Dynamic Sorting of Lipids and Proteins in Multicomponent Membranes

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(Received 18 June 2012; published 6 November 2012)

Dynamic sorting of lipids and proteins in cellular membranes plays a critical role in establishing and maintaining distinct compositions in various organelles. Recent experiments found that the lipid sorting in a membrane tube highly depends on the pulling speed at the tip. However, the mechanism of this velocity dependence has not yet been revealed. In this Letter, we found that when a membrane is deformed rapidly, the lipid flow induced by fast membrane shape change will significantly affect the sorting results. The competition between the curvature-driven lipid sorting and the pulling-induced lipid flow leads to novel behaviors. When a membrane tube is pulled out from a liquid ordered (L_o) domain at a constant speed, slow pulling leads to the formation of a liquid disordered (L_d) tube, while fast pulling results in a L_o tube. Interestingly, in a membrane tube pulled at an intermediate speed, alternate L_d and L_o domains appear in the tube. The sorting dynamics and the corresponding pulling force were systematically studied. The results of this study could lead to a better understanding of the dynamic sorting and traffic of lipids and proteins in living cells.

DOI: 10.1103/PhysRevLett.109.198101 PACS numbers: 87.16.−b, 46.70.Hg, 87.15.Zg

In cellular membranes, sorting and traffic of lipids and proteins are essential to maintain the distinct compositions in the various membrane compartments of the cell. However, the sorting mechanism is still poorly understood. It has been suggested that lipids and proteins can be sorted to specific locations according to membrane curvature [1–6]. In model membranes, experiments have shown that the mixture of lipids in ternary system can separate into a liquid ordered (L_o) phase and a liquid disordered (L_d) phase [5–10]. The measured bending rigidity of the L_o phase is almost 2 times bigger than that of the L_d phase [5,11]. Consequently, in equilibrium, the L_o phase mainly appears in the low curvature regions of membranes so that the bending energy can be minimized.

A recent experiment [11] found that the lipid sorting in membrane tubes is not only determined by curvature but also highly dependent on the pulling speed at the tip. Two movies in Ref. [11] show that slow pulling leads to the formation of a L_d tube, while fast pulling results in a L_o tube. However, why the sorting result depends on the pulling speed has not yet been revealed in the theoretical study of Ref. [11]. In most conventional treatments [11–19] about multicomponent membranes, either the membrane shape is fixed, or the lipid velocity induced by membrane shape change is assumed to be small. So the Péclet number $\mathrm{Pe} = UL/D$ is small, and the lipid flow is negligible compared to diffusion. However, the situation is dramatically different if the deformation of membranes is fast. For a membrane tube with diameter $L \sim 0.1\ \mu$m, pulled at the speed $U \sim 10\ \mu$m/s, we have $\mathrm{Pe} \sim 1$ if the diffusivity of lipids $D \sim 1\ \mu\mathrm{m}^2/\mathrm{s}$. Apparently, the lipid flow induced by membrane shape change should not be neglected for this typical pulling experiment. In this Letter, we will show how this lipid flow significantly affects the sorting results.

We assume that the membrane is an incompressible binary fluid mixture composed of species A and B [2,4,11,14]. The mixture could represent a lipid-lipid or protein-lipid system. An order parameter is defined as $\phi = (c_A - c_1)/(c_2 - c_1)$, where c_A is the concentration of A and c_1 and c_2 are the concentrations of A in phase 1 and phase 2, respectively, on a flat membrane. In the context of the ternary system, phase 1 ($\phi = 0$) indicates a L_d phase and phase 2 ($\phi = 1$) indicates a L_o phase. The domain structure is modeled by Ginzburg-Landau free energy

$$E_1 = \int\left[\frac{\lambda}{2}\phi^2(1-\phi)^2 + \frac{\mu}{2}g^{\alpha\beta}\frac{\partial\phi}{\partial u_\alpha}\frac{\partial\phi}{\partial u_\beta}\right]\sqrt{g}d^2u, \quad (1)$$

where λ and μ are positive constants. u_α are the coordinates on the surface. $g^{\alpha\beta}$ is the contravariant metric tensor, and g is the determinant of the metric tensor. In these expressions, Greek letters indicate index 1 or 2.

