RAPID COMMUNICATION

Curvature-induced lipid segregation

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(Received 3 April 2015; revised manuscript received 8 April 2015; published online 10 April 2015)

We investigate how an externally imposed curvature influences lipid segregation on two-phase-coexistent membranes. We show that the bending-modulus contrast of the two phases and the curvature act together to yield a reduced effective line tension. On largely curved membranes, a state of multiple domains (or rafts) forms due to a mechanism analogous to that causing magnetic-vortex formation in type-II superconductors. We determine the criterion for such a multi-domain state to occur; we then calculate respectively the size of the domains formed on cylindrically and spherically curved membranes.

Keywords: curvature, phase separation, lipid membrane, rafts

PACS: 87.16.dt, 64.75.St, 61.30.Dk **DOI:** 10.1088/1674-1056/24/6/068701

Lipid membranes play important roles in maintaining cell integrity and intracellular trafficking.[1,2] At high temperatures, the three main components of the membranes (saturated lipids, unsaturated phospholipids, and cholesterols) form a homogeneous mixture; below a critical demixing temperature, the three components segregate into two coexistent fluid phases, a saturated-lipid-enriched liquid-ordered (L_o) phase and unsaturated-phospholipid-enriched liquid-disordered (L_d) phase. Small L_o domains formed on cell membranes are also referred to as rafts.[3] The L_o phase (or rafts) has a larger bending modulus than the L_d phase.[4–6] There have been a large body of studies[7–18] investigating how membrane curvature influences the segregation of membrane lipids. All these studies are based on a physical mechanism that the L_o phase because of its larger resistance to bending prefers flatter regions and drives the L_d phase to more curved regions, i.e., these studies have implicitly assumed what matters is the variation of curvature rather than the curvature itself.

In this letter, we investigate how a uniform externally imposed curvature may influence lipid segregation on an L_o–L_d coexistent membrane. We show that, with the presence of a bending-modulus contrast, the curvature of the membrane induces the lipids to tilt away from the membrane normal and yields a reduced effective line tension between the lipid domains; when the curvature is larger than a certain critical value, lipid segregation leads to the formation of multiple L_o/L_d domains (or rafts) of a microscopic lengthscale rather than a complete separation of the two phases. We determine the criterion for the multi-domain pattern to occur; we then calculate respectively the size of the domains formed on cylindrically curved and spherically curved lipid membranes. We point out that although inspired by lipid systems the results obtained in this letter apply to any membranes that contain two distinct smectic phases.

Nonzero curvatures of lipid membranes can be either induced by their intrinsic spontaneous curvatures or imposed externally. We consider the latter case. (More precisely, we are interested in membranes whose curvatures are different from their spontateous values, including flat membranes with nonzero spontaneous curvatures (SC). Although this paper focuses on membranes having zero SC, the results achieved here can be easily generalized (with suitable modifications) to those with nonzero SC. In particular, when applied to flat membranes with nonzero SC, our results explain why the size of *in vivo* rafts is only a few nanometers.) Examples of lipid membranes with an externally imposed curvature includes *in vivo* membranes attached to BAR domains of proteins[19] and *in vitro* membranes attached to rigid substrates. There are two possible extreme types of lipid configurations resulting from an imposed curvature: (i) the lipids are perpendicular to the membrane surface and the energy cost originates from the splay energy cost of lipid orientation; and (ii) the lipids are parallel to each other, and the energy penalty is induced by the deviation of lipids' orientations from the membrane normals,

*Project supported by the Hundred-Talent Program of the Chinese Academy of Sciences (FY) and the National Science Foundation of USA via Grant DMR-1106014 (RLBS, JVS).

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or more microscopically, by the relative sliding of the lipids with respect to one another.

In general, the lipid configuration of a curved membrane should be a superposition of the aforementioned two types. The corresponding elastic energy density thus includes two parts, the splay energy and the tilt energy penalty,

$$f = \frac{1}{2}K(\nabla' \cdot \boldsymbol{n})^2 - \frac{1}{2}C(\boldsymbol{n} \cdot \boldsymbol{N})^2, \tag{1}$$

where $\boldsymbol{n}$ and $\boldsymbol{N}$ are unit vectors representing, respectively, lipid orientation and membrane normal, ∇' represents the two-dimensional derivative on a curved surface,[20] K is the splay coefficient, and C is a coefficient measuring how strongly $\boldsymbol{n}$ is locked to $\boldsymbol{N}$. The combination of K and C gives a characteristic length $\xi = \sqrt{K/C}$, which is the penetration length of lipid tilting. For a single-phase membrane, given that ξ is usually much smaller than the membrane size, most of the lipids are perpendicular to the membrane surface, and the bending modulus of the membrane is mostly determined by the splay coefficient K. A bending-modulus contrast in the literature thus corresponds to a splay-coefficient contrast.

