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# Composite membrane deformation on the mesoscopic length scale

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The physics of soft materials can be investigated using nuclear spin-lattice relaxation, which depends on the spectral densities of motion in the MHz range. For the first time, NMR relaxation has been used to study influences of the acyl length, polar head groups, a cosurfactant, and cholesterol on the viscoelastic properties of membrane lipids. The results imply the concept of elastic deformation is relevant on lengths ≈ the bilayer thickness and less, involving a broad spectrum of collective modes which contribute to the forces between lipid bilayers.

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The properties of flexible amphiphilic layers composed of phospholipids are of fundamental interest to physicists with regard to their microstructures [1–5], and play a role in key biological functions [6]. These and other fascinating soft materials can be studied using nuclear spin-lattice relaxation (for reviews, see Refs. [7,8]). In this Rapid Communication, we report NMR relaxation data that provide knowledge of the viscoelastic properties of lipid bilayers, including their dependence on composition. The results imply that the concept of elastic deformation of membranes is applicable on the mesoscopic length scale, approaching the molecular dimensions, with quasicoherent modes on the order of the bilayer hydrocarbon thickness and less [9]. We suggest the entire spectrum of fluctuations is important for repulsions due to entropic confinement, including long wavelength undulations as well as higher frequency excitations.

Our development is based on a composite membrane deformation model [10], which explains the combined angular and frequency dependencies of the nuclear spin relaxation rates ($R_{1Z}$) of fluid bilayer lipids. A special feature is that quasicoherent order fluctuations give a relaxation enhancement vis-à-vis simple hydrocarbon fluids. We have postulated [11] that the bilayer interior can be modeled in terms of a director field $\mathbf{n}(\mathbf{r})$. Given the numerous degrees of freedom, the system is treated as a continuous medium, where collective motions are related to the elastic moduli. Use of the equipartition theorem together with integration over the modes gives a characteristic frequency ($\omega$) dependence of the relaxation [12]. The experimentally found $\omega^{-1/2}$ dependence of $R_{1Z}$ suggests that collective fluctuations of the individual segments occur in the MHz range [10]. More generally, one should consider the modes in relation to the interbilayer separation. Figure 1 depicts the types of bilayer deformations for a free membrane. The elastic shape fluctuations are modeled as unconstrained splay, twist, and bend excitations, together with effective axial rotations of the lipids [10]. In the case of splay deformations, the divergence $\nabla \cdot \mathbf{n}(\mathbf{r}) \neq 0$. For twist deformations, the curl is along the local director such that $\mathbf{n}(\mathbf{r}) \cdot \nabla \times \mathbf{n}(\mathbf{r}) \neq 0$. For bend the curl is perpendicular, viz. $\mathbf{n}(\mathbf{r}) \times \nabla \times \mathbf{n}(\mathbf{r}) \neq 0$. Such a continuum picture represents a significant departure from previous molecular theories for lipid bilayer relaxation. The absence of molecular details is both the strength and weakness of the approach.

Now in $^2$H NMR spectroscopy of lipid bilayers (reviewed in [7,8]), the observables are the order parameters $|S_{\mathrm{CD}}^{(i)}|$ as a function of the acyl chain position ($i$), and the corresponding $R_{1Z}^{(i)}$ relaxation rate profile. These manifest a hierarchy of motions which contribute to the dynamical roughness of the bilayer. Using the notation of Ref. [10], the order parameters are $S_{\mathrm{CD}}^{(i)} \equiv 1/2\langle 3\cos^2 \beta_{PD}^{(i)} - 1\rangle$, where $\beta_{PD}^{(i)}$ is the angle between the principal axis system ($P$) of the electric field gradient (EFG), viz. the $i$th C—$^2$H bond, and the macroscopic bilayer normal (director frame, $D$). The order parameters represent the motional amplitudes, whereas the NMR relaxation also includes the motional rates. As a rule the dynamics can involve (i) segmental motions of the flexible phospholipids, (ii) molecular motions, and (iii) collective motions. For disaturated phospholipids in the liquid-crystalline ($L_\alpha$) state, the dependence of the NMR relaxation on temperature and frequency disfavors a noncollective model, involving molecular motions or superimposed internal chain rotations. One is left with collective motions as a source of the relaxation [10,11].

