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Magnetically Oriented Bicelles with Monoalkylphosphocholines: Versatile Membrane Mimetics for Nuclear Magnetic Resonance Applications

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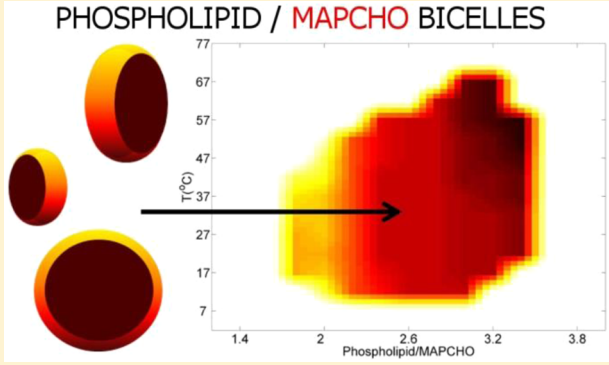
Supporting Information

ABSTRACT: Bicelles (bilayered micelles) are model membranes used in the study of peptide structure and membrane interactions. They are traditionally made of long- and short-chain phospholipids, usually dimyristoylphosphatidylcholine (D14PC) and dihexanoyl-PC (D6PC). They are attractive membrane mimetics because their composition and planar surface are similar to the native membrane environment. In this work, to improve the solubilization of membrane proteins and allow their study in bicellar systems, D6PC was replaced by detergents from the monoalkylphosphocholine (MAPCHO) family, of which dodecylphosphocholine (12PC) is known for its ability to solubilize membrane proteins. More specifically 12PC, tetradecyl- (14PC), and hexadecyl-PC (16PC) have been employed. To verify the possibility of making bicelles with different hydrophobic thicknesses to better accommodate membrane proteins, D14PC was also replaced by phospholipids with different alkyl chain lengths: dilauroyl-PC (D12PC), dipalmitoyl-PC (D16PC), distearoyl-PC (D18PC), and diarachidoyl-PC (D20PC). Results obtained by ^{31}P solid-state nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC) at several lipid-to-detergent molar ratios (q) and temperatures indicate that these new MAPCHO bicelles can be formed under a variety of conditions. The quality of their alignment is similar to that of classical bicelles, and the low critical micelle concentration (CMC) of the surfactants and their miscibility with phospholipids are likely to be advantageous for the reconstitution of membrane proteins.



INTRODUCTION

Bilayered micelles, or so-called bicelles, were introduced in the 1990s and quickly gained popularity because of their similarities with biological membranes.[1,2] They are composed of long-chain phospholipids organized in a bilayer stabilized by short-chain lipids or detergents in the high-curvature region of discs or perforated vesicles. The planar region made of long-chain phospholipids constitutes a favorable environment for studying molecular interactions as well as the structure of membrane peptides and proteins with different biophysical techniques such as nuclear magnetic resonance (NMR), circular dichroism, and fluorescence.[3−9] Bicelles can also be used to obtain protein crystals for X-ray crystallography[10,11] and have potential pharmaceutical applications.[12−16] In particular, bicelles have proven to be an ideal mimetic for solid-state NMR because they provide an ideal support for integral membrane proteins in a near native environment. Moreover, they are not limited by the solubility, the size of the macromolecules or complex, or the requirement of crystals.[17] The importance of lipid interactions to the folding, structure and functioning of membrane proteins has long been recognized,[18−20] in particular, the hydrophobic mismatch and lateral packing pressure due to the curvature of the lipids or detergents in model membranes.[20−23] The insertion and folding of membrane proteins is indeed more favorable with long-chain lipids.[21]

To better mimic the complexity and diversity of biological membranes, various alkyl chain lengths, degrees of unsaturation, and overall net charge have been proposed in the preparation of bicelles. Bile salt 3-(cholamidopropyl) dimethylammonio-2-hydroxyl-1-propane sulfate (CHAPSO) used with D14PC in the 1990s[1] was quickly replaced by D6PC, which was more structurally similar to phospholipids.[2] Other lipids

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with various headgroups as well as cardiolipin, gangliosides, and sphingomyelin can be incorporated into bicelles to mimick a variety of biological membranes, as reviewed elsewhere.[9] Studies have been carried out to improve the bicelles' stability by changing the length of the detergent using dipentanoyl-PC (D5PC) and diheptanoyl-PC (D7PC), the length and unsaturation of the phospholipid using dilauroyl-PC (D12PC), dipalmitoyl-PC (D16PC) and palmitoyloleoyl-PC (POPC),[24−26] or by using ether lipids.[27] Triton X-100 detergent demonstrates a better magnetic alignment and stability of membrane proteins in the bilayer,[28] whereas the addition of cholesterol, cholesterol 3-sulfate, and/or hexadecyltrimethylammonium bromide (CTAB) stabilizes the bilayer and increases the temperature range at which bicelles form.[29] To obtain bicelles at low concentrations that are more suitable for solution NMR, detergents with a low critical micelle concentration (CMC) were used such as dodecyl-PC (12PC) and several cyclohexyl-1-butyl-PC's.[30−32]

The objective of our work was to develop orientable PC-based bicellar systems with various hydrophobic chain lengths to accommodate membrane proteins and peptides. We focused on phosphatidylcholines because they are abundant in eukaryotic membrane cells.[9] Namely, a series of lipids ranging from D12PC to D20PC (diarachidonoylPC) were combined with monoalkylphosphocholine (MAPCHO) detergents. Specifically, dodecyl- (12PC), tetradecyl- (14PC), and hexadecyl (16PC) phosphocholines were employed. The detergent 12PC is often used to solubilize membrane proteins and has previously been used with D14PC to prepare isotropic and magnetically oriented bicelles.[30,31,33] When compared to 12PC, 14PC micelles have shown their ability to maintain the activity of dialkylglycerol kinase (DAGK) because of their better match with the hydrophobic span of the transmembrane domain of this protein.[34] In this work, we present the characterization of MAPCHO bicelles using ^{31}P and ^{2}H solid-state NMR and discuss the molar ratios and hydrophobic chain length of the phospholipids and detergents in relation to the magnetic alignment of the bicelles. Finally, data on the critical bicelle concentration (CBC) of the different binary systems, by analogy with the CMC, is provided using isotropic bicelles.

MATERIALS AND METHODS

Materials. Phospholipids 1,2-dilauryl-*sn*-glycero-3-phosphocholine (D12PC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (D14PC), deuterated 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine-d_{54} (D14PC-d_{54}), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (D16PC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (D18PC), and 1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine (D20PC) as well as detergents *n*-dodecylphosphocholine (12PC), deuterated *n*-dodecylphosphocholine-d_{38} (12PC-d_{38}), *n*-tetradecylphosphocholine (14PC), and *n*-hexadecylphosphocholine (16PC) were obtained from Avanti Polar Lipids (Alabaster, AL, USA) and used without further purification. Ytterbium(III) nitrate pentahydrate and ^{2}H-depleted water were purchased from Sigma-Aldrich (Oakville, ON, Canada). Deuterium oxide (D_2O) was obtained from CDN Isotopes (Pointe-Claire, QC, Canada).

Sample Preparation. Samples used for solid-state NMR experiments were prepared by suspending the appropriate weight of detergent and phospholipid in nanopure water (pH 4.5) or ^{2}H-depleted water. The total concentration used was 400 mM, well above the CMC of all constituents (Tables S1−S3). Samples had phospholipid/MAPCHO molar ratios (q) ranging from 1 to 3.6 with 80% (w/v) hydration. Samples were then submitted to about 10 cycles of freezing (liquid N_2), thawing (60 °C), and vortex shaking. The alignment of bicelles was flipped with the normal of the bilayer parallel to the magnetic field by adding the lanthanide salt $YbCl_3$ at a concentration of 2.5 mM[31,35] for the binary systems D14PC/14PC and D16PC/14PC.

For solution NMR, bicelles containing 12PC were prepared at a total lipid and detergent concentration of 100 mM and q ratios of 0.5 and 1, whereas 14PC-based samples were made at 20 mM and a q ratio of 0.5. Samples were made using D_2O and submitted to cycles of freeze/thaw/vortex shaking. Serial dilutions were then performed.

For isothermal titration calorimetry (ITC) experiments, D14PC lipid vesicles were prepared in nanopure water at 24 mM, incubated at 40 °C for 14 h, and submitted to seven cycles of freezing, thawing, and vortex shaking. Vesicles were then extruded 30 times through membranes of 0.2 μm pores using a LiposoFast-Basic extruder from Avestin (Ottawa, ON, Canada). Solutions of detergents (16PC and 14PC) were prepared in nanopure water at 1.4 mM, well above their CMC. Both lipid and detergent solutions were degassed for a minimum of 10 min.

