

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

*Maiwenn Beaugrand<sup>†</sup>, Alexandre A. Arnold<sup>†</sup>, Antoine Juneau<sup>†</sup>, Aline Balieiro Gambaro<sup>†</sup>,  
Dror E. Warschawski<sup>†,‡</sup>, Philip T. F. Williamson<sup>§</sup> and Isabelle Marcotte<sup>†\*</sup>*

<sup>†</sup>Département de Chimie, Université du Québec à Montréal, P.O. Box 8888, Downtown Station,  
Montreal, H3C 3P8, Canada

<sup>#</sup>UMR 7099, CNRS - Université Paris Diderot, IBPC, 13 rue Pierre et Marie Curie, F-75005  
Paris, France

<sup>§</sup>Centre for Biological Sciences/Institute of Life Sciences, Highfield Campus, University of  
Southampton, Southampton, SO17 1BJ, United Kingdom

\* Corresponding author: [marcotte.isabelle@uqam.ca](mailto:marcotte.isabelle@uqam.ca)

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## 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 of their composition and planar surface similar to the native membrane environment. In this work, to improve the solubilisation 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  $^{31}\text{P}$  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 in a variety of conditions. The quality of their alignment is similar to the one of classical bicelles, while 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 1990's and quickly gained in popularity due to their similarities with biological membranes<sup>1,2</sup>. 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 to study 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<sup>3-9</sup>. Bicelles can also be used to obtain protein crystals for X-ray crystallography<sup>10,11</sup> and have potential pharmaceutical applications<sup>12-16</sup>. In particular, bicelles have proven to be an ideal mimetic for solid-state NMR as 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<sup>17</sup>. This enables the study of membrane peptides and proteins in a near-native membrane environment. The importance of lipid interactions to the folding, structure and functioning of membrane proteins has long been recognized<sup>18-20</sup>, in particular the hydrophobic mismatch and lateral packing pressure due to the curvature of the lipids or detergents in model membranes<sup>20-23</sup>. The insertion and folding of membrane proteins is indeed more favorable with long-chain lipids<sup>21</sup>.

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. The bile salt 3-(cholamidopropyl) dimethylammonio-2-hydroxyl-1-propane sulfate (CHAPSO) used with D14PC in the 1990s<sup>1</sup> was quickly replaced by D6PC – more structurally

similar to phospholipids<sup>2</sup>. Other lipids 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<sup>9</sup>. Studies have been carried out to improve 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 palmitoyl-oleoyl-PC (POPC)<sup>24-26</sup>, or by using ether lipids<sup>27</sup>. Triton X-100 detergent demonstrates a better magnetic alignment and stability of membrane proteins in the bilayer<sup>28</sup>, while addition of cholesterol, cholesterol 3-sulfate and/or hexadecyl-trimethylammonium bromide (CTAB) stabilizes the bilayer and increases the temperature range at which bicelles form<sup>29</sup>. To obtain bicelles at low concentrations and more suitable for solution NMR, detergents with low critical micelle concentration (CMC) were used such as dodecyl-PC (12PC) and several cyclohexyl-1-butyl-PC<sup>30-32</sup>.

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 since they are abundant in eukaryotic membrane cells<sup>9</sup>. 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<sup>30,31,33</sup>. When compared to 12PC, 14PC micelles have shown their ability to maintain the activity of dialkylglycerol kinase (DAGK) due to their better match with the hydrophobic span of the transmembrane domain of this protein<sup>34</sup>. In this work, we

present the characterization of MAPCHO bicelles using  $^{31}\text{P}$  and  $^2\text{H}$  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), 1,2-dimyristoyl-sn-glycero-3-phosphocholine- $\text{d}_{54}$  (D14PC- $\text{d}_{54}$ ), 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- $\text{d}_{38}$  (12PC- $\text{d}_{38}$ ), 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  $^2\text{H}$ -depleted water were purchased from Sigma Aldrich (Oakville, ON, Canada). Deuterium oxide ( $\text{D}_2\text{O}$ ) 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  $^2\text{H}$ -depleted water. The total

concentration used was 400 mM, well above the CMC of all constituents (Tables S1, S2 and 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 freeze (liquid N<sub>2</sub>), thaw (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<sub>3</sub> at a concentration of 2.5 mM<sup>31,35</sup> for the binary systems D14PC/14PC and D16PC/14PC.

