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Magnetically-orientable Tween-based model membranes for NMR studies of proteins

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ABSTRACT

We present a new membrane mimetic system using a membrane softening detergent commonly known as Tween 80 (TW80), to form oriented systems for solid-state NMR applications. TW80 is a fatty acid ester (oleate) of sorbitan polyethoxylate and a mild non-ionic surfactant. Phosphatidylcholine (PC)/TW80 model membrane systems were characterized by solid-state NMR and FTIR spectroscopy. ^{31}P and ^{2}H NMR spectra showed that DMPC (14:0) and DPPC (16:0) self-assemble with TW80 to form oriented structures, and maintain alignment over a wide range of molar ratios and temperatures. The addition of lanthanide ions revealed that the membrane alignment can be flipped from parallel to perpendicular with respect to the magnetic field direction. Using ^{15}N solid-state NMR and a labeled model transmembrane peptide, we showed that TW80-based membranes can be employed to determine the peptide orientation in the magnetic field, which is useful for structural determination. Altogether, our work showed that TW80 could be exploited for direct and efficient membrane protein extraction and to enhance membrane and membrane protein orientation without using a detergent removal step. This approach could be extended to a wide range of membranes including native ones.

1. Introduction

The membrane acts as a semi-permeable barrier, the main role of which is to separate and compartmentalize the cell. It also plays an important role in many biological processes such as protein scaffolding and cell signaling. It is therefore essential to investigate its biological components individually, such as lipids and proteins, at a molecular level. To do so, a variety of model membranes can be used, such as micelles, liposomes, or bicelles, depending on biological and experimental requirements, and several criteria should be considered when choosing a membrane mimetic system [1]. First, the reconstitution environment should mimic as closely as possible the native one, in order to maintain innate structures and functions. Morphology, size, and composition of model membranes will dictate the NMR experiment to be used, i.e., solution or solid-state (SS) NMR. In addition, the temperature range of stability should be considered to preserve the sample over time during the NMR experiments.

Detergents, such as sodium dodecyl sulfate (SDS) and n-dodecyl-β-D-maltoside (DDM), play an important role in biochemistry, particularly for membrane protein (MP) extraction, purification and handling [2,3]. In an aqueous environment, these surfactants spontaneously form micelles and allow maintaining MPs in solution [3]. Although their morphology differs from that of biomembranes, micelles are often used for MP structural studies by solution NMR because of their fast tumbling, which increases spectral resolution [1,4–6]. For MP reconstitution into liposomes, detergents need to be removed, which can be tedious, time consuming, and result in losing a large amount of MPs.

Several compromise models have been proposed for NMR studies of MPs, combining lipids and detergents. One such model are bicelles, or bilayered micelles, since their composition and local morphology resemble those of biomembranes [1]. Commonly comprised of dimyristoyl- (DMPC) and dihexanoyl- (DHPC) phosphatidylcholines as the long and short chain lipids, respectively, DMPC can be substituted by a variety of saturated and unsaturated lipids with various headgroups, as

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reviewed elsewhere [1]. Bicelles can also be prepared by mixing lipids with non-lipid surfactants such as CHAPSO [7], Triton X-100 [8,9] or monoalkylphosphocholines [10], and oriented membranes can be prepared by using amphipathic peptides or polymers [11–13]. Their spontaneous alignment in the magnetic field under certain conditions (e.g. temperature, lipid concentration and ratio) makes bicelles interesting model membranes for SS-NMR applications [1]. Notably aligned bicelles allow the determination of MP orientation with respect to the membrane by SS-NMR [14–17]. Bicelles aligned with the bilayer normal perpendicular to the magnetic field are obtained when the temperature is above the phospholipids' gel-to-fluid phase transition (T_m), and when the long-to-short-chain lipid ratio (q) and lipid concentrations are high enough ($q > 2.3$, 3–60% *w/v* lipids in aqueous solution) [1]. Addition of small amounts of paramagnetic lanthanide ions switches the bicelle normal alignment to parallel by alterating of the overall magnetic susceptibility anisotropy of the membrane [18–20]. This strategy improves spectral resolution and facilitates the determination of MP orientation, since it becomes independent of its azimuthal orientation and axial diffusion rate.

In this work, we examined the possibility of combining the membrane softening ability of detergents with the phospholipids propensity to align in a magnetic field, in order to form oriented model systems for SS-NMR study. The first magnetically-oriented liposomes were made of *Escherichia coli* lipid extracts, or mixtures of lipids, with or without detergents [7,21–24]. Here, we propose to employ polysorbate 80 - commercially known as Tween 80® (TW80) - to soften liposomes, thus improving their magnetic alignment. TW80 is a fatty acid ester (oleate) of sorbitan polyethoxylate with a multi-headed structure of four hydrophilic moieties, one of which is extended by an alkyl chain (Fig. 1A). TW80 is a mild non-ionic surfactant employed as an emulsifier in the food industry, and as an excipient in the pharmaceutical industry, but also for extracting soluble and membrane-bound proteins while maintaining their functions [25–30]. A lesser-known use for TW80 is as a bilayer-softening component to generate transferosomes. These elastic liposomes are utilized as transdermal delivery systems to enhance drug permeation in a tissue of interest, such as the skin [31,32].

We tested TW80 with two lipids that bear a choline headgroup, found in biological membranes, and known to maintain MP activity [33], namely DMPC and dipalmitoyl-phosphatidylcholine (DPPC) (Fig. 1B and C). We optimized the adequate lipid-to-surfactant ratios for membrane orientation, and measured the quality of magnetic orientation for each ratio. ^{31}P SS-NMR is a useful tool as it allows the observation of changes in the morphology and orientation of model membranes [34]. Since the electronic environment surrounding the phosphate group is anisotropic, the resulting chemical shift will depend on the orientation of a phospholipid with respect to the external magnetic field (B_0). The chemical shift anisotropy (CSA) represents the superposition of all chemical shifts for the distribution of orientations of the phospholipids in the samples. It also informs on the dynamic properties of the phospholipids, as increased lipid motions will result in spectral averaging of the powder pattern. If the sample is oriented, only a single well-resolved ^{31}P resonance remains for a given oriented phospholipid. ^{2}H SS-NMR allows probing the hydrophobic core of the membrane when lipids with deuterated acyl chains (DMPC-d_{54} and DPPC-d_{62}) are used. The doublet spacing of individual CD_2 bond resonances, also called quadrupolar splitting ($\Delta\nu_Q$), is proportional to the order parameter of the acyl chain at this position [35].

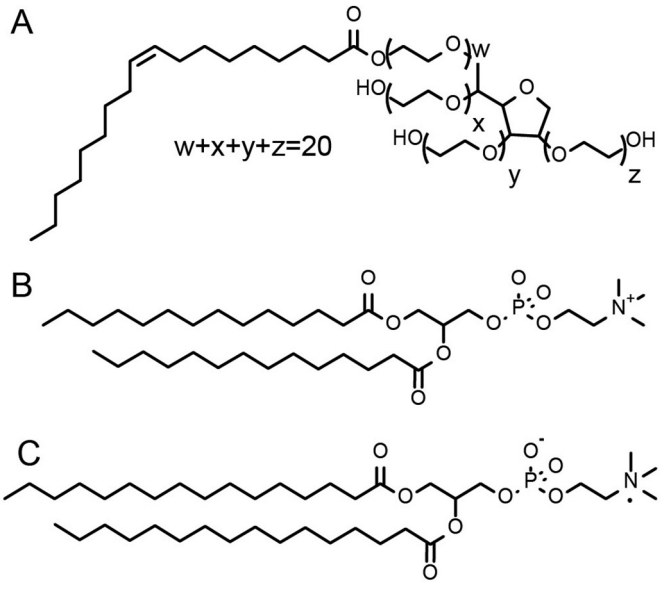

Fig. 1. Molecular structures of (A) Tween 80, (B) dimyristoylphosphatidylcholine, and (C) dipalmitoylphosphatidylcholine.

