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Pressure-induced ordering in mixed-lipid bilayers

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Isothermal application of hydrostatic pressure to liquid crystalline phospholipid bilayers increases chain segment orientational order and thus chain extension. By using pressure to perturb chain order in single-component bilayers and bilayers comprising a binary mixture of lipids, it is possible to compare the relative influences of intrinsic lipid properties and collective bilayer properties on chain orientational order. Deuterium nuclear magnetic resonance was used to investigate the response of saturated chain orientational order to pressure in single-component and two-component liquid crystal bilayers of lipids having saturated chains of different lengths (dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine) or having one saturated chain and one unsaturated chain (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine). For bilayers containing a single lipid species, the isothermal response of average chain orientational order to pressure decreased with increasing difference between measurement temperature and that lipid's ambient-pressure gel-to-liquid-crystalline phase transition temperature. For all of the lipids observed, the range of orientational order displayed by the saturated chain methylene groups was approximately conserved as pressure was applied. In binary mixtures, the difference between the average saturated chain orientational orders of the two bilayer components was approximately conserved as pressure was increased. These observations provide some insight into how the response of the bilayer to pressure is distributed over interacting components with different intrinsic properties and illustrate the sensitivity of the effective chain ordering potential in the bilayer interior to bilayer composition.

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I. INTRODUCTION

Phospholipids dispersed in water form bilayers that are important models for biological membranes and interesting anisotropic soft materials. At high temperature and ambient pressure, lipid bilayers exist in the liquid crystalline state which is characterized by fast, axially symmetric reorientation of acyl chain segments. At lower temperatures or higher pressures, bilayers can undergo a transition into a more ordered phase. For bilayers containing a single lipid component, the transition between the more ordered gel phase and the less ordered liquid crystalline phase is sharp and the transition temperature depends on molecular properties including lipid acyl chain length and the degree of chain unsaturation.

The effect of pressure on lipid bilayers can provide insight into the way in which bilayer collective properties are determined by interactions between bilayer components. Knowledge of the response of bilayer properties to pressure can also be relevant to the understanding of how bilayer composition is related to the ability of marine organisms to adapt to large variations in hydrostatic pressure. The application of hydrostatic pressure generally stabilizes the more ordered phases of lipid bilayers. The temperatures for the main gel-to-liquid crystal transitions of disaturated phosphatidylcholines like dimyristoyl phosphatidylcholine (DMAC) and dipalmitoyl phosphatidylcholine (DPPC) rise by about 0.2 °C per MPa [1–4]. Transitions between ordered phases shift at different rates with increasing pressure so that application of pressure can stabilize ordered phases that are not accessible at ambient pressure [1,3–5].

Lipid bilayers are soft, anisotropic materials, and the application of hydrostatic pressure can modify the properties of a particular phase. If bilayers in the liquid crystalline phase are cooled at constant pressure, chain orientational order increases until the transition to a more ordered phase intervenes. The ordering that can be accommodated by the liquid crystalline phase before the transition intervenes decreases with increasing pressure. As a result, the jump in chain orientational order at the liquid crystal to gel transition of a given lipid bilayer increases with increasing pressure [2].

If temperature is held constant, application of pressure to liquid crystalline bilayers increases chain segment orientational order and thus chain extension [2,4,6,7]. Driscoll *et al.* [7] noted that the effect of pressure on orientational order of the DPPC methyl group decreased with increasing temperature. The effect of pressure on average DMPC chain order has also been found to decrease with increasing separation between the measurement temperature and the ambient-pressure transition temperature for that lipid [2]. A self-consistent field theory calculation showed a similar dependence, on temperature, of the slope of orientational order versus pressure for dipalmitoyl phosphatidylethanolamine (DPPE) [8].

Hydrostatic pressure is transmitted uniformly throughout the fluid bilayer structure. If pressure is applied to a liquid crystalline bilayer containing a mixture of lipids, the chain orientational order for all lipids will increase but the response of a given lipid species to pressure may be modified by interactions with surrounding lipids with a greater or lesser tendency to order in response to applied pressure. The response of a given lipid species to pressure when it is part of a binary mixture compared to its response within a single-component bilayer is expected to provide some indication of the extent to which chain order in a given lipid is coupled to that of other bilayer components.

In the work reported here, deuterium nuclear magnetic resonance (NMR) was used to observe the isothermal pressure dependence of chain orientational order for three lipid species in single-component bilayers and in binary mixtures with the saturated chain or chains of one or the other component fully deuterated. The phospholipid species used were 1-palmitoyl-2-oleoyl-*sn*-3-glycero-phosphocholine (POPC), DMPC, and DPPC. The binary mixtures investigated were equimolar mixtures of lipids with ambient-pressure gel-to-liquid-crystal transition temperatures differing by about 20° (POPC/DMPC and DMPC/DPPC) or by about 40° (POPC/DPPC). Observations were carried out at 20°–25° above the ambient-pressure transition temperature of the component with the highest transition temperature. For each pair of lipids, the pressure dependence of average saturated chain orientational order was determined for each lipid in a single-component bilayer and separately for each lipid component in the equimolar mixed bilayer. The results provide insight into the way in which the response to pressure is distributed over different lipid components in a mixture of lipids with different apparent susceptibilities to pressure-induced ordering.

II. MATERIALS AND EXPERIMENTAL METHODS

For deuterium NMR observations, POPC was fully deuterated on its saturated chain (POPC-d_{31}), and the disaturated phosphatidylcholines were fully deuterated on both saturated chains (DMPC-d_{54} and DPPC-d_{62}). The three deuterated lipids were purchased from Avanti Polar Lipids. Normal DMPC and DPPC were purchased from Sigma. Samples of normal POPC were obtained from both suppliers. All lipids were used without further purification.

The ambient-pressure main transition temperatures for single-component bilayers of POPC, DMPC, and DPPC are approximately $-3\,^\circ\mathrm{C}$, $24\,^\circ\mathrm{C}$, and $41\,^\circ\mathrm{C}$, respectively. This set of lipids comprises two disaturated phosphatidylcholines with differing chain lengths (DMPC and DPPC) and a phosphatidylcholine with one saturated and one unsaturated chain (POPC). For the four samples containing POPC-d_{31}, POPC-d_{31}/DMPC (1:1), POPC/DMPC-d_{54} (1:1), or DMPC-d_{54} bilayers, spectra were obtained over a series of pressures with the sample temperature fixed at $45\,^\circ\mathrm{C}$. For the four samples containing DMPC-d_{54}, DMPC-d_{54}/DPPC (1:1), DMPC/DPPC-d_{62} (1:1), or DPPC-d_{62} bilayers, spectra for different pressures were obtained at $65\,^\circ\mathrm{C}$. Samples containing POPC-d_{31}, POPC-d_{31}/DPPC (1:1), POPC/DPPC-d_{62} (1:1), or DPPC-d_{62} bilayers were also studied at $65\,^\circ\mathrm{C}$.

