Theory for the bending anisotropy of lipid membranes and tubule formation

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We study the spontaneous symmetry breaking of the bending rigidity of lipid membranes in two principal directions by a Landau mean-field theory. When the temperature is below the tilting temperature (T_c), the coupling between molecular orientation and membrane local curvature square leads to an increase of the bending rigidity in the tilting direction and therefore a spontaneous symmetry breaking in two principal directions. The asymmetry (Δ) of the bending rigidity undergoes a continuous change upon cooling and grows as $T_c - T$ for $T < T_c$. We discuss this anisotropical effect on the tilt structure of the ripple phase $P_{\beta'}$ of a nearly flat membrane and the sphere-to-tubule transition in a dilute solution of lipids. The transition between the spherical vesicle phase and the tubular phase is predicted to be first order. [S1063-651X(99)15705-2]

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Lipid molecules in water can self-assemble into bilayer membranes to shield their nonpolar hydrocarbon tails from contact with surrounding water. In many ways, these lipid bilayer membranes are excellent model systems of biological membranes. Aside from their structural resemblance, many physical properties of lipid bilayers are very similar to those of biomembranes, such as membrane thickness, surface tension, refraction index, water permeability, and bending rigidity. Furthermore, lipid bilayers can be prepared in various forms, such as planar membranes, stacks of lipid bilayers, multilamellar vesicles (MLV's), small unilamellar vesicles (SUV's), and large unilamellar vesicles (LUV's). Thus lipid membranes provide a unique opportunity for us to investigate various biological functions of biomembranes, such as exocytosis and endocytosis. In addition to a basic understanding of biomembranes, encapsulation of drugs and DNA by liposomes also provides a powerful tool in controlled drug delivery and release.

The study of lipid membranes has attracted considerable attention. Helfrich has developed a theory of the curvature elasticity of lipid bilayers [1] for studying planar membranes as well as vesicles. Most past theoretical work has been restricted to membranes with isotropic bending rigidity. The assumption of isotropic membranes is sensible for systems at temperatures above the tilting temperature (T_c) where the long axes of constituent lipids are parallel to the layer normal. For temperatures below T_c, lipid molecules are tilted relative to the layer normal, which leads to the $L_{\beta'}$ phase or the $P_{\beta'}$ phase [2–4]. In this case, membranes could develop anisotropy since the isotropy of molecular orientation is spontaneously broken. It has been suggested that the anisotropy of membranes could possibly lead to a tubular phase [5]. Indeed, tubules have been observed in various systems in which the isotropy of the membrane is broken [6]. Although there are many theories that attempt to explain the formation of tubules [7–9], none of them has proved to be conclusive.

In this Brief Report, we study the spontaneous symmetry breaking of the bending rigidities of lipid membranes in two principal directions by a continuum Landau theory in which the molecular orientation couples to the membrane local curvature square. The origin of this coupling can be easily understood from Fig. 1 in which the orientation of lipid molecules and their projection on the local tangent plane are shown. Here l is the bilayer thickness, a is the diameter of their head groups, and ml is the projected length on the local tangent plane. It is easy to see that the in-plane isotropy of the membrane is broken in Fig. 1(b). The anisotropy of the bending rigidity can be represented by a coupling term between the molecular tilt and local curvature like $\lambda m^a m^b K_{ac} K_b^c$ where λ is the coupling constant, K_{ab} is the curvature tensor, and the summation convention is assumed. The coupling constant is the energy difference to bend the membrane parallel to the tilt **m** and perpendicular to **m** and should be positive since it is more difficult to bend the membrane in the tilt direction. If we take the tilt direction as one of the principal axes (x_1 axis), this coupling term can be simplified to $\lambda |\mathbf{m}|^2 (\partial_1^2 \mathbf{r})^2$ where $\mathbf{r}$ is the position vector of the membrane. Other coupling terms permitted by symmetry are possible but will be ignored for simplicity. Such a coupling enhances the bending rigidity of the membrane in the tilting direction and the symmetry in the two principal directions is spontaneously broken when lipid molecules are tilted upon cooling. We discuss the anisotropical effect on the tilt structure of the ripple phase $P_{\beta'}$ of a nearly flat membrane