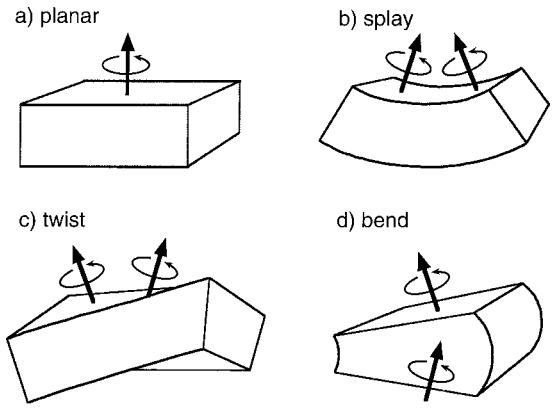


FIG. 1. Excitations of a fluid lipid bilayer within the continuum elastic approximation. (a) Planar bilayer; (b) splay, (c) twist, and (d) bend deformations, together with axial rotations about the local director.

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The spin-lattice relaxation is due to orientational fluctuations of the individual C—$^2$H bonds, which induce transitions between the various Zeeman levels. According to Bloch-Wangsness-Redfield theory, $R_{1Z}$ is given to second order by [8]

$$R_{1Z}=\frac{3}{4}\pi^2\chi_Q^2[J_1(\omega_D)+4J_2(2\omega_D)], \tag{1}$$

where $\chi_Q$ is the static quadrupolar coupling constant. Here $J_m(\omega)=\int G_m(t)\exp(-i\omega t)dt$, where $G_m(t)$ are rank-2 autocorrelation functions of the perturbing Hamiltonian ($m=1,2$), and $\omega_D$ is the deuteron Larmor frequency. Note the EFG fluctuations can encompass rapid local motions of the *static* EFG tensor, e.g., due to *trans-gauche* isomerizations of the acyl chains, as described by a fast order parameter $S_f^{(2)}$. Additional slower motions of the remaining *residual* EFG tensor can also occur, as described by an order parameter $S_s^{(2)}$. The slow motions can arise from quasicoherent fluctuations of a three-dimensional (3D) (splay, twist, and bend) or 2D (splay) nature, together with acyl rotations. These lead to $S_{\mathrm{CD}}=S_f^{(2)}\tilde{S}_{\mathrm{int}}^{(2)}S_s^{(2)}$ where $\tilde{S}_{\mathrm{int}}^{(2)}$ is a geometric factor [10].

The irreducible spectral densities [10] are then

$$J_m(\omega)=J_m^{\mathrm{col}}(\omega)+J_m^{\mathrm{mol}}(\omega)+J_m^{\mathrm{mol\text{-}col}}(\omega), \tag{2}$$

in which the small contribution from segmental motions is neglected. The first term describes collective 3D membrane deformations, and assumes a single elastic constant $K$ for splay ($K_{11}$), twist ($K_{22}$), and bend ($K_{33}$) fluctuations. For a lipid bilayer (smectic-*A*) this is clearly an heuristic approximation, as one expects $K_{11}<K_{22}$, $K_{33}$ for longer wavelengths. However, it is premature to discuss individual NMR active modes in our view. To linear order [12], the contribution from order fluctuations is [11]

$$J_m^{\mathrm{col}}(\omega)=\frac{5}{2}S_{\mathrm{CD}}^2 D\omega^{-(2-d/2)}[|D_{-1m}^{(2)}(\Omega_{DL})|^2 + |D_{1m}^{(2)}(\Omega_{DL})|^2], \tag{3}$$