Canham-Helfrich energy [20,21] is modified to study shape changes of multicomponent membranes [3]:

$$E_2 = \int\left[\frac{\kappa(\phi)}{2}(2H)^2 + \kappa_G(\phi)K + \sigma(\phi)\right]\sqrt{g}d^2u, \quad (2)$$

where H and K are mean and Gaussian curvature, respectively, $\kappa(\phi)$ and $\kappa_G(\phi)$ are bending and Gaussian rigidities, respectively, and $\sigma(\phi)$ is surface tension. $\kappa(\phi)$ depends on the composition, since its value is quite different in the L_o and L_d phases [22]. Following Ref. [3], we assume $\kappa(\phi) = \kappa_0[1 + \alpha_1(\phi^2 - 1)]$. A positive α_1 indicates that phase 2 (L_o) is stiffer than phase 1 (L_d). In general, the surface tension could also be different in the two phases [7,11]. Therefore, we further assume $\sigma(\phi) = \sigma_0[1 + \alpha_2(\phi^2 - 1)]$ and the constant α_2 can be positive or negative [3]. Here, we neglect the $\kappa_G(\phi)K$ term in Eq. (2). The coupling of Gaussian curvature and lipid composition shifts the position

of a phase boundary [23] and results in a thinner neck which could facilitate fission [24,25]. We also neglect the pressure difference across the membrane, since it has only a negligible effect on tube formation in big vesicles [26]. These effects will not be discussed in our work.

The total free energy of the system can be written as $E = E_1 + E_2 = \int [f(\phi) + \frac{\mu}{2} g^{\alpha\beta} \frac{\partial \phi}{\partial u_\alpha} \frac{\partial \phi}{\partial u_\beta}] \sqrt{g} d^2u$, where $f(\phi) = \frac{\lambda}{2}\phi^2(1-\phi)^2 + \frac{\kappa(\phi)}{2}(2H)^2 + \sigma(\phi)$. To simplify the problem, we assume that the density and diffusivity of two species of lipid are the same. The difference between the chemical potentials of the two species [27–29] is defined by $Q \equiv (1/\sqrt{g})\frac{\delta E}{\delta \phi} = \frac{\partial f}{\partial \phi} - \mu \nabla^2 \phi$, where $\nabla^2 \equiv (1/\sqrt{g})\frac{\partial}{\partial u^\alpha}(g^{\alpha\beta}\sqrt{g}\frac{\partial}{\partial u^\beta})$. Here Q can be interpreted as the energy decrease or increase as the lipid composition changes a unit value. Therefore, the diffusion can be driven either by the nonuniform distribution of lipids $\nabla^2\phi$ or by the curvature difference through $\frac{\partial f}{\partial \phi}$.

Figure 1(a) illustrates the mechanism by which high curvature drives a transition from the L_o phase to the L_d phase. Both phases are stable for a flat membrane. However, the L_o phase becomes less and less favorable as the mean curvature increases, until eventually the L_d phase is the only stable phase. The potential will become a single-well potential with a minimum at $\phi = 0$ if $\kappa_0\alpha_1(2H)^2 + 2\sigma_0\alpha_2 > \lambda/8$. So sufficiently high curvature can drive the formation of a high-flexibility L_d phase [3,6]. This conclusion is based on the implicit assumption that H is not the function of ϕ. The assumption is correct when a membrane is attached to a curved substrate [6]. In this case, the membrane curvature is fully determined by the substrate curvature. So curvature can influence the lipid composition, but the lipid composition cannot affect membrane curvature. However, for a freestanding lipid vesicle, the lipid composition and membrane curvature are fully coupled. It is very difficult to solve such coupled equations analytically. To reduce the complexity and get some

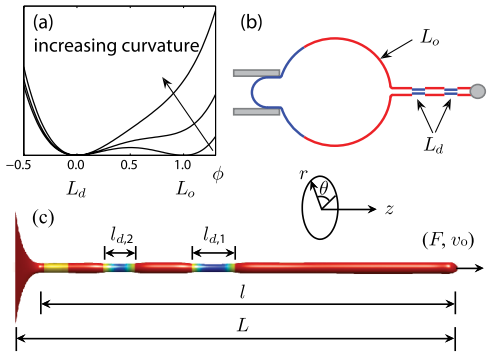

FIG. 1 (color online). (a) High curvature drives the transition from a double-well potential to a single-well potential [3]. (b) A typical membrane tube pulling experiment. Multiple L_d domains (blue) may appear when a tube is pulled out from a L_o domain (red) rapidly. (c) Simulation results for the formation and growth of multiple L_d domains in a tube that is pulled at a speed $\bar{v}_0 = 0.2$.

insight, we can consider the stability of a membrane tube with uniform lipid composition. We found that, in such a tube, the spinodal decomposition occurs when $0.21 < \phi < 0.79$ (see the discussion in Supplemental Material [34]).