Samples contained 30–50 mg of lipid. All binary lipid mixtures were approximately equimolar (± 3 mol %). For binary mixture samples, lipids were weighed and then dissolved in redistilled ethanol. The solvent was then removed by rotary evaporation at a temperature above the expected hydrated bilayer main transition temperature. Binary mixtures were then further dried by pumping overnight using a system equipped with a liquid nitrogen trap. Single-component samples were not dissolved but were evacuated overnight to ensure dryness. Dried samples were hydrated by adding $\sim 200\ \mu$l of potassium phosphate monobasic buffer (pH=6.9–7) to the flask containing dried lipid and rotating the flask for approximately 30 min in a bath warmed to above the main transition temperature for the sample bilayers. Following suspension, samples were gently centrifuged for no more than 45 s to reduce the suspension volume slightly and then transferred to a flexible polyethylene container which was closed by heat sealing.

Deuterium NMR spectra were obtained using a 3.5 T superconducting magnet in conjunction with a locally built spectrometer and variable pressure probe [9]. The coil and tube containing a given sample suspension were enclosed within a beryllium-copper cell which was pressurized with hydraulic oil (AW ISO grade 32). A Bourdon tube gauge calibrated against a dead weight gauge was used to measure pressure in the cell.

Spectra were acquired using a quadrupole echo sequence [10]. The $\pi/2$ pulses were 2.5–4 μs long. The separation between $\pi/2$ pulses in the quadrupole echo sequence was 35 μs. Oversampling [11] was used to obtain effective dwell times of 4 μs. For most samples, between 7000 and 10 000 transients were averaged to obtain the free induction decays that were Fourier transformed to obtain sample spectra.

For deuterons attached to bonds undergoing fast, axially symmetric reorientation, the orientational order parameter is

$$S_{CD}=\tfrac{1}{2}\langle 3\cos^2\theta_{CD}-1\rangle, \tag{1}$$

where θ_{CD} is the angle between the carbon-deuterium bond and the rotational axis of the molecule. The average is over accessible chain conformations. For multilamellar vesicles, the bilayer normal can assume all orientations and the resulting spectrum is a Pake double with prominent edges arising from lipids reorienting about bilayer normals perpendicular to the applied magnetic field. For deuterons with a given orientational order parameter, the prominent edges of the Pake doublet are split by

$$\Delta\nu=\frac{3}{4}\frac{e^2qQ}{h}S_{CD}, \tag{2}$$

where $e^2qQ/h=167$ kHz is the quadrupole coupling constant for carbon-deuterium bonds.

For deuterons with fully deuterated chains, the observed spectrum is a superposition of Pake doublets. In the liquid crystalline phase, order parameters are highest for deuterons at the headgroup end of the chain and decrease with increasing proximity to the bilayer center where the amplitude of reorientation is greater. The resulting dependence of orientational order parameter on position along the chain gives rise to an orientational order parameter profile characterized by a plateau region corresponding to deuterons near the headgroup end of the chain with similar order parameters.

Quadrupole splittings for specific positions along the chain can be extracted from the unoriented sample spectrum by transforming it to the oriented sample spectrum. Methods that have been developed to facilitate this transformation are referred to as ‘‘de-Pake-ing’’ algorithms [12–15]. For this work, spectra corresponding to the oriented sample were obtained using an algorithm based on the method described by McCabe and Wassal [16]. The average orientational order parameter for all methylene deuterons on a chain was obtained from the average splitting

$$\langle \nu \rangle = \frac{\int \nu f_D(\nu)\, d\nu}{\int f_D(\nu)\, d\nu}, \tag{3}$$

where $f_D(\nu)$ is the spectrum corresponding to a single orientation obtained by applying the de-Pake-ing transformation to the unoriented spectrum. The integral covers that portion of the spectrum containing signal from methylene chain deuterons. The approximate dependence of orientational order on position along a perdeuterated acyl chain, known as a smoothed orientational order parameter profile, can also be extracted from the spectrum corresponding to an oriented sample [17,18]. For the work reported here, orientational order parameter profiles were approximated from spectra corresponding to oriented samples by integrating over the spectral features corresponding to the methylene deuterons and then plotting frequency versus normalized, integrated intensity. Each profile was calculated from oriented-sample spectra obtained using both the Wassal and McCabe fast Fourier transform algorithm [16] and the non-negative least squares de-Pake-ing algorithm described by Whittal and co-workers [15]. Differences in the baselines produced by the two de-Pake-ing algorithms were reflected in details for resulting profiles. As long as comparisons were based on profiles obtained in the same way, however, the effect of pressure on the approximate profile for a given mixture component was not sensitive to the choice of de-Pake-ing algorithm.

III. RESULTS AND DISCUSSION

The lipid pairs and specific observation temperatures were chosen to facilitate particular comparisons. The main transition temperatures for POPC and DMPC differ by approximately 26 °C. Complete deuteration of saturated acyl chains lowers the main transition temperatures by 2–4 °C. The observation temperature for this system, 45 °C, was chosen to ensure that samples remained in the liquid crystalline phase up to pressures of at least 140 MPa. In this pair of lipids, the component with the higher melting temperature was a phosphatidylcholine with two 14-carbon saturated chains, DMPC. The component with the lower transition temperature, POPC, had one 16-carbon saturated chain and one 18-carbon chain with one double bond.

For the bilayers containing DMPC and/or DPPC, which has two 16-carbon saturated chains, the observation temperature was 65 °C. This was chosen to be well above the main transition temperature 41 °C for DPPC bilayers at ambient pressure. In this lipid pair, DMPC was the shorter component as well as the component with the lower single-component main transition temperature. The third lipid pair was POPC and DPPC. The observation temperature for this set of samples was also 65 °C. For all of the binary mixtures studied, the upper boundary of gel/liquid crystal coexistence remained below the measurement temperature for all pressures used.