Nuclear Magnetic Resonance. All solid-state NMR experiments were carried out on a hybrid solution/solid-state Varian Inova Unity 600 (Agilent, Santa-Clara, CA, USA) spectrometer operating at frequencies of 599.95 MHz for ^{1}H, 246.86 MHz for ^{31}P, and 92.125 MHz for ^{2}H and equipped with a 4 mm broadband/^{1}H dual-frequency magic-angle-spinning (MAS) probehead. For ^{31}P NMR spectra, a phase-cycled Hahn echo pulse sequence[36] was used with gated broadband proton continuous wave decoupling at a field strength of 50 kHz. A $\pi/2$ pulse of 3 μs, an interpulse delay of 33 μs, a recycle delay of 5 s, an acquisition time of 20 ms, and a dwell time of 5 μs were used, and 64 to 512 scans were acquired. Spectra were externally referenced with respect to the signal of 85% phosphoric acid set to 0 ppm, which rendered chemical shifts of free 12PC at 0.125 mM and 14PC at 0.025 mM at 0.330 and 0.324 ppm, respectively. ^{2}H NMR spectra were obtained using a solid echo pulse sequence[37] with a $\pi/2$ pulse length of 3 μs, an interpulse delay of 45 μs, and a repetition delay of 500 ms. Typically, 2400 scans were acquired. For ^{31}P and ^{2}H NMR experiments, preacquisition delays of at least 10 min were used between temperature steps. Spectra were acquired at least in duplicate at temperatures ranging from 7 to 82 °C depending on the phospholipid/detergent system. All spectra were processed using MNova software (Mestrelab Research, Santiago de Compostela, Spain).

All solution ^{31}P NMR experiments were carried out on an Avance III HD 600 MHz spectrometer (Bruker, Milton, ON, Canada) equipped with a 5 mm double-resonance probe. A single-pulse experiment was employed with a $\pi/2$ pulse of 15 μs, a recycle delay of 5 s, and an acquisition time of 1 s with broadband proton continuous wave decoupling at a field strength of 5 kHz. A preacquisition delay of 10 min was used before each experiment to ensure thermal equilibration of the samples. Spectra were acquired at least in duplicate with 8 to 8192 scans at 37 °C. They were internally referenced using a sealed capillary containing phosphate ions at pH 11 in D_2O, which was previously referenced with respect to 85% H_3PO_4 at 3.38 ppm. All spectra were processed with the Bruker TopSpin 3.2 interface.

Isothermal Titration Calorimetry. ITC reconstitution experiments were performed using a MicroCal VP-ITC (GE Healthcare, Baie d'Urfé, QC, Canada) at 17, 47, 57, and 72 °C. The 24 mM lipid suspension was injected into the 1.4 mM solution of detergent. One injection of lipid suspension of 1 μL was followed by 49 injections of 5 μL performed with a 600 s delay between each injection using 307 rpm stirring. Because of the nature of the experiment, no fitting was performed.

RESULTS AND DISCUSSION

Versatile Magnetically Oriented Bicelles with Low Free Surfactant Concentration. Several binary lipid mixtures were prepared by changing the chain length of the MAPCHO detergents (12PC, 14PC, and 16PC) for all of the phospholipids investigated, namely, D12PC, D14PC, D16PC, D18PC, and D20PC. The mixtures were studied over a wide

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range of temperatures (7−82 °C) and phospholipid/MAPCHO molar ratios ($1 \leq q \leq 3.6$). Figure 1 presents a

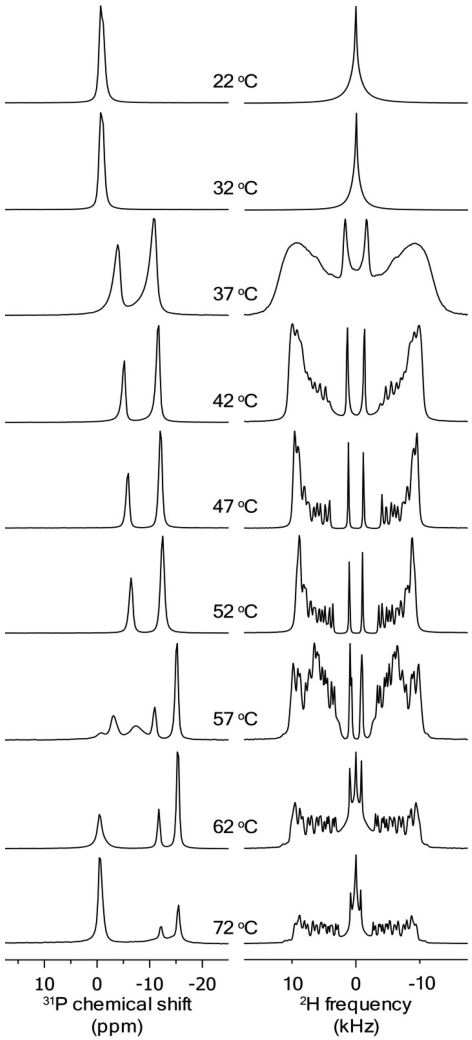

Figure 1. Evolution of the (A) ^{31}P and (B) ^{2}H solid-state NMR spectra of D16PC-d_{62}/14PC bicelles with q = 2 as a function of temperature.

characteristic example of one of the systems studied, namely, D16PC/14PC. The magnetic alignment is revealed by two well-resolved peaks on the ^{31}P solid-state NMR spectra between 42 and 52 °C, similar to classical D14PC/D6PC bicelles where the peaks at ca. −5 and −12 ppm are mainly assigned to 14PC and D14PC, respectively.[38] This alignment is confirmed by well-resolved doublets on the ^{2}H solid-state NMR spectra of D16PC-d_{62} in the same range of temperatures, which are characteristic of bilayers oriented with their normal perpendicular to the magnetic field. Quadrupolar splitting values are very close to those measured on classical D14PC/D6PC bicelles. At 47 °C, for example, these values vary between 2.3 and 26.4 kHz for the CD_3 terminal and methylene groups in close proximity to the ester link, respectively.

Characteristic ^{31}P solid-state NMR spectra of the various studied lipid mixtures and temperatures can be found in the Supporting Information (Figures S1 and S2). Complementary experiments were also performed with deuterated MAPCHO, and preliminary data has been obtained using commercially available deuterated 12PC-d_{38} with D14PC phospholipids, confirming the partial orientation of these detergent molecules when bicelles are aligned (Figures S3 and S4).

Ideally, bicelles would align with their bilayer normal perfectly perpendicular to the magnetic field. However, in practice this is not the case, and the deviation around this static orientation is called the *static* mosaic spread. This distribution of orientations is reflected in the width of the long-chain lipid's ^{31}P or ^{2}H resonances, a smaller mosaic spread yielding a narrower line. Each bicelle furthermore oscillates rapidly around its average orientation, within a cone that is called the *dynamic* mosaic spread. This rapid oscillation modifies the chemical shift or the quadrupolar splitting of the ^{31}P or ^{2}H NMR resonances, respectively: an oscillation on a smaller angle is shifting the ^{31}P resonance upfield or increasing the ^{2}H quadrupolar splitting toward a position corresponding to the ideal bicelle orientation, perpendicular to the magnetic field. An evaluation of static[39,40] and dynamic[38,40] mosaicities therefore gives a better evaluation of bicelle alignment to guide their use as model membrane systems. A detailed description of the experimental evaluation of both static and dynamic mosaicities is given in the Supporting Information. Values of dynamic and static mosaic spreads for all systems are compiled in Tables S1−S3, with properties such as the melting temperature (T_m) of the phospholipids and the CMC of the detergents. Static mosaicities fall below 5° for the best-oriented systems; the quality of the orientation of MAPCHO bicelles is therefore as good as that of classical bicelles.[41] The best dynamic mosaicities are also very good, for example, 6° with the D14PC/14PC system at 77 °C.

The molar ratios and temperatures at which bicelles align are summarized in Figure 2 for 12PC and in Figure S5 for 14PC and 16PC. In these figures, dynamic mosaicities are color coded, with a darker shade indicating better alignment that should be favored when choosing a bicelle system. MAPCHO bicelles could be formed with 12PC and phospholipids with chain lengths from 12 to 16. Our results for D14PC/12PC bicelles are in agreement with Nolandt et al.[31] With 14PC, bicelles could be formed with phospholipids having 14- to 16-carbon-long chains. Finally, aligned bicelles containing 16PC were formed only with D18PC at q = 1.6 and 57 °C. As shown in Figure S2C, the poor quality of the spectra and the limited q and temperature range of orientation discouraged the exploration of more 16PC-based bicelles.

In the study of transmembrane protein structures by solid-state NMR, for example, to determine the tilt of a transmembrane α-helix, it is interesting to align the bilayer normal parallel to the magnetic field. The addition of lanthanides to bicelles has been shown to flip the orientation of the bilayer normal from perpendicular to parallel to the magnetic field.[35] Using this strategy, alignment flipping has been demonstrated with the D14PC/12PC system by ^{31}P solid-state NMR.[31] We have verified that it was also possible for D16PC/14PC (Figure 3) and D16PC/12PC (data not shown); the possibility to flip the bicelles thus appears to be a general property of MAPCHO bicelles.

