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Role of Tilt Order in the Asymmetric Ripple Phase of Phospholipid Bilayers

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We present the electron density map of the asymmetric ripple phase of dilauroylphosphatidylcholine. We find that the *primary* feature characterizing the "asymmetry" of the rippled bilayers is the difference in the bilayer thickness in the two arms, and not the asymmetry of the bilayer height profile as is generally assumed. This difference in the bilayer thickness can be attributed to a mean tilt of the hydrocarbon chains of the lipid molecules along the direction of the ripple wave vector. We propose a Landau theory for this phase which takes into account the anisotropic elastic properties of a bilayer with tilt order.

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The ripple or $P_{\beta'}$ phase is seen in some phospholipids under high hydration. In this phase the lipid bilayers have a one-dimensional height modulation [1]. In the temperature-humidity phase diagram of these lipids the ripple phase is sandwiched between the L_α and the $L_{\beta'}$ phases, which are lamellar phases with flat bilayers. In the L_α phase the hydrocarbon chains of the lipid molecules are molten and the in-plane order is liquidlike. In the lower temperature $L_{\beta'}$ phase the chains are predominantly in the all-*trans* conformation and are tilted with respect to the bilayer normal [2–4].

Most of the structural features of the ripple phase have been obtained from freeze fracture electron microscopy and x-ray diffraction (XRD) experiments. The commonly observed structure is characterized by a two-dimensional oblique unit cell which lacks a mirror plane normal to the ripple wave vector. This is referred to as the asymmetric ripple phase. The wavelength of the ripples is typically in the range of 12-16 nm and the peak-to-peak amplitude is about 2 nm. The modulated bilayers in this phase usually have a sawtoothlike profile, with a larger bilayer thickness in the longer arm of the ripple [5–8]. In principle any one of these two features (namely, a sawtoothlike bilayer shape or different values of the bilayer thickness in the two arms) is sufficient to destroy the mirror plane normal to the ripple wave vector, but in almost all the systems studied so far both these features are present simultaneously.

Various theories of the ripple phase have been proposed [9–14], but none of them accounts for all the experimental observations. In these theories the asymmetry of the rippled bilayers is assumed to be related to an asymmetric bilayer height profile. Lubensky and MacKintosh [12] have proposed a phenomenological Landau theory (the LM model) which brings out the importance of the coupling between the curvature of the bilayer and the divergence of the order parameter $\mathbf{m}$ (Fig. 1). The interbilayer interactions are ignored since the rippled structure is also observed in unilamellar vesicles [15]. The LM model predicts only a symmetric height profile in the ripple phase of bilayers made up of achiral molecules. Further, the chiral version of the LM model predicts an asymmetric height profile with *zero mean tilt.* In the absence of a mean tilt along the ripple wave vector, the bilayer thickness is the same in the two arms of the ripple, both in chiral and achiral models. These predictions are in conflict with the results of XRD experiments on enantiomeric (chiral) and racemic (achiral) samples of dimyristoylphosphatidylcholine [16–18] which show that the ripple shape is asymmetric in chiral as well as in achiral systems. Evidently, factors other than molecular chirality determine the structure of the ripple phase.

The aim of this Letter is to present (i) a phospholipid-water system in which the asymmetry of the ripple phase arises from the difference in the bilayer thickness in the two arms of the ripple, and *not from a sawtoothlike bilayer height profile,* (ii) a phenomenological Landau theory for the asymmetric ripple phase in bilayers composed of *achiral* molecules, which attributes this asymmetry to the existence of a *nonzero mean tilt* of the hydrocarbon chains of the lipid molecules. In the experimental system described, the height profile of the ripples is almost symmetric. *Thus the difference in the thickness of the two arms is the primary feature of asymmetric ripples,* a fact that has not been appreciated in the literature. Our theory explicitly takes into account the anisotropic elastic properties of a bilayer with tilt order. The mean-field phase diagram obtained from this model shows three types of ripple phases, in particular, a phase in which the magnitude of the tilt order parameter oscillates about a nonzero mean. This

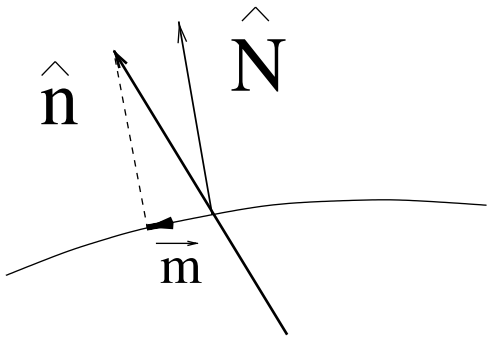

FIG. 1. The unit vector $\hat{\mathbf{n}}$ represents the orientation of the long axis of the lipid molecules. The projection of $\hat{\mathbf{n}}$ on the bilayer plane is the order parameter $\mathbf{m}$. $\hat{\mathbf{N}}$ is the local bilayer normal.