For solution NMR, bicelles containing 12PC were prepared at total lipid and detergent concentration of 100 mM and  $q$  ratios of 0.5 and 1, while 14PC-based samples were made at 20 mM and a  $q$  ratio of 0.5. Samples were made using D<sub>2</sub>O 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 14h, and submitted to 7 cycles of freezing, thawing and vortex shaking. Vesicles were then extruded 30 times through membranes of 0.2  $\mu$ m 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 minutes.

## **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 <sup>1</sup>H, 246.86 MHz for <sup>31</sup>P, 92.125 MHz for <sup>2</sup>H, and equipped with a 4-mm broadband/<sup>1</sup>H dual-frequency magic-angle-spinning (MAS) probehead. For <sup>31</sup>P NMR spectra, a

phase-cycled Hahn echo pulse sequence<sup>36</sup> was used with gated broadband proton continuous wave decoupling at a field strength of 50 kHz. A  $\pi/2$  pulse of 3  $\mu$ s, an interpulse delay of 33  $\mu$ s, a recycle delay of 5 s, an acquisition time of 20 ms and a dwell time of 5  $\mu$ s 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.  $^2\text{H}$  NMR spectra were obtained using a solid echo pulse sequence<sup>37</sup> with a  $\pi/2$  pulse length of 3  $\mu$ s, an interpulse delay of 45  $\mu$ s and a repetition delay of 500 ms. Typically 2400 scans were acquired. For  $^{31}\text{P}$  and  $^2\text{H}$  NMR experiments, pre-acquisition delays of at least 10 minutes were used between temperature steps. Spectra were acquired at least in duplicate at temperatures ranging from 7°C to 82°C depending on the phospholipid/detergent system. All spectra were processed using MNova software (Mestrelab Research, Santiago de Compostela, Spain).

All solution  $^{31}\text{P}$  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  $\mu$ s, 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  $\text{D}_2\text{O}$ , which was previously referenced with respect to 85%  $\text{H}_3\text{PO}_4$  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°C, 47°C, 57°C 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  $\mu$ l was followed by 49 injections of 5  $\mu$ l performed with a 600 s delay between each injection, using a 307 rpm stirring. Due to the nature of the experiment, no fitting was performed.

## **RESULTS AND DISCUSSION**

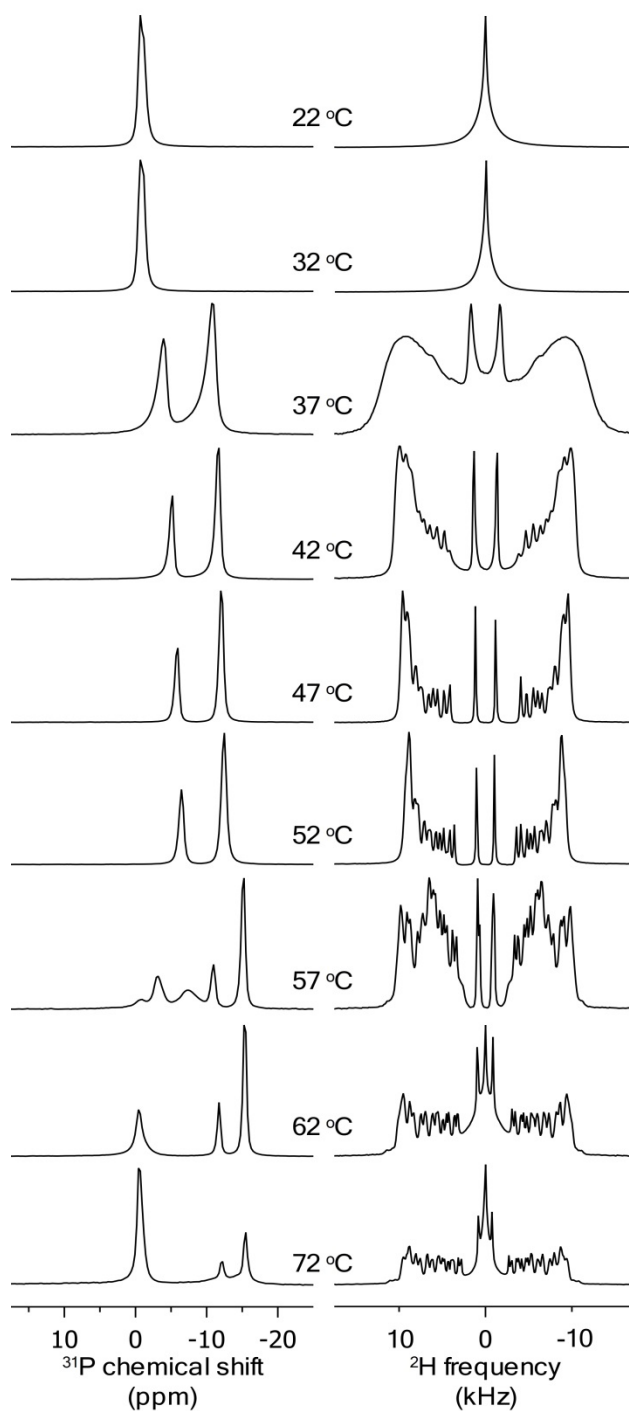
### **Versatile magnetically-oriented bicelles with low free surfactant concentration**

