Phase transitions in lipid bilayers

Wladimir A. Benalcazar

Abstract

Despite the simplicity in their structure, lipid bilayers can arrange in a rich variety of phases, including rippled phases that exhibit supramolecular periodic structures. A number of theoretical models have been produced to characterize these phases over the last three decades, some of which show consistency with x-ray diffraction data. Here I describe a phenomenological Landau free energy theory of lipid bilayers, whose phases show reasonable agreement with experiments, and which predicts the existence of some novel phases, unobserved so far (to the best of my knowledge).

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1 Introduction

Lipid bilayers (LB) constitute the basic structure of biological membranes, and are ubiquitous in living organisms. Lipids consist of a hydrophilic polar head group joined to a hydrophobic hydrocarbon chain (see fig. 1). When embedded in water at sufficiently high concentration, lipids assemble in lamellar structures that break translational symmetry and posses various degrees of internal ordering, which, in turn, may affect the shape of the membrane itself.

Since decades ago, experiments have identified a number of phases in the arrangement of lipid bilayers [1], with the most salient ones being the so-called L_α, $L_{\beta'}$, and $P_{\beta'}$ phases. The first one is a smectic A phase, found at high temperatures, in which the lipids form lamellar structures with an orientation perpendicular to the membrane surface (see fig. 1(C)). The second phase is found at low temperatures, and is a smectic C phase, in which the molecules are tilted with respect to the normal of the membrane surface. At moderate temperatures, the rippled phase $P_{\beta'}$ is found. This phase is characterized by corrugations of the membrane surface with well defined periodicity. An experimental phase diagram is given in fig. 2.

Many theories have been developed over the last decades to understand this problem [2, 3, 4, 5]. An interesting possibility is that, in fact, the phase diagram may be much richer that initially found. Theory predicts that this richness arises from the 2 dimensional nature of the orientation order parameter, and its coupling to the membrane curvature. The first factor gives rise to different modulations phases (that is, modulations in the orientation of the molecules); and the second gives rise to symmetric or asymmetric ripples in the membrane. This last aspect has been experimentally inferred from 2D x-ray diffraction and freeze-fracture crystallography [4].

In what follows I will describe the theory that best reproduces the experimental phase diagram of fig. 2. This theory is built upon previous ones and gives account of the variety of rippled phases mentioned above. At the core of this theory lays the notion that the curvature of the membrane is a spontaneous self-adjustment that results when the moleculecular orientations present divergence. This theory is a phenomenological Landau theory with a two dimensional order parameter.

2 Landau theory

Let us begin by defining the order parameter $\vec{m}$ as

$$\vec{m} = \vec{n} - \left(\vec{N}.\vec{n}\right)\vec{N} \tag{1}$$

where $\vec{N}$ is the unitary vector normal to the membrane surface and $\vec{n}$ is the unitary vector pointing in the direction in which the molecule is oriented (see fig. 3). Notice that $\vec{m}$ is a two dimensional OP.

Now, the free energy is built respecting the symmetries of the problem

$$f_T = f_m + f_c \tag{2}$$

The first contribution to the free energy in eq. (2) represents the energy from the tilt elasticity, and involves only quadratic and quartic terms due to rotational symmetry around an azimuthal angle ϕ (i.e. an angles that rotates the OP within the plane of the membrane surface):

$$f_m = \frac{c_1}{2}\left(\vec{\nabla}.\vec{m}\right)^2 + \frac{c_2}{2}\left(\vec{\nabla}\times\vec{m}\right)^2 + \frac{D}{2}\left(\nabla^2\vec{m}\right)^2 + \frac{t}{2}m^2 + um^4 \tag{3}$$

where the terms D and u are always positive and preserve stability against c_1, c_2 or t, which can be both positive, negative, or zero. Notice that, because the OP is a vector, no cubic term is allowed. This term is a Lifshitz free energy [6], that produces modulated phases if c_1 or c_2 is negative.