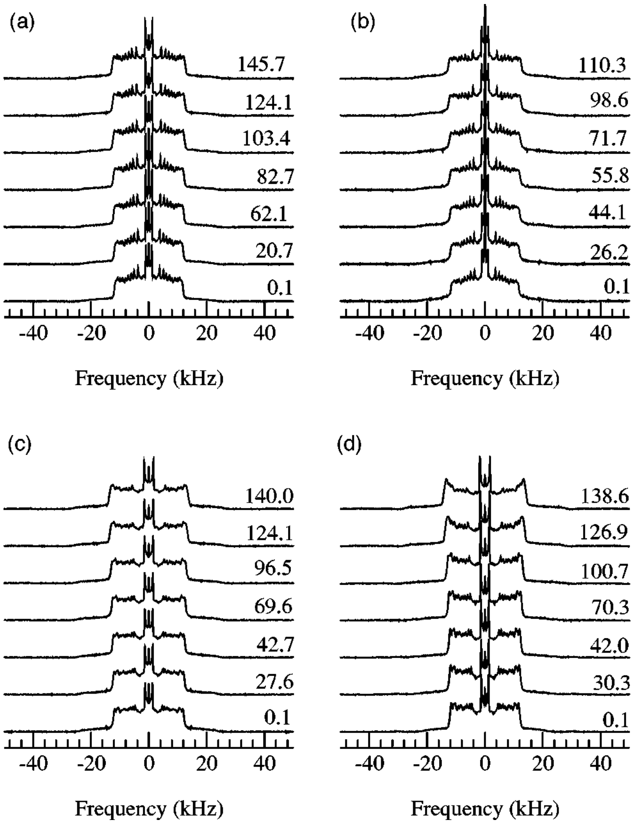

FIG. 1. Deuterium NMR spectra of (a) POPC-d_{31} (b) POPC-d_{31}/DMPC (1:1), (c) POPC/DMPC-d_{54} (1:1), and (d) DMPC-d_{54} at 45 °C and selected pressures up to ~145 MPa. Spectra are labeled by pressure in MPa.

Figure 1 shows spectra of POPC-d_{31}, POPC-d_{31}/DMPC, POPC/DMPC-d_{54} and DMPC-d_{54} at 45 °C and selected pressures. Similar series of spectra were obtained at 65 °C for bilayers of DMPC-d_{54}, DPPC-d_{62}, and 1:1 mixtures of DMPC with DPPC, with each component deuterated in turn and for bilayers of POPC-d_{31}, DPPC-d_{62}, and 1:1 mixtures of POPC with DPPC, with each component deuterated in turn. For each of the samples studied, the increase of quadrupole splittings with pressure reflects the increase, with pressure, of orientational order for all saturated chain segments in a sample.

To facilitate comparison of the chain order pressure dependences, all of the powder spectra obtained were transformed to yield the corresponding oriented sample spectrum from which individual quadrupole splittings could be more easily extracted. Representative de-Paked spectra for POPC-d_{31}, POPC-d_{31}/DMPC, POPC/DMPC-d_{54} and DMPC-d_{54} at 45 °C are shown in Fig. 2. The distribution of splittings in the de-Paked spectra are characteristic of the dependence of orientational order on position along unsaturated chains in phospholipid bilayers. The prominent doublet having the largest splitting arises from deuterons near the headgroup end of the chain where motions are most constrained. Orientational order changes slowly with position along this portion of the chain. In a plot of orientational order versus position along the chain, this gives rise to a characteristic plateau region. Orientational order, and thus doublet splitting, de-

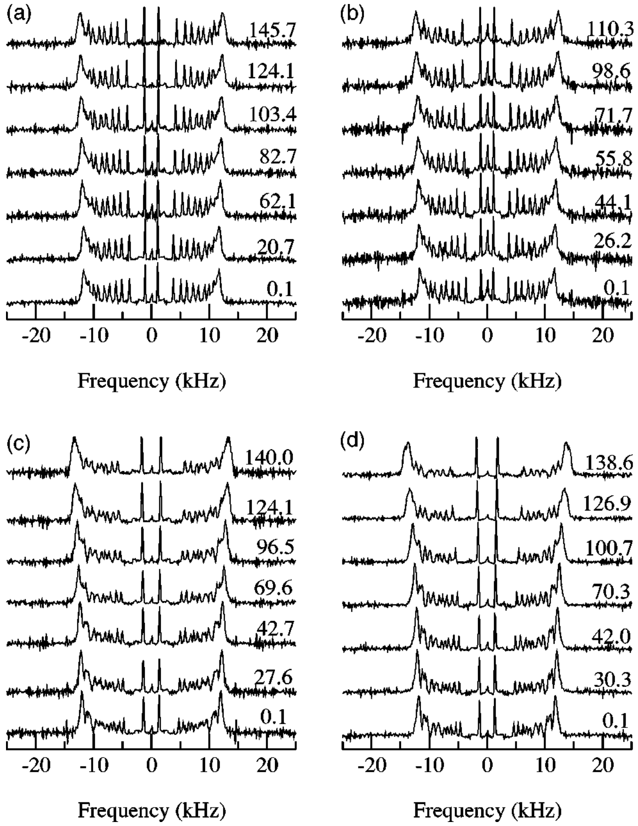

FIG. 2. Spectra corresponding to oriented samples obtained by de-Pake-ing of powder spectra for (a) POPC-d_{31} (b) POPC-d_{31}/DMPC (1:1), (c) POPC/DMPC-d_{54} (1:1), and (d) DMPC-d_{54} at 45 °C and selected pressures up to ~145 Mpa. Spectra are labeled by pressure in MPa.

creases with increasing proximity to the bilayer center where chain motion amplitudes are greater. For this region of the spectrum, doublets from individual chain segments can be resolved. For the disaturated lipids DMPC-d_{54} and DPPC-d_{62}, the splittings for some corresponding segments on the *sn*-1 and *sn*-2 chains can be slightly different and may be resolvable. The doublet with the smallest splitting arises from the methyl group at the chain end. The splitting for this doublet reflects additional averaging of the quadrupole interaction by fast rotation about the methyl group symmetry axis.