where the high-frequency cutoff [10,11] is neglected and $m=1,2$. (Inclusion of higher order director fluctuations [13] gives an additional correction for $m=2$.) Here $D_{m'm}^{(2)}(\Omega)$ is a Wigner rotation matrix element, and the Euler angles $\Omega_{DL}$ describe the transformation from the director ($D$) to the laboratory ($L$) frame. The above formula corresponds to the overdamped regime, such that each of the modes relaxes with a single exponential time constant. For 3D director fluctuations, $d=3$ yielding an $\omega^{-1/2}$ frequency dispersion. Following Ref. [12], the viscoelastic constant is $D=3k_BT\sqrt{\eta}/5\pi\sqrt{2K^3}(S_s^{(2)\mathrm{col}})^2$, where $K$ is the effective elastic constant for 3D excitations and $\eta$ is the corresponding viscosity. One can also consider 2D membrane deformations ($d=2$), e.g., smectic undulation waves (splay), resulting in $D\rightarrow D'$ with an $\omega^{-1}$ dependence, which is not observed experimentally at MHz frequencies [11]. The $J_m^{\mathrm{mol}}(\omega)$ term in Eq. (2) corresponds to effective rotations of the flexible lipids, and finally $J_m^{\mathrm{mol\text{-}col}}(\omega)$ to a geometrical cross-term. According to theory [10], the spectral densities scale in closed form with the square of the order parameter $S_{\mathrm{CD}}$ as a characteristic signature, assuming $S_s^{(2)}$ and $\tilde{S}_{\mathrm{int}}^{(2)}$ do not depend appreciably on chain position.

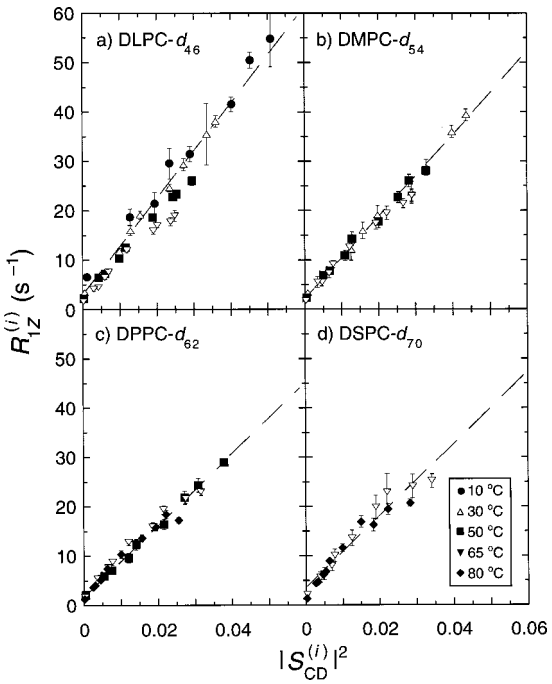


FIG. 2. Square-law functional dependence of spin-lattice relaxation rates $R_{1Z}^{(i)}$ and order parameters $|S_{\mathrm{CD}}^{(i)}|$ along the acyl chains (index $i$) for a homologous series of PCs in the $L_\alpha$ phase, showing influence of the acyl length (bilayer thickness). Data are for unoriented dispersions containing 50 wt. % $H_2O$ (67 mM phosphate buffer, pH 7.0) at 55.4 MHz and various temperatures ($T\geqslant T_m+6$ °C): (a) DLPC-$d_{46}$, (b) DMPC-$d_{54}$, (c) DPPC-$d_{62}$, and (d) DSPC-$d_{70}$, with acyl lengths of $n=12$, 14, 16, and 18 carbons, respectively.