For an L_o–L_d coexistent membrane, the total energy includes the contributions from both phases. We will use subscripts "o" and "d" to distinguish the quantities of these two phases. For convenience in the expressions, we will also use the subscript i, with $i = 1$ representing the phase with smaller volume fraction and $i = 2$ representing the one with larger volume fraction; in a case of no ambiguity, we will use a symbol without subscript to represent the corresponding quantities of both phases. The total energy of a two-phase-coexistent membrane should also include a phase-boundary energy cost, which equals the product of the line tension t and the phase-boundary length. To minimize the boundary energy, the two phases on a flat membrane completely separate from each other, with each phase forming a large single domain. However, for a curved membrane, such a single domain may become unstable and split into multiple domains.

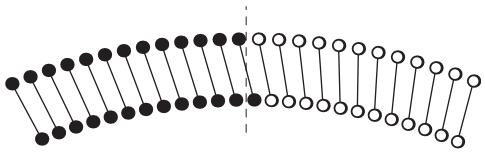


Fig. 1. Illustration of external-curvature-induced lipid-tilting in the phase-boundary region. The solid and hollow dimers represent, respectively, L_o and L_d lipids. The dashed line represents the membrane normal at the phase boundary.

We now present the reason for the splitting of large single domains and determine the corresponding instability criterion. As illustrated in Fig. 1, when subjected to an externally imposed curvature, the L_o lipids tilt away from the membrane normals and become more parallel to each other so as to reduce the splay energy cost, given that K_o is larger than K_d. The anchoring energy, i.e., the second term in Eq. (1), constraints the tilting to occur within a narrow region of size $\sim \xi$ around the phase boundary. The energy change induced by such tilting can be computed. To simplify calculation, we assume that the size of the large single domains resulting from a complete phase separation is much larger than ξ and that the imposed curvature is much smaller than $1/\xi$. Thus, in the aforementioned phase-boundary region, the splay $\nabla' \cdot \boldsymbol{n}_i$ can be approximated by $2H + (-)^i \partial\gamma_i/\partial s_i$, where H is the mean curvature, s_i is the distance between the phase boundary and the lipid location in phase i (i.e., the arc length of the geodesic perpendicular to the phase-boundary line), and γ_i is the angle between the membrane normal and the orientation of lipids of phase i. Note that the lipids now tilt in the plane defined by the membrane normal and the tangential vectors of these geodesics. The factor $(-)^i$ is added into the expression of $\nabla' \cdot \boldsymbol{n}_i$ because of the opposite directionality of s_1 and s_2. Substituting the approximate expression of $\nabla' \cdot \boldsymbol{n}_i$ into Eq. (1) and taking the functional derivative yields $\gamma_i = \gamma_b \exp(-s_i/\xi_i)$, where γ_b represents the deviation angle at the phase boundary. The energy change resulting from the lipid tilting can then be obtained by minimizing over γ_b the sum of the energies of L_o and L_d. The combination of this energy change and the line tension t produces a reduced effective line tension:

$$t_e = t - \frac{2H^2(K_o - K_d)^2}{\sqrt{C_o K_o} + \sqrt{C_d K_d}}. \tag{2}$$

Equation (2) clearly shows t_e can be lowered either by increasing H or the contrast between K_o and K_d or by decreasing C_o and C_d.

The instability criterion of the single-domain state can then accordingly be obtained by simply setting $t_e = 0$. We can further simplify this criterion to an approximate form: $t/H^2 \lesssim K\xi$. Given that K is of order 10^{-19} J for most lipid systems[20] and ξ can be assumed to be comparable to the membrane thickness and is thus of order 1 nm,[21,22] the criterion is then $t/H^2 \lesssim 10^{-28}$ J · m. Depending on the compositions and temperature, the line tension t on a lipid membrane usually varies between 1 pN and 0.01 pN (or even smaller).[5,6,23,24] For $t \sim 1$ pN, the critical curvature H_c is thus of order 10^8 m^{-1}; for $t \sim 0.01$ pN (for example, in systems with the presence of hybrid lipids,[25]) H_c is of order 10^7 m^{-1}.

As t_e becomes negative, the large single domains resulting from a complete phase separation become unstable and split into multiple small domains. In the following paragraphs, we calculate the size of the lipid domains formed on cylindrically curved and spherically curved membranes, respectively.