One advantage of MAPCHO bicelles over classical bicelles is the very low CMCs of this type of surfactant (Tables S1−S3). Indeed, the presence of a significant amount of free surfactant can be a drawback, in particular when samples need to be diluted.[42] By studying the effect of dilution on ^{31}P NMR spectra, the concentration of free detergent (or the critical bicellar concentration, CBC) can be determined.[41,42] Examples of such spectra are shown in the Supporting Information

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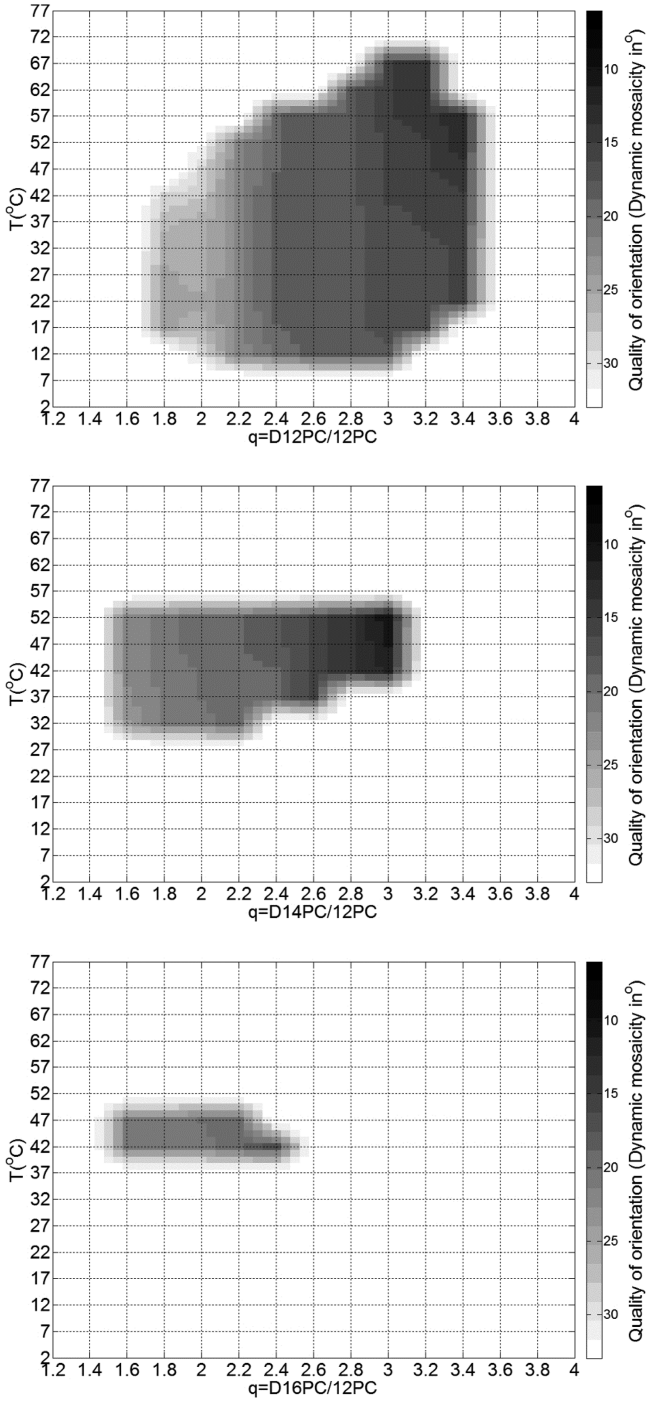

Figure 2. Compilation of molar ratio q and temperature ranges at which 12PC systems with D12PC, D14PC, or D16PC phospholipids align in the magnetic field. The quality of orientation is measured by the dynamic mosaicity and is coded in gray, a darker shade indicating better alignment. Note that some values have been interpolated. Exact experimental values are given Table S1.

(Figure S6), and CBC values are listed in Table 1, calculated as described by Beaugrand et al.[42] For a given molar ratio q of 0.5 and long-chain phospholipid D14PC, the CBCs with D6PC, 12PC, and 14PC are 7.5, 0.86, and 0.07 mM, respectively. The magnitude of the CBC follows the CMC of corresponding detergents (15, 1.5, and 0.12 mM, respectively[43]) and it is thus clearly advantageous to use MAPCHO bicelles when dilute conditions are necessary. For a given molar ratio q of 1 and

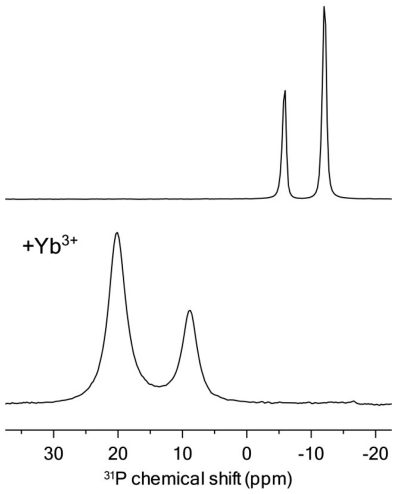

Figure 3. ^{31}P NMR spectra of D16PC/14PC bicelles (q = 2, 400 mM) at 47 °C (top) without and (bottom) with 2.5 mM lanthanide ions Yb^{3+}.

Table 1. Critical Bicelle Concentrations (CBC) of MAPCHO Bicelles at 37 °C

lipid	detergent	molar ratio q	CBC (mM)
D12PC	12PC	1	0.75 ± 0.01
D14PC	12PC		0.78 ± 0.01
D16PC	12PC		0.82 ± 0.01
D14PC	12PC	0.5	0.86 ± 0.02
	14PC		0.07 ± 0.01
D14PC	D6PC	0.5	7.5 ± 0.5

detergent 12PC, the CBCs for D12PC, D14PC, and D16PC are 0.75, 0.78, and 0.82 mM, respectively. By increasing the length of the phospholipid, the CBC slightly increases, indicating a decrease in miscibility when the chain-length mismatch increases.[42] For a given detergent 12PC and phospholipid D14PC, the CBC slightly decreases when q increases from 0.5 to 1, an effect also observed for classical bicelles and attributed to a higher segregation between D14PC and D6PC as q increases.[42]

Behavior as a Function of Temperature. As already described by Triba et al.,[24,38] it is possible to identify different characteristic transition temperatures for bicelle systems by ^{31}P and ^{2}H solid-state NMR as a function of temperature. The best-known transition for the phospholipids is the melting temperature (T_m) that corresponds to the transition between gel and liquid-crystalline phases. All T_m values of the lipids studied in this work are listed in Tables S1−S3. In bicelle systems, another important transition corresponds to the temperature at which they align in the magnetic field.[38] This temperature is superior to T_m in classical bicelles. Figures 1, S1, and S3 display the temperature behavior for a given molar ratio q of bicelles for the different MAPCHO systems, as observed by ^{31}P and ^{2}H solid-state NMR, and Figure 4 displays the temperature behavior as observed by ITC. Below T_m, lipids and detergent molecules do not mix, as shown by the absence of energy transfer in the ITC curve when lipids are added to a detergent solution (Figure 4A). Samples are milky (data not shown), and NMR spectra of MAPCHO bicelles show a narrow resonance centered at 0 ppm in both ^{31}P and ^{2}H NMR spectra, indicative of fast-tumbling detergent structures, as well as a broad lipid vesicles powder pattern, which is particularly

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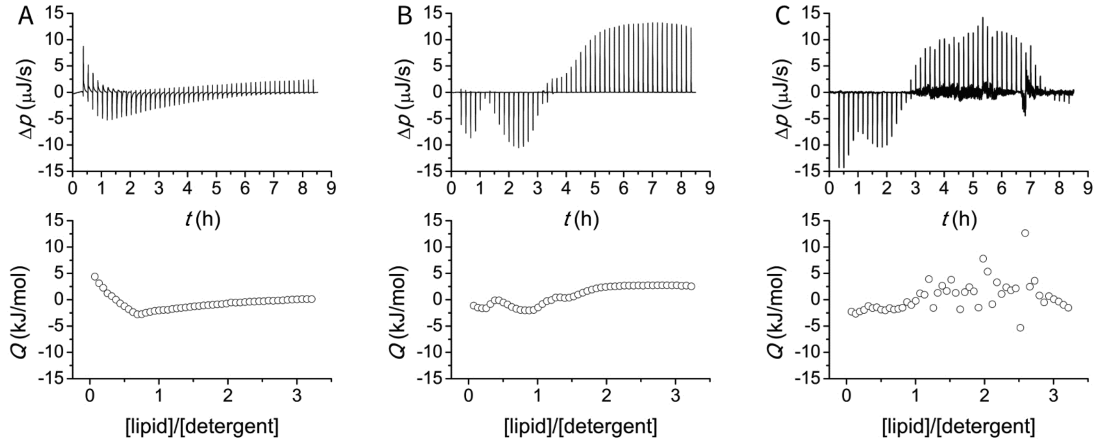

Figure 4. Isothermal titration calorimetry reconstitution curves of D14PC vesicles at 24 mM titrated in solutions of 14PC MAPCHO micelles at 1.4 mM and (A) 17 °C ($<T_m$), (B) 57 °C ($>T_m$, $<T_v$), and (C) 72 °C ($>T_v$). Top: raw differential heat as a function of time. Bottom: integrated heat as a function of the q ratio.

intense when detergents and phospholipids have the same alkyl chain length (D12PC/12PC and D14PC/14PC) (Figures 1 and S1B).

Close to T_m, two broad resonances emerge from the ^{31}P NMR spectra and a broad doublet emerges from the ^{2}H NMR spectra (Figure 1), as was seen for D14PC/D6PC when magnetic alignment starts.[38] Above T_m, samples are transparent and viscous (data not shown), and well-defined ^{31}P resonances and ^{2}H doublets are observable (Figures 1 and S1), characteristic of aligned bicelles and reduced mosaicities with increasing temperature.[2,38] The low-field (higher ppm values) ^{31}P peak has been ascribed to short-chain phospholipids (or detergents in our case) mainly on the edges or holes, and the high-field (lower ppm values) resonance corresponds to long-chain phospholipids mainly in the bilayer.[38] Similar information can be extracted from ^{2}H NMR spectra of bicelles with deuterated MAPCHO, exhibiting residual quadrupolar couplings when bicelles are aligned (Figure S3). These quadrupolar splittings increase with temperature as more 12PC-d_{38} partitions into the oriented bilayers. In this temperature range, lipids and detergent molecules mix, as confirmed by the energy absorbed by the detergent solution when lipids are added to it (Figure 4B), and the objects formed are stable over a large range of q values.