Consider a typical membrane tube pulling experiment [Fig. 1(b)]. The Reynolds number $\mathrm{Re} = \rho UL/\eta$ is about 10^{-6} for a membrane tube with diameter $L \sim 0.1$ μm, pulled at the speed $U \sim 10$ μm/s, given that the density of water $\rho = 10^3$ kg/m^3 and the viscosity of water $\eta = 0.001$ Pa $\cdot$ s. So the inertial force of lipid membrane is negligible compared to the viscous force. Furthermore, the ratio of the viscous force to the elastic force of the membrane can be estimated by $\eta UL^2/\kappa_0$ [30]. By using the above parameters and $\kappa_0 = 10$–$20k_BT$, the above ratio should be 0.001–0.01. This means the viscous force can also be neglected. Therefore, both the inertial and viscous force are negligible compared to the elastic force of membrane, and the membrane shape is simply determined by $\delta E/\delta r = 0$. Notice that this conclusion is valid only for the velocity regime we are interested in, tens of microns per second. If the pulling speed is 1–2 orders of magnitude higher, the viscous force applied by surrounding fluids [31] and the friction between two leaflets [32] must be considered.

If we assume the membrane is incompressible, its mass density should be a constant. Therefore, from the covariant version of the Reynolds transport theorem for a moving surface with internal flows [30], the mass conservation of lipids can be described by

$$\nabla_\alpha V^\alpha = 2HV_n, \tag{3}$$

where V^α and V_n are the tangent and normal components, respectively, of the velocity $\mathbf{V} = V^\alpha \mathbf{t}_\alpha + V_n \mathbf{n}$. Here $\mathbf{t}_\alpha$ and $\mathbf{n}$ are the tangent vectors and unit normal vector of the surface, respectively. If the membrane is static ($V_n = 0$) or flat ($H = 0$), Eq. (3) can be reduced to the regular incompressibility equation $\nabla_\alpha V^\alpha = 0$ on a 2D surface. By considering the diffusion of lipids, the mass conservation of one species of lipid molecules yields a generalized Cahn-Hilliard equation about ϕ for a moving membrane with internal flows:

$$\frac{\partial \phi}{\partial t} + V^\alpha \nabla_\alpha \phi = M\nabla^2\left[\frac{\partial f}{\partial \phi} - \mu\nabla^2\phi\right], \tag{4}$$

where M is a generalized diffusion constant. The derivation of Eqs. (3) and (4) is given in the Supplemental Material [34].

In this problem, we can define two length scales $R = \sqrt{\kappa_0/2\sigma_0}$ and $d = \sqrt{\mu/\lambda}$, which represent the radius of the membrane tube in the L_o phase and the width of the phase boundary, respectively. We can also define two time scales $\tau_1 = R^4/M\kappa_0$ and $\tau_2 = R/v_0$, where v_0 is the pulling speed at the tube tip. τ_1 is the time scale of diffusion, and τ_2 is the time needed for the membrane tube to move distance R at speed v_0 due to the pulling at the tip. The pulling speed at the tube tip can be normalized as

$\bar{v}_0 = v_0\tau_1/R = R^3 v_0/(M\kappa_0)$, which can be interpreted as the ratio of the two time scales, i.e.,

$$\bar{v}_0 = \tau_1/\tau_2 = R^3 v_0/(M\kappa_0). \quad (5)$$

This dimensionless pulling speed can also be interpreted as the Péclet number in fluid mechanics which is the ratio of the rate of convection to the rate of diffusion. In our model, the length scale R is determined by κ_0 and σ_0, and the dimension of M is different from the classical diffusion constant [27–30]. So this dimensionless constant differs from the conventional definition of the Péclet number.

By using the front-fixing method [33] for moving boundary problems, the above coupled equations can be solved [34]. If a tube is pulled out from a L_d domain, no lipid sorting happens, since L_d domain has lower bending rigidity. Therefore, we consider only the case that a tube is pulled out from a flat L_o domain at a constant speed.

First we consider two limit cases. If $\bar{v}_0$ is very big, the diffusion process is much slower than the pulling process. Lipids are "frozen" on the surface, and the diffusion can be neglected. So the whole membrane is still in the L_o phase. In contrast, if $\bar{v}_0$ is very small, the membrane shape changes so slowly that the pulling can be regarded as a quasistatic process. The high curvature of the tube will drive the formation L_d phase. So the whole tube becomes L_d phase, while the flat membrane region connected to the tube is still in the L_o phase. Movies S1 and S2 in Supplemental Material [35] show the two limit cases, and the corresponding movies from experiments can be found in Ref. [11].