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structure is similar to that inferred from the electron density map (EDM) of dilauroylphosphatidylcholine (DLPC) (Fig. 2), calculated from the data given in Ref. [3].

The phases of the reflections are obtained by fitting the observed magnitudes of the structure factors ($|F_o|$) with those calculated (F_c) from a model for the bilayer electron density distribution [5,7]. The calculated phases are then combined with the corresponding $|F_o|$ and inverse Fourier transformed to obtain the EDM. The $|F_o|$'s and the F_c's obtained from the best fit are listed in Table I. Phases of some of the reflections given in Table I are different from those obtained by Tardieu *et al.* [3] using a pattern recognition method. The present set of phases gives a smoother and hence a better EDM (Fig. 2) compared to that of Ref. [3]. The map shows rippled bilayers with a height profile which is almost symmetric, in contrast to sawtoothlike shapes seen in other systems [5–7]. The bilayer thickness is different in the two arms and the electron density in the headgroup region is higher in the thicker arm. There is also an offset between the corresponding kinks in the two leaflets of the bilayer. These three features are present in the ripple phase of all the phospholipids studied and can be consistently attributed to an average tilt of the hydrocarbon chains with respect to the normal to the mean bilayer plane, along the ripple wave vector [7,8]. In Fig. 2 the lengths of both arms are comparable to the bilayer thickness. In all the other maps available in the literature the length of the thinner arm of the ripple is comparable to the bilayer thickness, whereas the thicker arm is about twice as long, thus making the bilayer height profile asymmetric.

The elastic free energy in our model is given by $F = \int f\, ds = \int [f_{\rm LM} + f_{nl}]\, ds$, where $f_{\rm LM} = f_m + f_c$ is the free energy density of the LM model, f_{nl} represents the additional symmetry-allowed terms, and the integral is over the surface of the bilayer. The Lifshitz free energy density

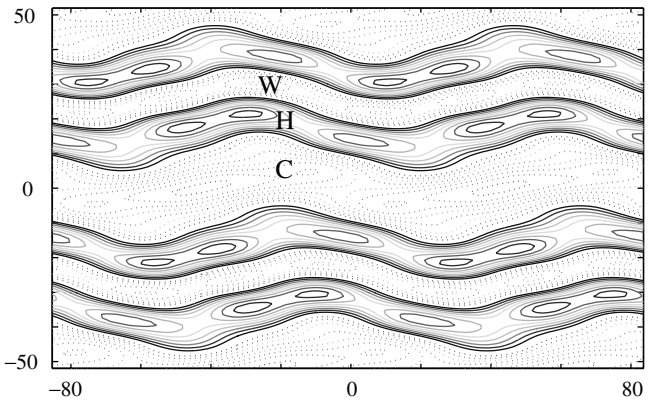

FIG. 2. EDM of the ripple phase of DLPC containing 77% by weight of water at −7 °C, calculated using the data of Ref. [3]. The ripple wavelength is 85.3 Å, the lamellar periodicity 51.9 Å, and the oblique angle $\gamma = 110°$. The regions in the map denoted by W, H, and C correspond to water and to the headgroups and hydrocarbon chains of the lipid molecules, respectively.

f_m is given by

$$f_m = \tfrac{1}{2}a|\mathbf{m}|^2 + \tfrac{1}{4}b|\mathbf{m}|^4 + \tfrac{1}{2}\tilde{c}_\parallel(\nabla\cdot\mathbf{m})^2 + \tfrac{1}{2}c_\perp(\nabla\times\mathbf{m})^2 + \tfrac{1}{2}\alpha(\nabla^2\mathbf{m})^2. \quad (1)$$

This produces modulated phases if either $\tilde{c}_\parallel$ or $c_\perp$ becomes negative. These two terms correspond, respectively, to splay and bend deformations of the $\mathbf{m}$ field. The curvature energy density of the bilayer is

$$f_c = \tfrac{1}{2}\kappa(\nabla^2 h)^2 - \gamma(\nabla^2 h)\,(\nabla\cdot\mathbf{m}), \quad (2)$$

where $h(x, y)$ is the height of the bilayer relative to a flat reference plane, κ is the bending rigidity, and γ couples the mean curvature to the splay in $\mathbf{m}$. The additional terms which we include in our model are

$$f_{nl} = \frac{\beta}{4}(\nabla\cdot\mathbf{m})^4 + \frac{\xi}{2}[(\mathbf{m}\cdot\nabla)\mathbf{m}]^2 + \frac{\zeta}{2}[(\mathbf{m}\times\nabla)\mathbf{m}]^2. \quad (3)$$