Weighted average splittings for the deuterated chains in each sample were extracted from the de-Paked spectra using Eq. (3). The relationship described by Eq. (2) was then used to obtain the average orientational order parameter for the deuterated chains in each sample at each measurement pressure. Figure 3 shows the pressure dependence of average saturated chain orientational order for observations of samples containing POPC and/or DMPC at 45 °C. The lines show linear fits to each set of observations. Observations for samples containing DMPC and/or DPPC and for samples containing POPC and/or DPPC at 65 °C were similarly analyzed. For all of the samples studied, average orientational order of the deuterium-labeled saturated chains increased linearly with applied hydrostatic pressure. The scatter in the POPC-d_{31}/DMPC data reflects noise apparent in the corresponding spectra [Fig. 2(b)] due to the small size of this particular sample. Scatter for other samples was reduced by the averaging of larger numbers of transients during spectral acquisition.

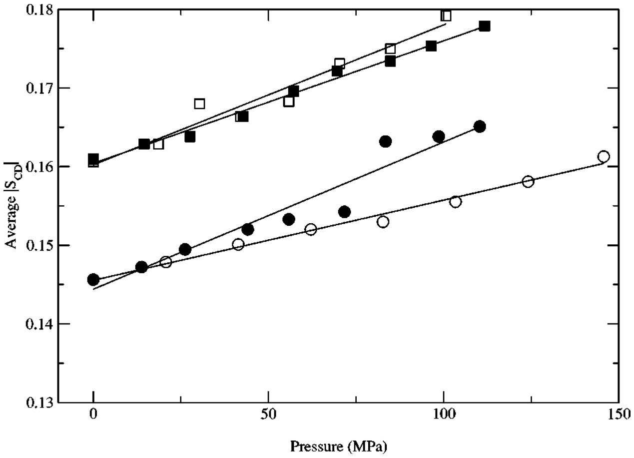

FIG. 3. Pressure dependence of average saturated chain orientational order for (○) POPC-d_{31}, (●) POPC-d_{31}/DMPC (1:1), (■) POPC/DMPC-d_{54} (1:1), and (□) DMPC-d_{54} at 45 °C.

Figure 4 summarizes slopes of the fits to average order parameter versus pressure for the four samples observed at 45 °C and the eight samples observed at 65 °C. For all of the

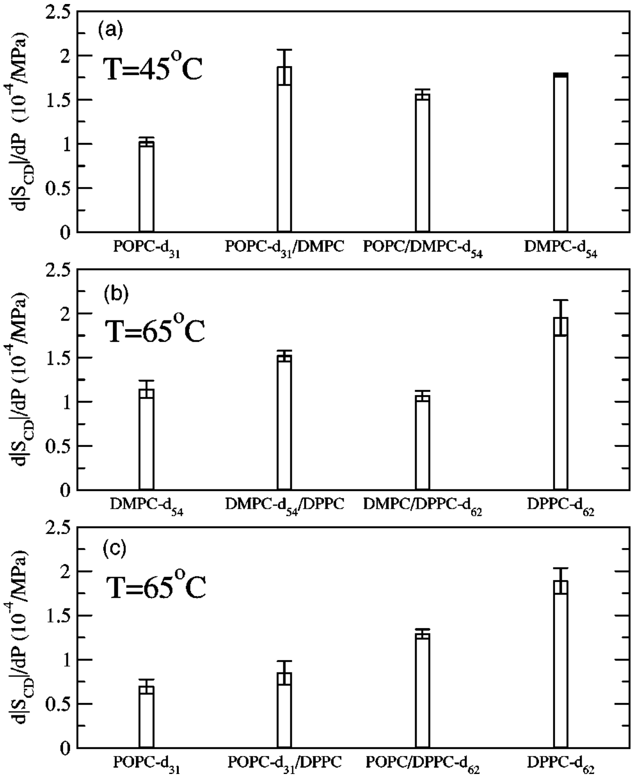

FIG. 4. Slopes of average methylene deuteron orientational order parameter vs pressure for (a) POPC-d_{31}, POPC-d_{31}/DMPC (1:1), POPC/DMPC-d_{54} (1:1), and DMPC-d_{54} at 45 °C; (b) DMPC-d_{54}, DMPC-d_{54}/DPPC (1:1), DMPC/DPPC-d_{62} (1:1), and DPPC-d_{62} at 65 °C; and (c) POPC-d_{31}, POPC-d_{31}/DPPC (1:1), POPC/DPPC-d_{62} (1:1), and DPPC-d_{62} at 65 °C.

samples studied, the slopes fall between 1×10^{-4} MPa^{-1} and 2×10^{-4} MPa^{-1}. Whitmore and Whitehead [8] reported similar slopes from a self-consistent mean field calculation of DPPE average orientational order parameter versus pressure.

The observations for single-component bilayers shown in Fig. 4 illustrate the way in which the response of chain orientational order to pressure depends on the separation between measurement temperature and the ambient-pressure main transition temperature for bilayers of a given lipid. Taking into account the effect of chain deuteration on the main transition temperature for single-component bilayers, both POPC-d_{31} at 45 °C and DMPC-d_{54} at 65 °C are about 45° above their ambient-pressure transition temperatures. Both display a similarly small response of orientational order to pressure at these temperatures. At 65 °C, POPC-d_{31} is furthest from its ambient pressure transition temperature and displays the smallest sensitivity to pressure. Alternately, DMPC-d_{54} at 45 °C and DPPC-d_{62} at 65 °C are about 25° and 28°, respectively, above their ambient-pressure main transition temperatures. For both lipids, the closer proximity to the corresponding ambient-pressure transition temperature increases the sensitivity of orientational order to applied pressure. The difference between the DMPC-d_{54} responses at 45 °C and 65 °C is particularly striking but it is interesting that the DMPC-d_{54} response is similar to those of both DPPC, with two longer saturated chains, and POPC, with one longer saturated and one longer unsaturated chain, for corresponding differences between the measurement temperature and the ambient-pressure transition. As noted above, Driscoll *et al.* [7] found that the slope of order parameter versus pressure for the DPPC methyl group decreased with increasing temperature and self-consistent mean field calculations of DPPE chain order showed a similar decrease in the slope of average orientational order parameter versus pressure with increasing temperature [8].

Figure 4 also shows the rate at which pressure orders the saturated chains of the deuterated components in the two binary mixtures at 45 °C and the four binary mixtures at 65 °C. Mixing with DMPC significantly amplifies the response of POPC-d_{31} to pressure at 45 °C and significantly reduces the response of DPPC-d_{62} to pressure at 65 °C. The response of DMPC-d_{54} to pressure is more modestly reduced by mixing with POPC at 45 °C and more modestly increased by mixing with DPPC at 65 °C. The response of DPPC-d_{62} to pressure at 65 °C is substantially reduced by mixing with POPC. The response of POPC-d_{31} to pressure at 65 °C is slightly increased by mixing with DPPC.