For a lipid membrane subjected to a uniform cylindrical curvature, we assume there is translational symmetry along $\boldsymbol{e}_z$, the direction of the long axis of the cylinder, and that the lipids align perpendicular to $\boldsymbol{e}_z$. In this case, a domain is a strip along $\boldsymbol{e}_z$, and the three-dimensional problem becomes a two-dimensional problem. The splay of the lipid orientation can then be expressed as $\nabla' \cdot \boldsymbol{n}_i = \cos\gamma_i[1+\partial\gamma_i/\partial\theta_i]/R_c$, where R_c is the radius of the cylinder and the θ's are the azimuthal angles representing the lipid location in a domain. In the center of a domain, i.e., at $\theta_i = 0$, the lipids align along the membrane normal with $\gamma_i = 0$. Substituting the expression of $\nabla' \cdot \boldsymbol{n}_i$ into Eq. (1) and then differentiating the energy density with respect to γ_i yields $\gamma_i = A_i \sinh(\theta_i R_c/\xi_i)$, where the A's are the amplitudes and the approximations $\xi_i \ll R_c$ and $\gamma_i \ll 1$ have been used. Approximately speaking, the deviation γ_i decays exponentially for domains of size much larger than ξ_i and linearly for those of a size smaller than ξ_i. The amplitudes A_1 and A_2 are not independent from each other — they are related by the continuity condition $\gamma_1(\theta_1^s) = \gamma_2(-\theta_2^s)$, where θ_1^s and θ_2^s are, respectively, the angular sizes of the L_1 and L_2 domains (from the domain centers to the domain edges). We then minimize the total energy (per unit length along the long axis of the cylinder) over the A's and obtain

$$E = \frac{2\pi}{\theta_1^s + \theta_2^s}\left[t - \frac{(K_1-K_2)^2/R_c^2}{2\sum_{i=1}^2 \sqrt{C_i K_i}\coth(\theta_i^s R_c/\xi_i)}\right], \qquad (3)$$

which clearly shows a competition between the energy penalty of the line tension and the energy gain of the director tilting induced by the difference between K_1 and K_2. The critical line tension t_c, below which the completely separated state becomes unstable, is given by the maximum value of the second term in the parenthesis in Eq. (3), which is the same as the one we can obtain from Eq. (2) by setting $t_e = 0$ and $H = 1/(2R_c)$.

At $t < t_c$, a stable multi-domain ground state is expected. The domain size can be determined by numerically minimizing Eq. (3) over θ_1^s (or θ_2^s) with the constraint $\theta_1^s/\theta_2^s = \phi/(1-\phi)$ applied, where ϕ is the volume fraction of phase 1. The results are given in Figs. 2(a)–2(d), which show that the domain size decreases with t and quickly reduces to order ξ_o. For $\phi = 1/2$ [see Fig. 2(a)], because of the permutation symmetry of the two lipid phases, the curves describing how the domain size varies with the normalized line tension t' $(= t/t_c)$ for the K's with a certain ratio v (i.e., $K_1 = vK_2$) overlap with the curves for the K's with a ratio of $1/v$ (i.e., $K_1 = K_2/v$). For $\phi < 1/2$, this permutation symmetry is broken: the L_o domains formed on membranes with the L_o phase having a volume fraction ϕ have a larger size than the L_d domains formed on the phase-permutated membranes, i.e., membranes with the L_d phase having a volume fraction ϕ. Moreover, the size difference increases as ϕ decreases [see Figs. 2(a)–2(d)]. The origin of this size difference and its variation with ϕ is given as follows. As can be seen from Fig. 1, with the presence of an external curvature, the L_o lipids provide tilt-driving forces while the L_d lipids resist tilt. Membranes with the L_d phase having volume fraction ϕ possess more L_o lipids and thus tend to have more phase-boundary regions so that tilt can occur. A preference for boundary regions means a preference for smaller domains, and therefore leads to the aforementioned domain-size difference and its variation with ϕ.