Another transition is observed at higher temperatures, when a peak appears at −15 ppm on the ^{31}P NMR spectra, which is characteristic of a 90° orientation in vesicles and is described as T_V by Triba et al.[38] In Figure 1, this occurs at 57 °C, and over a certain temperature range, these new vesicles coexist with remaining misaligned bicelles, as seen from the complex ^{31}P and ^{2}H NMR spectra, until those bicelles disappear, as seen at 62 °C. In this type of situation, ITC reconstitution experiments cannot inform us of the shape of the complex formed but reveal that lipid and detergent molecules mix until the objects formed are saturated with lipids. Any further addition of lipid vesicles will coexist in the solution, without mixing with the detergent complexes, as seen on the ITC curve by a decrease in the energy absorption at higher q values (Figure 4C). The samples become liquid again but lose transparency (data not shown), consistent with the presence of vesicles coexisting with other complex structures containing detergent molecules, such as bicelles and fast-tumbling objects (Figures 1 and S1).

When compared to traditional D14PC/D6PC (DMPC/DHPC) bicelles, the MAPCHO bicelles explored in this work display similar transitions but a larger temperature range of alignment. D14PC/D6PC bicelles typically align between 30 and 50 °C, as reviewed elsewhere,[5,44] whereas the MAPCHO systems with D14PC can orient between 27 and 77 °C (Figure 2 and Table S1), which makes them better systems for the study of aligned membrane molecules.

Behavior as a Function of Molar Ratio *q*. Figures 5, S2, and S4 show ^{31}P and ^{2}H NMR spectra at different q ratios, at temperatures where most MAPCHO-based lipid systems are aligned. In a similar manner to temperature, it is possible to identify transitions according to the molar ratio q for systems that display perpendicularly aligned bicellar objects. Therefore, q_m describes the minimal ratio at which alignment occurs, i.e., when two well-resolved resonances are observed on the ^{31}P NMR spectra. We can also define a q_v when a powder pattern starts to show on the spectra, the best alignment therefore being between q_m and q_v. D14PC/D6PC bicelles typically align for q ratios between ∼2.5 and 7.5, as reviewed elsewhere.[5,44] By comparison and although MAPCHO bicelles explored in this work start to align at lower q molar ratios, the q_m−q_v interval in which aligned objects are found is smaller. As an example, system D16PC/14PC has q_m and q_v values of 1.2 and 2.6, respectively. In the case of D12PC/12PC, q_m and q_v are 1.7 and 3.6, respectively, as seen in Figure 2.

The magnetic alignment of a bilayer is due to the anisotropy of the diamagnetic susceptibility of the phospholipids. However, a sufficient number of phospholipids are required to counterbalance thermal agitation; i.e., the planar region needs to be above a certain threshold size. One might therefore infer that MAPCHO bicelles at q values as low as 1.6 for 12PC or q = 1.2 for 14PC have already reached this threshold size for orientation, which is attained only for the D14PC/D6PC system at q = 2.5. This is a probable consequence of a higher degree of mixing between those monoalkylphosphocholines and diacylphosphocholines of similar chain lengths than between diacylphosphocholines of very different lengths. Similarly, bicelles turn into vesicles at q_v when they are too large to remain planar, hence when a new threshold size is reached, which is around 2.6 for MAPCHO bicelles instead of 7.5 for traditional bicelles. In summary, for the same q values

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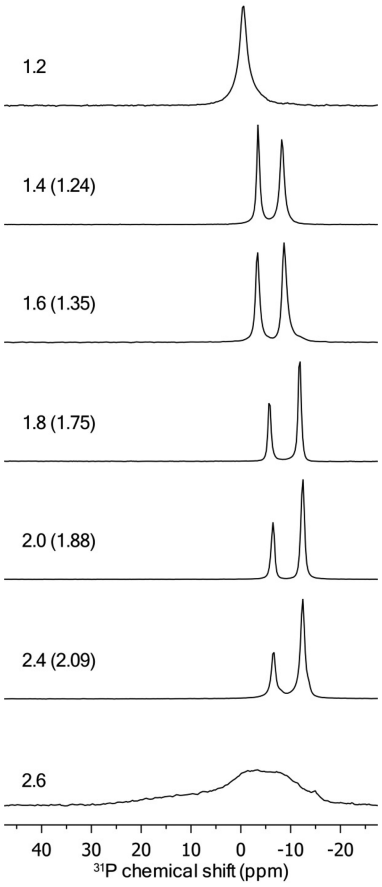

Figure 5. Evolution of the ^{31}P solid-state NMR spectrum of D16PC/14PC bicelles at 52 °C as a function of molar ratio q. The peak integration ratio is shown in parentheses.

between q_m and q_v, MAPCHO bicelles appear to be larger than classical bicelles.

In theory, the bicelles' resonance integration ratio should be equal to q. In practice, the deviation from this expected value is assigned to the presence of either a short-chain surfactant in the bicelle planar section or long-chain lipids in the bicelle edges.[38] It is noteworthy that in the case of MAPCHO bicelles an intermediate type of oriented system appears at low q ratios (for example, q = 1.4 or 1.6 in Figure 5). It is characterized by two well-resolved peaks, with chemical shifts moved to lower fields as compared to those of classical bicelles and with a strong deviation of the integration ratio from the expected value (by 10 to 30%). These observations are consistent with a bicelle system that would not be very well aligned, and with a large proportion of long-chain phospholipids that would be in the high-curvature edge area. This high degree of mixing between 12PC and lipids has been reported by Draney et al.[32] Initial data using deuterated 12PC-d_{38} supports this high degree of mixing, which furthermore increases when the temperature is increased (Figure S3) or q is reduced (Figure S4). Note that whereas the quadrupolar splittings of the phospholipid diminish with decreasing q because of increased bicelle tumbling (Figure S4B), the splittings of the detergent increase because of its greater partitioning into the oriented bilayer (Figure S4C). However, more work would be necessary to accurately quantify this degree of mixing.

At higher q values of 1.8 or 2.0 (Figure 5), the integration ratio of those two peaks deviates only by around 5%, consistent with the formation of better aligned and segregated bicelles. Although these intermediate systems would not be very interesting for structural studies, this property of MAPCHO bicelles may have interesting applications in the reconstitution of membrane proteins. When solubilized in MAPCHO detergents, a membrane protein could be reconstituted by progressively adding phospholipids, i.e., increasing q ratio. Although for low q values the miscibility between a phospholipid and surfactant is high, it gradually diminishes and the protein can either remain in the micelle or switch its environment in favor of a lipid bilayer. Considering that the lipid bilayer better mimics the natural membrane with respect to its curvature, hydrophobic thickness, and lateral pressure, the membrane protein will most likely leave the micelle and gently be brought from the surfactant to a bilayer environment. The reconstitution of membrane proteins into MAPCHO bicelles would therefore be easier than in classical bicelles.

Effect of Detergent and Phospholipid Chain Length Difference on MAPCHO Bicelles. Data can be analyzed by looking at the effect of varying the MAPCHO detergent on bicelle formation with a given phospholipid or on the phospholipid for a given MAPCHO detergent. However, it appears that these effects can be unified by considering the difference in chain length between the phospholipid and surfactant. As shown in Figure 2 and Table S1, 12PC can form perpendicularly aligned bicelles with phosphatidylcholines with chain lengths ranging from 12 to 16 carbons, and 14PC can form lipids whose chain lengths range from 14 to 16 carbons (Figure S1B and Table S2). Finally, 16PC can only form bicelles with D18PC (Figure S1C and Table S3). MAPCHO bicelles could not be formed when the surfactant hydrophobic length was greater than the lipid bilayer (14PC and D12PC, for example). In all cases, increasing the chain length difference resulted in a decrease in the temperature range in which bicelles could be formed. For example, D14PC/14PC systems orient over a temperature range of 50 °C as compared to 20 °C for D14PC/12PC bicelles. The range of q ratios at which MAPCHO bicelles align is also reduced when the chain mismatch increases. For example, D12PC/12PC bicelles align from q = 1.8 to 3.4, D14PC/12PC aligns from q = 1.6 to 3.0, and finally D16PC/12PC aligns only between q = 1.6 and 2.4.

Before dwelling on the differences in chain lengths, it is noteworthy that MAPCHO bicelles are formed with a variety of long-chain lipids and therefore a diversity of hydrophobic thicknesses that can accommodate a variety of membrane proteins. This again shows the versatility of these systems for structural biology applications. In addition, our data indicate that in the case of MAPCHO bicelles, it is ideal to use a surfactant with hydrophobic chains having the same number of carbons as those of each phospholipid hydrophobic chain. A certain amount of deviation from the same length rule is possible. The maximum difference at which MAPCHO bicelles formed was four carbons, although this was possible only in the case of D16PC/12PC.