The second contribution to the free energy in (2) represents the energy associated with generating a curvature in the membrane, and has the form:

$$f_c = \frac{\kappa}{2}\left(\nabla^2 h\right)^2 - \gamma\left(\nabla^2 h\right)\left(\vec{\nabla}.\vec{m}\right) \tag{4}$$

The parameter $h = h(x_1, x_2)$ is the height of the membrane relative to an arbitrary point (see fig. 4(B)). Notice that there is a coupling between molecular tilt represented by the OP and the membrane curvature, represented by h. This coupling is meant to represent the steric interactions between neighboring molecules, and is illustrated in the caricature of fig. 4.

The equilibrium curvature is be given by solving the Lagrange equation, taking into account that there is no dependence of h in f_m,

$$\frac{\partial f_c}{\partial h} - \nabla^2\frac{\partial f_c}{\partial\left(\nabla^2 h\right)} = 0 \rightarrow \nabla^2 h = \frac{\gamma}{\kappa}\left(\vec{\nabla}.\vec{m}\right) \tag{5}$$

which leads to the equilibrium value of the curvature free energy

$$f_c = -\frac{\gamma^2}{2\kappa}\left(\vec{\nabla}.\vec{m}\right)^2 \tag{6}$$

Putting this value back in the expression for the total free energy, it simplifies to

$$f_T = \frac{1}{2}\left(c_1 - \frac{\gamma^2}{2\kappa}\right)\left(\vec{\nabla}.\vec{m}\right)^2 + \frac{c_2}{2}\left(\vec{\nabla}\times\vec{m}\right)^2 + \frac{D}{2}\left(\nabla^2\vec{m}\right)^2 + \frac{t}{2}m^2 + um^4 \tag{7}$$

and so, the free energy at equilibrium curvature has the same form as the tilt elasticity free energy with new parameter c_1' equal to

$$c_1' = c_1 - \frac{\gamma^2}{2\kappa} \tag{8}$$

It is convenient to shift the problem to Fourier space. By using $\vec{\nabla} \to -i\vec{k}$, and employing the same notation for the variables, which are now functions of the wavevector $\vec{k}$, the free energy becomes

$$f_T = \frac{c_1'}{2}\left(\vec{k}.\vec{m}\right)^2 + \frac{c_2}{2}\left(\vec{k}\times\vec{m}\right)^2 + \frac{D}{2}\left(k^2 m\right)^2 + \frac{t}{2}m^2 + um^4 \tag{9}$$

Now, to develop a basic understanding of the phase diagram, let us restrict the study of this problem to the simplest case where $\vec{m}$ and $\vec{k}$ are parallel. Then $(\vec{k}.\vec{m}) = km$ and $(\vec{k}\times\vec{m}) = 0$, and we are left with

$$f_T = \frac{c_1'}{2}k^2m^2 + \frac{D}{2}k^4m^2 + \frac{t}{2}m^2 + um^4 \tag{10}$$

The extremal points of this free energy are found from

$$\frac{\partial f_T}{\partial k} = 0 \to \begin{cases} m = 0 \\ k = 0 \\ k = \sqrt{\frac{-c_1'}{2D}} \end{cases} \tag{11}$$

$$\frac{\partial f_T}{\partial m} = 0 \to \begin{cases} m = 0 \\ m = \sqrt{-\frac{\left(c_1'k^2 + Dk^4 + t\right)}{4u}} \end{cases} \tag{12}$$

Now, from eq. (11) one can see that $k \neq 0$ only for $c_1' < 0$, or $\gamma^2/\kappa > c_1$. Thus, we must have a transition from a ripple phase to a uniform phase

which is related to the rigidity parameter γ^2/κ. To find out which phases are involved in this transition, consider the case $c_1' > 0$, so that $k = 0$, then eq. (12) is reduced to $m = \sqrt{-t/4u}$, which implies that one can have $m \neq 0$ only for $t < 0$.

Joining these two conditions one concludes that for $\gamma^2/\kappa < c_1$ there are two phases: smectic A ($m = 0$) for $t > 0$, denoted in the introduction as L_α, and smectic C ($m =$ constant $\neq 0$) for $t < 0$, which we denoted before as $L_{\beta'}$. As the membrane rigidity term γ^2/κ is reduced, the $L_{\beta'}$ phase becomes unstable to a ripple phase $P_{\beta'}$ with decreasing wavelength given by $k = 2\pi/\lambda = \sqrt{-c_1'/2D}$. This weakening of the rigidity occurs when the hydration increases.