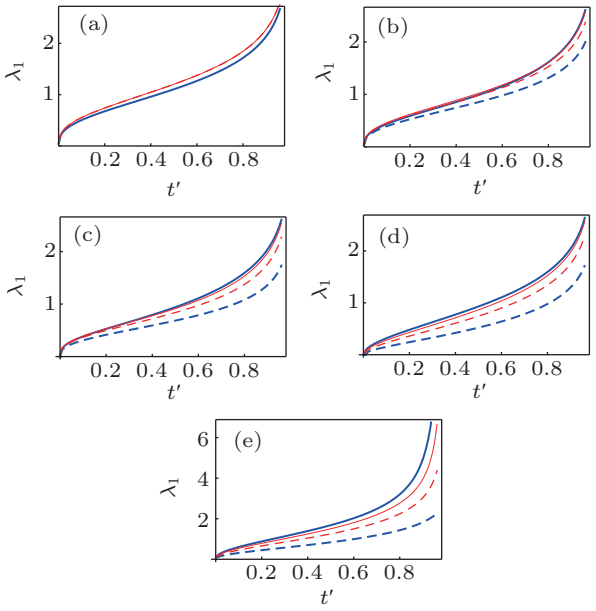


Fig. 2. (color online) (a)–(d) Relations between the L_1-domain size (measured in unit ξ_o, $\lambda_1 = \theta_1^s R_c/\xi_o$) and the normalized line tension $t' = t/t_c$ on a cylindrically curved membrane for (a) $\phi = 0.5$, (b) $\phi = 0.4$, (c) $\phi = 0.3$, and (d) $\phi = 0.01$; (e) relations between $\lambda_1 = \theta_1^s R_s/\xi_o$ and t' on a spherically curved membrane for $\phi = 0.01 (\ll 1)$. The (blue) thick solid and dashed lines correspond to $K_1 = 2K_2$ and $K_1 = K_2/2$, and the (red) thin solid and dashed lines correspond to $K_1 = 1.2K_2$ and $K_1 = K_2/1.2$, respectively. We have set $C_1 = C_2$.

In addition to the numerical results, we also present in the following analytical results for the small t limit so as to clearly elucidate the dependence of the domain size (i.e., θ_1^s and θ_2^s) on the parameters. We consider two cases separately: i) the volume fractions of the two phases are of same order, i.e., $\phi \sim 1/2$; and ii) one phase has a much smaller volume fraction than the other, i.e., $\phi \ll 1/2$. In the case of $\phi \sim 1/2$, when $t \ll t_c$, we have $\theta_i^s \ll \xi_i/R_c$ for both phases, and the deviation angles γ_1 and γ_2 both decay (approximately) linearly from the phase boundary to the domain centers. We thus approximate $\coth(x)$ in Eq. (3) as $1/x + x/3$. We then differentiate the energy given in Eq. (3) with respect to θ_1^s, with the relation between θ_1^s and θ_2^s applied, and obtain

$$\theta_1^s \approx \left(\frac{3t}{R_c\tilde{C}}\right)^{1/3}\left[\frac{1+r\phi/(1-\phi)}{1-r}\right]^{2/3}, \qquad (4)$$

where $\tilde{C} = C_1 + C_2(1-\phi)/\phi$ and $r = K_2/K_1$. We mention the following three noteworthy points contained in Eq. (4): (I) a large contrast between the K's (i.e., large $|r-1|$) gives a small

domain; (II) although the angular size θ_1^s decreases as R_c increases, the linear domain size ($R_c\theta_1^s$) increases with R_c; (III) at $\phi = 1/2$, the permutation symmetry is conserved, viz, the value of θ_1^s does not change under the replacement $r \to 1/r$. We now turn to the case of $\phi \ll 1/2$. In this case, we have $\theta_1^s \ll \xi_1/R_c$ but $\theta_2^s \gg \xi_2/R_c$; γ_1 decays linearly and γ_2 decays exponentially from the phase boundary to the domain center. We thus replace $\coth(\theta_1^s R_c/\xi_1)$ and $\coth(\theta_2^s R_c/\xi_2)$ in Eq. (3) by $\xi_1/(\theta_1^s R_c)$ and $\coth(\infty)$, respectively. Minimizing Eq. (3) then yields

$$\theta_1^s \approx \left(\frac{2t}{\sqrt{C_2 K_2}}\right)^{1/2} \frac{1}{|1-r|}, \tag{5}$$

which is inversely proportional to $|r-1|$ and independent of the radius R_c (the linear domain size is thus linearly proportional to R_c). In addition, the angular size is now more sensitive to the variation of t than the angular size given in Eq. (4) is.

We proceed to address lipid segregation on a spherically curved membrane. A complete phase separation now leads to the formation of a single (curved) disk of L_1 lipids embedded in an L_2-lipid sea. The instability criterion of the single-disk pattern can be obtained from Eq. (2) by setting $t_e = 0$ and $H = 1/R_s$. For a negative t_e, a multi-domain pattern forms. In the case of $\phi \sim 1/2$, the shape of the formed multiple domains is complicated, and this complication makes an analytic calculation of the system energy inaccessible. We will study the $\phi \sim 1/2$ case in a numerical approach in a future publication and consider here only the case of $\phi \ll 1/2$. In this case, we have a pattern of multiple L_1 disks embedded in an L_2 sea.