Several binary lipid mixtures were prepared by changing the chain length of the MAPCHO detergents (12PC, 14PC and 16PC) for all the phospholipids investigated, namely D12PC, D14PC, D16PC, D18PC and D20PC. The mixtures were studied over a wide range of temperatures (7-82°C) and phospholipid/MAPCHO molar ratios ( $1 \leq q \leq 3.6$ ). Figure 1 presents a characteristic example of one of the systems studied, namely D16PC/14PC. The magnetic alignment is revealed by two well-resolved peaks on the  $^{31}\text{P}$  solid-state NMR spectra between 42 and 52°C – similarly to classical D14PC/D6PC bicelles where the peaks at *circa* -5 and -12 ppm are mainly assigned to 14PC and D14PC, respectively<sup>38</sup>. This alignment is confirmed by well-resolved doublets on the  $^2\text{H}$  solid-state NMR spectra of D14PC- $\text{d}_{54}$  in the same range of temperatures, which are characteristic of bilayers oriented with their normal perpendicular to the magnetic field. Quadrupolar splittings values are very close to those measured on classical

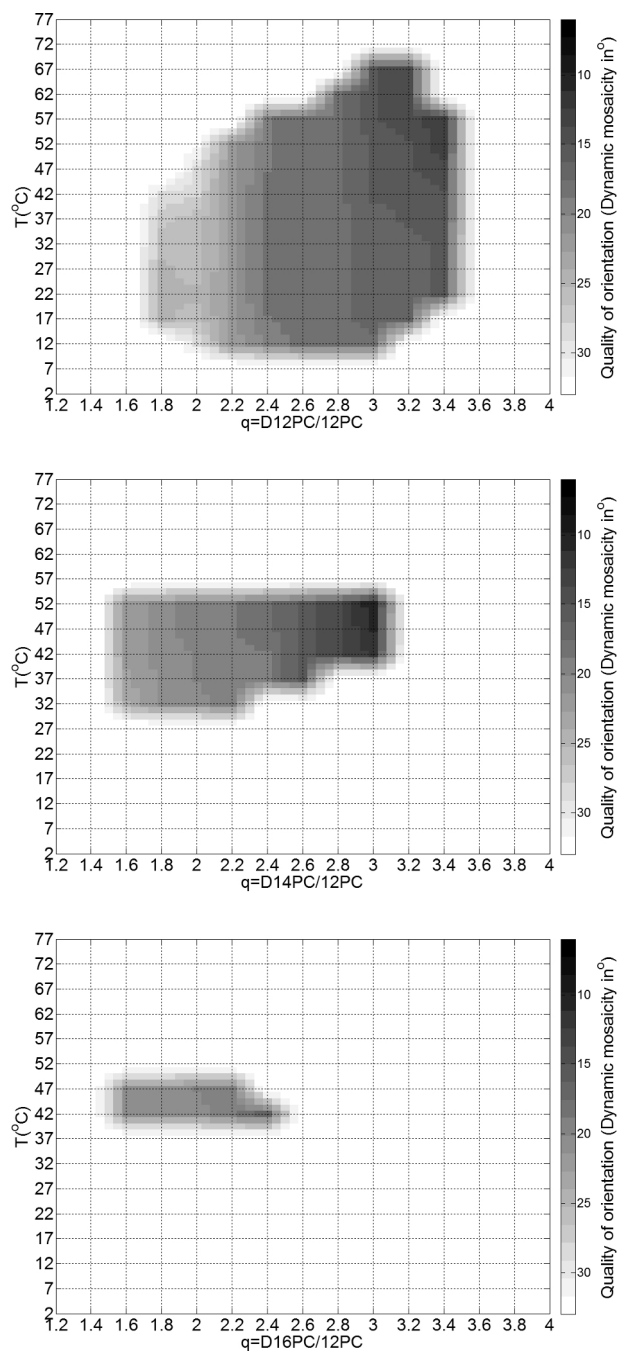


D14PC/D6PC bicelles. At 47°C for example, these values vary between 2.3 kHz and 26.4 kHz, for the CD<sub>3</sub> terminal and methylene groups in close proximity to the ester link, respectively.

Characteristic <sup>31</sup>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 the commercially available deuterated 12PC-d<sub>38</sub> with D14PC phospholipids, confirming the partial orientation of these detergent molecules when bicelles are aligned (Figures S3 and S4).