Our results show that TW80 increases the elasticity of PC bilayers, allowing them to deform and align in the magnetic field. An advantage of our approach is that the same surfactant is used to extract and reconstitute MPs, and to enhance the membrane magnetic orientation, thus eliminating the detergent removal step before carrying out SS-NMR experiments. To show the applicability of such model membranes, they were used for the reconstitution of a model transmembrane peptide. Our results show that the magnetic orientation of the Tween-based bilayers can be flipped using lanthanide salts, thus facilitating the determination the peptide orientation. Because the softening ability of surfactants is a general property, our approach could in principle be generalized to other surfactants [36]. In addition, the softening of liposomes is less restrictive than the ability of a given mixture to form bicelles. Although lipids with unsaturated acyl chains are more difficult to align in a magnetic field [37–39], their cooperativity could confer an overall magnetic susceptibility anisotropy enabling the alignment of elongated liposomes made of mixtures of saturated and unsaturated lipids, as shown previously [21,40]. With our approach, the softening and orientation of a variety of liposomes is thus conceivable, including native membranes.

2. Materials and methods

2.1. Materials

Protonated and deuterated dimyristoyl- and dipalmitoylphosphatidylcholine (DMPC, DMPC-d_{54}, DPPC, DPPC-d_{62}) were purchased from Avanti Polar Lipids (Alabaster, AL, USA), while OmniPur polyoxyethylene (20) monooleate (TW80) was obtained from EMD Millipore (Billerica, MA, USA). Deuterium-depleted water and ytterbium (III) nitrate pentahydrate (99.9% purity) were purchased from Sigma Aldrich (Oakville, ON, Canada), and deuterium oxide (D_2O) from CDN isotopes (Pointe-Claire, QC, Canada).

2.2. Peptide synthesis and purification

A 20 amino-acid membrane peptide close to the KALP peptide family was synthesized. Its sequence is Ac-GKKLALALA*LALAALALKKA-NH_2, with the 9th amino acid (the alanine with a star) being ^{15}N-labeled. The peptide synthesis was carried out on solid support using Fmoc-chemistry and 2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) as coupling reagent. The peptide was cleaved from the Rink Amide AM resin with a mixture of trifluoroacetic acid (TFA), ethanedithiol, phenol, and water. It was then precipitated with ethyl ether, solubilized in water to be lyophilized. The peptide was dissolved in 100% acetic acid and diluted to 35% to be purified by a reverse-phase high performance liquid chromatography (RP-HPLC) on a preparative C_{18} column (Phenomenex). Collected fractions were analyzed by RP-HPLC using an Aeris peptide XB C_{18} column (Phenomenex) and by mass

spectrometry using a LC/MS-TOF (Agilent). Fractions corresponding to the desired peptide with purity higher than 95% were pooled and lyophilized.

2.3. Solid-state NMR

2.3.1. NMR sample preparation

PC/TW80 model membranes were prepared by mixing freeze-dried DMPC or DPPC with a viscous TW80 solution, followed by a series of four freeze (liquid N_2)/thaw (50 °C)/vortex cycles. The lipid concentration was maintained well above the critical micelle concentration of TW80 (12 nM). Solid-state NMR samples were prepared with deuterium-depleted water at 80% (*w/v*) hydration. Samples of lanthanide-doped membranes were prepared at the minimal ytterbium (Yb^{3+}) concentration necessary for parallel alignment, therefore molar ratios (PC/Yb^{3+}) were of 105 for DMPC and 88 for DPPC. All samples were at least duplicated.

For the peptide-containing membranes, the peptide was first solubilized in a TW80 solution, with or without Yb^{3+}, in D_2O, and this solution was added to dry DMPC, followed by at least four freeze/thaw/vortex cycles. Both samples had a ratio DMPC/TW80 of 3, a ratio of DMPC/peptide of 30, and the Yb^{3+} containing sample had a DMPC/Yb^{3+} ratio of 55. Typically, this resulted in samples with approximately 10 mg of DMPC, 7 mg of TW80, 1 mg of peptide, 70 µl of D_2O and 0 or 0.1 mg of Yb^{3+}.

2.3.2. NMR experiments

^{31}P and ^{2}H solid-state NMR experiments were recorded on a Varian Inova Unity (Agilent, Santa Clara, CA, USA) spectrometer operating at a magnetic field of 14.1 T (corresponding to frequencies of 599.95 MHz for ^{1}H, 242.84 MHz for ^{31}P, and 92.09 MHz for ^{2}H). A 4-mm broadband/^{1}H dual-frequency magic-angle-spinning (MAS) probe head was employed. Additional ^{31}P, ^{2}H and ^{15}N SS-NMR experiments were carried out on a Bruker Avance III HD (Billerica, MA, USA) spectrometer operating at a magnetic field of 9.4 T (frequencies of 400.02 MHz for ^{1}H, 161.92 MHz for ^{31}P, 61.41 MHz for ^{2}H, and 40.55 MHz for ^{15}N), using a double resonance 4-mm MAS probe. Data were processed using either Topsin (Bruker) or MestReNova software (Mestrelab Research, Santiago de Compostela, Spain).

^{31}P SS-NMR spectra were recorded using a phase-cycled Hahn echo pulse sequence [41] with gated broadband proton continuous wave decoupling at a field strength of 50 kHz and an interpulse delay of 40 µs. Typically, 1024 scans were acquired with a recycle delay of 5 s. ^{2}H NMR spectra were obtained using a solid echo pulse sequence [42], with an interpulse delay of 30 µs and typically 2048 scans were recorded with a recycle delay of 0.5 s. For all ^{2}H and ^{31}P SS-NMR experiments, the 90° pulse length was between 2.5 and 4 µs.

^{15}N SS-NMR spectra were recorded using a simple cross-polarization (CP) pulse (of around 40 kHz for 1.5 ms) sequence with continuous wave proton decoupling at 80 kHz. A total of 30,000 scans were generally acquired with a recycle delay of 5 s (total time of approximately 46 h). The ^{1}H 90° pulse length was 3 µs. Before acquiring the ^{15}N spectrum, the sample was equilibrated at 67 °C for 20 min, then brought to the temperature with the best alignment (37 °C without Yb^{3+}, and 47 °C with Yb^{3+}), for another 20 min. A ^{31}P spectrum was recorded to verify the membrane alignment before the ^{15}N spectrum was acquired.

^{31}P SS-NMR spectra were externally referenced with respect to 85% phosphoric acid set to 0 ppm, while ^{15}N SS-NMR spectra were externally referenced with respect to ammonium chloride powder set to 39.3 ppm [43].

2.3.3. ^{31}P spectrum fitting

We wrote a MATLAB program to determine the deformation of the PC/TW80 model membranes from the ^{31}P spectrum lineshape. The extent of vesicle deformation is described with a *c/a* ratio, where *c* and *a* are the major and minor axes for the ellipsoid, respectively (Fig. 2).