In our experimental NMR relaxation studies, we investigated a series of phospholipids in the $L_\alpha$ state, with the general structure $(\mathrm{RCOO})\mathrm{CH_2}(\mathrm{RCOO})\mathrm{CHCH_2O\text{-}X}$, where R denotes the fatty acyl chains and X the polar head group. The influences of the acyl length (bilayer thickness), lipid polar head groups (interfacial area per molecule), addition of a cosurfactant, and incorporation of cholesterol were studied in terms of the bilayer viscoelastic properties. The effort to obtain these results was substantial, and entailed chemical synthesis and $^2$H-isotopic labeling of phospholipids, solid-state NMR studies at different magnetic field strengths, and testing of theoretical models. For phosphatidylcholine (PC), the polar head group is $\mathrm{PO_3^-CH_2CH_2N^+(CH_3)_3}$. Use of acyl chain-perdeuterated phospholipids ($n=12$ to 18 carbons) allowed us to observe simultaneously the entire hydrocarbon region of the bilayer. As shown in Fig. 2, a square-law functional dependence of the $R_{1Z}^{(i)}$ rates on $|S_{\mathrm{CD}}^{(i)}|$ along the chains describes the data for the homologous PCs for $T\geqslant 6$ °C above the main phase transition temperature ($T_m$). The slopes of Figs. 2(a)–2(d) suggest the bending rigidity $\kappa$ ($\approx K_{11}t$) depends only weakly on temperature in the $L_\alpha$

state, increasing with the bilayer thickness $t$. Our conclusions agree with earlier studies of temperature [14] and chain length [15] effects on the elastic properties of PC bilayers. The fact that a broad range of data can be superimposed in terms of a nearly universal square-law functional dependence is striking.

For bilayers of DMPC-$d_{54}$, we obtain $D=1.02\times10^{-5}\,\mathrm{s}^{1/2}$, and by treating higher order terms [10] we estimate that $K\approx1.2\times10^{-11}\,\mathrm{N}$ and $\eta\approx0.71\,\mathrm{P}$. An heuristic calculation assuming $t\approx4$ nm gives $\kappa\approx K_{11}t\approx0.5\times10^{-19}\,\mathrm{J}$, i.e. $\approx11k_BT$, which implies a fairly soft bilayer in accord with micromechanical measurements ($\kappa$ may be length-scale dependent) [16]. For the other PCs $t$ increases by $\approx1.2$ nm for $C_{12}$ to $C_{18}$ chains [15], giving a $\approx30\%$ calculated increase in $\kappa$. The angular amplitude of the fluctuations of the local director ($N$) relative to the average director ($D$), assuming $|S_s^{(2)\mathrm{col}}|\approx1-3/2\langle\beta_{ND}^2\rangle\approx0.6$, is $\langle\beta_{ND}^2\rangle^{1/2}\approx30°$. Then, from the relation [10,12,13] that $\langle\beta_{ND}^2\rangle\approx k_BTq_c/\pi^2K$, the cutoff wavelength is $\lambda_c=2\pi/q_c\approx8$ Å, less than the bilayer thickness $t$ and close to the lateral lipid dimensions [1]. Clearly, an aspect of our work involves extension of the concept of membrane elasticity to relatively short distances. Using continuum mechanics [9], one can also estimate the area elastic modulus $K_a$ from the relation $\kappa=K_at^2/48$, giving $\approx150\,\mathrm{mJ\,m}^{-2}$, in the range for fluid bilayers [16]. (The individual monolayers are treated as homogenous thin films with zero 2D shear modulus [9].) Clearly, our NMR relaxation approach should be distinguished from previous NMR lineshape studies [17,18], which probe membrane elasticity at lower frequencies. Modeling the lipid bilayer as an ordered fluid thus yields our conjecture that $q$-modes of substantial amplitude influence the relaxation within the MHz regime, with wavelength components on the order of the bilayer thickness and smaller, i.e., the mesoscopic length scale.