If the pulling speed is comparable with the diffusion speed, some interesting phenomena appear. For example, when $\bar{v}_0 = 0.1$, first a L_o tube is pulled out [Fig. 2(a)]. The average value of ϕ will decrease due to the curvature-driven diffusion. The lowest value of ϕ appears around the junction between the tube and vesicle, since it is the nearest high curvature region connected to the lipid reservoir. When this lowest value of ϕ is smaller than 0.79, the spinodal decomposition occurs and one L_d domain appears (see Movie S5 [35]). The L_d domain size grows almost linearly with time [Fig. 2(b)]. However, the normalized domain size, i.e., the ratio of the L_d domain length to the total tube length, grows nonlinearly and eventually reaches 1 [Fig. 2(c)] so that the whole tube becomes L_d phase (see Movie S3 [35]). This means the diffusion speed is relatively fast in this case and the right interface of the L_d domain finally catches up with the tube tip.

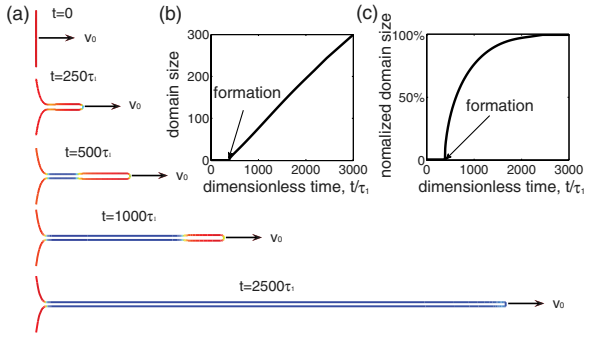

FIG. 2 (color online). (a) The time evolution of the membrane shape and lipid composition at $\bar{v}_0 = 0.1$ (also see Movie S3 [35]). Red and blue represent L_o and L_d phases, respectively. (b) The L_d domain size and (c) normalized domain size vs dimensionless time. For all the simulations in this work, we use the same parameters: $\alpha_1 = 0.5$, $\alpha_2 = 0$, $\lambda R^2/\kappa_0 = 3$ and $\mu/\kappa_0 = 1$.

If the pulling is a little faster ($\bar{v}_0 = 0.2$), L_d domains are formed one after another around the junction between the tube and vesicle [Fig. 3(a)]. The growth of the first four L_d domains is shown in Fig. 3(b). All domains follow the same pattern: formation, growth, shrinkage, and coalescence or disappearance. Whenever the lowest ϕ decreases to 0.79 around the neck region, a new L_d domain is formed (Movie S6 [35]). We can observe L_d domains travel to the tube tip, since they move faster than the pulling speed. Because of the confinement of the boundary and the limitation of the pulling speed, the interface close to the tube tip moves slower than the other interface as the L_d domain approaches the tip. Consequently, the L_d domain at the tube tip will coalesce with the neighboring domain to form a larger L_d domain (see Movie S4 [35]). This prediction has be verified by recent experiments [36].

The pulling forces can be calculated as $F = \partial E/\partial l$ [4,26]. Before membrane tube formation, the response of the membrane is linear. The slope of this region is fully determined by the surface energy [37]. After a force barrier, a membrane tube is formed and the pulling force is saturated [Fig. 4(a)]. The saturated forces for the two limit cases ($\bar{v}_0 = 10$ and $\bar{v}_0 = 0.01$) are given by $F = 2\pi\sqrt{2\kappa_0\sigma_0}$ and $F = 2\pi\sqrt{2\kappa_0\sigma_0(1-\alpha_1)(1-\alpha_2)}$, respectively. When $\bar{v}_0 = 0.1$, the pulling force decreases with the growth of the L_d phase and finally reaches a constant value after the whole tube becomes L_d phase. For $\bar{v}_0 = 0.15$ and 0.2, the pulling forces lie between the two limit cases and approach a constant with small oscillations. The oscillation comes from the formation and coalescence of L_d domains.

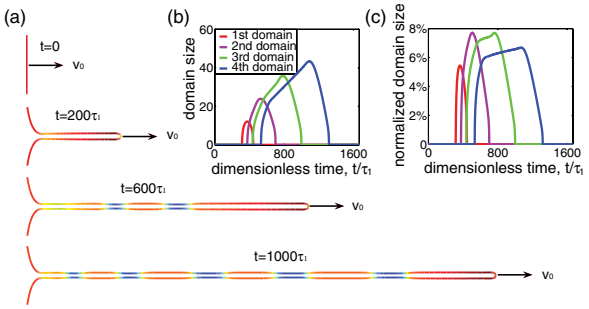

FIG. 3 (color online). (a) The time evolution of the membrane shape at $\bar{v}_0 = 0.2$ (more details in Movie S4 [35]). (b) The domain size and (c) normalized domain size of the first four L_d domains vs dimensionless time.