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



**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 coded in gray, a darker color indicating a better alignment. Note that some values have been interpolated. Exact experimental values are given Table S1.

Ideally, bicelles would align with their bilayer normal perfectly perpendicular to the magnetic field. In practice this is however not the case and the deviation around this *static* orientation is called the static mosaic spread. This distribution of orientations is reflected on the width of the long chain lipid's  $^{31}\text{P}$  or  $^2\text{H}$  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  $^{31}\text{P}$  or  $^2\text{H}$  NMR resonances, respectively: an oscillation on a smaller angle is shifting the  $^{31}\text{P}$  resonance upfield or increasing the  $^2\text{H}$  quadrupolar splitting, towards a position corresponding to the ideal bicelle orientation, perpendicular to the magnetic field. An evaluation of static<sup>39,40</sup> and dynamic<sup>38,40</sup> mosaicities therefore gives a better evaluation of bicelles' 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, S2 and S3, with properties such as the melting temperature ( $T_m$ ) of the phospholipids and the CMC of the detergents. Static mosaicities fall below  $5^\circ$  for the best oriented systems; the quality of the orientation of MAPCHO bicelles is therefore as good as the one of classical bicelles<sup>41</sup>. The best dynamic mosaicities are also very good, for example  $6^\circ$  with the D14PC/14PC system at  $77^\circ\text{C}$ .

