Theory of the asymmetric ripple phase in achiral lipid membranes

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We present a phenomenological theory of phase transitions in achiral lipid membranes in terms of two coupled order parameters – a scalar order parameter describing *lipid chain melting*, and a vector order parameter describing the *tilt of the hydrocarbon chains* below the chain-melting transition. Existing theoretical models fail to account for all the observed features of the phase diagram, in particular the detailed microstructure of the *asymmetric ripple phase* lying between the fluid and the tilted gel phase. In contrast, our two-component theory reproduces all the salient structural features of the ripple phase, providing a unified description of the phase diagram and microstructure.

Phospholipids self-assemble in water to form a rich variety of spatially modulated phases [1]. The simplest of these is the 1-dimensionally modulated fluid lamellar phase (L_α) consisting of periodic stacks of lipid bilayer membranes separated by water, where the hydrocarbon chains are floppy with liquid-like in-plane order. Changing the temperature or water content induces a sequence of symmetry breaking transitions characterized by unique microstructures.

On reducing the temperature below the chain melting (main) transition (T_m), the L_α phase of phosphatidylcholines (PCs) transforms to a gel phase ($L_{\beta'}$), characterized by fully-stretched $all - trans$ chains which are tilted with respect to the bilayer normal [2–4]. In addition, an *asymmetric ripple phase* ($P_{\beta'}$) is found to occur in between the L_α and $L_{\beta'}$ phases in many PCs at high water content [1, 2, 5].

Extensive studies using a variety of experimental techniques [1, 6–18], reveal that the $P_{\beta'}$ phase is characterized by a periodic saw-tooth height modulation of the bilayers having an amplitude of ~ 1 nm and a wavelength of ~ 15 nm, and a bilayer thickness that is different in the two arms of the ripple (fig. 1) [9, 10]. As a result, the rippled bilayers lack a mirror plane normal to the rippling direction. While in principle, this discrete symmetry breaking can arise from an asymmetry in either *shape* (unequal lengths of the two arms) or *bilayer thickness* (unequal bilayer thickness in the two arms), in practice these asymmetries seem to appear simultaneously.

At first it was believed that the origins of the asymmetric ripple lay in the chirality of lipid molecules [20]. However, subsequent experiments using racemic mixtures showed this was not the case [8, 15]. More recently, all-atom molecular dynamics simulations of lipid bilayers have observed that the degree of chain ordering is different in the two arms of the ripple [21]. The occurrence of the ripple phase only in those lipids that exhibit a $L_{\beta'}$ phase at lower temperatures [22], and in isolated bilayers [23], suggests an intimate connection between chain tilt and the ability of the bilayers to form ripples.

Several theoretical models have been proposed to describe the sequence of phase transitions in such lipid bilayers and the microstructure of the ripple phase [20, 24–34]. None of them accounts for all the observations. We list three key features that should be explained by any theory of the *ripple phase in achiral bilayers* : (1) occurrence of $P_{\beta'}$ phase between L_α and $L_{\beta'}$ phases, separated by two first-order transitions; (2) unequal bilayer thickness in the two arms of the ripple; and (3) unequal lengths of the two arms.

In this paper we present a phenomenological Landau theory to describe the ripple phase in an isolated, achiral lipid bilayer. Our free energy expression is written in terms of two order parameters: a scalar order parameter ψ and a 2-D vector order parameter $\mathbf{m}$ (fig.2). ψ describes the melting of the bilayer and is the difference in the bilayer thickness [28, 29] between the fluid (L_α) and ordered ($P_{\beta'}$ and $L_{\beta'}$) phases. Since the
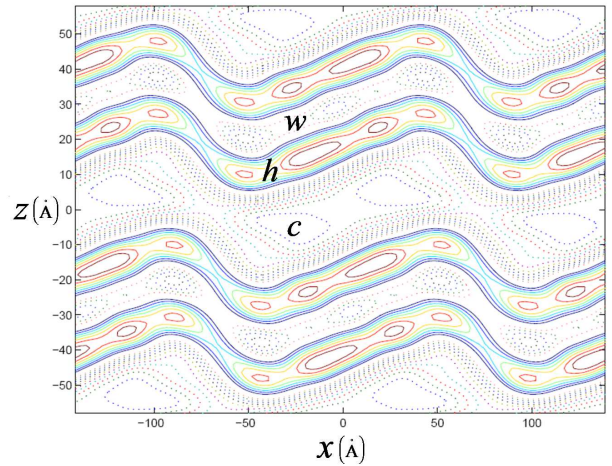