We first give the explicit form of the energy as a function of the deviation angle γ and the equation determining how γ varies from the center of a domain to the domain edge. We adopt a spherical-coordinate description for the positions of the lipids, with the point of zero polar angle (i.e., $\theta = 0$) corresponding to the center of an L_1 domain, and we assume γ only depends on the polar angle θ but not the azimuth angle. Thus, for the total energy, we have

$$\begin{aligned} E = 2\pi N_d \Big\{ & R_s t \sin\theta_1^s \\ & + R_s^2 \int_0^{\theta_1^s} d\theta \sin\theta \left[\frac{1}{2} K_1 (\nabla' \cdot \boldsymbol{n}_1)^2 + \frac{1}{2} C_1 \sin^2\gamma_1\right] \\ & + R_s^2 \int_{\theta_1^s}^{\theta^u} d\theta \sin\theta \left[\frac{1}{2} K_2 (\nabla' \cdot \boldsymbol{n}_2)^2 + \frac{1}{2} C_2 \sin^2\gamma_2\right] \Big\}, \end{aligned} \tag{6}$$

$$\nabla' \cdot \boldsymbol{n}_i = \frac{1}{R_s}\Big\{ \cos\gamma_i(\theta)\left[2 + \frac{\partial\gamma_i(\theta)}{\partial\theta}\right] + \cot\theta \sin\gamma_i(\theta)\Big\},$$

where R_s is the radius of the sphere, θ_1^s and $N_d = 2\phi/(1-\cos\theta_1^s)$ are respectively the angular size and the total number of the L_1 domains. The upper limit θ^u of the second integration equals to π in the single-domain case; and its value in the multi-domain case will be addressed later. Differentiating E with respect to γ, under the assumption that γ is small, yields

$$\frac{\partial^2\gamma_i}{\partial\theta^2} + \frac{\partial\gamma_i}{\partial\theta}\cot\theta - \gamma_i\left(\frac{R_s^2}{\xi_i^2} - 4 + \csc^2\theta\right) = 0. \tag{7}$$

We now proceed to determine the domain size, i.e., the size of the L_1 disks. Given the constraint $\phi \ll 1/2$, the distance between the disks becomes much larger than the disk size; and, in the L_2 sea, only a narrow annulus around the disks contributes to the system energy. Furthermore, because the domain size is small, the deviation angle is nonzero only at small θ's. We thus replace $\cot\theta$ and $\csc\theta$ in Eq. (7) by $1/\theta$, and obtain $\gamma_1 \propto \mathrm{BI}_1(b_1\theta)$ and $\gamma_2 \propto \mathrm{BK}_1(b_2\theta)$, where $b_i = \sqrt{(R_s/\xi_i)^2 - 4} \approx R_s/\xi_i$, and $\mathrm{BI}_n(x)$ and $\mathrm{BK}_n(x)$ are, respectively, the modified Bessel functions of the first and second kind. To calculate the energy, we then follow a procedure similar to that for the cylindrical case and obtain

$$E = 8\pi\phi\left[\frac{R_s t}{\theta_1^s} - \frac{2(K_1-K_2)^2}{\mathrm{W}(\theta_1^s)}\right], \tag{8a}$$

$$\mathrm{W}(\theta_1^s) := K_1 b_1 \theta_1^s \frac{\mathrm{BI}_0(b_1\theta_1^s)}{\mathrm{BI}_1(b_1\theta_1^s)} + K_2 b_2 \theta_1^s \frac{\mathrm{BK}_0(b_2\theta_1^s)}{\mathrm{BK}_1(b_2\theta_1^s)}. \tag{8b}$$

Minimizing this energy yields the domain size. Figure 2(e) shows the numerical results, which are qualitatively similar to the results of cylindrically curved membranes with small ϕ [see Fig. 2(d)].

We conclude with a remark that the multi-domain-inducing mechanism addressed in this paper is different from that using elastic repulsions to stabilize multiple domains on a lipid vesicle,[5,26], but analogous to the mechanism via which a large magnetic field induces the formation of magnetic vortices in type-II superconductors.[27] The tilt penetration length ξ in this work is the counterpart of the magnetic field penetration length in superconductors. What corresponds to the superconducting coherence length is the correlation length of the density fluctuation of the various membrane constituents.[24] We have implicitly assumed this correlation length is smaller than ξ, viz., we have assumed a sharp boundary between the two coexisting phases. We will present elsewhere how an externally imposed curvature influences lipid segregation on membranes with this assumption violated, for example, on membranes with the temperature close to a critical demixing point.

Acknowledgment

We thank A. Travesset, P. M. Goldbart, T. C. Lubensky, and A. C. Shi for helpful discussions.

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