The response of chain orientational order to applied hydrostatic pressure in the liquid crystalline phase reflects the soft and anisotropic nature of the bilayer. In effect, the bilayer is slightly more susceptible to a reduction in area per lipid than to a reduction in bilayer thickness. The reduction in overall bilayer volume in response to the application of pressure is thus dominated by a reduction in chain area which, in turn, is reflected by an increase in chain order.

In a mixture of two lipids with very different ambient-pressure main transition temperatures, the responses of the two components must be consistent both with the uniformity of pressure throughout the bilayer and with interactions between the chains of a given lipid species and its surrounding bilayer environment. One factor that might influence average orientational order of a particular chain is the difference between its average extension and the average extension of chains surrounding it. A number of authors have argued that the contribution to chain extension away from the bilayer surface by a given segment of saturated chain takes the form $\langle l\rangle = a + b|S_{CD}|$ where a and b are constants [19–23]. For the purposes of this discussion, it is sufficient to consider the simple model introduced by Schindler and Seelig [19] which assumes that rotations about carbon-carbon bonds of the acyl chain allow each segment to assume one of three possible orientations with respect to the bilayer normal. In this approximation, the extension of a chain consisting of n segments is

$$\langle L\rangle \approx l_0\left[\frac{n}{2} + \sum |S_{CD}|\right], \tag{4}$$

where $l_0 = 0.125$ nm and the sum is over the n segments. This can be rewritten in terms of the average orientational order parameter for chain segments as

$$\langle L\rangle \approx n l_0\left[\tfrac{1}{2} + \langle |S_{CD}|\rangle\right]. \tag{5}$$

Figure 5 shows the pressure dependence of chain extensions calculated in this way for samples containing POPC and DMPC at 45 °C and the samples containing DMPC and DPPC at 65 °C. The difference between the average extensions of the saturated chains for DMPC-d_{54} and DPPC-d_{62} in the mixture at 65 °C is about 0.16 nm at ambient pressure. In the mixture, DMPC orders more easily with pressure than DPPC but that ordering is applied to 12 segments in DMPC as opposed to 14 segments for DPPC. The result is that the extensions of DMPC and DPPC tend to track each other and even converge as pressure is increased. The responses of DMPC and DPPC to pressure appear to adjust so as to maintain their difference in chain orientational order as pressure is increased.

The situation for the mixture of POPC and DMPC appears to be rather different and its interpretation is somewhat complicated by the lack of order parameter data for the unsaturated chain. For this mixture, the component with the lower ambient-temperature main transition temperature has longer chains, one of which contains a double bond. The average extension of the POPC saturated chain in the mixture at ambient pressure is about 0.14 nm longer than that of the DMPC chains. The POPC saturated chain is two segments longer than the DMPC chains so its higher rate of ordering with increasing pressure in the mixture implies that the difference in extension between the POPC saturated chain and the DMPC saturated chains grows slightly with increasing pressure. Taken together, the two sets of data shown in Fig. 5 indicate that the differences in saturated chain extension for the two components of these binary mixtures are dominated by the numbers of segments in each saturated chain and that the relative responses of chain order to pressure for the two components in a binary mixture are

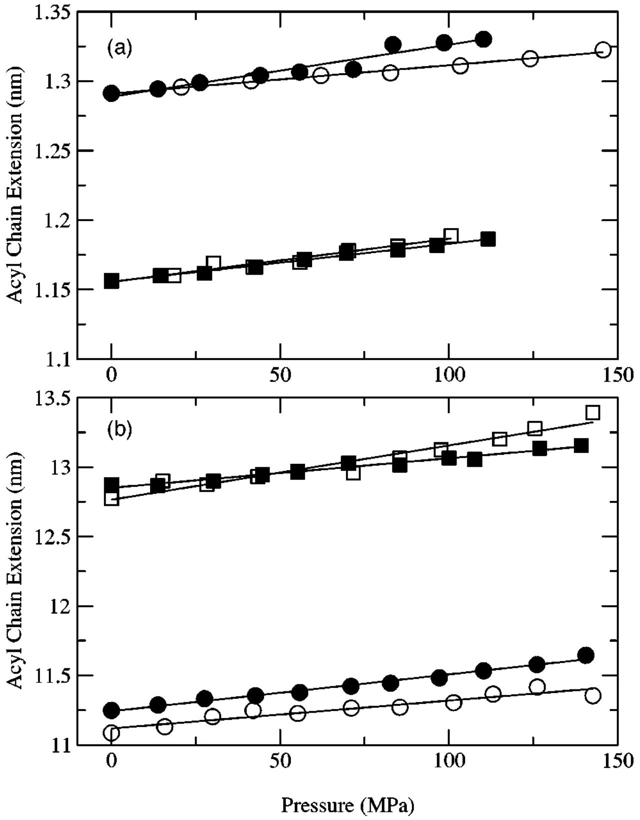

FIG. 5. (a) Pressure dependence of saturated chain extension for (○) POPC-d_{31}, (●) POPC-d_{31}/DMPC (1:1), (■) POPC/DMPC-d_{54} (1:1), and (□) DMPC-d_{54} at 45 °C. Pressure dependence of saturated chain extension for (○) DMPC-d_{54}, (●) DMPC-d_{54}/DPPC (1:1), (■) DMPC/DPPC-d_{62} (1:1), and (□) DPPC-d_{62} at 65 °C.

not related in a simple way to the mismatch in their chain extensions.

While the observations summarized in Figs. 4 and 5 demonstrate that mixing with another lipid component modifies the response of chain order to pressure for a given lipid component, they do not provide a clear indication of how the responses of the two components in binary mixture might be coupled. In order to investigate how the response to pressure is distributed along the chain, and thus hopefully to gain some more insight into the coupling of component responses in the binary mixture, spectra corresponding to oriented samples, obtained by the de-Pake-ing transformation, were compared for ambient pressure and high pressure.