The first term in f_{nl} is a higher order stabilizing term [19]. The next two terms describe the symmetry-allowed anisotropy in the elasticity of the $\mathbf{m}$ field. With a nonzero mean tilt, these terms distinguish between gradients of $\mathbf{m}$ along $\mathbf{m}$ and gradients of $\mathbf{m}$ orthogonal to $\mathbf{m}$ [20]. Note that there are no terms with pseudoscalar coefficients in our model—the molecules constituting the bilayer are achiral.

The height profile of the bilayer is related to the tilt via the Euler-Lagrange equation, $\nabla^2 h = (\gamma/\kappa)\nabla\cdot\mathbf{m}$. As in the LM model, eliminating the height field h from the free energy density f leads to the effective free energy density $f_{\rm eff}$ with a reduced splay elastic constant $c_\parallel = \tilde{c}_\parallel - \gamma^2/\kappa$. In what follows, the ripple wave vector is always taken to be along the x axis, whereas the mean tilt can be along either the x or the y axis. Following [12] we choose the ansatz, $m_x = m_{0x} + m_{1x}\cos(qx) + m_{2x}\cos(2qx)$; $m_y = m_{oy} + m_{1y}\cos(qx)$. This ansatz is general enough to describe the symmetries of the various

TABLE I. The observed ($|F_o|$) (from Ref. [3]) and the calculated (F_c) structure factors for the ripple phase of DLPC containing 77% by weight of water at −7 °C.

h	k	$\lvert F_o\rvert$	F_c	h	k	$\lvert F_o\rvert$	F_c
0	1	5.5	−1.3	2	1	19.1	+19.6
1	0	36.4	−30.5	2	2	6.9	−9.7
1	−1	10	−6.3	3	−1	16.3	+11.5
0	2	3.2	+0.3	3	−2	10	+4.1
1	1	5.5	+9.9	3	0	5.7	+10.3
1	−2	3.9	−0.1	3	1	9.7	−11.1
2	−1	22.4	−18.5	3	2	8.3	+7.9
1	2	3.0	−3.4	4	−1	9.6	−10.5
2	0	30	−32.6	4	0	7.6	−4.3
2	−2	21	−3.3				

modulated phases appearing in the model [21]. Depending on the values of the coefficients in the above ansatz, the following types of ripple phases can be obtained: (a) the P_β phase ($P^{(2)}_{\beta'}$ phase of [12]), where $m_{0x} = m_{2x} = m_{oy} = m_{1y} = 0$ and $m_{1x} \neq 0$. This phase has symmetric ripples with no mean tilt, i.e., the tilt oscillates about a zero mean (Fig. 4b), (b) the $P^t_{\beta'}$ phase ($P^{(1)}_{\beta'}$ phase of [12]); $m_{0x} = m_{2x} = m_{1y} = 0, m_{oy} \neq 0, \quad m_{1x} \neq 0,$ and the ripples are symmetric with a nonzero mean tilt in the direction transverse to the rippling direction (Fig. 4a), (c) the $P^l_{\beta'}$ phase, which has a nonzero mean tilt in the direction of rippling; $m_{0x} \neq 0, m_{1x} \neq 0, m_{2x} \neq 0,$ and $m_{oy} = m_{1y} = 0$ (Fig. 4c). In addition to the above one-dimensionally modulated phases, we have also considered the two-dimensionally modulated square lattice phase. For this phase we choose the ansatz $m_x = m_1 \cos(qx)$ and $m_y = m_1 \cos(qy)$.