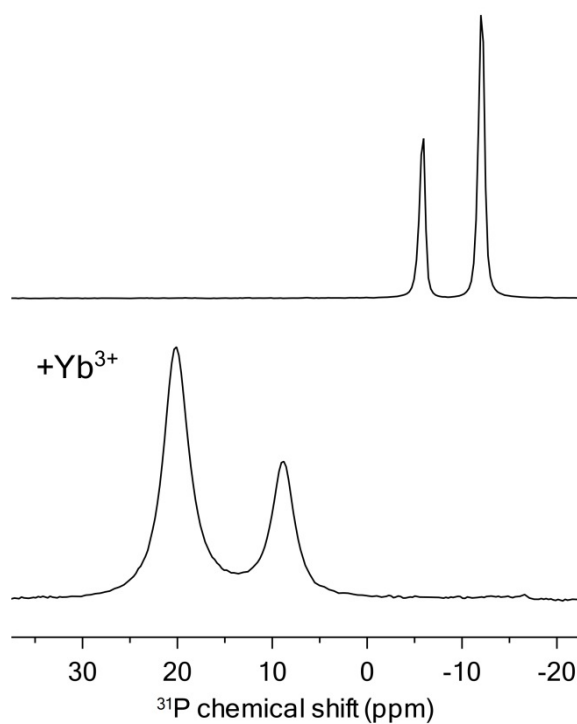
The molar ratios and temperatures at which bicelles align are summarized in Figure 2 for 12PC and in Figure S5 for 14PC and 16PC. In these figures, dynamic mosaicities are color coded, a darker shade indicating a better alignment which should be favored when choosing a bicelle system. MAPCHO bicelles 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.<sup>31</sup>. With 14PC, bicelles could be formed with phospholipids having 14 to 16 carbon long chains. Finally, aligned bicelles containing 16PC were only formed 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  $\alpha$ -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<sup>35</sup>. Using this strategy, alignment flipping has been demonstrated with the D14PC/12PC system by  $^{31}\text{P}$  solid-state NMR<sup>31</sup>. 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 surfactants (Tables S1, S2 and S3). Indeed, the presence of a significant amount of free surfactant can be a drawback in particular when samples need to be diluted<sup>42</sup>. By studying the effect of dilution on  $^{31}\text{P}$  NMR spectra, the concentration of free detergent (or critical bicellar concentration, CBC) can be determined<sup>41,42</sup>. Examples of such spectra are shown in the supporting information (Figure S6), and CBC values are listed in Table 1, calculated as described by Beaugrand et al.<sup>42</sup>. For a given molar ratio  $q$  of 0.5 and long-chain phospholipid D14PC, the CBC 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<sup>43</sup>) and it

is thus clearly advantageous to use MAPCHO bicelles when diluted conditions are necessary. For a given molar ratio  $q$  of 1 and detergent 12PC, the CBC 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<sup>42</sup>. 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<sup>42</sup>.



**Figure 3.**  $^{31}\text{P}$  NMR spectra of D16PC/14PC bicelles ( $q = 2$ ; 400 mM) at 47°C (A) without and (B) with 2.5 mM of lanthanide ions  $\text{Yb}^{3+}$ .

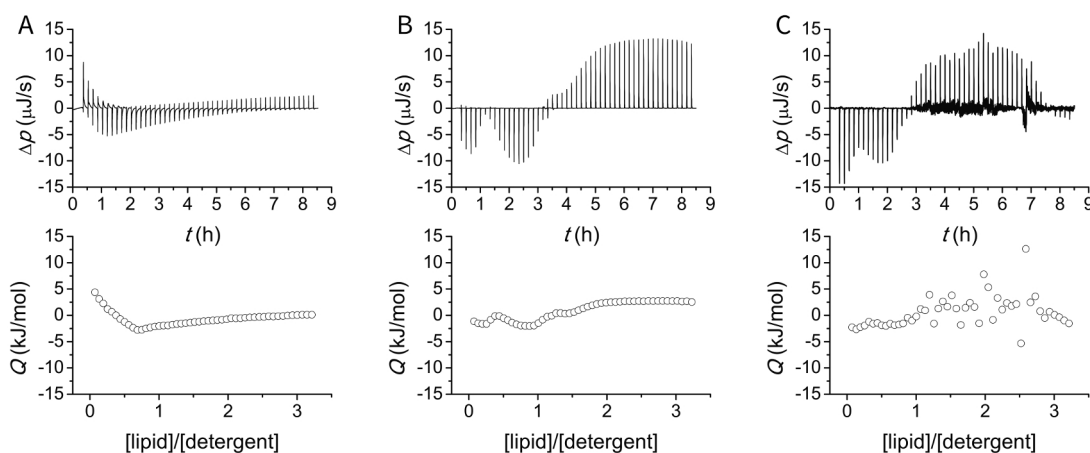
**Table 1.** Critical bicelle concentrations (CBC) of MAPCHO bicelles at 37°C.