Now, for $c_1' < 0$ we may have $k \neq 0$. Replacing $k = \sqrt{-c_1'/2D}$ in the non-zero solution for m in eq. (12) one gets

$$m = \frac{1}{2}\sqrt{\frac{1}{u}\left(\frac{c_1'^2}{4D} - t\right)} \tag{13}$$

therefore, $m \to 0$ as $t \to (c_1')^2/4D$ from below. The transition is from the ripple phase $P(\beta)$ at low t to the smectic A phase $L\alpha$ at high t. This phase boundary line meets the L_α-L_β boundary line at the Lifshitz point $(\gamma^2/\kappa, t) = (C, 0)$.

All the above mentioned characteristics for the phases L_α, $L_{\beta'}$ and $P_{\beta'}$, plus others described (qualitatively) below, are shown in the phase diagram of fig. 5. Notice the qualitative resemblance of this diagram with the experimental diagram of fig. 2.

3 Symmetric phases, asymmetric phases and chirality

Recall that the OP is a two-dimensional vector. In the previous section I simplified the problem to the case where $\vec{m}$ and $\vec{k}$ were parallel. However, a number of interesting phases can be found if that assumption is relaxed. Let us do that by defining the OP as

$$\begin{aligned} \vec{m} &= (m_x, m_y) \\ m_x &= m_1^L \cos(qx) \\ m_y &= m_0 + m_1^T \sin(qx) \end{aligned} \tag{14}$$

where no constant longitudinal component of the OP is included because this problem does not leave any stable phases for such a component. When the ripples are assumed to be only along the x direction, three phases can be found in in achiral LB: $P^{(1)}_{\beta'}$, $P^{(2)}_{\beta'}$, and $P^{(3)}_{\beta'}$, all of which are associated to symmetric ripples (i.e. each membrane is symmetric under reflection through the midplane followed by a translation). Both $P^{(1)}_{\beta'}$ and $P^{(2)}_{\beta'}$ have the same membrane symmetry, but the $P^{(1)}_{\beta'}$ phase has order parameter with both parallel and orthogonal components, while $P^{(1)}_{\beta'}$ has only parallel component. In $P^{(3)}_{\beta'}$ the order parameter makes a complete revolution with identical period has the ripple period, and it is said to be a *spiral phase*. More precisely, we have

$$\begin{array}{lcl} P^{(1)}_{\beta'} & \rightarrow & m_0 \neq 0,\, m_1^L \neq m_1^T = 0 \\ P^{(2)}_{\beta'} & \rightarrow & m_0 = m_1^T = 0,\, m_1^L \neq 0 \\ P^{(3)}_{\beta'} & \rightarrow & m_0 = 0,\, m_1^T = m_1^L \neq 0 \end{array} \tag{15}$$

A graphical representation of these three phases is shown in fig. 6 (phase $P^{(3)}_{\beta'}$ is shown in part (C) over an asymmetric ripple in the membrane; this is proper of chiral LB, see below).

Even though the present theory showed that ripples should be symmetric, experiments often report asymmetric ripples. To account for this, one needs to include a chiral term to the free energy

$$f_c' = f_c + \epsilon_{ik}(\nabla_i \nabla_j h) m_j, m_k \tag{16}$$

Then one finds that above mentioned phases can also exist in chiral LB, however, $P^{(3)}_{\beta'}$ looses its ripple symmetry in chiral systems, and its denomination is changed to $P^{(3*)}_{\beta'}$. In addition, chiral LB can develop two more phases: $P^{(4*)}_{\beta'}$ and $P^{(5*)}_{\beta'}$, both with asymmetric ripples. Just as $P^{(2)}_{\beta'}$, $P^{(4*)}_{\beta'}$ has an OP parallel to the direction of the ripples. $P^{(5*)}_{\beta'}$ differs from $P^{(4*)}_{\beta'}$ in that its period is doubled. Illustrations of the phases $P^{(3*)}_{\beta'}$ and $P^{(4*)}_{\beta'}$ are shown in fig. 6

Finally, the present theory allows for a stable two-dimensional ripple structure, with ondulations both along x and y. Its existence is still to be found, as I think are some of the above mentioned phases, since I could not locate studies that given experimental account of them.