FIG. 1: Electron density map of the ripple phase of dimyristoylphosphatidylcholine calculated from x-ray diffraction data [19]. Bands labeled h and c correspond to the headgroup and hydrocarbon chain regions of the bilayer; w denotes the water layer separating the bilayers.

bilayer thickness is determined by the conformations of the hydrocarbon chains, ψ can also be interpreted in terms of differences in chain conformations between the fluid and ordered phases [30]. $\mathbf{m}$ is the projection of the molecular axis on the bilayer plane [20]. A third order parameter h, describing the height of the bilayer, can be integrated out of the expression for the total free energy density. This model is found to capture all the three salient features of the ripple phase listed above.
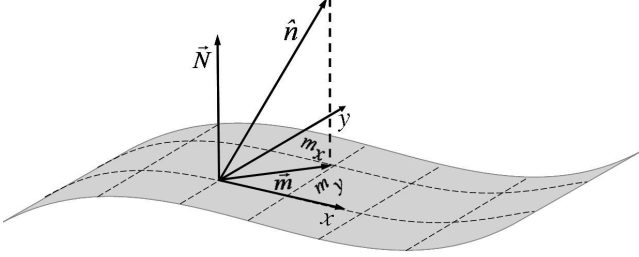

FIG. 2: The unit vector $\hat{n}$ represents the orientation of the long axis of the lipid molecules relative to the bilayer normal $\vec{N}$. $\vec{\mathbf{m}} = \vec{n} - (\vec{N} \cdot \hat{n}) \hat{N}$ is the projection of $\hat{n}$ on the bilayer plane.

The total free energy per unit area is taken to be the sum of three terms; the stretching free energy density f_s, the tilt free energy density f_t, and the curvature free energy density f_c. For an isolated lipid bilayer f_s is given by [34],

$$f_s = \frac{1}{2} a_2 \psi^2 + \frac{1}{3} a_3 \psi^3 + \frac{1}{4} a_4 \psi^4 + \frac{1}{2} C (\nabla \psi)^2 \\ + \frac{1}{2} D (\nabla^2 \psi)^2 + \frac{1}{4} E (\nabla \psi)^4 \qquad (1)$$

where $\psi(x,y) = \frac{\delta(x,y) - \delta_0}{\delta_0}$, $\delta(x,y)$ being the membrane thickness at position (x,y) in the bilayer measured with respect to a flat reference plane, and δ_0 the constant thickness of the membrane in the L_α phase. ψ is taken to be positive for $T < T_m$ due to the stretching of the chains. This is valid in general, even if the chains are tilted below T_m. Explicit temperature dependence is assumed to reside solely in the coefficient of $\psi^2 : a_2 = a_2'(T - T^*)$, T^* being a reference temperature. a_3 is taken to be negative, so that the continuous transition at T^* is preempted by a first order melting transition at $T_m = T^* + \frac{2a_3^2}{9a_2'a_4}$. The coefficient C can either be positive or negative, but a_4, D and E are always positive to ensure stability. With $C > 0$, the equilibrium phases are always homogeneous in space; either as L_α or L_β ($L_{\beta'}$). However, with $C < 0$ modulated phases are possible with some characteristic wave vector q_0. The $(\nabla \psi)^4$ term is included, since in the context of a one dimensional model with a scalar order parameter, it has been shown that such a term is necessary to stabilize a modulated phase with a non zero mean value of the order parameter [35].