Some readers may think it surprising that our NMR results for soft membrane bilayers, involving flexible lipid molecules with many degrees of freedom, can be interpreted using fairly simple concepts drawn from the physics of materials. Within our framework, the membrane lipids are effectively tethered to the aqueous interface via their polar head groups, and the bilayer interior is essentially liquid hydrocarbon. The reason why the $R_{1Z}$ relaxation is governed by collective order fluctuations is that the local segmental motions of the lipids are very fast ($\approx10^{-11}$ s), with spectral densities extending to very high frequencies. Hence relatively slow, quasicoherent order fluctuations provide a frequency-dependent enhancement versus simple $n$-paraffinic liquids, e.g., $n$-hexadecane [8].

We also investigated bilayers containing phosphatidylethanolamine (PE), with the polar head group $PO_3^-CH_2CH_2NH_3^+$. Figure 3 shows the $R_{1Z}^{(i)}$ rates and the $|S_{\mathrm{CD}}^{(i)}|$ values are correlated reasonably well over the entire temperature range by a square-law functional dependence. Yet a completely universal behavior is not observed for the entire series of phospholipids investigated. Rather, compared to DPPC-$d_{62}$ alone, Fig. 3(a), increasing the mole fraction of DPPE-$d_{62}$ yields a progressive reduction in $R_{1Z}^{(i)}$ for a given order parameter $|S_{\mathrm{CD}}^{(i)}|$, Figs. 3(b) and 3(c). Use of the viscoelastic constant for DMPC-$d_{54}$ as a reference [10] leads to $K_{\mathrm{DPPE}}=(D_{\mathrm{DMPC}}/D_{\mathrm{DPPE}})^{2/3}K_{\mathrm{DMPC}}\approx4.0\times10^{-11}\,\mathrm{N}$, about a three-fold *increase* in $K$ due to the presence of PE head groups. Assuming $t\approx5$ nm results in $\kappa\approx43k_BT$, and a correspondingly larger value of the area expansion modulus $K_a$. To explain these observations, we note that for PEs the reduction in the interfacial area per lipid gives an increase in acyl ordering. We propose that a reduction in entanglement of the acyl chains (configurational entropy) yields an increase in bilayer stiffness, and a possibly greater rate of effective axial chain rotations. The result is a weaker repulsive force for PE-containing bilayers, due to quasicoherent elastic modes, with a reduced hydration of the membrane dispersion and a concomitantly smaller interlamellar separation [19]. This would give evidence for a connection between bilayer properties on the mesoscopic scale, as studied by NMR relaxation, and macroscopic properties of the bulk material.