4 Discussion and Conclusion

A phenomenological Landau free energy theory has shown relative success in characterizing the phases and phase transitions of lipid bilayers. At its core is the notion that a divergence in the orientation of the molecules has an energy cost that is reduced by bending the membrane surface. The theory accounts for the possibility of asymmetric ripples, which have been inferred experimentally and computationally [5]. Other phases deduced in this theory differentiate among themselves not by the symmetry of the membrane, but by the orientation of its molecules relative to the vector normal to the membrane surface. Other approaches do not predict this variety of phases [2, 3], and even experiments have not shown such classification, to the best of my knowledge. This is most likely due to the fact that current microscopy techniques cannot image lipid bilayers with the required level of detail fast enough. New advances in biological microscopy techniques, such as those based in coherent anti-stokes Raman scattering [7, 8] may help elucidate this questions, as they are specially sensitive to lipids and do not need the introduction of exogenous agents (e.g. fluorophores), and thus can permit a better manipulation of the hydration and temperature.

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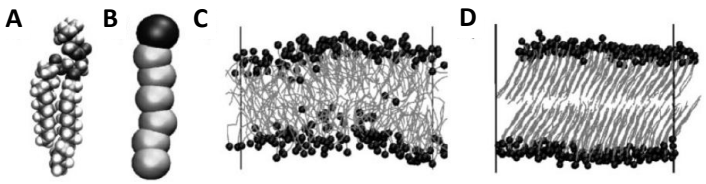

Figure 1: Lipids and lipid bilayers (LB). (A) All atom model of DPPC, (B) coarse grained version bead-spring model used in simulations, (C) LB in liquid phase L_α, (D) LB in tilted gel phase $L_{\beta'}$. Fig. adapted from ref. [5].

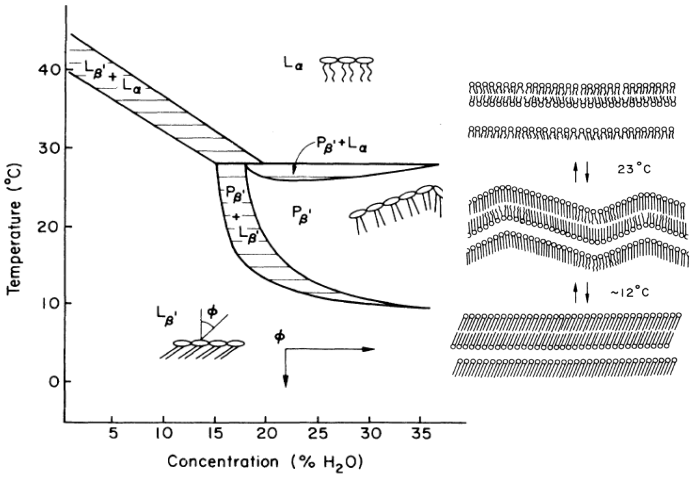

Figure 2: Experimental phase diagram for DMPC, plotted as a function of temperature and hydration. Solid lines indicate first order transitions. Arrows indicate directions of increasing tilt in the $L_{\beta'}$ phase. The rightmost schematic shows, from top to bottom, the forms of the phases L_α, $P_{\beta'}$ and L'_β. Fig. adapted from ref. [3].

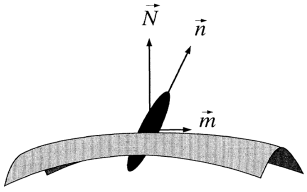

Figure 3: Definition of the two-dimensional order parameter $\vec{m} \equiv \vec{n} - (\vec{N}.\vec{n})\vec{N}$, where $\vec{n}$ is the orientation of the lipid and $\vec{N}$ is the orientation of the normal to the membrane surface. Fig. adapted from ref. [4].