The tilt free energy density can be written as,

$$f_t = \frac{1}{2} b_2 |\mathbf{m}|^2 + \frac{1}{4} b_4 |\mathbf{m}|^4 + \tilde{\Gamma}_1 (\nabla \cdot \mathbf{m})^2 \\ + \Gamma_2 (\nabla^2 \mathbf{m})^2 + \Gamma_3 (\nabla \cdot \mathbf{m})^4 + \Gamma_4 \psi |\mathbf{m}|^2 \\ + \Gamma_5 (\mathbf{m} \cdot \nabla \psi)^2 + \Gamma_6 (\mathbf{m} \times \nabla \psi)^2 \\ + \Gamma_7 (\nabla^2 \psi) (\nabla \cdot \mathbf{m})^2 + \Gamma_8 (\nabla \psi)^2 (\nabla \cdot \mathbf{m})^2 \qquad (2)$$

The first four terms in eqn.(2) are the usual terms in the expansion of the free energy in terms of a vector order parameter. The $(\nabla \cdot \mathbf{m})^4$ term is included to be consistent with the $(\nabla \psi)^4$ term introduced in f_s. The next term represents the coupling between ψ and $\mathbf{m}$, which is responsible for the appearance of tilted phases in this model, as b_2 is taken to be positive. If $\Gamma_4 > 0$, the stable phase below T_m is L_β with $|\mathbf{m}|=0$. On the other hand, tilted phases can form if $\Gamma_4 < 0$. The succeeding two terms take into account the anisotropy of the tilted bilayer. The next two terms represent higher order couplings between modulations in ψ and in $\mathbf{m}$, allowed by the symmetry of the system.
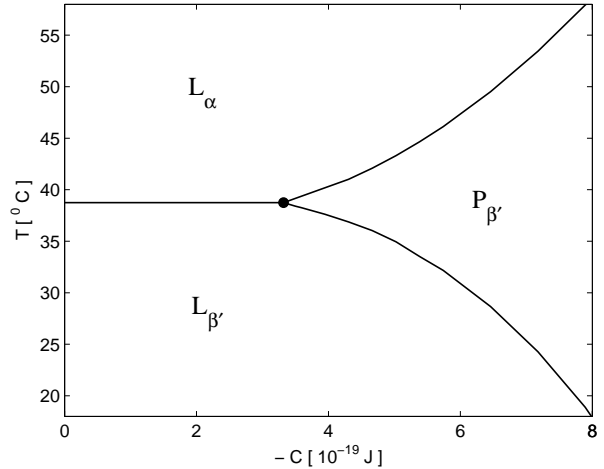

FIG. 3: Phase diagram in the $T - C$ plane calculated from the model. $a_2' = 159.42 \, k_B$, $T^* = 260.0$ K. Values of the other coefficients in units of $k_B T^*$ are: a_3 = -306.5, a_4 = 613.15, b_2 = 0.2, b_4 = 200.0, D = 557.41, E = 600.0, Γ_1 = 0.010, Γ_2 = 1.80, Γ_3 = 500.0, Γ_4 = -3.0, Γ_5 = -20.0, Γ_6 = -20.0, Γ_7 = -500.0, Γ_8 = -750.0. Both the main-transition $(L_\alpha \rightarrow L_{\beta'}; L_\alpha \rightarrow P_{\beta'})$ and pre-transition $(P_{\beta'} \rightarrow L_{\beta'})$ are first order.

