AND 2-COMPONENT PHOSPHOLIPID-MEMBRANES, Journal of Physical Chemistry 91(1) (1987) 95-101.
[88] B. Gruenewald, S. Stankowski, A. Blume, CURVATURE INFLUENCE ON THE COOPERATIVITY AND THE PHASE-TRANSITION ENTHALPY OF LECITHIN VESICLES, FEBS Lett. 102(2) (1979) 227-229.
[89] D. Marsh, A. Watts, P.F. Knowles, COOPERATIVITY OF PHASE-TRANSITION IN SINGLE-BILAYER AND MULTI-BILAYER LIPID VESICLES, Biochimica Et Biophysica Acta 465(3) (1977) 500-514.