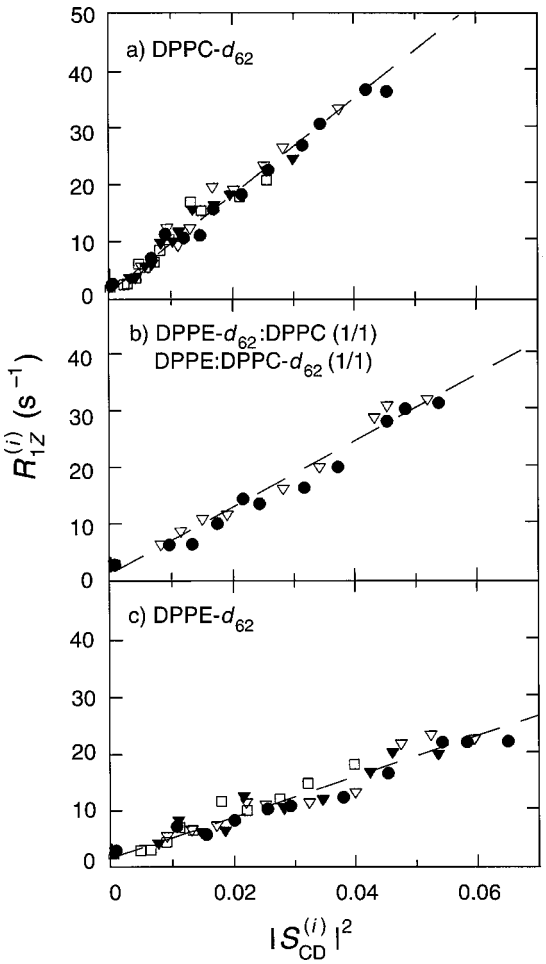


FIG. 3. Dependence of $R_{1Z}^{(i)}$ on square of $|S_{\mathrm{CD}}^{(i)}|$ for phospholipid bilayers in the $L_\alpha$ phase, showing effect of phosphoethanolamine head groups (interfacial area per molecule). Results are shown for unoriented dispersions containing 50 wt.% $H_2O$ (20 mM MOPS buffer, pH 7.1) at 46.1 MHz and various temperatures: (a) DPPC-$d_{62}$, 42–80 °C, (b) DPPE-$d_{62}$: DPPC (1/1) and DPPE:DPPC-$d_{62}$(1/1), 65 °C, and (c) DPPE-$d_{62}$, 60–80 °C.

The physical relevance of our findings is further suggested by the opposite influences of a nonionic surfactant,

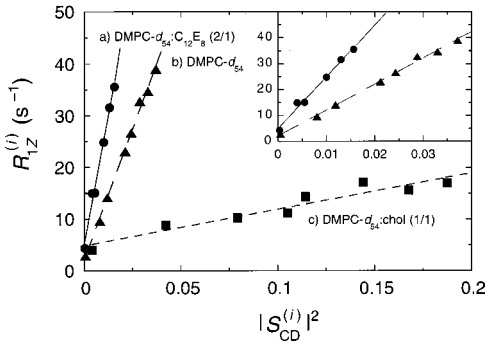


FIG. 4. Square-law dependence of $R_{1Z}^{(i)}$ on $|S_{CD}^{(i)}|^2$ for bilayers of DMPC-$d_{54}$, summarizing influences of a nonionic cosurfactant, $C_{12}E_8$, in the $L_\alpha$ phase, and cholesterol in the liquid-ordered phase. Results are shown for bilayers aligned at $\theta=90°$ containing excess $H_2O$ at 46.1 MHz: (a) DMPC-$d_{54}$:$C_{12}E_8$ (2/1) at 42 °C, (b) DMPC-$d_{54}$ at 40 °C, and (c) DMPC-$d_{54}$:cholesterol (1/1) at 40 °C. The inset shows an expansion of the data for DMPC-$d_{54}$:$C_{12}E_8$ (1/2) vs DMPC-$d_{54}$.

$C_{12}E_8$, and cholesterol, as shown in Fig. 4. Previous studies of macroscopic bending deformations have revealed that a cosurfactant favors a decrease in $\kappa$, whereas cholesterol leads to an increase [2]. The presence of $C_{12}E_8$ in the DMPC-$d_{54}$ bilayer (1/2 molar ratio), Fig. 4(a), yields a larger slope of the square-law plot versus DMPC-$d_{54}$ alone, Fig. 4(b) (cf. also inset), which we interpret as due to softening of the bilayer. We find that $K_{\mathrm{DMPC/C_{12}E_8}}\approx 0.75\times 10^{-11}$ N, nearly a two-fold *decrease*, with $\kappa\approx 6k_BT$. The increased entropic repulsion would result in greater swelling of the membrane dispersion, as found for nonionic surfactants [20]. By contrast, a square-law plot for DMPC-$d_{54}$:cholesterol (1/1) is shown in Fig. 4(c), evincing a reduction in slope compared to DMPC-$d_{54}$ alone. Here we obtain $K_{\mathrm{DMPC/chol}}\approx 9.3\times 10^{-11}$ N, roughly an eight-fold *increase* [21]. Assuming $t\approx 5$ nm gives $\kappa\approx 100k_BT$ for the bending rigidity, a dramatic stiffening. Similar conclusions have been reached from NMR transverse relaxation studies [18]. The fact that bilayer additives influence the $R_{1Z}$ data paralleling earlier results for larger distance scales [2,17] supports our hypothesis [10] that quasicoherent modes are already present on the order of the bilayer thickness and less, and that all modes should be considered unless one can show they are decoupled from the physical properties of interest.