It is tempting to derive some general thermodynamical laws from such a rule of thumb, and the relative shapes of those molecules most certainly play a role in the stabilization of such macromolecular complexes. Nevertheless, it is useful to remember that the packing shape concept, developed by Israelachvili et al.[45] for one type of lipid, stems from a more general analysis of various forces responsible for lipid aggregation: surface tension, partition coefficient, curvature elasticity, steric repulsions, hydration forces, electrostatic and

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hydrophobic interactions, and so forth. It is therefore not straightforward to transpose the classical packing shape concept developed for one type of lipid to mixtures of surfactant and lipids, even by considering the volume of a disordered monoalkylphosphocholine to be roughly the same as for a dialkylphosphocholine with chains half as long. A new theoretical framework should therefore be developed in the future to be able to predict the shapes of the complex formed by these molecules, depending on the q ratio and temperature.

CONCLUSIONS

We have shown that mixtures of phospholipids with varying hydrophobic thicknesses (from 12 to 18 carbons) could form magnetically oriented bicelles with MAPCHO surfactants. The quality of the perpendicular alignment is similar to that obtainable with classical phospholipid-based bicelles. The orientation of the bilayer region of these bicelles could be changed from parallel to perpendicular to the magnetic field by adding lanthanides. MAPCHO bicelles align in a broader temperature range than classical bicelles and offer greater stability upon dilution because of the low CMC of the surfactants. Both 12PC-d_{38} and 14PC-d_{42} are commercially available in fully perdeuterated form and are more cost-effective than the classical D6PC-d_{35}. Moreover, detergents such as 12PC and 14PC can be used directly in the purification process of membrane proteins. By progressively adding phospholipids to the surfactant-membrane protein mixture and by exploiting the gradual segregation of surfactants and lipids in MAPCHO bicelles, membrane proteins could safely be reconstituted in a bicelle bilayer environment.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b03099.

Description of the calculation of static and dynamic mosaicities, together with additional ^{31}P and ^{2}H NMR spectra of phospholipid/MAPCHO systems as a function of temperature, q ratio or concentration. Results are also compiled as graphs and tables, reporting the conditions under which oriented bicelles form and their degree of alignment. (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting information for

Magnetically-oriented Bicelles with Monoalkylphosphocholines – Versatile Membrane Mimetics for Nuclear Magnetic Resonance Applications

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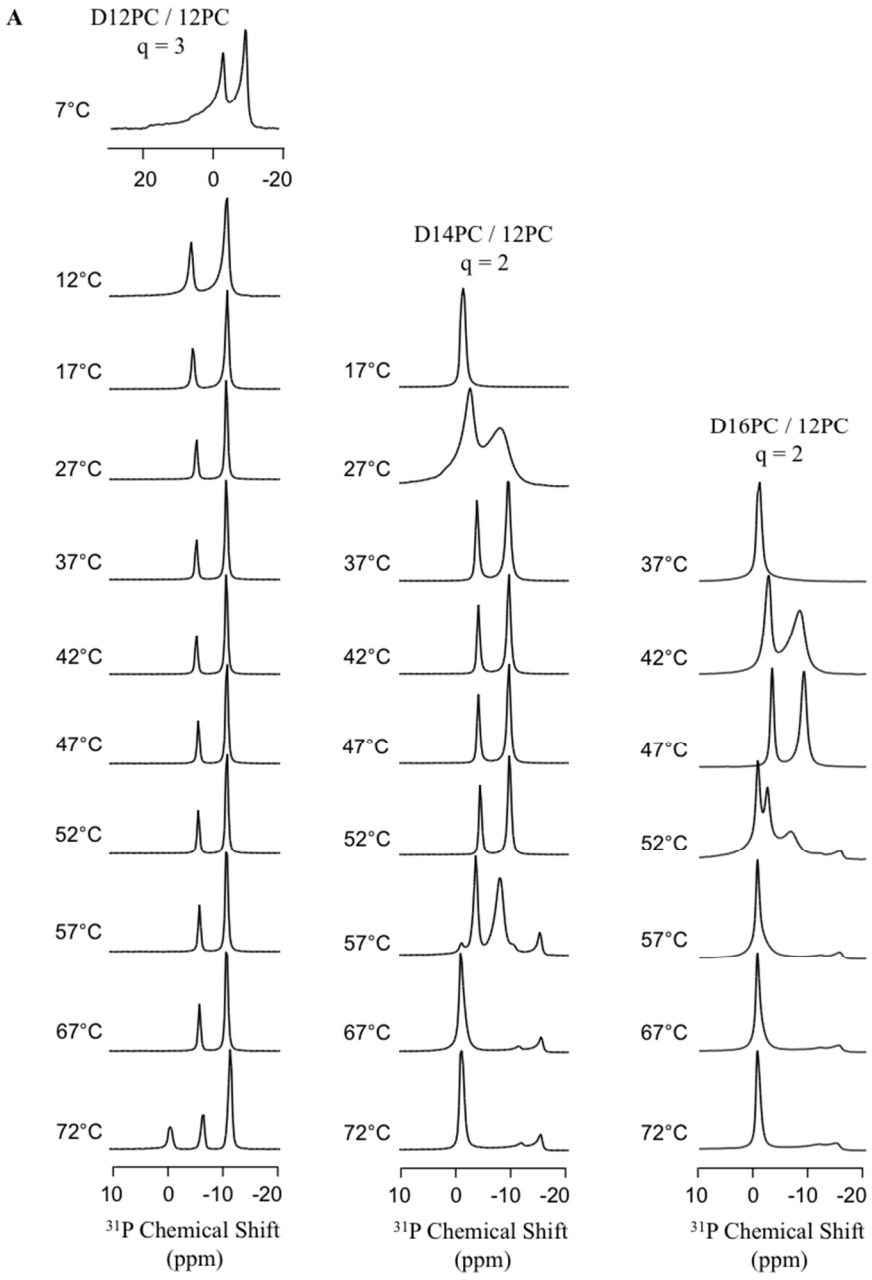
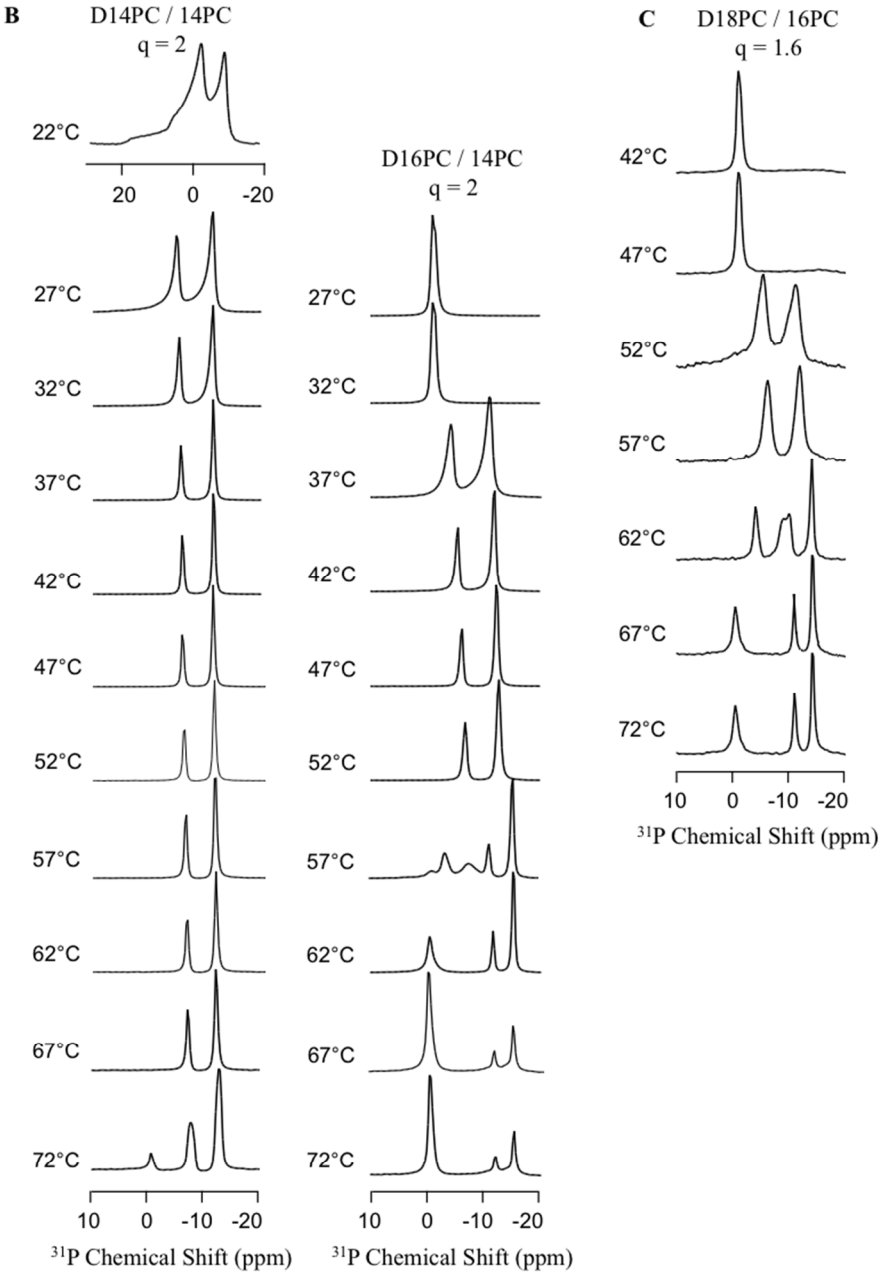


Figure S1. A) ^{31}P solid-state NMR spectra of 12PC mixtures with phospholipids D12PC, D14PC and D16PC at temperatures between 7 and 72˚C.