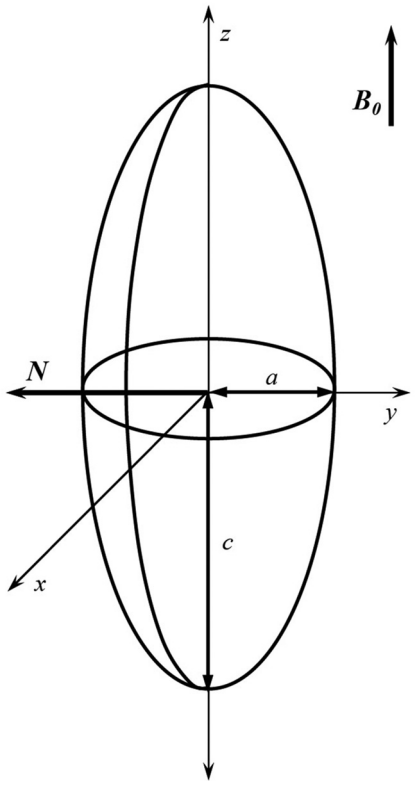

Fig. 2. Geometric model of an ellipsoid where *c* and *a* are the major and minor axis, respectively, B_0 is the external magnetic field, and *N* the membrane normal.

The *c/a* value depends on the elastic properties of the membrane as described previously [44,45]:

$$c - a = r_0^3 \frac{H^2 \Delta\chi}{12K} \quad (1)$$

where r_0 is the radius of non-deformed liposome in cm, $\Delta\chi$ is the magnetic susceptibility of phospholipid molecules, *K* is the curvature-elastic modulus in dynes, and *H* is the intensity of the magnetic field in gauss.

To simulate ^{31}P spectra for fitting with experimental ^{31}P spectra, Δ (CSA), ν_P (chemical shift at 90° orientation) and β (resonance width) were obtained from simulated spectra fitted to experimental spectra of pure PC vesicles (DMPC or DPPC multilamellar vesicles, MLVs). The ellipsoid ratio (*r*), which is the *c/a* ratio, was then adjusted to obtain the best-fit spectrum.

2.4. Fourier transform infrared spectroscopy

Samples for infrared spectroscopy were prepared by weighting the desired amount of perdeuterated DMPC or DPPC and TW80. The total mass of phospholipids and detergent was 4 mg, and the phospholipid/TW80 molar ratio (q) was 3 or 4 for DMPC, and 3 or 9 for DPPC. Samples were then hydrated with 11 µl of D_2O, resulting in a total proportion of 20% (*w*/w) lipids in water. Vesicles were formed by performing 5 cycles of vigorous vortexing, freezing (liquid N_2), and thawing (50 °C). All samples were duplicated and were highly reproducible.

Experiments were performed using a Nicolet Magna 560 FTIR spectrometer (Thermo Scientific, Madison, WI) equipped with a nitrogen-cooled MCT A detector. Temperatures were adjusted using a home-build temperature controller (± 1 °C) and the stabilization time for each temperature was 3 min. Samples were deposited between two CaF_2 windows (Spectral Systems, Hopewell Junction, NY) separated by

a Mylar film spacer of 13 μm (Goodfellow Cambridge Ltd., Huntingdon, U.K.). Spectra were recorded via the acquisition of 128 interferograms at a resolution of 4 cm^{-1} using a Happ-Genzel apodization on the Grams/7 AI software (Galactic Industries Corp., Salem, NH). A D_2O spectrum was subtracted from each series of spectra, and the 3100–2700 region was baseline-corrected with a cubic function.

The phase transition (melting) temperature (T_m) is the inflexion point of the IR thermotropic curve when plotting the intensity of the symmetric stretching vibration (ν_sCD_2) at (~2090 cm^{-1}) of deuterated lipids, and was obtained by measuring the maximum of the first derivative of the curve. Indeed the acyl chains of the PC lipids used in the IR experiments were deuterated (DMPC-d_{54} and DPPC-d_{62}), which is known to decrease T_m by 3 to 5° compared to protonated lipids [46,47].

3. Results and discussion

We first optimized, using ^{31}P and ^{2}H SS-NMR, the phospholipid/TW80 molar ratios (q) and temperatures at which the bilayers spontaneously align in the magnetic field with the membrane normal perpendicular to B_0. As summarized in Table S1A, DMPC/TW80 systems align at molar ratios ranging from $q = 2$ to $q = 5$, while DPPC/TW80 systems orient between $q = 2$ to $q = 13$. This is evidenced by ^{31}P SS-NMR with the appearance of sharp signal at -15 ppm, which corresponds to the 90° edge of a pure PC spectrum (Fig. 3). Note that a small residual powder pattern is also visible, especially at high q ratio for both DMPC and DPPC systems. A lower proportion of TW80 is necessary to align DPPC bilayers as compared to DMPC.

A resonance is observed at 0 ppm at all q ratios for DMPC/TW80 systems, but only at low q ratios for DPPC/TW80. This isotropic resonance most likely reflects the presence of rapidly reorienting structures, probably pure or mixed micelles. The magnetic alignment of the membranes is further evidenced on the ^{2}H SS-NMR spectra (Fig. 4) by narrow lines, and the highly reduced 0° shoulders. The reversible transition from liposomes to mixed micelles is a process that is well documented [48]. As the detergent content increases, it partitions into the membrane until a saturation point where detergent-saturated bilayers and lipid-detergent mixed micelles coexist. This saturation point appears to be reached earlier for DMPC than DPPC.

All systems remained aligned well above T_m (Table S1). Conversely, just below T_m, at 17 °C and 37 °C for DMPC/TW80 and DPPC/TW80, respectively, both ^{31}P and ^{2}H powder spectra are poorly resolved, implying the formation of large size aggregates with reduced elasticity (Fig. 4). At even lower temperatures a single isotropic peak is obtained, probably corresponding to a population of mixed micelles [49].

The PC/TW80 membrane systems do not behave like bicelles since the central peak and 90° edge of the ^{31}P NMR spectra hardly shift either with q or temperature [49], and a very small residual powder pattern is almost always visible. Moreover, as the TW80 concentration increases, hardly any changes in $\Delta\nu_Q$ of ^{2}H are observed for both DMPC and DPPC systems, indicating that the lipid order is not much affected (Fig. S1 and Table S2). This is very different from what was observed by Goni et al. with other PC/detergent systems, i.e., PC/TX-100, where $\Delta\nu_Q$ decreased while the detergent content increased [9]. We believe that our aligned PC/TW80 systems form oriented liposomes with a prolate-type ellipsoid morphology instead of flat disks. For lower q values or temperatures, these oriented liposomes can coexist with mixed micelles (see Fig. 3 and Fig. S1). To use these systems to align MPs in the magnetic field, the presence of mixed micelles can be reduced by using higher molar ratios, such as $q = 3$ to $q = 5$ for DMPC/TW80, or $q = 7$ to $q = 9$ for DPPC/TW80, and working at temperatures at least 15 °C over T_m.