The curvature energy density of the bilayer can be written as [20, 31],

$$f_c = \frac{1}{2} \kappa (\nabla^2 h)^2 - \gamma (\nabla^2 h) (\nabla \cdot \mathbf{m}) \qquad (3)$$

where $h(x,y)$ is the height of the bilayer relative to a flat reference plane, κ is the bending rigidity of the membrane, and γ couples the mean curvature to splay in $\mathbf{m}$.
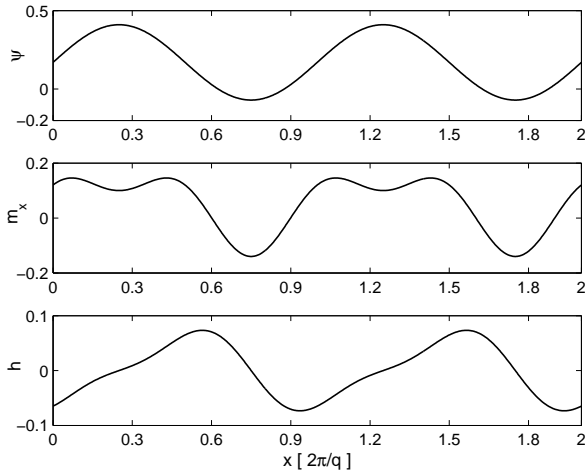
FIG. 4: Spatial variation of the different order parameters in the $P_{\beta'}$ phase at $T = 310.5$ K and C= -4.84 $\times 10^{-19}$ J.
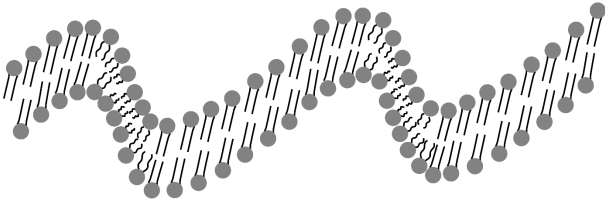

FIG. 5: Schematic of the bilayer profile obtained from the model. The bilayer thickness is different in the two arms of the saw-tooth-like ripples.

The equilibrium height profile of the bilayer $h(x,y)$ is related to the tilt $\mathbf{m}$ via the Euler-Lagrange equation,

$$\nabla^2 h = \frac{\gamma}{\kappa} \, (\nabla \cdot \mathbf{m}) \qquad (4)$$

Eliminating h from the free energy density f leads to the effective energy density f_{eff} with a reduced splay elastic constant $\Gamma_1 = \tilde{\Gamma}_1 - \gamma^2/(2\kappa)$.

To determine the mean field phase diagram we choose the following ansatz for ψ and $\mathbf{m}$,

$$\psi = \psi_0 + \psi_1 sin(qx)$$
$$m_x = m_{0x} + m_{1x} \, cos\,(qx) + m_{2x} \, sin\,(qx)$$
$$+ \, m_{3x} \, cos\,(2qx) + m_{4x} \, sin\,(2qx)$$
$$m_y = m_{0y} \qquad (5)$$

We do not consider two-dimensionally modulated ripples, since they do not appear to be generic; there being, as far as we know, only one report of such a structure [37]. Spatial modulation of m_y is neglected as we do not keep terms proportional to $(\nabla \times \mathbf{m})$ in eqn.(2) for reasons discussed below. Higher order Fourier components of m_x are retained in order to account for the ripple asymmetry. Three different ripple structures can be described with this ansatz: (1) the P_β with no mean

tilt ($m_{0x} = m_{0y} = 0$), (2) the $P_{\beta'}^T$ with a mean tilt along y ($m_{0x} = 0$, $m_{0y} \neq 0$) , and (3) the $P_{\beta'}^L$ with a mean tilt along x ($m_{0x} \neq 0$, $m_{0y} = 0$). Of these only the $P_{\beta'}^L$ structure has an asymmetric height profile [33].