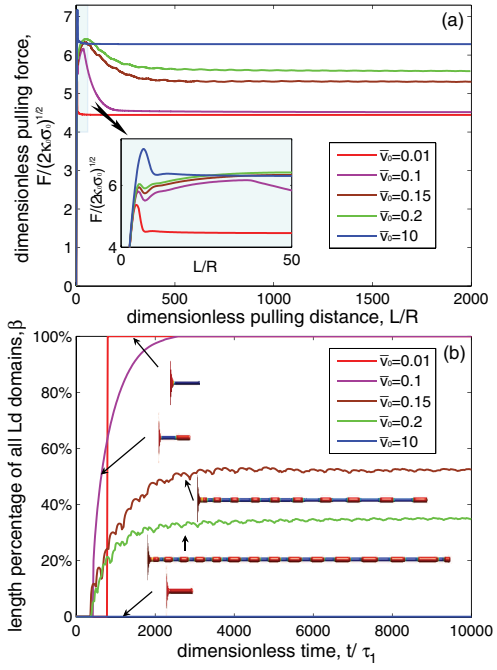

FIG. 4 (color online). (a) The dimensionless pulling force vs the dimensionless pulling distance for various pulling speeds. The inset shows the force barrier during membrane tube formation. (b) The length percentage of all L_d domains vs dimensionless time. The insets are the typical membrane shapes for various $\bar{v}_0$.

The length percentage of all L_d domains can be defined as $\beta = \sum_i l_{d,i}/l$, where l is the tube length and $l_{d,i}$ is the size of the ith L_d domain at a specific time [Fig. 1(c)]. When the pulling speed is very big ($\bar{v}_0 = 10$), the whole membrane is still in the L_o phase so that β is always zero [Fig. 4(b)]. When $\bar{v}_0$ is very small ($\bar{v}_0 = 0.01$), one L_d domain forms right after the membrane tube is formed, and thereafter the whole membrane is in L_d phase. So β is a step function from 0 to 100 percent. When $\bar{v}_0 = 0.1$, only one L_d domain forms and β increases nonlinearly with time and finally reaches 100 percent. When $\bar{v}_0 = 0.15$ or 0.2, multiple L_d domains are formed, and β approaches a constant that depends on $\bar{v}_0$. Similar to the pulling force, there are some small oscillations resulting from the formation and coalescence of L_d domains. Notice that if $\bar{v}_0$ is very small ($\bar{v}_0 = 0.01$), the formation of the L_d domain is limited by the pulling speed, since it requires the tube formation. Therefore, the formation time of the L_d domain is bigger than other cases [see the red curve in Fig. 4(b)] and determined by the tube formation time, while the formation time of the first L_d domain is almost the same in other cases because the formation time is set by the diffusion, not the pulling speed, when the pulling speed is big. In this case, the formation time, or waiting time, can be simply estimated as $T \sim L^2/M\kappa_0 \sim \tau_1 L^2/R^4$, where L is the membrane radius on the left boundary. In the simulation, we use $L = 10R$. Therefore, the waiting time $T \sim 10^2\tau_1$, which is consistent with our numerical results (Figs. 2–4).

To study how curvature-driven diffusion itself affects the sorting process, we pull out a tube from a L_o domain very quickly and then decrease $\bar{v}_0$ to zero to exclude the effects of lipid flow induced by membrane shape change. Since the initial pulling speed is very big, the whole membrane is still in the L_o phase as we have shown above. After the pulling is stopped, one L_d domain forms around the junction and grows nonlinearly with time [Fig. 5(a)]. The pulling force decays as the L_d domain grows until the whole tube is occupied by the L_d phase [Fig. 5(a)], which is consistent with experimental results (Fig. S5 of Ref. [11]). Interestingly, we found that the pulling force is approximately linear with the length percentage of the L_d domain [Fig. 5(b)]. This conclusion is also valid when the pulling speed is a nonzero constant [Fig. 5(b)].

To get more insight about pulling force, we can develop a simplified model. To simplify the problem, we focus on the pulling process after the tube is formed, so that we do not have to consider the force barrier during tube formation and neglect the catenoid region. Furthermore, we assume that the energy of phase boundaries is small compared with the energy of each phase. Therefore, in terms of the length percentage of all L_d domains $\beta = \sum_i l_{d,i}/l$, the total energy of the system, $E_1 + E_2$ [see Eqs. (1) and (2)], is simplified to

$$E = 2\pi r_d l\beta\left(\sigma_d + \frac{\kappa_d}{2r_d^2}\right) + 2\pi r_o l(1-\beta)\left(\sigma_o + \frac{\kappa_o}{2r_o^2}\right), \quad (6)$$