Lipid	Detergent	Molar ratio q	CBC (mM)
D12PC	12PC	1	$0.75 \pm 0.01$
D14PC	12PC		$0.78 \pm 0.01$
D16PC	12PC		$0.82 \pm 0.01$
D14PC	12PC 14PC	0.5	$0.86 \pm 0.02$ $0.07 \pm 0.01$
D14PC	D6PC	0.5	$7.5 \pm 0.5$

### Behavior as a Function of Temperature

As already described by Triba et al.<sup>24,38</sup>, it is possible to identify different characteristic transition temperatures for bicelle systems by  $^{31}\text{P}$  and  $^2\text{H}$  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  $T_m$  of the lipids studied in this work are listed in Tables S1, S2 and S3. In bicelle systems, another important transition corresponds to the temperature at which they align in the magnetic field<sup>38</sup>. 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  $^{31}\text{P}$  and  $^2\text{H}$  solid-state NMR, and Figure 4 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  $^{31}\text{P}$  and  $^2\text{H}$  NMR spectra, indicative of fast-tumbling detergent structures, as well as a broad lipid vesicles

powder pattern, which is particularly intense when detergents and phospholipids have the same alkyl chain length (D12PC/12PC and D14PC/14PC) (Figures 1 and S1B).



**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 ( $>T_v$ ). Above: raw differential heat as a function of time. Below: integrated heat as a function of q ratio.

Close to  $T_m$ , two broad resonances emerge from the  $^{31}\text{P}$  NMR spectra and a broad doublet from  $^2\text{H}$  NMR spectra (Figure 1), as was seen for D14PC/D6PC when magnetic alignment starts<sup>38</sup>. Above  $T_m$  samples are transparent and viscous (data not shown) and well-defined  $^{31}\text{P}$  resonances and  $^2\text{H}$  doublets are observable (Figures 1 and S1), characteristic of aligned bicelles and reduced mosaicities with increasing temperatures<sup>2,38</sup>. The low-field (higher ppm values)  $^{31}\text{P}$  peak has been ascribed to short-chain phospholipids (or detergents in our case) mainly on the edges or holes, while the high-field (lower ppm values) resonance corresponds to long-chain phospholipids mainly in the bilayer<sup>38</sup>. Similar information can be extracted from  $^2\text{H}$  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  $^{31}\text{P}$  NMR spectra, which is characteristic of a  $90^\circ$  orientation in vesicles, and is described as  $T_v$  by Triba et al.<sup>38</sup>. In Figure 1, this occurs at  $57^\circ\text{C}$  and, over a certain temperature range, these new vesicles coexist with remaining misaligned bicelles, as seen from the complex  $^{31}\text{P}$  and  $^2\text{H}$  NMR spectra, until those bicelles disappear, as seen at  $62^\circ\text{C}$ . In this type of situation, ITC reconstitution experiments cannot inform us on 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).