An analysis of the phospholipid thermotropic phase behaviour in Fourier transform infrared (FTIR) spectroscopy was performed to verify the oriented liposomes hypothesis. As shown in Fig. 5, the CD_2 symmetric stretching vibration (ν_sCD_2) frequency of the deuterated lipids (DMPC-d_{54}, DPPC-d_{61}) as a function of temperature increased with TW80 content (Fig. 5), indicating an increase in conformational

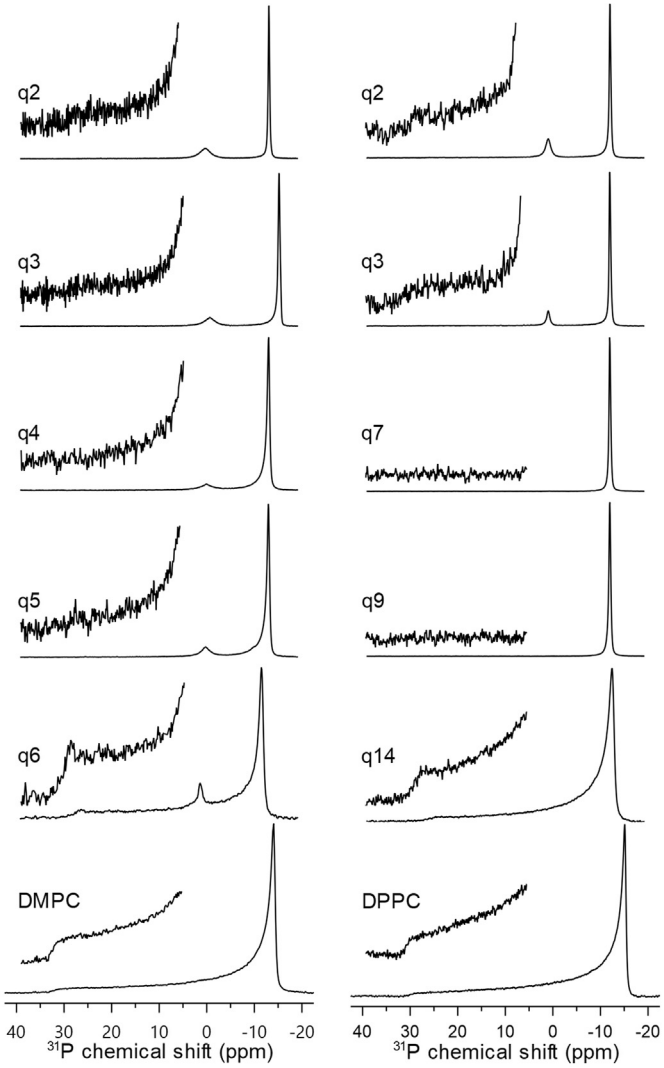

Fig. 3. ^{31}P NMR spectra of DMPC/TW80 (left) and DPPC/TW80 (right) at 37 °C and 57 °C respectively for different molar ratios (q). Pure phospholipid spectra (bottom) are shown for comparison. Magnifying factor of pure lipid and non-oriented insets (DMPC/TW80 q = 6 and DPPC/TW80 q = 14) is 10×. All other insets for oriented molar ratio are magnified 100×.

disorder of the lipid acyl chains, especially with DMPC [50]. In addition, a higher TW80 content broadens the gel-to-liquid crystalline phase transition, implying that TW80 slightly disrupts the cooperative nature of the lipids. Nevertheless, this disruption is small, especially for DPPC, indicating that PC and TW80 molecules are not much segregated. The larger effect observed on DMPC *vs*. DPPC can be explained by the chain length of TW80, which is closer to that of DPPC, thus facilitating the miscibility of TW80 with DPPC [51]. This is consistent with the broader q range at which DPPC/TW80 systems align (Fig. S1). Finally, the T_m can also be measured by FTIR, and is also not significantly affected by the presence of TW80 (Table S3). This is again different from the observations of Goni et al., where TX-100 lowered the T_m of DPPC and DMPC by ~10 °C, indicating again a much stronger effect of TX-100 on lipids as compared to the mild TW80 [9]. Altogether, these observations are all compatible with PC/TW80 systems forming oriented liposomes, rather than bicelle disks.

As presented in Eq. (1), the orientation of the Tween-based liposomes depends on the diamagnetic susceptibility anisotropy of the phospholipids ($\Delta\chi$) conferred by the acyl chains and, to a lesser extent, the ester groups ([52] and references therein). PC molecules have a negative $\Delta\chi$ value; therefore, their orientation perpendicular to the

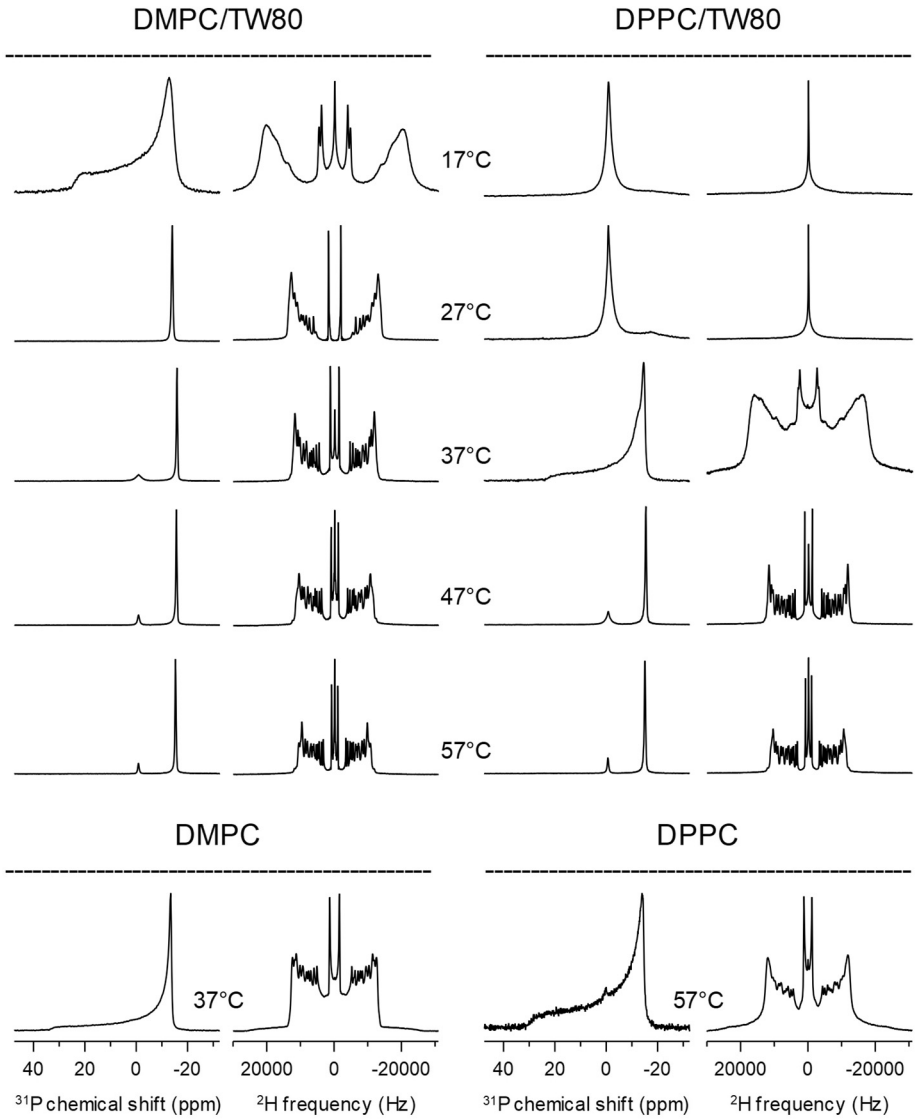

Fig. 4. ^{31}P (first and third columns) and ^{2}H (second and last columns) NMR spectra of DMPC/TW80 (left) and DPPC/TW80 (right) with $q = 3$ at different temperatures. Pure lipid spectra of DMPC (bottom, left) and DPPC (bottom, right) are shown for comparison.