Figures S1. B) ^{31}P solid-state NMR spectra of 14PC mixtures with phospholipids D14PC and D16PC at temperatures between 22 and 72˚C, and of **C)** 16PC mixtures with phospholipids D16PC at temperatures between 42 and 72˚C.

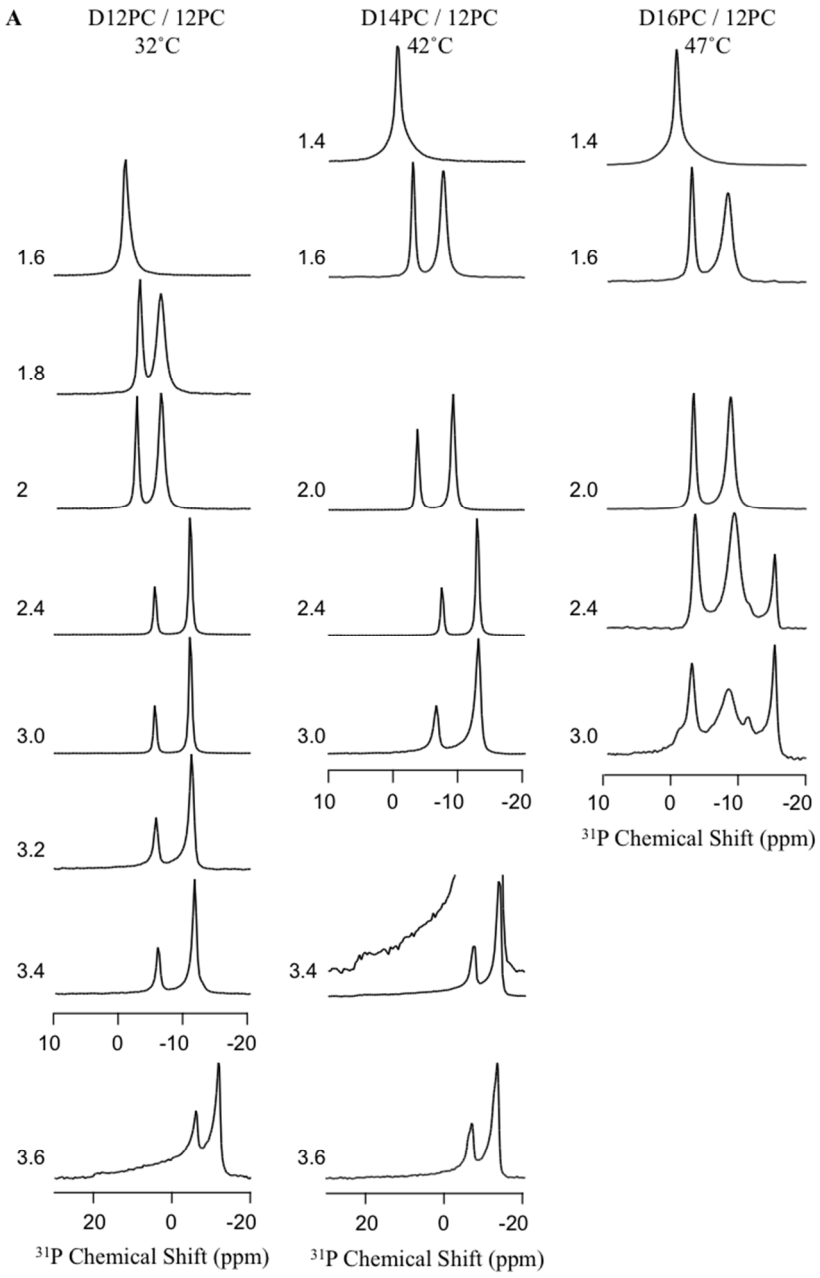
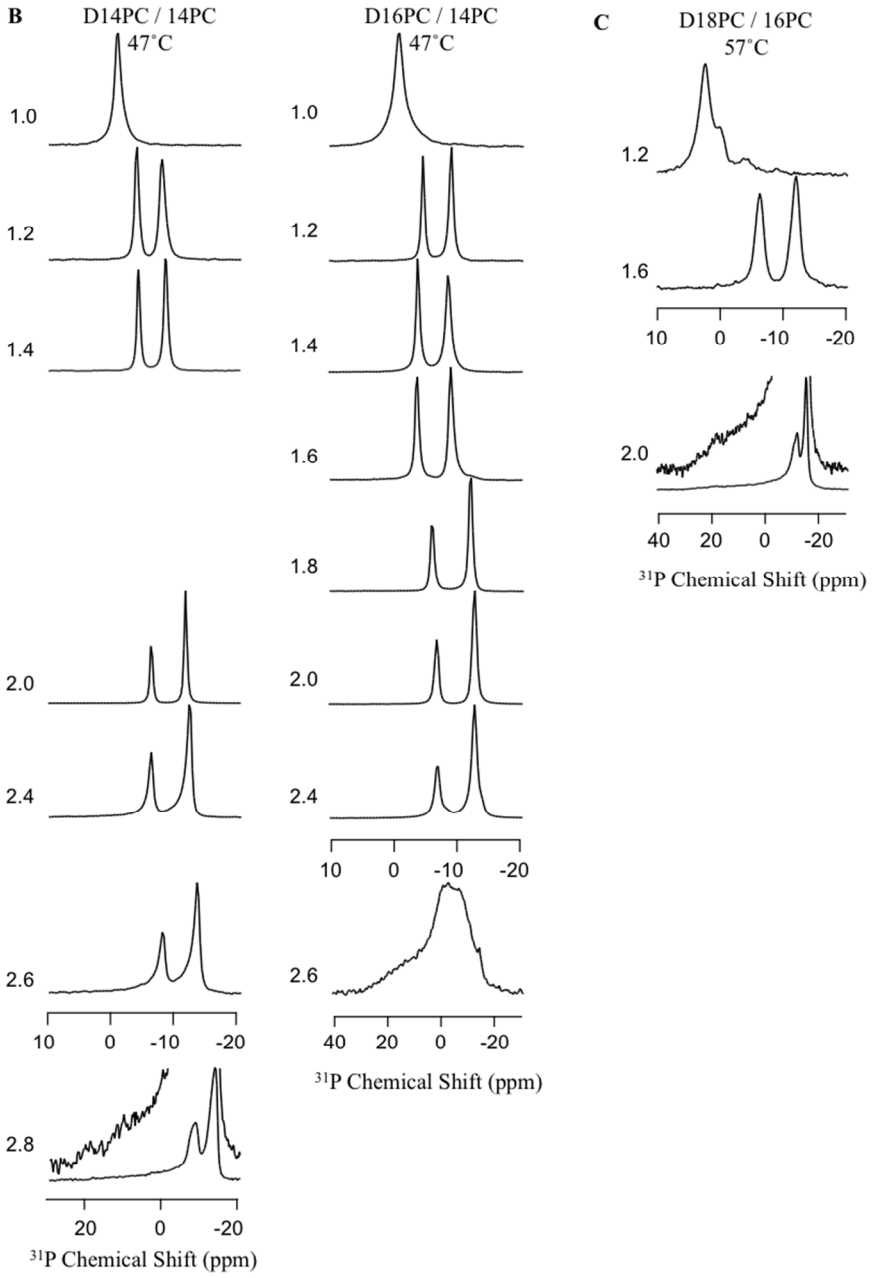


Figure S2. A) ^{31}P solid-state NMR spectra of 12PC mixtures with D12PC, D14PC and D16PC at different molar ratio q between 1.4 and 3.6. Enlarged spectra above the figures are added when necessary.



Figures S2. B) ^{31}P solid-state NMR spectra of 14PC mixtures with D14PC and D16PC at different molar ratios q between 1 and 2.8, and of **C)** 16PC mixtures with D18PC at different molar ratios q between 1.2 and 2. Enlarged spectra above the figures were added when necessary.

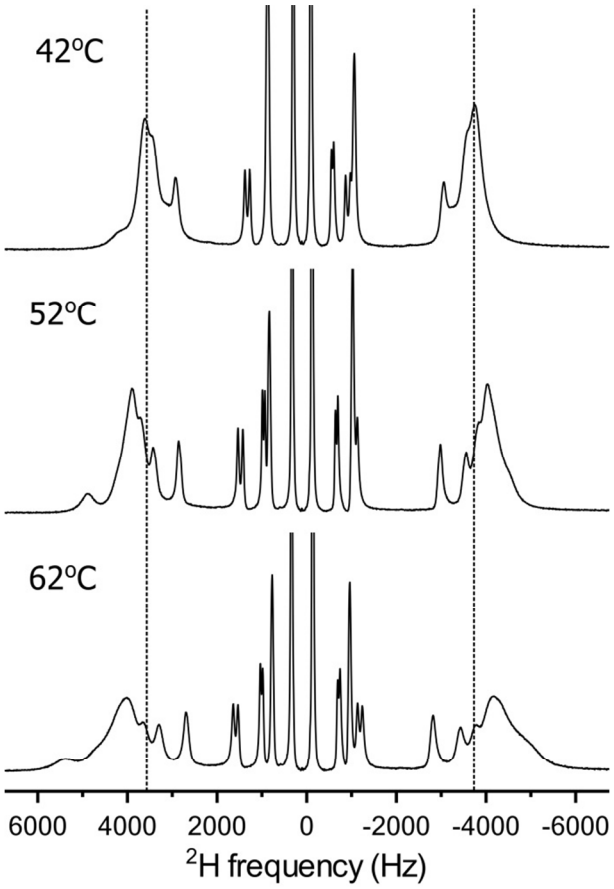


Figure S3. ^{2}H NMR spectra of D14PC/12PC-d_{38} bicelles with q=2.4, at different temperatures.