The phase diagram in the C-T plane obtained from numerical minimization of the effective free energy density averaged over one spatial period, $< f_{eff} > = (q/2\pi) \int_0^{2\pi/q} f_{eff} \, dx$, is given in fig. 3. It is calculated for a choice of parameter values, which reproduce closely the main- and pre-transition temperatures of dipalmitoylphosphatidylcholine at $C = - 4.84 \times 10^{-19} J$. The values of T^*, a_2, a_3, a_4, and D are similar to those used in refs. [29] and [34]. As can be seen from fig. 3, there are three distinct regions in the phase diagram corresponding to three different phases: L_α ($\psi_0 = \psi_1 = m_{0x} = m_{0y} = m_{1x} = m_{2x} = m_{3x} = m_{4x} = 0$), $L_{\beta'}$ ($\psi_0 \neq 0, m_{0x} \neq 0, m_{0y} \neq 0; \psi_1 = m_{1x} = m_{2x} = m_{3x} = m_{4x} = 0$), and $P_{\beta'}^L$ ($\psi_0 \neq 0, \psi_1 \neq 0, m_{0x} \neq 0, m_{2x} \neq 0, m_{3x} \neq 0; m_{1x} = m_{4x} = m_{0y} = 0$).

It is interesting that of the three possible ripple structures only the asymmetric $P_{\beta'}^L$, which is similar to the experimentally observed structure, is present in this phase diagram [40]. The first-order transition lines which separate these three phases meet at the Lifshitz point located at $C_{Lp} = -3.32 \times 10^{-19}J$ and $T = 38.75°C$. For $C > C_{Lp}$, the first order $L_{\beta'} \rightarrow L_\alpha$ transition line is parallel to the C axis and occurs at $T_m = 38.75°C$. But for $C < C_{Lp}$, the intermediate $P_{\beta'}$ phase is found, separated from the other two phases by first-order transition lines. Further, the region occupied by the $P_{\beta'}$ phase expands at the expense of the other two as C becomes more negative.

Typical spatial variation of the order parameters in the ripple phase is shown in fig. 4. The height profile is asymmetric and resembles very closely those seen in experiments (fig. 1) [9, 10, 18]. ψ_1 is almost $\pi/2$ out of phase with h, so that it is positive (negative) along the longer (shorter) arm of the ripple, resulting in different bilayer thicknesses in the two arms, again in agreement with experimental observations (fig. 1) [9, 10]. Fig. 5 shows a schematic of the structure of the bilayer inferred from these results. It is clear that the model presented here accounts for all the salient features of the ripple phase listed in the introduction.

The essential term in the free energy expression responsible for the asymmetric ripples is the one with the coefficient Γ_8, since similar structures can be obtained by setting $\Gamma_5 = \Gamma_6 = \Gamma_7 = 0$, as long as Γ_8 is < 0. Thus the present model spontaneously picks out a non-zero value of the mean tilt along the rippling direction q, even in the absence of any explicit in-plane anisotropy of the bending rigidity. This is in contrast to the model presented in refs. [20] and [31], where the mean tilt occurs in a direction normal to q, resulting in symmetric ripples in the case of achiral bilayers; the bending rigidity has to be explicitly taken to be lower along the tilt

direction in order to obtain a non-zero mean tilt along q and to stabilize asymmetric ripples within this model [33]. If Γ_7 is made sufficiently positive in the present model, ψ_1 and h become almost in phase, so that the bilayer thickness is modulated within each arm of the ripple. It might be possible to tune this parameter by a suitable choice of an impurity which would prefer to smoothen variations in ψ; in such cases we predict the existence of this new ripple phase.

We have included only terms proportional to $(\nabla \cdot \mathbf{m})$ in the expression for f_t. In general there will also be terms proportional to $(\nabla \times \mathbf{m})$, which lead to a ripple structure with a non-zero winding number in the model presented in refs. [20] and [31]. However, such a structure can be expected to be energetically very unfavorable in an achiral bilayer, since it is not consistent with parallel close-packing of the chains demanded by van der Waals interaction.

A straightforward extension of this model would be to use a better description of the chain-melting transition, instead of the reduced bilayer thickness ψ employed here. Possible order parameters include components of the in-plane density wave, as considered in the theory of weak crystallization [38], and those of herringbone order, used to describe positional ordering in monolayers [39]. However, such an attempt would be useful only if the details of chain ordering in this phase, presently unknown, can be experimentally established.

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