magnetic field direction is more energetically favourable, and aided by the collective response of the PC molecules packed in ordered structures [52]. The extent of liposome deformation in the magnetic field into prolate-shaped ellipsoid also depends on their elastic properties (K), as shown in Eq. (1). This elasticity is conferred by Tween molecules, employed as bilayer-softening agents to prepare deformable liposomes [31]. We used ^{31}P NMR spectral fitting [44] to determine the degree of deformation of the PC/TW80 systems. Characteristic simulated spectra can be found in the supplementary information (Figs. S2–S4). Spherical systems with c/a close to 1 were observed at high q values, above q = 6 for DMPC/TW80, and q = 14 for DPPC/TW80 (Fig. 6). Under these conditions, aggregates no longer align in the magnetic field. At intermediate molar ratios, deformation starts and reaches approximately $c/a = 16$ for DMPC/TW80 and $c/a = 25$ for DPPC/TW80. Conversely, highly elongated systems, with c/a above 50, were observed at very low q ratios, below q = 3 for DMPC/TW80, and q = 4 for DPPC/TW80.

Eq. (1) also shows that an increased magnetic field intensity (H) should increase the ellipsoid ratio (r). This was verified by comparing ^{31}P spectra recorded at 400 and 600 MHz, and fitting them to determine the extent of deformation (Fig. 7). As expected for oriented liposomes, the ellipsoid ratios are higher ($r = 15$) at 600 MHz than at 400 MHz ($r = 5$).

As mentioned in the introduction, MP orientation is easier to determine in "flipped" systems, where the membrane normal is parallel to B_0, for example by adding small amounts of trivalent lanthanide ions [16]. As shown by ^{31}P and ^{2}H SS-NMR (Fig. 8), addition of ytterbium ions (Yb^{3+}) to DMPC/TW80 at a PC/Yb^{3+} molar ratio of 105 "flipped" the bilayer normal from perpendicular ($\theta = 90^{\circ}$) to parallel ($\theta = 0^{\circ}$) with respect to B_0. Parallel alignment is revealed by the main ^{31}P resonance broadening and shifting from -15 ppm to +20 ppm, and by a doubling of the ^{2}H quadrupolar splittings, such as the external doublet, which goes from 26 to 52 kHz. While the "flipped" orientation is unambiguous, the new liposome shape cannot be determined from our calculated NMR spectra which rely on an analytical solution only valid for prolate ellipsoids. We do note that the spectra are compatible with an oblate ellipsoid which would result from the "flipping" of the prolate ellipsoids.

Magnetically-oriented model membranes have mostly been used for peptide structure determination by ^{15}N SS-NMR, although they can also be used in CD and other biophysical techniques [1]. We have therefore verified if the PC/TW80 model membranes can be used to determine the orientation of a transmembrane peptide using a KALP-like peptide [53]. Incorporating this peptide in the TW80-based membrane mimetics was straightforward, and did not modify their alignment

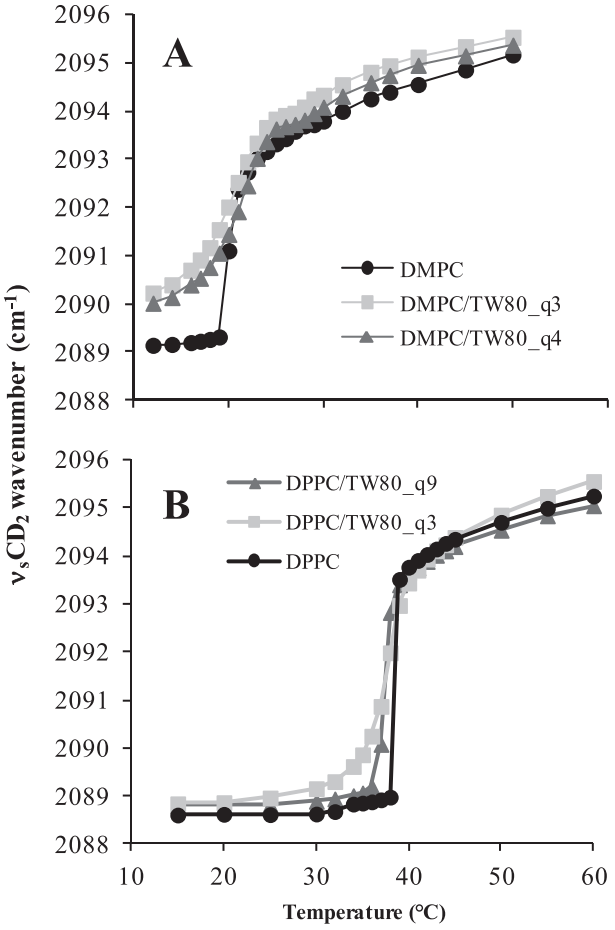

Fig. 5. Temperature dependence of CD_2 symmetric stretching vibration ($\nu_s CD_2$) for PC/TW80 samples by FTIR spectroscopy: (**A**) DMPC, and (**B**) DPPC, with and without TW80 at different molar ratios.

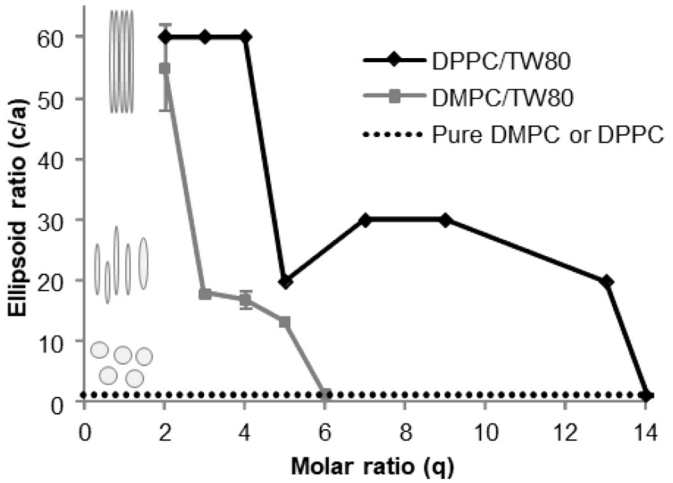

Fig. 6. Best-fit ellipsoid ratios (c/a) of DMPC/TW80 at 37 °C (grey) and DPPC/TW80 at 57 °C (black) plotted against the surfactant molar ratio q and at a field of 14.1 T. The experimental ellipsoid ratio of pure PC (dotted line) was added for comparison.

properties, as confirmed by ^{31}P spectra with and without peptide (data not shown). Of course, PC/TW80 model membranes are slow-tumbling objects with large correlation times, therefore more appropriate for solid-state than for solution NMR.