De-Paked spectra for different pressures were first compared by scaling splittings for high-pressure spectra such that their maximum splittings matched those of the corresponding ambient-pressure spectra. This comparison of ambient-pressure and scaled high-pressure spectra (not shown) demonstrated that orientational order parameters for different positions along the acyl chains did not scale uniformly. In general, the relative increase in order parameter with increasing pressure was greater for methylene deuterons having smaller splittings than for those corresponding to the plateau portion of the orientational order parameter profile. As a result, the difference between the highest and lowest methylene orientational order parameters along the chain was roughly conserved as pressure was raised.

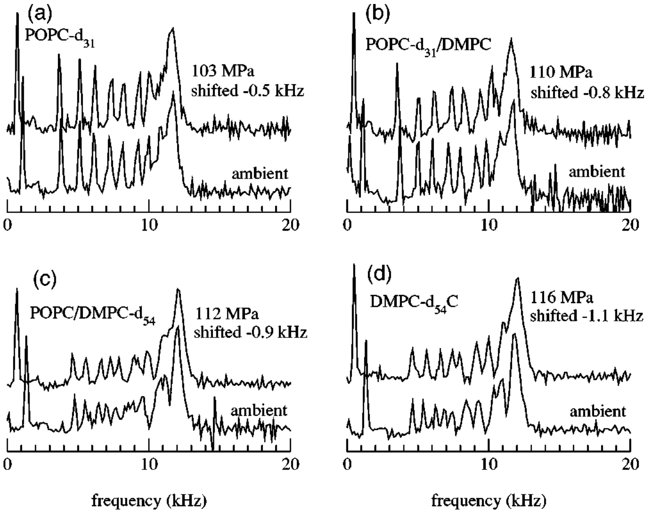

FIG. 6. Right halves of de-Paked spectra for ambient pressure and ~110 MPa for (a) POPC-d_{31}, (b) POPC-d_{31}/DMPC (1:1), (c) POPC/DMPC-d_{54} (1:1), and (d) DMPC-d_{54} at 45 °C. The high-pressure spectra have been shifted to the left by (a) 0.5 kHz, (b) 0.8 kHz, (c) 0.9 kHz, and (d) 1.1 kHz in order to align the upper edges of the spectral features arising from the saturated chain plateau deuterons.

Because spittings were not found to scale in a simple way with pressure, alternate comparisons were considered. Figure 6 shows right halves of de-Paked spectra at ambient pressure and roughly equivalent high pressures for POPC-d_{31}, POPC-d_{31}/DMPC (1:1), POPC/DMPC-$d_5$4 (1:1), and DMPC-$d_5$4 at 45 °C. High-pressure spectra have been shifted to the left so as to align the upper edges of the spectral features arising from plateau deuterons at each pressure. As pointed out by Nagle [21], orientational order in the plateau region of the chain is more reliably correlated with area per lipid, and thus with overall chain packing, than is the average orientational order over the entire chain. The amount by which the high-pressure spectrum must be shifted to align the upper edge of the plateau with the corresponding feature at ambient pressure is thus an indication of the extent to which the area per saturated chain has been reduced by the application of pressure. In Fig. 6, the shifts for single-component bilayers of POPC-d_{31} and DMPC-d_{54} are the smallest and largest, respectively, for that lipid pair. This is consistent with the measurement temperature being further from the ambient-pressure transition temperature of POPC than from that of DMPC. The shifts for each deuterated component in the binary mixture fall between the shifts for the single-component bilayers and are similar in magnitude.

As noted above, the high-pressure spectra for a given lipid component in Fig. 6 cannot be related to the corresponding ambient-pressure spectrum through a uniform scaling of the splittings. Indeed, it is apparent from Fig. 6 that the range of methylene splittings is approximately conserved as pressure is raised and chain order increases. The methyl group splitting, on the other hand, does not increase by a corresponding amount. Comparisons of the high- and low-pressure spectra in Fig. 6 show that even though the smallest and largest methylene splittings are increased by similar amounts with the application of pressure, the splittings corresponding to methylene groups near the center of the chain increase more with pressure than those at either end of the chain. This is most apparent in the DMPC-d_{54} spectra [Figs. 6(c) and 6(d)] but can also be seen in the POPC-d_{31} spectra. In terms of the orientational order parameter profile, this corresponds to a slight flattening of the plateau with increasing pressure.

The relationships between the ambient-pressure and high-pressure spectra for the POPC/DPPC and DMPC/DPPC systems at 65 °C are similar to those seen in Fig. 6. For POPC-d_{31} and DPPC-d_{62}, the upper edges of the plateau features for ~140 MPa can be aligned with the corresponding ambient pressure features by shifts of 0.7 kHz and 1.3 kHz, respectively. In both of the POPC/DPPC mixtures, the high-pressure spectrum for the deuterated component in the binary mixture must be shifted by 1.0 kHz in order to align the edge of the plateau feature with the corresponding ambient-pressure feature. For DMPC-d_{54} and DPPC-d_{62}, the upper edges of the plateau features for ~140 MPa can be aligned with the corresponding ambient-pressure features by shifts of 0.8 kHz and 1.3 kHz, respectively. The high-pressure spectra for DMPC-d_{54} and DPPC-d_{62} in the DMPC/DPPC mixtures must be shifted by 1.1 kHz and 1.0 kHz, respectively, to align the edge of the plateau feature with the corresponding ambient-pressure feature. The ranges of methylene splittings for a given component are still approximately conserved as pressure is raised but the alignment is not quite as close as is seen in Fig. 6 for the POPC/DMPC system at 45 °C. Examination of data presented Driscoll *et al.* [7] shows that the range of DPPC methylene order parameters observed in their work also remained roughly constant up to ~150 MPa.

Orientational order parameter profiles provide a convenient way in which to compare the responses to pressure of the lipid components in these systems. Driscoll *et al.* [7] constructed orientational order parameter profiles for resolvable doublets using DPPC-d_{62} powder spectra at 75 °C for ambient pressure, 60 MPa and 120 MPa. Methylene deuterons beyond the plateau showed similar increases in order with applied pressure. Because all plateau deuterons were assigned the same order, this approach would not be expected to show small changes in the shape of the order parameter profile with pressure.

Because the pressure-induced changes in splitting are small, we chose to extract approximations of the order parameter profiles from de-Paked spectra using a transformation that did not depend on detailed assignment of spectral intensity to specific chain segments. This approach is similar to other methods for extraction of smoothed orientational order parameter profiles [17,18] but yields a continuous plot of splitting versus normalized position along the chain. The de-Paked spectra were transformed by first integrating from the lower edge of the methylene doublet having the smallest splitting to the upper edge of the plateau feature. The resulting integral was then normalized and inverted to yield a plot of frequency versus normalized integrated intensity along the methylene region of the chain. Because the normalized integral of intensity can be directly related to position along the chain, the shape of the resulting continuous curve closely approximates the discrete smoothed order parameter profile that would be extracted from the corresponding spectrum. The methyl group is excluded from this analysis because of its different intensity per segment.