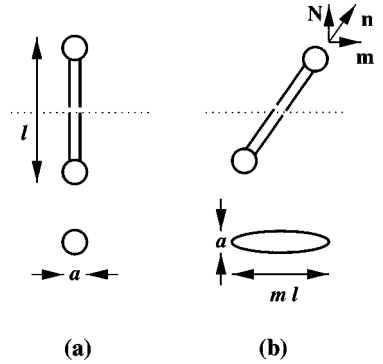

FIG. 1. Schematic representations of molecular orientations on a lipid membrane (up) and their projections on the local tangent plane (down): (a) above T_c and (b) below T_c. The characteristic sizes are a, head group diameter; l, bilayer thickness; and ml, the projected size on the local tangent plane.

and the sphere-to-tubule transition in a dilute solution of lipids. Due to the enhanced rigidity in the tilt direction, the tilt direction of the ripple phase is expected to be roughly perpendicular to the ripple wave vector, in the limit of large λ. In a dilute solution of lipids, below T_c, we predict a tubular phase with a monodisperse radius $(2\kappa/\tau)$ and widely varied lengths by assuming a finite line tension τ. For diacetylenic lipid systems, the tubule radius is typically 0.3–1 μm, which gives a line tension (edge energy per unit length) of diacetylenic lipid membranes of $\tau \sim 10^{-7}$ dyn. Our theory also predicts a first-order transition from the spherical vesicle phase to the tubular phase upon cooling.

Considering a two-dimensional bilayer membrane with uniform molecular tilt, the free energy of the membrane in our model can be expressed as

$$F=\int dx_1 dx_2 (f_b+f_m),$$

$$f_b=\frac{1}{2}\kappa(\partial_1^2\mathbf{r}+\partial_2^2\mathbf{r})^2+\bar{\kappa}(\partial_1^2\mathbf{r})(\partial_2^2\mathbf{r}), \tag{1}$$

$$f_m=\lambda(\partial_1^2\mathbf{r})^2|\mathbf{m}|^2+t|\mathbf{m}|^2+u|\mathbf{m}|^4,$$

where f_b is the local curvature energy density and f_m is the Landau expansion of the local free energy density with respect to the tilting order parameter $\mathbf{m}$. We have arbitrarily chosen the x_1 axis in the tilting direction. As shown in Fig. 1, the tilt field $\mathbf{m}$ is the projection of the molecular orientation $\mathbf{n}$ onto the local tangent plane, i.e., $\mathbf{m}=\mathbf{n}-\mathbf{n}\cdot\mathbf{N}$, where $\mathbf{N}$ is the membrane normal unit vector. Here κ is the bending rigidity, $\bar{\kappa}$ is the Gaussian rigidity, $t=(T-T_c)/T_c$ is the reduced temperature, and $u>0$ is the coefficient of the high-order term $\mathbf{m}^4$ which stabilizes the tilting order parameter. The coupling constant λ is expected to increase with l/a. For a nearly flat fluid membrane with thermal fluctuations, we adopt the ansatz $\mathbf{r}=(\xi_1 x_1,\xi_2 x_2,h(x_1,x_2))$ [10] where $h(x_1,x_2)$ is the membrane height profile relative to a reference x_1-x_2 plane. At $t>0$, the tilt field $\mathbf{m}$ vanishes everywhere and $f_m=0$. At $t<0$, the minimization of f_m leads to

$$|\mathbf{m}|^2=-\frac{t+\lambda(\partial_1^2 h)^2}{2u} \tag{2}$$

and

$$f_b+f_m=\frac{1}{2}\kappa'(\partial_1^2 h)^2+\frac{1}{2}\kappa(\partial_2^2 h)^2+\kappa(\partial_1^2 h)(\partial_2^2 h)+\bar{\kappa}(\partial_1^2 h)(\partial_2^2 h), \tag{3}$$