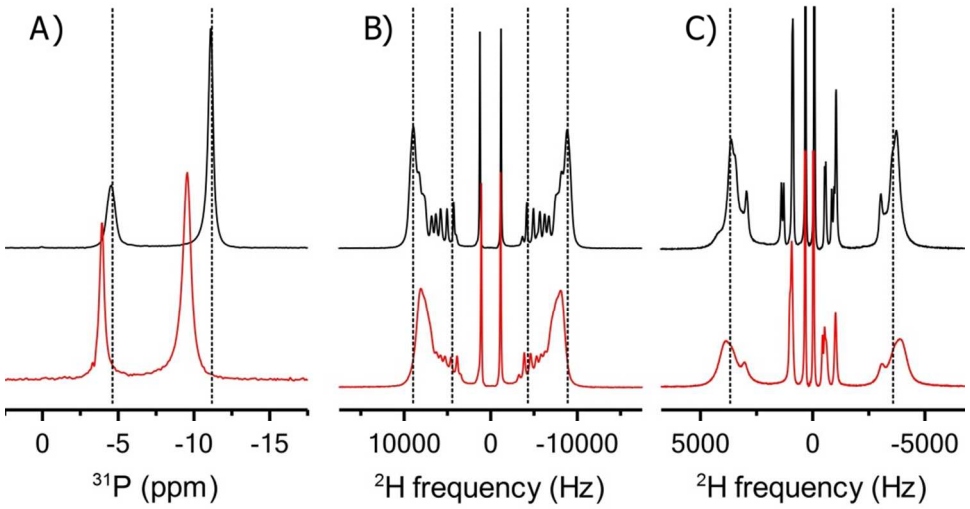


Figure S4. A) ^{31}P NMR spectra of D14PC/12PC bicelles, **B)** ^{2}H NMR spectra of D14PC-d_{54}/12PC bicelles, and **C)** ^{2}H NMR spectra of D14PC/12PC-d_{38} bicelles, with q=2.4 (black) and q=1.8 (red) at 42°C. Note the horizontal scale difference between (B) and (C). Dotted vertical lines are plotted to help visualize the differences in chemical shifts and quadrupolar splittings.

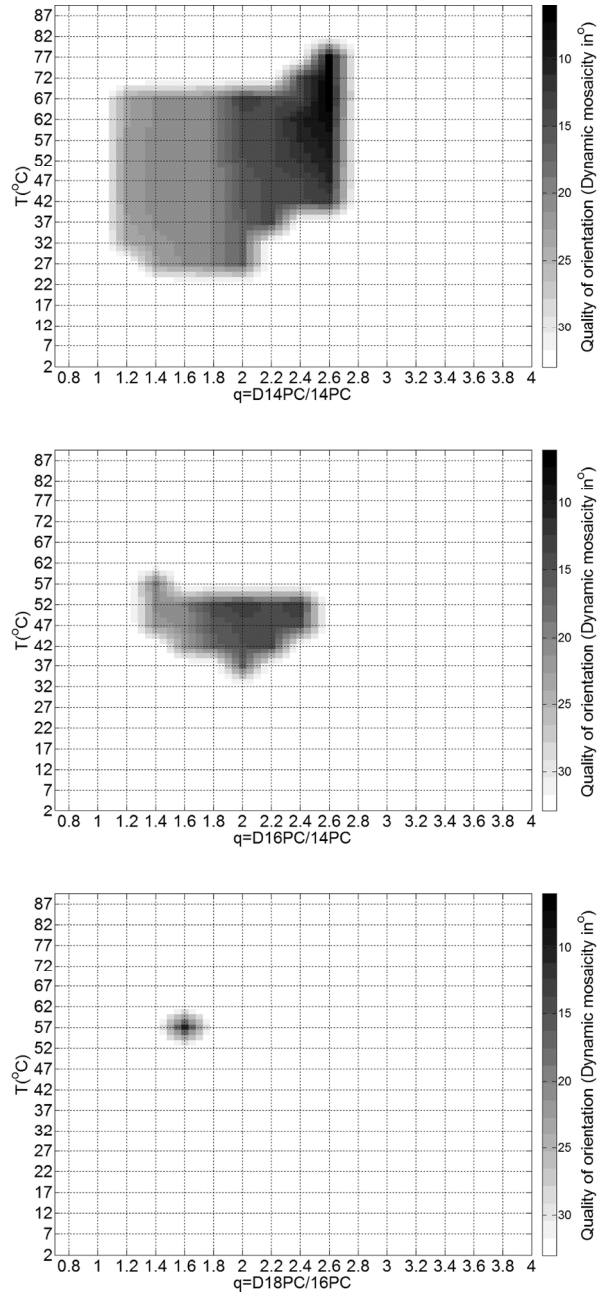


Figure S5. Compilation of molar ratio q and temperature ranges at which 14PC or 16PC systems with D14PC, D16PC or D18PC phospholipids align in the magnetic field. The quality of orientation is measured by the dynamic mosaicity and coded in gray, a darker color indicating a better alignment. Note that some values have been interpolated. Exact experimental values are given Tables S2 and S3.

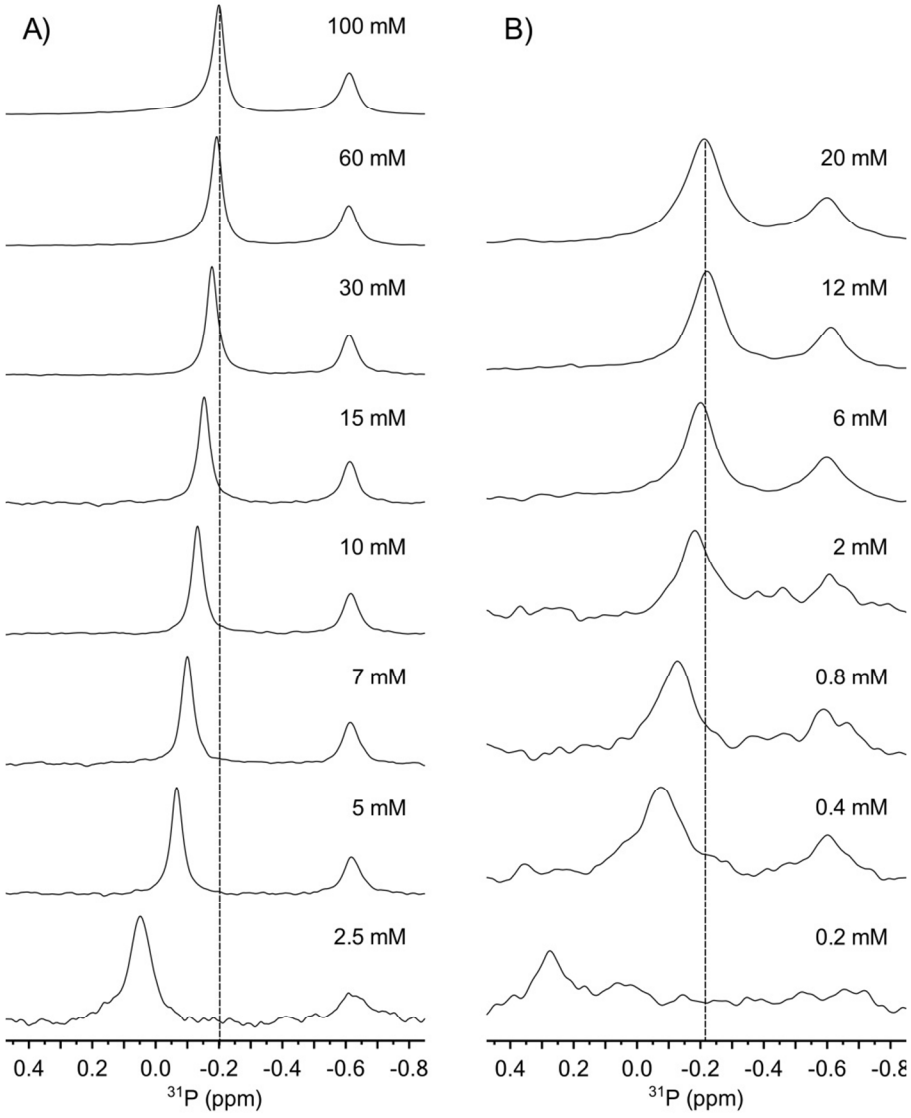


Figure S6. Examples of ^{31}P NMR spectra used to determine the critical bicellar concentration (CBC). A) Spectra for D14PC/12PC at a molar ratio q=0.5 and T=37 °C, B) spectra for D14PC/14PC at a molar ratio q=0.5 and T=37 °C. Vertical dotted lines are plotted to help ascertain the small chemical shift changes. A sealed capillary containing phosphate ions at pH=11 in D_2O previously referenced with respect to 85% H_3PO_4 at 3.38 ppm was used as an internal reference (not visible on figure). Note the differences in total lipid concentrations indicated for each spectrum.