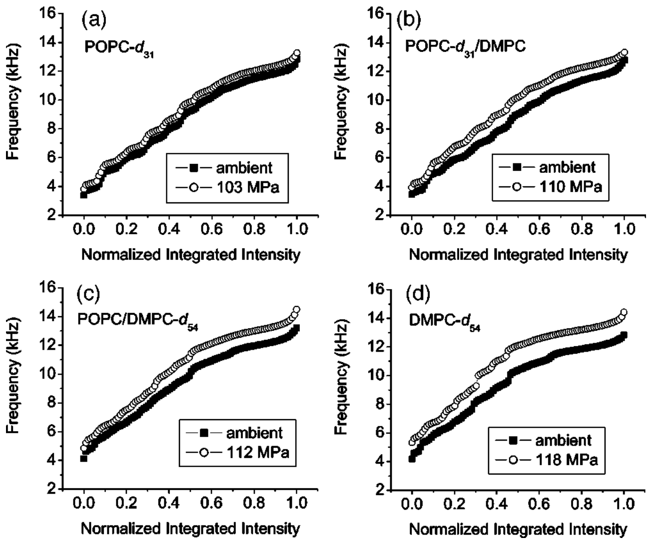

FIG. 7. Frequency vs normalized integrated intensity across the methylene region of the de-Paked spectra at ambient pressure (solid symbol) and ~110 MPa (open symbol) for (a) POPC-d_{31}, (b) POPC-d_{31}/DMPC (1:1), (c) POPC/DMPC-d_{54} (1:1), and (d) DMPC-d_{54} at 45 °C.

Figures 7, 8, and 9 show ambient-pressure and high-pressure plots of frequency versus normalized integrated intensity for deuterated chains in the POPC/DMPC system at 45 °C, the DMPC/DPPC system at 65 °C, and the POPC/DPPC system at 65 °C, respectively. These approximate orientational order parameter profiles were obtained from spec-

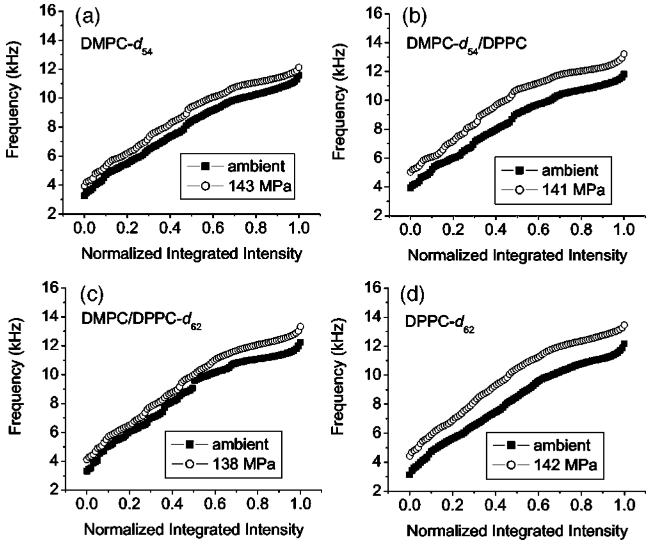

FIG. 8. Frequency vs normalized integrated intensity across the methylene region of the de-Paked spectra at ambient pressure (solid symbol) and ~140 MPa (open symbol) for (a) DMPC-d_{54}, (b) DMPC-d_{54}/DPPC (1:1), (c) DMPC/DPPC-d_{62} (1:1), and (d) DPPC-d_{62} at 65 °C.

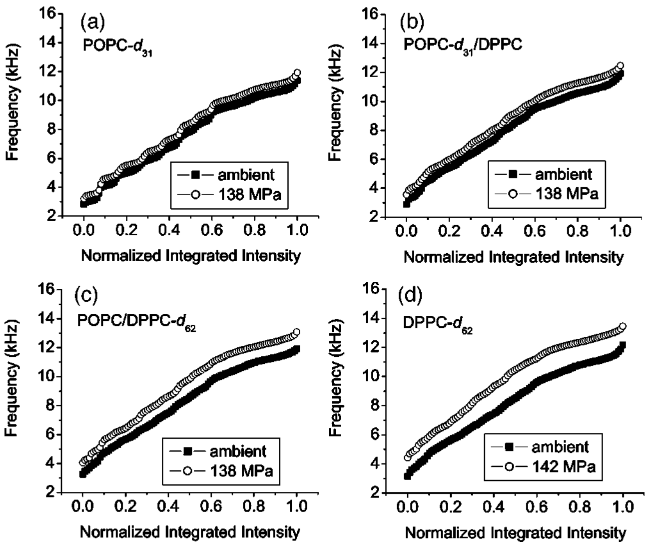

FIG. 9. Frequency vs normalized integrated intensity across the methylene region of the de-Paked spectra at ambient pressure (open symbol) and ~140 MPa (solid symbol) for (a) POPC-d_{31}, (b) POPC-d_{31}/DPPC (1:1), (c) POPC/DPPC-d_{62} (1:1), and (d) DPPC-d_{62} at 65 °C.

tra that were de-Paked using a non-negative least squares algorithm [15]. Profiles obtained using the Wassall and McCabe fast Fourier transform algorithm [16] displayed similar effects of pressure on approximate orientational order parameter profile for a given bilayer component.

Figure 7 shows that for single-component bilayers, the application of pressure has a substantially greater effect on the plateau order for DMPC-d_{54} than for POPC-d_{31}. For DMPC-d_{54} and for both deuterated lipids in the binary mixtures, comparison of the ambient- and high-pressure profiles illustrates the larger effect of pressure on chain order near the midpoint of the chain than at either end. As inferred from the comparison of the de-Paked spectra, the shift of the profiles for the two deuterated components in the binary mixture are similar to each other and fall between the shifts observed for the two single-component bilayers. Figures 8 and 9 display similar trends.

The observation that the plateau regions of both components in a binary mixture display similar increases in orientational order on the application of pressure suggests that the chains respond in such a way as to maintain an approximately constant difference in chain order between the two components as pressure is increased. This provides some insight into the ways in which chains of different components couple in mixed-lipid bilayers.