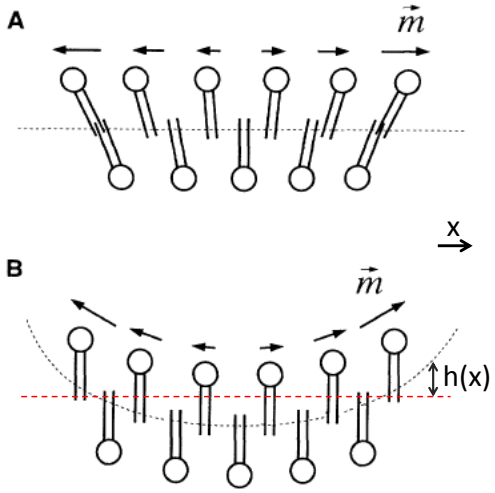

Figure 4: Caricature of the microscopic coupling mechanism between the molecular tilt and membrane curvature. (A) divergence of the molecular tilt $\vec{m}$ gives rise to (B) the spontaneous curvature of the membrane, characterized by the height $h(x)$. Fig. adapted from ref. [4].

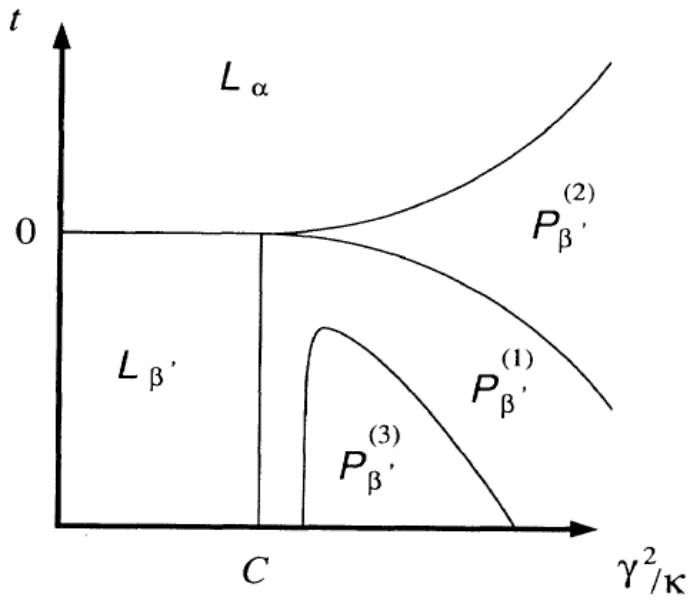

Figure 5: Theoretical mean-field phase diagram for one-dimensional ripple structures. Besides the uniform phases L_α ($\vec{m} = 0$, or smectic A) and L_β ($\vec{m} = \text{constant} \neq 0$, or smectic C), three non-uniform phases are present: $P^{(1)}_{\beta'}$ (symmetric), $P^{(2)}_{\beta'}$ (symmetric), and $P^{(3)}_{\beta'}$ (symmetric if achiral and asymmetric if chiral). There is a 1^{st} order phase transition between $P^{(1)}_{\beta'}$ and the spiral phase $P^{(3)}_{\beta'}$. The point $(\gamma^2/\kappa, t) = (C, 0)$ is a Lifshitz point. Fig. adapted from ref. [4].

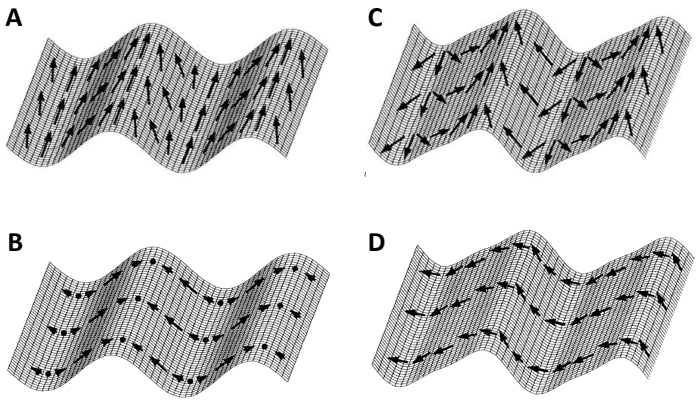

Figure 6: Representation of the vector order parameter $\vec{m}$, indicated by arrows in the symmetric (A) $P_{\beta'}^{(1)}$ and (B) $P_{\beta'}^{(2)}$ phases and in the asymmetric, chiral (C) $P_{\beta'}^{(3*)}$ and (D) $P_{\beta'}^{(4*)}$ phases. Fig. adapted from ref. [4].