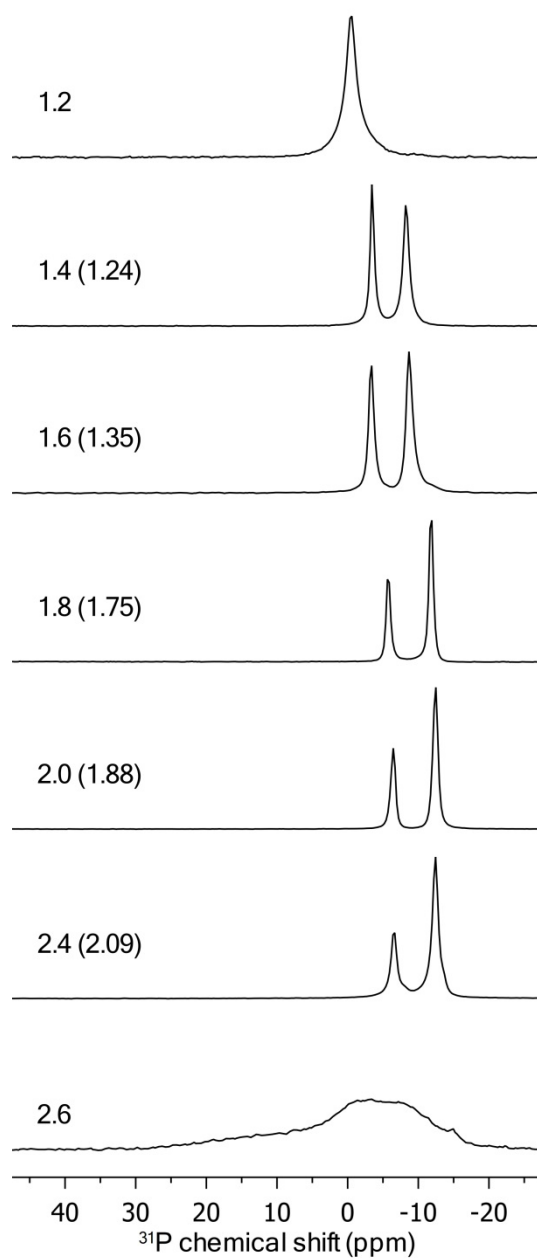
When compared to traditional D14PC/D6PC (DMPC/DHPC) bicelles, the MAPCHO bicelles explored in this work display similar transitions, but a larger temperature range of alignment. D14PC/D6PC bicelles typically align between  $30$  and  $50^\circ\text{C}$ , as reviewed elsewhere<sup>5,44</sup>, while the MAPCHO systems with D14PC can orient between  $27$  and  $77^\circ\text{C}$  (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  $^{31}\text{P}$  and  $^2\text{H}$  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 perpendicular 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  $^{31}\text{P}$  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  $\sim 2.5$  and  $7.5$ , as reviewed elsewhere<sup>5,44</sup>. 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, the 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 on Figure 2.

The magnetic-alignment of a bilayer is due to the anisotropy of 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 only attained 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 between  $q_m$  and  $q_v$ , MAPCHO bicelles appear to be larger than classical bicelles.



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

In theory, bicelles' resonance integration ratio should be equal to  $q$ . In practice, the deviation from this expected value is assigned to the presence of either short-chain surfactant in the bicelle planar section, or long-chain lipids in the bicelle edges<sup>38</sup>. It is noteworthy that in the case of MAPCHO bicelles, an intermediate type of oriented systems appears at low  $q$  ratios (see 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 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 had been reported by Draney et al.<sup>32</sup>. Initial data using deuterated 12PC- $d_{38}$  supports this high degree of mixing which furthermore increases when temperature is increased (Figure S3) or  $q$  is reduced (Figure S4). Note that while the quadrupolar splittings of the phospholipid diminish with decreasing  $q$  due to increased bicelle tumbling (Figure S4B), the splittings of the detergent increase, due to its greater partitioning into the oriented bilayer (Figure S4C). More work would however 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 only deviates 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). While for low  $q$  values the miscibility between phospholipid and surfactant is high, it gradually diminishes and

the protein can either remain in the micelle or switch environment in favor of a lipid bilayer. Considering that the lipid bilayer better mimics the natural membrane, its curvature, hydrophobic thickness or 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 analysed either by looking at the effect of varying the MAPCHO detergent on the bicelle formation with a given phospholipid, or of 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 perpendicular aligned bicelles with phosphatidylcholines with chain lengths ranging from 12 to 16 carbons, and 14PC with 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 of 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 while D14PC/12PC align from  $q=1.6$  to 3.0 and finally D16PC/12PC only aligns 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, 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 4 carbons, although this was only possible in the case of D16PC/12PC.

It is tempting to derive some general thermodynamical laws from such 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.*<sup>45</sup> for one type of lipids, 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 hydrophobic interactions etc. 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 one 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.

## CONCLUSION

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 the one 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, due to the low CMC of the surfactants. Both 12PC-d<sub>38</sub> and 14PC-d<sub>42</sub> are commercially available in fully perdeuterated form and are more cost-effective than the classical D6PC-d<sub>35</sub>. Moreover, detergents such as 12PC or 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.

## SUPPORTING INFORMATION

Description of the calculation of static and dynamic mosaicities can be found in the supporting information, together with additional <sup>31</sup>P and <sup>2</sup>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 in which oriented bicelles form, and their degree of alignment. This material is available free of charge via the internet at <http://pubs.acs.org>.

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