where the renormalized bending rigidity in the x_1 direction is $\kappa'=\kappa-t\lambda/2u$. Immediately, we see that the symmetry of the bending rigidity in the x_1 direction and x_2 direction is broken as $t<0$. Here we define the asymmetry of the bending rigidity as $\Delta\equiv\kappa'-\kappa$. The asymmetry vanishes at $t\geq 0$ and is linear in $-t$ for $t<0$. This corresponds to a continuous phase transition in the spontaneous symmetry breaking of the bending rigidity with respect to temperature. Since the asymmetry is predicted to be linear in $|t|$ and λ, one expects that the rigidity in the tilt direction is much enhanced for a thick membrane at low temperatures. The persistence length in the tilt direction is enhanced by a factor $e^{|t|\lambda/2ukT}$. For such a membrane, ripple phases undulating along the tilt direction are not favored compared to those undulating along the other direction. Previously we have proposed a theory [4] for the ripple phase of lipid bilayers without including the coupling between the molecular orientation and local curvature square. We predict the existence of various tilt structures with similar one-dimensional ripple shapes. Taking the anisotropic effect into consideration, our $P_{\beta'}^{(1)}$ phase, whose tilt direction is roughly perpendicular to the wave vector, will be favored in the limit of large λ. Indeed, this is consistent with the experimental results of Hentschel and Rustichelli [3].

Furthermore, we consider this symmetry breaking phenomenon in a dilute solution of lipids. Particularly, we focus on the transition from the spherical vesicle phase to the tubular phase upon cooling. For a dilute lipid solution, closed vesicles are preferred in order to minimize their edge energy [11]. Minimizing the edge energy leads to a spontaneous curvature or equivalently a preferred area difference of two monolayers of vesicles which can be expressed as $\Delta A \simeq l \oint dA[(\partial_1^2\mathbf{r})+(\partial_2^2\mathbf{r})]$ as long as the bilayer thickness is much smaller than the overall vesicle size [12]. The effective spontaneous curvature can be derived by considering the relative free energy of vesicles to flat membranes with finite surface area. Setting the surface energy of a flat membrane to be zero, the local surface energy density of a vesicle can be expressed as

$$f_b=\frac{1}{2}\kappa(\partial_1^2\mathbf{r}+\partial_2^2\mathbf{r})^2+\bar{\kappa}(\partial_1^2\mathbf{r})(\partial_2^2\mathbf{r})-\frac{\tau}{2}(\partial_1^2\mathbf{r}+\partial_2^2\mathbf{r}), \tag{4}$$

where the integration of the third term gives the edge energy of the corresponding flat membranes. Here we have explicitly broken the bilayer symmetry in order to minimize the edge energy of the vesicles. For a sphere with radius R_0, its corresponding flat membrane has an edge energy $4\pi R_0\tau$. For a tubule with radius $R_0/2$ and length L, it can be obtained by merging $N_s=L/(4R_0)$ spheres and its corresponding flat membranes have an edge energy of $\pi L\tau$. The fact that the tubule radius is half of the sphere radius is obvious from Eq. (5). Including the configurational entropy of vesicles will lead to a broader distribution in the sphere radius but not in the tubule radius since the tubule length can vary widely. Minimizing the local free energy density (f_b+f_m) with respect to $\mathbf{m}$ at $t<0$, we have

$$f_b+f_m=\frac{1}{2}\kappa\left[(\partial_1^2\mathbf{r})+(\partial_2^2\mathbf{r})-\frac{\tau}{2\kappa}\right]^2+\bar{\kappa}(\partial_1^2\mathbf{r})(\partial_2^2\mathbf{r})-\frac{t\lambda}{2u}(\partial_1^2\mathbf{r})^2+\text{const.} \tag{5}$$