where κ_d (κ_o), σ_d (σ_o), and r_d (r_o) are the bending rigidity, surface tension, and radius, respectively, of L_d (L_o) domains. The equations $\partial E/\partial r_d = 0$ and $\partial E/\partial r_o = 0$ yield the radius of each phase: $r_d = \sqrt{\kappa_d/(2\sigma_d)}$ and $r_o = \sqrt{\kappa_o/(2\sigma_o)}$. The pulling force can be simply given by

$$F = \partial E/\partial l = F_o - \beta(F_o - F_d), \quad (7)$$

where $F_d = 2\pi\sqrt{2\kappa_d\sigma_d}$ and $F_o = 2\pi\sqrt{2\kappa_o\sigma_o}$ are the pulling forces for the pure L_d and L_o phase, respectively. So the pulling force is linear with β, which in turn is determined by diffusion of lipids and the lipid flow induced by pulling. If $\bar{v}_0$ is very small, the membrane tube is completely occupied by the L_d phase. So $\beta = 1$ and the

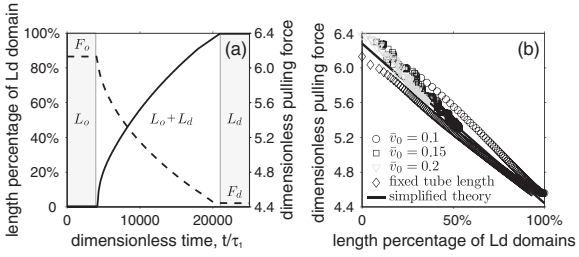

FIG. 5. (a) The increasing of the L_d domain size (solid curve) and the decay of the pulling force (dashed curve) in a membrane tube with fixed length. The whole tube is in the L_o phase initially. F_d and F_o are the pulling forces for the pure L_d and L_o phase, respectively. (b) The pulling force is approximately linear with the length percentage of all L_d domains.

pulling force is F_d. If $\bar{v}_0$ is very big, the whole tube is still in the L_o phase. So $\beta = 0$ and the pulling force is F_o. The prediction in Eq. (7) agrees with simulation results for various pulling speeds very well [Fig. 5(b)]. Notice that Eq. (7) is based on the assumption that the energy is a single-valued continuous function of l. So this is not applicable to the experiment where stepwise tube length extension was used [11].

In conclusion, we have shown how the competition between the pulling-induced lipid flow and the curvature-driven lipid sorting determines the formation, growth, and motion of the L_d domains when membrane tubes are pulled out from a L_o domain with various pulling speeds. The time evolutions of membrane shape and lipid composition within different regimes of pulling speed were investigated. In all cases, the pulling force is approximately linear with the length percentage of all L_d domains. We further derived a simple expression for the pulling force needed to pull out a multicomponent membrane tube.

This curvature-driven dynamic sorting mechanism might be crucial for many biological processes in living cells. For example, lipid domains have already been found in the membrane regions exhibiting controlled curvatures and fast shape changes, such as membrane protrusions (filopodia) and adhesion points [38–40], flagellar and ciliary membranes [41,42], pollen tube tips [43], and bacterial cell poles and septum [44].

We thank Thomas R. Powers and Tobias Baumgart for useful discussions on the work.

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Dynamic sorting of lipids and proteins in multi-component membranes: Supplemental Material

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(Dated: October 9, 2012)

I. THE DERIVATION OF GOVERNING EQUATIONS

The mass conservation of lipids can be described by the covariant version of Reynolds transport theorem for a moving surface with internal flows [1],

$$\frac{d}{dt}\int_{S(t)}\Phi\, dS=\int_{S(t)}\left[\frac{d\Phi}{dt}+\Phi\ \nabla_\alpha V^\alpha-2\Phi H V_n\right]\, dS, \tag{S1}$$

where Φ is an arbitrary scalar quantity and $\frac{d\Phi}{dt}=\partial_t\Phi+V^\alpha\partial_\alpha\Phi$ is the total time derivative. V^α and V_n are the tangent and normal components of the velocity $\mathbf{V}=V^\alpha\mathbf{t}_\alpha+V_n\mathbf{n}$, where $\mathbf{t}_\alpha$ and $\mathbf{n}$ are the tangent vectors and unit normal vector of the surface, respectively.

The mass density ρ of an incompressible membrane should be a constant. By replacing Φ by ρ in Eq. S1 and using $\frac{d\rho}{dt}=0$, we have [1]

$$\nabla_\alpha V^\alpha=2HV_n. \tag{S2}$$

This equation is similar to the incompressibility equation $\nabla\cdot\mathbf{V}=0$ in fluid mechanics.