Description of the calculations of static and dynamic mosaicities:

Static mosaicity

The shape of the ^{31}P resonance spectrum allowed us to estimate the *static* mosaic spread (ζ), which is the standard deviation of the gaussian distribution of the bicelle orientation angle (β) from the main orientation at 90° (β_0). The probability to find bicelles whose normal is at an angle β with respect to the magnetic field is thus given by[1,2]:

$$P(\beta) = \frac{1}{\zeta\sqrt{2\pi}} e^{\frac{-(\beta-\beta_0)^2}{2\zeta^2}} \qquad \text{(S1)}$$

A spectrum with such an angular distribution can be simulated using dedicated MATLAB scripts and the calculated spectrum fitted to the experimental one to determine the static mosaic spread.

S_{bil} and dynamic mosaicity.

The ^{31}P resonance frequency (δ_{obs}) of the long-chain phospholipid in bicelles[2,3] is given by:

$$\delta_{obs} = \delta_{iso} + \frac{1}{2}(3cos^2\theta - 1)\delta \times S_{bil} \qquad \text{(S2)}$$

where δ_{iso} is the isotropic chemical shift, θ is the angle between the bilayer normal and the magnetic field direction, δ is the anisotropy of the chemical shielding tensor, and S_{bil} is the order parameter describing the motions of the bilayer normal compared to its average orientation.

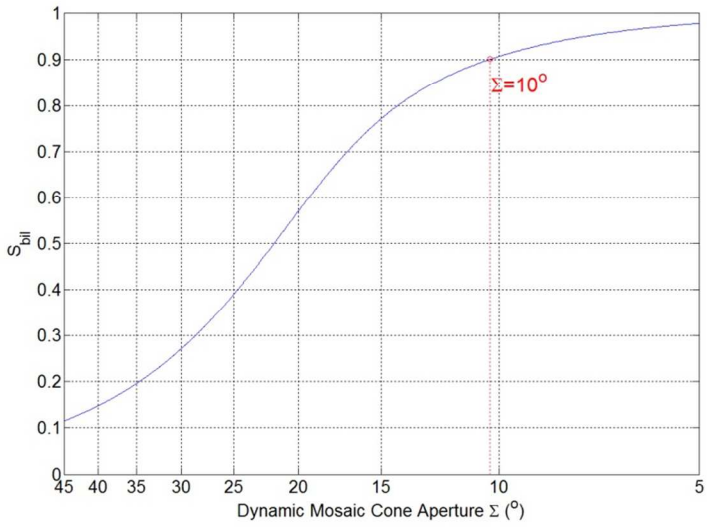
In a magnetic field B_0, bicelles have their bilayer normal perpendicular on average to the direction of B_0 (i.e. $\theta = 90°$). Since bicelles undergo rapid fluctuations, S_{bil} is less than 1, and it can be determined by measuring δ_{obs} on the spectra:

$$S_{bil} = 2\frac{(\delta_{obs}-\delta_{iso})}{\delta} \qquad \text{(S3)}$$

The deviation of the bilayer normal orientation is a Gaussian distribution in a cone of aperture Σ, called the *dynamic* mosaic spread, which depends on S_{bil}:

$$S_{bil} = \frac{\int_0^{90°} e^{\frac{sin^2\theta}{2\Sigma^2}} \times \frac{1}{2}(3cos^2\theta - 1) \times sin\theta \times d\theta}{\int_0^{90°} e^{\frac{sin^2\theta}{2\Sigma^2}} \times sin\theta \times d\theta} \qquad \text{(S4)}$$

This Gaussian distribution can be represented graphically to help determine the dynamic mosaic spread corresponding to a given S_{bil}[4], as shown below:



Graphical representation of S_{bil} as a function of the dynamic mosaic cone aperture Σ. For Σ=10°, S_{bil}=0.9.

Tables compiling the conditions in which oriented bicelles form, and their degree of alignment.

Crosses indicate experiments that were carried out but corresponding to a system with no visible alignment. Numbers correspond to the dynamic mosaicity, when only two peaks were visible on the spectra. Static mosaicity is indicated by font color: red, orange and green indicate magnetically-oriented systems with a longitudinal bilayer deviation of less than 15°, 10° and 5° from the magnetic field direction, respectively. The melting temperature, T_m[5], and the critical micelle concentration, CMC[6,7], are respectively indicated next to the phospholipid and detergent names.

Table S1. Compilation of ^{31}P NMR results for 12PC bicelles.

MAPCHO	Phospholipid															
12PC (1.5 mM)	D12PC (-2.1 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.6	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1.8	X	X	25	25	25	26	27	29	X	X	X	X	X	X	X	X
2.0	X	29	26	25	26	26	26	28	29	32	X	X	X	X	X	X
2.2																
2.4	X	20	19	18	18	18	18	18	19	19	19	X				
2.6																
2.8																
3.0	X	18	17	17	17	17	17	16	16	16	15	15	15	X		
3.2	X	X	17	17	17	17	16	16	15	15	14	14	14	X		
3.4	X	X	X	16	16	16	15	15	14	13	13	X	X	X		
3.6	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

MAPCHO	Phospholipid															
12PC (1.5 mM)	D14PC (23.9 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.4				X	X	X	X	X	X	X	X	X	X	X	X	X
1.6				X	X	24	23	22	22	22	X	X	X	X	X	X
1.8																
2.0				X	X	21	20	20	19	19	X	X	X	X	X	X
2.2																
2.4				X	X	X	19	19	18	18	X	X				
2.6																
2.8																
3.0				X	X	X	X	12	11	10	X	X	X	X	X	
3.2					X	X	X	X	X	X	X	X				
3.4				X	X	X	X	X	X	X	X	X				
3.6				X	X	X	X	X	X	X	X	X	X			

MAPCHO	Phospholipid															
12PC (1.5 mM)	D16PC (41.4 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.4						X	X	X	X	X	X	X	X	X		
1.6						X	X	21	21	X	X	X	X	X		
1.8																
2.0						X	X	21	20	X	X	X	X	X		
2.2																
2.4						X	X	17	X	X		X				
2.6						X	X	X	X	X	X	X	X	X		
2.8						X	X	X	X	X	X	X	X	X		
3.0						X	X	X	X	X	X	X	X	X		

MAPCHO	Phospholipid															
12PC (1.5 mM)	D18PC (55.3 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.6								X	X	X	X	X				
1.8																
2.0								X	X	X	X	X	X	X		

Table S2. Compilation of ^{31}P NMR results for 14PC bicelles.

MAPCHO	Phospholipid															
14PC (0.12 mM)	D12PC (-2.1 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
2.0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

MAPCHO	Phospholipid															
14PC (0.12 mM)	D14PC (23.9 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.0				X	X	X	X	X	X	X	X	X				
1.2				X	X	24	23	22	22	22	22	23	23	X	X	
1.4				X	23	22	21	21	21	21	21	21	21	X	X	X
1.6																
1.8																
2.0				X	17	17	17	16	16	15	14	14	13	X		
2.2																
2.4				X	X	X	X	14	14	12	11	10	9	9		
2.6				X	X	X	X	13	11	10	9	8	7	7	6	X
2.8					X	X	X	X	X	X	X	X	X	X	X	X
3.0				X	X	X	X	X	X	X	X	X	X	X	X	X
3.2																
3.4				X	X	X	X	X	X	X	X	X	X	X	X	X

MAPCHO	Phospholipid															
14PC (0.12 mM)	D16PC (41.4 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.0						X		X		X	X	X		X		
1.2						X	X	X	X	X	X	X	X			
1.4						X	X	X	22	21	20	X	X	X		
1.6						X	X	22	21	20	X	X				
1.8						X	X	17	16	14						
2.0						X	17	15	14	13	X	X	X	X		
2.2																
2.4						X	X	X	14	13	X	X	X	X		
2.6						X	X	X	X	X	X	X				

MAPCHO	Phospholipid															
14PC (0.12 mM)	D18PC (55.3 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.2								X	X	X	X	X	X			
1.4																
1.6								X	X	X	X	X	X			
1.8																
2.0								X	X	X	X	X	X	X		

Table S3. Compilation of ^{31}P NMR results for 16PC bicelles.

MAPCHO	Phospholipid															
16PC (0.003 mM)	D14PC (23.9 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.0																
1.2																
1.4																
1.6				X	X	X	X	X	X	X	X	X	X	X	X	X
1.8																
2.0				X	X	X	X	X	X	X	X	X	X	X	X	X

MAPCHO	Phospholipid															
16PC (0.003 mM)	D16PC (41.4 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.0																
1.2							X	X	X	X	X	X				
1.4																
1.6						X	X	X	X	X	X	X	X	X		
1.8																
2.0						X	X	X	X	X	X	X	X	X		

MAPCHO	Phospholipid															
16PC (0.003 mM)	D18PC (55.3 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.0								X	X	X	X	X	X	X		
1.2								X	X	X	X	X	X	X		
1.4																
1.6								X	X	X	11	X	X	X		
1.8																
2.0								X	X	X	X	X	X	X		

MAPCHO	Phospholipid															
16PC (0.003 mM)	D22PC (66.4 °C)															
q\Temp. (°C)	7	12	17	22	27	32	37	42	47	52	57	62	67	72	77	82
1.0																
1.2										X	X	X	X	X	X	
1.4																
1.6										X	X	X	X	X	X	
1.8																
2.0										X	X	X	X	X	X	

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