The model peptide contained a single ^{15}N nucleus on an alanine in the middle of the sequence, a challenge for SS-NMR to detect, hence the long acquisition time. Nitrogen-15 resonance frequency depends on the $^{15}N-^{1}H$ orientation relative to the magnetic field, which can range between 50 and 200 ppm. A powder distribution of peptide orientations would therefore result in a broad spectrum of several thousands of hertz. A ^{15}N nucleus in an α-helix perpendicular to the magnetic field is expected to provide a narrow line below 100 ppm (as opposed to isotropic values *ca.* 110–130 ppm), and at around 200 ppm if it is parallel to the magnetic field [54,55]. DMPC/TW80 model membranes containing our model peptide at 37 °C showed with no ambiguity a single and narrow ^{15}N resonance at 86 ppm, compatible with a transmembrane peptide embedded in membranes with their normal perpendicular to the magnetic field (Fig. 9A). The peptide in those same membranes with Yb^{3+} and at 47 °C showed a single and narrow ^{15}N

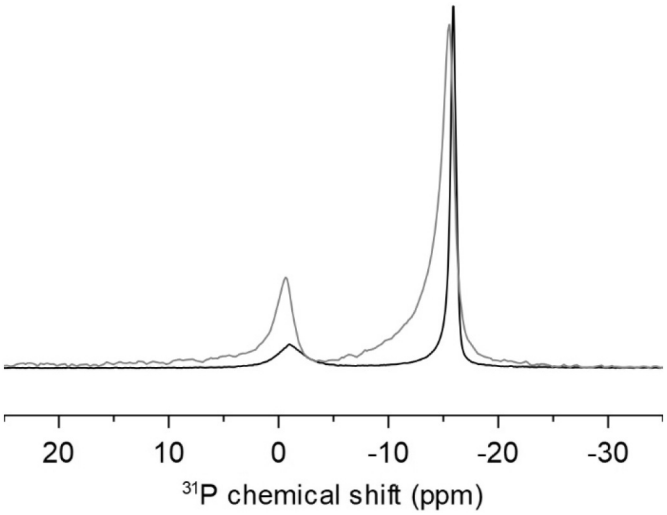

Fig. 7. ^{31}P NMR spectra of DMPC/TW80 q = 3 at 37 °C showing the extent of magnetically induced deformation at 161.92 MHz (9.4 T, grey) and 242.84 MHz (14.1 T, black).

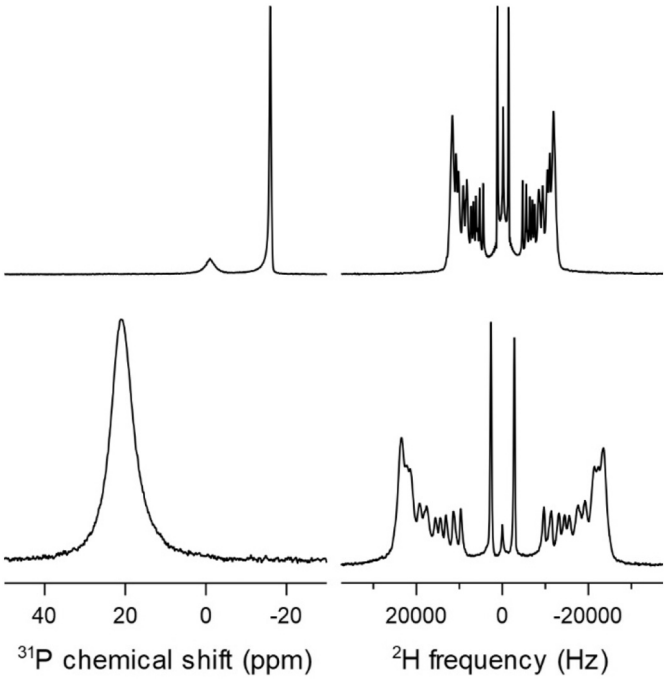

Fig. 8. ^{31}P (left) and ^{2}H (right) NMR spectra of DMPC/TW80 q = 3 at 37 °C without (top) and with (bottom) lanthanide ions using a DMPC/Yb^{3+} molar ratio of 105:1.

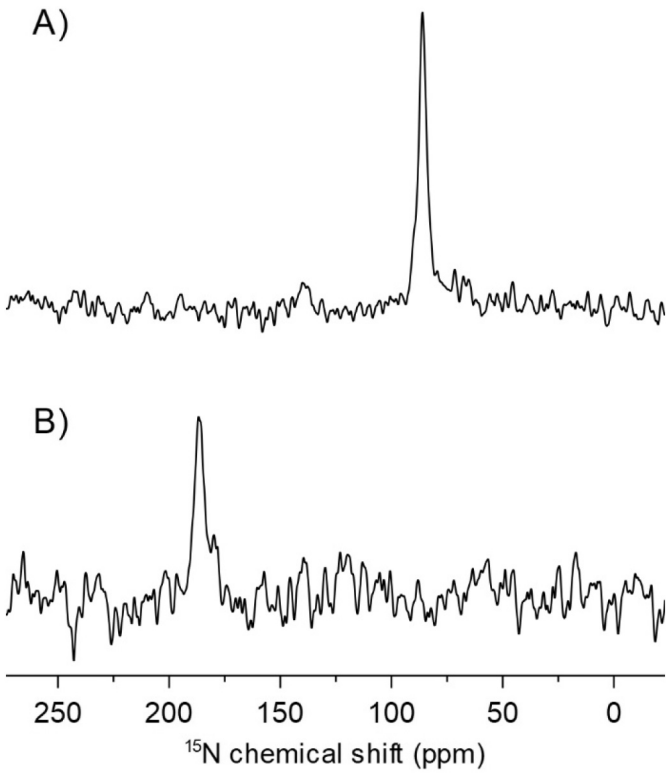

Fig. 9. ^{15}N NMR spectra of (A) DMPC/TW80 q = 3 with the model peptide at 37 °C, and (B) DMPC/TW80 q = 3 with the model peptide and Yb^{3+} at 47 °C.

resonance shifted to 187 ppm, compatible with a transmembrane peptide embedded in parallel-aligned membranes (Fig. 9B). This is a textbook example of a peptide traversing a membrane which orientation shifts by 90° upon addition of lanthanide ions [15,19].

Several studies have demonstrated the usefulness of detergent-saturated vesicles for the partitioning of MPs such as bacteriorhodopsin, multidrug transporter LmrP and small multidrug resistance protein EmrE [48,56,57]. Our results showed that such mixed liposomes can deform and align in the magnetic field of an NMR spectrometer, thus offering the possibility of a wide range of applications in SS-NMR, from determining the orientation of a membrane peptide to a full MP structure determination. As previous studies have shown [18,19], MPs will be better studied in "flipped" systems with lipids parallel to the magnetic field direction, and we have shown here that flipping was possible in PC/TW80 model membranes with Yb^{3+}. Mixed liposomes are expected to be stable in a wider temperature and composition range than bicelles, and should be able to incorporate lipids with various headgroups or acyl chains, as well as cholesterol. In order to maximize the population of oriented liposomes in the sample, we recommend working at least 15 °C over the gel-to-fluid main phase transition temperature.