The phase diagram in the a-$c_\parallel$ plane obtained from the numerical minimization of the effective free energy is shown in Fig. 3, for $\xi < \zeta$. In this case it is easier to create distortions in the tilt order along the direction of the tilt rather than in the perpendicular direction [22]. We find two major differences from the phase diagram obtained from the LM model. First, the square lattice phase, which has not been seen in experiments, is not stable in the present model. Second, the $P^l_{\beta'}$ phase is now stable in part of the parameter space. In this phase m_{2x} is always nonzero and hence the ripple profile is asymmetric. However, this asymmetry is rather small; m_{2x} is typically 2 orders of magnitude smaller than m_{1x}. On the other hand, the presence of a nonzero mean tilt makes the bilayer thickness in the two arms different, and hence the bilayer lacks a mirror plane normal to the ripple wave vector, as in the EDM shown in Fig. 2. However, this structure is not chiral as it has a mirror plane normal to y. The coordinates of the $P^l_{\beta'}$-$P^t_{\beta'}$-$L_{\beta'}$ triple point depend on the ratio (α/β) of the coefficients of the two stabilizing terms. As this ratio is decreased, the triple point moves towards the origin. As shown by Jacobs *et al.* [19] in the context of a one-dimensional model with a scalar order parameter, the $(\nabla \cdot \mathbf{m})^4$ term is essential to stabilize a modulated phase with a nonzero mean value of the order parameter. In our model, where the order parameter is a two-dimensional vector, this term alone is not sufficient to stabilize such a modulated phase with nonzero mean tilt ($P^l_{\beta'}$); we also have to take into account the anisotropic elasticity of the bilayer with tilt order. Note that the $L_{\beta'}$ phase is now stable till $(c_\parallel + \xi m_o^2)$ or $(c_\parallel + \zeta m_o^2)$ becomes negative. In the mean-field phase diagram of our model for $\xi > \zeta$ we do not find the square lattice ripple phase of the LM model. The bilayer profiles in the three ripple phases obtained from our model are illustrated in Fig. 4.

It is clear from the above discussion that the effective bending modulus of the bilayer must be smaller in the direction of the mean tilt in order to have asymmetric ripples. We may now speculate on the origin of this kind of anisotropy in the effective bending modulus. The tilt of the hydrocarbon chains with respect to the bilayer normal is achieved by relative sliding of the chains along their long axes. If the chains are constrained to be parallel, as seems to be the case in lipid bilayers, the curvature of the bilayer is produced by gradients in this sliding displacement. In this case the director field $\hat{\mathbf{n}}$ is uniform and the deformations in the $\mathbf{m}$ field arise solely from those in the h field. *Thus the molecular tilt as well as the bilayer curvature result from the same basic molecular mechanism*. When the chains are tilted, the overlap between two adjacent chains along the tilting direction decreases. Therefore, the net restoring force between the chains can be expected to be

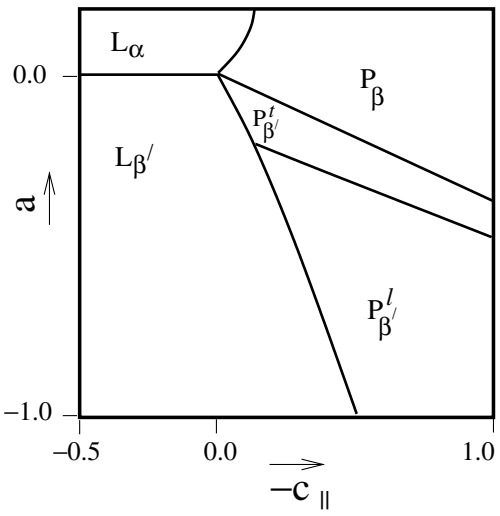

FIG. 3. The mean-field phase diagram for $\xi < \zeta$: $b = 1.0$, $\alpha = 0.01$, $\beta = 4.0$, $\xi = 0.1$, and $\zeta = 0.7$.

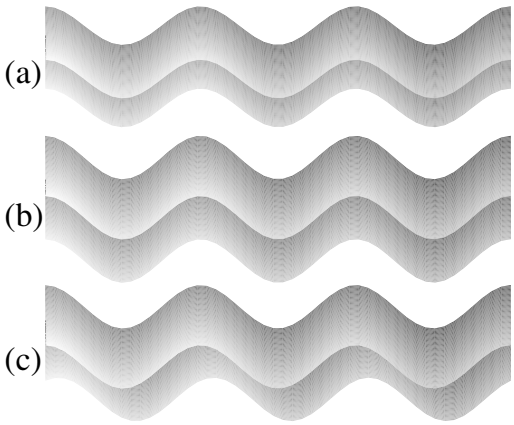

FIG. 4. A schematic of the bilayer profiles in the different ripple phases obtained from the model. (a) $P^t_{\beta'}$, (b) P_β, and (c) $P^l_{\beta'}$. In (c) the bilayer thicknesses in the two arms of the ripple are different due to the mean tilt of the chains along the ripple wave vector, $\mathbf{q}$. In (a) the mean tilt is transverse to $\mathbf{q}$ and hence the bilayer thickness is lower than that in (b), which has no mean tilt.

lower in the tilt direction, making it easier for the bilayer to bend in this direction.

In this Letter we have emphasized the role of the mean tilt of the phospholipid chains in the ripple phase. Detailed work on the height profile of the ripple phase (where we include higher order terms coupling the membrane curvature to **m** [23]) is in progress and will be published elsewhere.

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