In Eq. (5), the term $\tau/(2\kappa)$ is equivalent to the so-called spontaneous curvature in the spontaneous curvature model [1]. Our model predicts the same effective spontaneous curvature for both spheres and tubules, which only depends on the bending rigidity and line tension. This prediction is consistent with numerical results of Jülicher and Lipowsky [13]. If the components of two monolayers of membranes are different from each other, additional spontaneous curvature

should be added to the free energy density. In addition, we include the free energy contributions from the entropy of N vesicles constrained in a volume V at temperature T, which is given by the Sackur-Tetrode equation as $F_{\text{entropy}}=-NkT\{3/2+\ln(v/\lambda_T^3)\}$ [14]. Here, $v\equiv V/N$, $\lambda_T\equiv h/\sqrt{2\pi mT}$ is the thermal wavelength of a vesicle with mass m, and h is Planck's constant. To study the instability of the spherical vesicle phase as the temperature decreases, we compare its overall free energy to that of possible equilibrium phases. To simplify our problem, for $|\bar{\kappa}|$ comparable with kT, we ignore nontrivial topologies and only consider spherical vesicles, tubules, and flat membranes. For $\bar{\kappa}>0$, the minimization of Eq. (5) gives

$$\partial_1^2\mathbf{r}=0,\qquad \partial_2^2\mathbf{r}=\frac{\tau}{2\kappa}, \tag{6}$$

which corresponds to a tube with radius $2\kappa/\tau$. A flat phase is less stable than the tubular phase since it has a higher free energy. Furthermore, as the temperature decreases further below T_c, the third term in Eq. (5) favors the tubular phase over the spherical vesicle phase. In the following we will only consider the transition of the spherical vesicle phase to the tubular phase as temperature decreases. We then define the free energy difference of these two phases as $\Delta F\equiv F_{\text{sphere}}-F_{\text{tubule}}$, which is given by

$$\frac{\Delta F}{N}\simeq\begin{cases}4\pi\bar{\kappa}-kT[3/2+\ln(v/\lambda)], & t>0,\\ 4\pi[-t\lambda/(2u)+\bar{\kappa}]-kT[3/2+\ln(v/\lambda)], & t<0,\end{cases} \tag{7}$$

where we have ignored the entropy of tubules which is small compared with that of spheres. For $\Delta F<0$ the equilibrium phase is the spherical vesicle phase, while for $\Delta F>0$ the equilibrium phase is the tubular phase. As predicted in this model, the phase boundary between the spherical vesicle phase and the tubular phase strongly depends on the values of $\bar{\kappa}$ and t. The tubular phase is favored at large $\bar{\kappa}$ and small t. Moreover, in this model, larger values of λ favor the tubular phase. Notice that, for a nonvanishing line field on a closed surface of a topological sphere, there are at least two singular points [15] which could lead to a rupture of the spheres upon cooling. The extra energy due to these singularities can further destabilize the spherical vesicle phase and therefore shift the phase boundary toward the spherical vesicle phase. We predict that the sphere-to-tubule transition is first order.

The above theory predicts the existence of a stable tubular phase. It is also of general interest to describe the equilibrium distribution of tubules with different radii and lengths [16]. Here we consider a dilute suspension of many tubules. The total Helmholtz free energy F_{tot} for a system of noninteracting tubules in a box of volume V is given by the ideal gas free energy $\beta F_{\text{tot}}=\Sigma_{M=M_{\min}}^{\infty}N_M[\ln(N_M)-1+F_M(V)]$, where N_M is the number of tubules of M lipids on each tubule and $F_M(V)$ is the free energy of a single tubule of M lipids whose center of mass is constrained in a volume V. The lower cutoff $M_{\min}$ is introduced to include the fact that the density of very small tubules will be strongly suppressed by molecular packing effects. We then obtain the equilibrium size distribution by minimizing F_{tot} with respect to N_M in the presence of a constraint on the total number of lipids. The size distribution is given by