In closing, this work provides striking evidence that membrane deformational fluctuations occur over a wide range of length- and time-scales, which depend on the bilayer lipid composition. Clearly it is of interest to investigate the correspondence to other techniques for studying membrane deformation, involving splay fluctuations at larger wavelengths, and to molecular dynamics simulations. Moreover, these findings may be of relevance to the often debated issue of the nature and functional form of the repulsive forces between amphiphilic layers.

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[1] M. Bloom, E. Evans, and O. G. Mouritsen, Q. Rev. Biophys. **24**, 293 (1991).

[2] E. Sackmann, in *Handbook of Biological Physics*, edited by R. Lipowsky and E. Sackmann (Elsevier, Amsterdam, 1995), Vol. 1, pp. 213–304.

[3] W. Pfeiffer, S. König, J. F. Legrand, T. Bayerl, D. Richter, and E. Sackmann, Europhys. Lett. **23**, 457 (1993).

[4] W. Helfrich, Z. Naturforsch. A **33a**, 305 (1978).

[5] J. N. Israelachvili and H. Wennerström, J. Phys. Chem. **96**, 520 (1992).

[6] M. F. Brown, Chem. Phys. Lipids **73**, 159 (1994).

[7] M. Bloom, C. Morrison, E. Sternin, and J. L. Thewalt, in *Pulsed Magnetic Resonance: NMR, ESR, Optics*, edited by D. M. S. Bagguley (Clarendon Press, Oxford, 1992), pp. 274–316.

[8] M. F. Brown and S. I. Chan, in *Encyclopedia of Nuclear Magnetic Resonance*, edited by D. M. Grant and R. K. Harris (Wiley, New York, 1996), Vol. 2, pp. 871–885.

[9] R. Goetz, G. Gompper, and R. Lipowsky, Phys. Rev. Lett. **82**, 221 (1999).

[10] A. A. Nevzorov, T. P. Trouard, and M. F. Brown, Phys. Rev. E **58**, 2259 (1998).

[11] A. A. Nevzorov and M. F. Brown, J. Chem. Phys. **107**, 10 288 (1997).

[12] P. G. de Gennes and J. Prost, *The Physics of Liquid Crystals* (Clarendon Press, Oxford, 1993).

[13] R. L. Vold, R. R. Vold, and M. Warner, J. Chem. Soc., Faraday Trans. 2 **84**, 997 (1988).

[14] P. Méléard, C. Gerbeaud, T. Pott, L. Fernandez-Puente, I. Bivas, M. D. Mitov, J. Dufourcq, and P. Botherel, Biophys. J. **72**, 2616 (1997).

[15] L. Fernandez-Puente, I. Bivas, M. D. Mitrov, and P. Méléard, Europhys. Lett. **28**, 181 (1994).

[16] W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans, Biophys. J. **79**, 328 (2000).

[17] F. Auguste, P. Barois, L. Fredon, B. Clin, E. J. Dufourc, and A. M. Bellocq, J. Phys. (France) **4**, 2197 (1994).

[18] G. Althoff, N. J. Heaton, G. Gröbner, R. S. Prosser, and G. Kothe, Colloids Surf. A **115**, 31 (1996).

[19] R. P. Rand and V. A. Parsegian, Biochim. Biophys. Acta **988**, 351 (1989).

[20] U. Olsson and H. Wennerström, Adv. Colloid Interface Sci. **49**, 113 (1994).

[21] T. P. Trouard, A. A. Nevzorov, T. M. Alam, C. Job, J. Zajicek, and M. F. Brown, J. Chem. Phys. **110**, 8802 (1999).