By considering the diffusion of lipids, the mass conservation of one species of lipid molecules yields another equation

$$\frac{d}{dt}\int_{S(t)}\phi\, dS=-\oint_{\partial S(t)}J^\alpha m_\alpha\, dL \tag{S3}$$

where $\mathbf{m}$ is the unit tangent vector perpendicular to the boundary $\partial S(t)$. And $\mathbf{J}=-M\nabla Q$ is the diffusive flux within the surface, where M is a generalized diffusion constant and $Q=\frac{1}{\sqrt{g}}\frac{\delta E}{\delta\phi}=\frac{\partial f}{\partial\phi}-\mu\nabla^2\phi$ is the chemical potential defined in the main text.

Notice that ϕ also satisfy Eq. S1. Therefore, combining Eq. S1 and Eq. S3, and using Eq. S2 and the covariant form of Green's theorem for a two-dimensional surface [1]

$$\int_{S(t)}\nabla_\alpha J^\alpha\, dS=\oint_{\partial S(t)}J^\alpha m_\alpha\, dL \tag{S4}$$

we have a generalized Cahn-Hilliard equation about order parameter ϕ for a moving surface with internal flows

$$\frac{\partial\phi}{\partial t}+V^\alpha\nabla_\alpha\phi=M\nabla^2\left[\frac{\partial f}{\partial\phi}-\mu\nabla^2\phi\right]. \tag{S5}$$

In this problem, we can define two length scales

$$R=\sqrt{\frac{\kappa_0}{2\sigma_0}}\qquad\text{and}\qquad d=\sqrt{\frac{\mu}{\lambda}}. \tag{S6}$$

The first length scale represents the radius of membrane tube in Lo phase. The factor of 2 is introduced for convenience. Indeed, assuming that the membrane is uniform ($\phi=1$) and minimizing Canham-Helfrich energy, we may verify the radius of membrane tube in Lo phase is R. The second length scale represents the width of the phase boundary.

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We can also define three time scales

$$\tau_1 = \frac{R^4}{M\kappa_0}, \qquad \tau_2 = \frac{R}{v_0} \qquad \text{and} \qquad \tau_3 = \frac{R^2}{M\lambda} \tag{S7}$$

where v_0 is the pulling speed at the tip of membrane tube, and τ_2 is the time needed for membrane tube to move distance R at speed v_0 due to the pulling at the tip. τ_1 is the time scale of diffusion if we use κ_0 as energy measure to normalize the equations. τ_3 is the time scale of diffusion determined by mixing free energy λ, if we use λR^2 as energy measure to normalize the equations.

By using τ_1 and R, Eq. S5 can be transferred to dimensionless forms:

$$\frac{\partial \phi}{\partial \bar{t}} + \bar{V}^\alpha \nabla_\alpha \phi = \nabla^2 \left[\bar{\lambda}\phi(1-\phi)(1-2\phi) + \alpha_1 \phi (2H)^2 + 2\alpha_2 \phi - \bar{\mu}\nabla^2 \phi \right]. \tag{S8}$$

where $\bar{t} = t/\tau_1$ and $\bar{V}^\alpha = V^\alpha \tau_1 / R$. Two dimensionless constants $\bar{\lambda} = \lambda R^2/\kappa_0$ and $\bar{\mu} = \mu/\kappa_0$ are defined here. The pulling speed at the tube tip can be normalized as $\bar{v}_0 = v_0\tau_1/R = R^3 v_0/(M\kappa_0)$, which can also be interpreted as the ratio of the two time scales, i.e., $\bar{v}_0 = \tau_1/\tau_2 = R^3 v_0/(M\kappa_0)$. For all the simulations in this paper, we use $\bar{\lambda} = 3$, $\bar{\mu} = 1$, $\alpha_1 = 0.5$, $\alpha_2 = 0$ and vary $\bar{v}_0$ to study how the dimensionless pulling speed affects the lipid sorting process.

II. COMPUTATIONAL DETAILS

The membrane shape, lipid velocity and the lipid composition can be solved from the coupled equations:

$$\begin{aligned} &\delta E/\delta r = 0, \\ &\nabla_\alpha V^\alpha = 2HV_n, \\ &\frac{\partial \phi}{\partial t} + V^\alpha \nabla_\alpha \phi = M\nabla^2 \left[\frac{\partial f}{\partial \phi} - \mu \nabla^2 \phi \right]. \end{aligned}$$

These equations are not closed equations. Usually we need another equation about force balance and introduce a pressure field within the membrane surface [1]. However, the situation is simplified in this problem since the membrane shape and velocity field are axisymmetric and V^1, the tangent velocity along the circumferential direction of the tube, vanishes. At the same time, the normal components of the velocity, V_n, is only determined by the motion of surface $\mathbf{X}(t)$. So V_n is known once the membrane shape is solved from Eq. S9. Therefore, the pressure and velocity are decoupled and we only need to solve the tangent velocity along the pulling direction V^2 from Eq. S2.