$$N_M(V)\simeq e^{-\beta\{\kappa[(1/R)-(\tau/2\kappa)]^2A+\mu M\}}, \tag{8}$$

where A is the surface area of tubules and μ is a Lagrange multiplier to constrain the total number of lipids in the system. In the thermodynamical limit, $\mu\to 0$. The radius (R) distribution is given by $\rho(R)\propto\exp[-\beta\kappa(1/R-\tau/2\kappa)^2A]$ which indicates a monodisperse distribution of tubules' radius ($R\sim 2\kappa/\tau$) since the line tension τ is the same for all vesicles. The length (L) distribution is given by $\rho(L)\propto\exp(-\beta\mu L)$ which shows no length dependence in the thermodynamical limit. For diacetylenic lipid systems, the tubule radius is typically 0.3–1 μm, and the tubule length is typically 50–200 μm and can be as long as 1200 μm. This corresponds to a line tension of diacetylenic lipid membranes, $\tau\sim 10^{-7}$ dyn, which is consistent with the estimation ($\tau\sim k_{11}$) from standard liquid crystal theory, where k_{11} is the splay elastic constant.

Tubules have been observed in a variety of systems, such as diacetylenic lipids, bile, surfactants, and glutamates [6]. The formation of tubules has attracted many theoretical considerations. In the literature, there have been three general approaches to the theory of tubule formation. First, de Gennes argued that a spontaneous electrostatic polarization can induce a narrow strip of membrane to buckle into a cylinder [7]. However, experimental results of Chappell and Yager have shown that electrolytes in solution do not affect the radius of tubules [17]. Second, the model proposed by Lubensky and Prost predicts a scaling $R\propto\sqrt{L}$ resulting from competition between the curvature energy and the edge energy [8]. Nevertheless, experimental results of Georger *et al.* have found no correlation between R and L [18]. More precisely, R is quite monodisperse while L varies widely. A third approach to tubule formation based on the chiral packing of molecules in a membrane has been proposed by Helfrich and Prost as well as many other authors [9]. Although this approach seems to be more consistent with the experimental results, the major drawback comes from the prediction of the radius dependence on the chirality of the membrane and molecular tilt. So far, experiments show no evidence that the tube radius diverges as the untilted phase is approached or the radius can be varied by changing the magnitude of the chirality [19]. In fact achiral amphiphiles can also form stable tubules [20]. Therefore there are still many open questions about the formation of tubules. Nevertheless, in a more recent work by Seifert *et al.* [21], achiral tubules of radius about κ/β can be obtained by unlocking the tilting directions of the two monolayers, where β is the coupling between the curvature tensor and the tilting directions and has dimensions of energy/length. This unlocking of the two tilting directions can be induced, for example, by an edge energy of the membrane in dilute solutions of lipids, and $\beta\sim\tau/2$ in our case. Our model has tried to answer the above questions and seems to be consistent with the experimental results so far. We note that chiral terms have not been included in the free energy for simplicity; they are believed to

be responsible for the helical patterns observed in some tubules and have been extensively discussed by many authors [9,22].

In summary, we have presented a Landau theory for the bending anisotropy of membranes upon cooling. The bending rigidity is enhanced in the tilt direction, but not affected in the other one. Our theory predicts a continuous phase transition of the asymmetry of the bending rigidity as the temperature decreases and the asymmetry grows as $T_c - T$ below T_c. This bending anisotropy can have a profound effect on the tilt structure of the ripple phase and we expect that the tilt direction of the $P_{\beta'}$ phase is roughly perpendicular to the ripple wave vector. We also discuss the transition between the spherical vesicle phase and the tubular phase. Spherical vesicles become unstable to tubules as molecular tilt develops below T_c. The transition from spheres to tubules is predicted to be first order. Those tubules have a radius $2\kappa/\tau$ which is independent of the chirality and molecular tilt, while their length can vary widely. These predictions seem to be consistent with experimental results. We also predict the line tension of diacetylenic lipid membranes to be about 10^{-7} dyn. To further test our theory, one can measure the asymmetry of the bending rigidity of a black membrane by using differential confocal microscopy (DCM) [23]. DCM uses one laser beam to create optical excitations of the membrane and another laser beam to measure the driven membrane motion. Due to the high resolutions (2 nm in depth resolution and 0.3 μm in lateral resolution) of DCM, the bending rigidity of the membrane in two principal directions can be accurately measured at various temperatures and one expects to see the asymmetry increasing as $T_c - T$ below T_c.

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