4. Conclusion

In this work, we showed that magnetically-oriented PC/TW80 are novel model membranes suitable for MP studies. Combined SS-NMR and FTIR data showed that these systems adopt a prolate liposome shape in which lipids and detergent molecules are not segregated. PC/TW80 systems are advantageous as the non-ionic detergent can be exploited to both extract MPs and trigger bilayer orientation, thus eliminating the tedious detergent removal step. This strategy could be applied to other membrane softening surfactants. Another asset of PC/TW80 model membranes is that they can achieve magnetic orientation while closely mimicking biomembranes since they are mostly made of lipids at high *q* ratios. They offer a wide range of molar ratios and temperatures at which NMR experiments can be performed, especially DMPC/TW80 above 27 °C, and DPPC/TW80 above 42 °C. Moreover, the magnetic alignment of these PC/TW80 membranes can be flipped by paramagnetic salt addition, thus facilitating the determination of transmembrane peptide orientation, as demonstrated with a KALP-like peptide. PC/TW80 membranes are promising mimetic systems that could be useful to the SS-NMR structural study of MPs in general.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbamem.2020.183379.

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SUPPORTING INFORMATION

Magnetically-Orientable Tween-Based Model Membranes for NMR Studies of Proteins

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Table S1. Summary of all ^{31}P NMR experiments carried out for PC/TW80 model membranes at different temperatures, without Yb^{3+} and for various molar ratios q (A), and with Yb^{3+} and for q=3 (B).

A

		DMPC/TW80																DPPC/TW80													
		Temperature (°C)																													
	Molar ratio (q)	17	22	25	27	32	37	42	47	52	57	62	67	72	77	87	92	17	22	25	27	32	37	39	42	47	52	57	62	67	72	77
Solution NMR	0.25	-	-	I	-	-	I	-	-	-	I	-	-	-	-	-	-	-	-	I	-	-	I		-	-	-	I	-	-	-	-
	0.5	-	-	I	-	-	I	-	-	-	I	-	-	-	-	-	-	-	-	I	-	-	I		-	-	-	I	-	-	-	-
	0.75	-	-	I	-	-	I	-	-	-	I	-	-	-	-	-	-	-	-	I	-	-	I		-	-	-	I	-	-	-	-
	1	-	-	I	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	I		-	-	-	I	-	-	-	-
	1.5	-	-	I	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	I		-	-	-	I	-	-	-	-
SS-NMR	0.5	N*	N*	N*	N*	N*	N*	N*	N*	N*	N*	-	N*	-	N*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.75	N*	N*	N*	N*	N*	N*	N*	N*	N*	N*	-	N*	-	N*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	N*	N*	N*	N*	N*	N*	N*	N*	N*	N*	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	N*	-	-	A*	-	A*	-	A*	-	A*	A*	A*	A*	A*	A	-	N	N	N	N	N	N	N	N	A	A	A	-	A	-	A
	3	N*	N*	-	A*	A*	A*	A*	A*	A*	A*	A*	A*	A	A*	-	-	N	N	N	N	N	N	-	A*	A*	A*	A*	-	-	-	-
	4	N*	N*	-	A*	A*	A*	A*	A*	A*	A*	A*	A*	A*	A*	A*	A	N	N	N	N	N	N	-	A	A	A	A	-	A	-	A
	5	N*	N*	-	A*	A*	A*	A*	A*	A*	A*	A*	A*	A*	A*	-	-	N	N	N	N	N	N	-	A	A	A	A	-	-	-	-
	6	N	-	-	N	-	N	-	N	-	N	-	N	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	-	A	A	A	A	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N*	A*	A*	A*	A*	A	-	A	-	A
	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	-	A	A	A	A	-	A	-	A
	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	-	N	N	N	N	-	-	-	-

A: Aligned
N: Not aligned
I: Isotropic
*: experiments also done by ^2H NMR

B

DMPC/TW80/Yb3+ q3							DPPC/TW80/Yb3+ q3										
Temperature (°C)																	
17	27	37	47	57	67	77	17	22	27	32	37	42	47	52	57	67	77
N*	A*	A*	A*	A*	A*	A*	N*	N*	N*	N*	N*	A*	A*	A*	A*	A*	A*

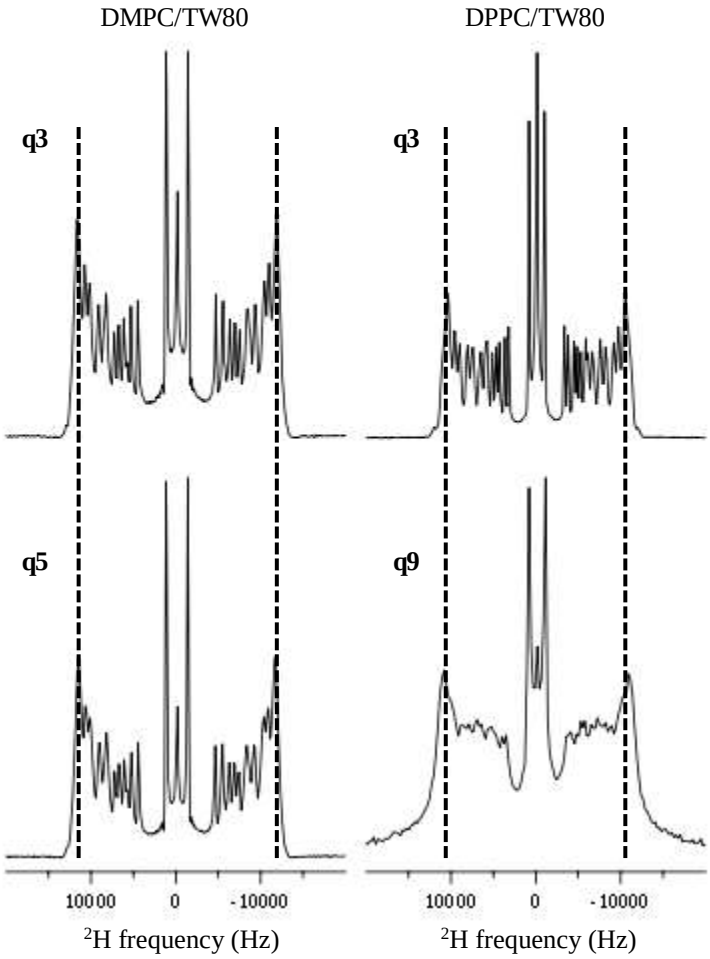

Figure S1. ^{2}H NMR spectra of DMPC/TW80 (37ºC) and DPPC/TW80 (57ºC) at selected molar ratios.

Table S2. Largest quadrupolar splitting ($\Delta\nu_Q$) values determined from ^{2}H NMR spectra of DMPC/TW80 and DPPC/TW80 at selected molar ratios.

Molar ratio (q)	$\Delta\nu_Q$ (kHz)	
	DMPC/TW80 (37°C)	DPPC/TW80 (57°C)
Pure lipid	24.9	24.3
3	23.6	22.1
5	23.2	-
9	-	21.8

Table S3. Phase transition temperatures (T_m) for DMPC-d_{54} and DPPC-d_{62} without and with TW80 at various molar ratios determined by FTIR.