Comparisons with the results of molecular field calculations have contributed significantly to improved understanding of how the experimentally observed dependence of orientational order on segment position along bilayer acyl chains arises from interactions between neighboring molecules. The calculation of bilayer orientational order parameter profiles from molecular field theory was introduced by Marčelja [24]. Various orientational order parameter calculations, many based on extensions of the molecular field approach, have since been presented [25–32]. The pressure dependence of bilayer chain orientational order has recently been investigated using a self-consistent mean field approach [8] and the slopes of average order versus pressure are in general agreement with the single-component bilayer observations reported here.

The effect of applied pressure on the orientational order parameter profile shape provides some information on how pressure-induced ordering is distributed along the saturated chain and represents an additional aspect of bilayer response to applied pressure that could be addressed theoretically. The difference between the response of a given lipid to pressure in a single-component bilayer and its response in a binary mixture and the relationship between the responses to pressure of the two components in a binary mixture both illustrate the way in which conformational behavior of a flexible bilayer component is determined by collective bilayer characteristics. This suggests that pressure dependence of chain order in mixed bilayers might be an interesting direction in which to extend bilayer studies by self-consistent mean field calculations.

IV. CONCLUSIONS

By comparing the response of particular lipids alone and in binary mixtures to perturbation by application of pressure, it has been possible to gain a number of insights into some factors influencing chain orientational order in single-component bilayers and binary mixtures of lipids. For bilayers containing a single lipid species, the isothermal response of chain orientational order to pressure decreased with increasing difference between measurement temperature and that lipid's ambient-pressure gel-to-liquid-crystalline phase transition temperature. In equimolar binary mixtures of lipids with different ambient-pressure transition temperatures, the ordering of each lipid component displayed a distinct dependence on pressure. The response to pressure of each lipid in the mixture also differed from that displayed in the corresponding single-component system. These observations demonstrate that the response to pressure of a given lipid is determined by the collective properties of the entire bilayer, which effectively determine an ordering potential, and not just on the intrinsic properties of a given lipid. In the equimolar mixtures studied here, the ordering in the saturated chain plateau region for either component was roughly intermediate between the plateau region ordering displayed by either component individually. Aside from a slightly greater response near the middle region of the chain, the ordering of a given saturated chain with pressure appears to be approximately uniform over its methylene region. The behavior described here provides a number of points against which theoretical models of pressure-induced bilayer chain ordering might be tested.

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[1] D.A. Driscoll, J. Jonas, and A. Jonas, Chem. Phys. Lipids **58**, 97 (1991).
[2] B.B. Bonev and M.R. Morrow, Phys. Rev. E **55**, 5825 (1997).
[3] H. Ichimori, T. Hata, H. Matsuki, and S. Kaneshina, Biochim. Biophys. Acta **1414**, 165 (1998).
[4] B.B. Bonev and M.R. Morrow, Can. J. Chem. **76**, 1512 (1998).
[5] B.B. Bonev and M.R. Morrow, Biophys. J. **70**, 2727 (1996).
[6] L.F. Braganza and D.L. Worcester, Biochemistry **25**, 2591 (1986).
[7] D.A. Driscoll, S. Samarasinghe, S. Adamy, J. Jonas, and A. Jonas, Biochemistry **30**, 3322 (1991).
[8] M.D. Whitmore and J.P. Whitehead, Can. J. Phys. **76**, 883 (1998).
[9] B.B. Bonev and M.R. Morrow, Rev. Sci. Instrum. **68**, 1827 (1997).
[10] J.H. Davis, K.R. Jeffrey, M. Bloom, M.I. Valic, and T.P. Higgs, Chem. Phys. Lett. **42**, 390 (1976).
[11] R.S. Prosser, J.H. Davis, F.W. Dahlquist, and M.A. Lindorfer, Biochemistry **30**, 4687 (1991).
[12] M. Bloom, J.H. Davis, and A.L. MacKay, Chem. Phys. Lett. **80**, 198 (1981).
[13] E. Sternin, M. Bloom, and A.L. MacKay, J. Magn. Reson. (1962-1992) **55**, 274 (1983).
[14] H. Schäfer and E. Sternin, Phys. Canada **53**, 77 (1997).
[15] K.P. Whittall, E. Sternin, M. Bloom, and A.L. MacKay, J. Magn. Reson. (1969-1992) **84**, 64 (1989).
[16] M.A. McCabe and S.R. Wassall, J. Magn. Reson., Ser. B **106**, 80 (1995).
[17] M. Lafleur, B. Fine, E. Sternin, P.R. Cullis, and M. Bloom, Biophys. J. **56**, 1037 (1989).
[18] M. Lafleur, P.R. Cullis, and M. Bloom, Biophys. J. **19**, 55 (1990).
[19] H. Schindler and J. Seelig, Biochemistry **14**, 2283 (1975).
[20] F.A. Nezil and M. Bloom, Biophys. J. **61**, 1176 (1992).
[21] J.F. Nagle, Biophys. J. **64**, 1476 (1993).
[22] L.L. Holte, S.A. Peter, T.M. Sinnwell, and K. Gawrisch, Biophys. J. **68**, 2396 (1995).
[23] B.D. Koenig, H.H. Strey, and K. Gawrisch, Biophys. J. **73**, 1954 (1997).
[24] S. Marčelja, Biochim. Biophys. Acta **367**, 165 (1974).
[25] F. Jähnig, J. Chem. Phys. **70**, 3279 (1979).
[26] K.A. Dill and P.J. Flory, Proc. Natl. Acad. Sci. U.S.A. **77**, 3115 (1980).
[27] D.W.R. Gruen, Biochim. Biophys. Acta **595**, 161 (1980).
[28] J.-P. Meraldi and J. Schlitter, Biochim. Biophys. Acta **645**, 183 (1981).
[29] J.-P. Meraldi and J. Schlitter, Biochim. Biophys. Acta **645**, 193 (1981).
[30] F.A.M. Leermakers and J.M.H.M. Scheutjens, J. Chem. Phys. **89**, 3264 (1988).
[31] F.A.M. Leermakers and J.M.H.M. Scheutjens, J. Chem. Phys. **89**, 6912 (1988).
[32] M.D. Whitmore, J.P. Whitehead, and A. Roberge, Can. J. Phys. **76**, 831 (1998).