Sample	Tm (°C)
DMPC	19.5
DMPC/TW80 q3	20.5
DMPC/TW80 q4	22.5
DPPC	38.5
DPPC/TW80 q3	37.5
DPPC/TW80 q9	37.5

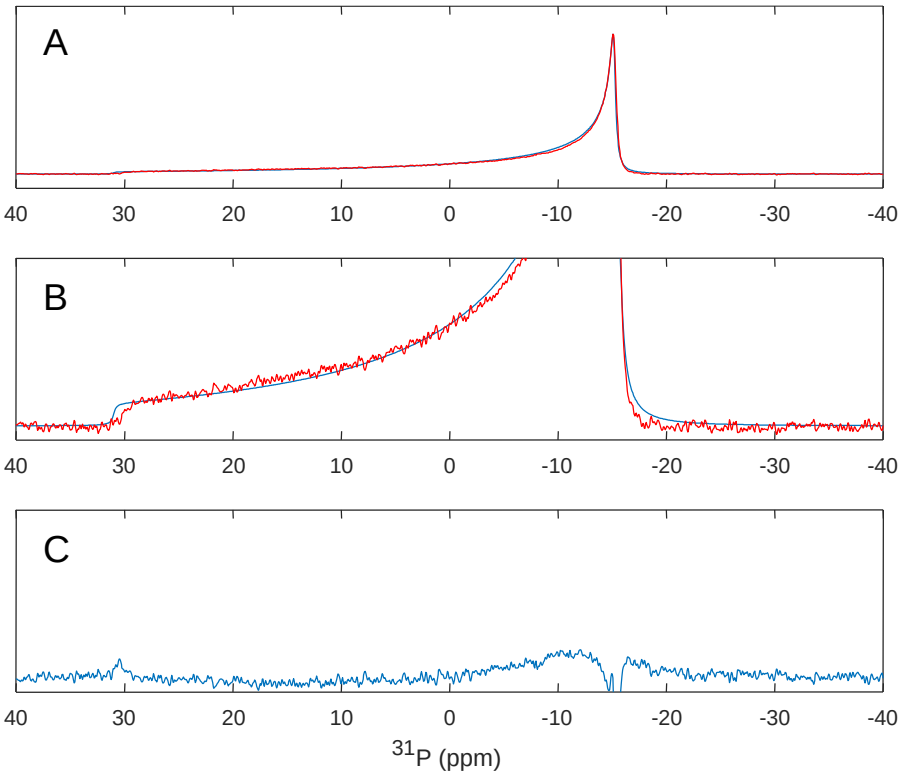

Figure S2. (A) Experimental (red) and simulated (blue) spectra of DPPC at 47°C with c/a=1.5. (B) Same as (A) with intensity multiplied by 10. (C) Residual intensity obtained after substracting the experimental spectrum from the best-fit simulated one.

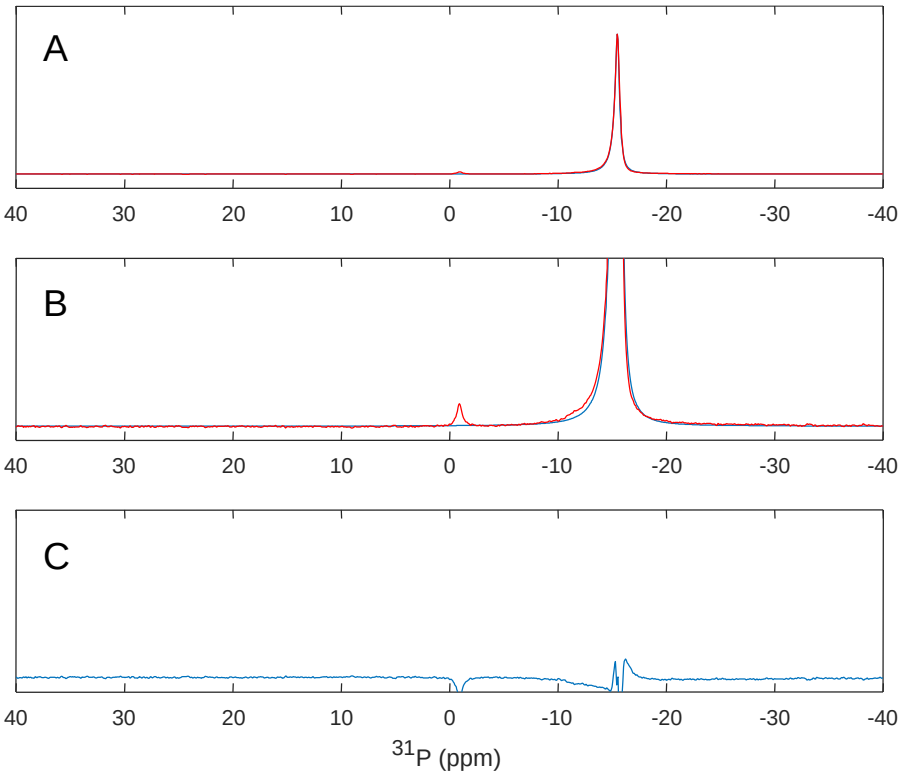

Figure S3. (A) Experimental (red) and simulated (blue) spectra of DPPC-TW80 q=9 at 47°C with c/a=12. (B) Same as (A) with intensity multiplied by 10. (C) Residual intensity obtained after substracting the experimental spectrum from the best-fit simulated one.

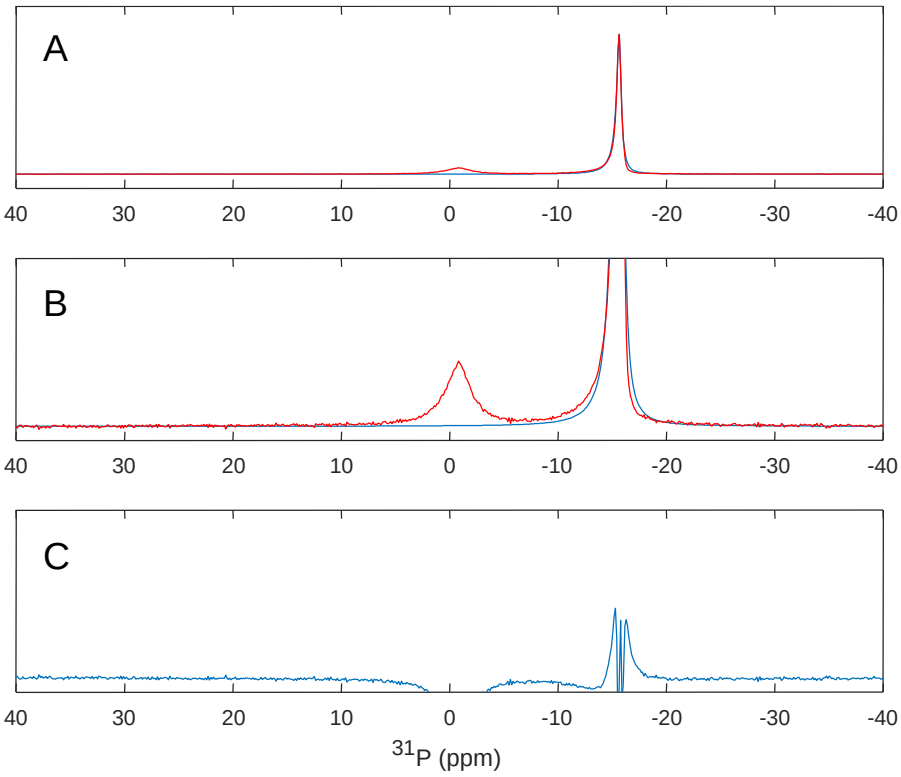

Figure S4. (A) Experimental (red) and simulated (blue) spectra of DMPC-TW80 q=3 at 37°C with c/a=10.5. (B) Same as (A) with intensity multiplied by 10. (C) Residual intensity obtained after substracting the experimental spectrum from the best-fit simulated one.