This is a moving boundary problem. Fortunately, the motion of the pulling point is known in this problem. If the tip of the membrane tube moves at a speed $v(t)$, the membrane can be normalized by the pulling distance $L(t) = \int_0^t v dt$. To solve the problem, we assume the membrane shape, composition distribution and velocity field are axisymmetric. By using the front-fixing method [2] for moving boundary problems, the position of the membrane can be given by $\mathbf{X} = [r(\eta, t)\cos\theta, r(\eta, t)\sin\theta, z(\eta, t)]$, where η is within a fixed interval $(0, 1)$. The boundary conditions for z are $z(0, t) = 0$ and $z(1, t) = L(t)$. Besides the motion of the pulling point, the phase boundaries between Ld phase and Lo phase also moves. In this phase field model, the lipid composition is represented by the order parameter ϕ. Therefore, the phase boundary can be easily tracked by the temporal and spatial variation of ϕ.

When a point force is applied, the governing equations S9, S2 and S5 are singular and the mean curvature goes to infinity at the tip (see the discussion of Ref. [3]). But the singularity can be eliminated by cutting off the tip with a small radius r_0 [3, 4]. In another word, the boundary condition at the tip is $r = r_0$, instead of $r = 0$. This treatment is also consistent with the experiment by M.Heinrich et al [5], where there is no end cap of the membrane tube because a bead was used to pull the membrane. We explored various cutting-off radius r_0. We found that a small Ld domain can be formed at the cap region of the tube when the cutting-off radius r_0 is small, but no Ld domain is formed when r_0 is big enough (close to the tube radius). Furthermore, we found the formation of Ld domains around the junction region between tube and the giant vesicle always exists no matter what the value of r_0 is used. Therefore, our model is different from the stripe-type phase separation induced by the higher curvature at the end cap of a cylindrical vesicle [6].

III. LOCALIZED SPINODAL DECOMPOSITION

In the main text, we argue that the double-well potential will become a single-well potential if $\kappa_0\alpha_1(2H)^2 + 2\sigma_0\alpha_2 > \lambda/8$. This conclusion is based on the implicit assumption that H is not the function of ϕ. The assumption is correct

when a membrane is attached to a curved substrate [7]. In this case, the membrane curvature is fully determined by the substrate curvature. So curvature can influence the lipid composition, but the lipid composition cannot affect membrane curvature. However, for a free-standing lipid vesicle, the lipid composition and membrane curvature are fully coupled. It's very difficult to solve such coupled equations analytically. To reduce the complexity and get some insight, we consider the stability of a membrane tube with uniform lipid composition ϕ_0. The free energy is given by

$$E = \left[\frac{\lambda}{2}{\phi_0}^2(1-\phi_0)^2 + \frac{\kappa(\phi_0)}{2}\frac{1}{r^2} + \sigma(\phi_0)\right] 2\pi r l \tag{S9}$$

where r and l are the radius and length of the membrane tube. $\delta E/\delta r = 0$ yields the equilibrium radius $r(\phi_0) = \sqrt{\frac{\kappa(\phi_0)}{2\sigma(\phi_0)+\lambda\phi_0^2(1-\phi_0)^2}}$. Therefore, f can be rewritten as

$$f(\phi_0) = \frac{\lambda}{2}{\phi_0}^2(1-\phi_0)^2 + \frac{\kappa(\phi_0)}{2}\frac{1}{r(\phi_0)^2} + \sigma(\phi_0) = \lambda{\phi_0}^2(1-\phi_0)^2 + 2\sigma(\phi_0) \tag{S10}$$

If we assume $\sigma(\phi_0) = \sigma_0$ so that surface energy is a constant, f is reduced to

$$f(\phi_0) = \lambda{\phi_0}^2(1-\phi_0)^2 + 2\sigma_0 \tag{S11}$$

In our simulation, we always use $\sigma(\phi_0) = \sigma_0$. Therefore, after consider the coupling between lipid composition and membrane curvature of a free-standing membrane tube, the free energy is always a double-well potential. It should be noted that along the curve of the double-well potential, not only lipid composition changes, but also membrane curvature (tube radius) changes (Eq. S11). So this is different from the assumption of Fig. 1(a) in the main text, where H is a constant for each curve.

The system is unstable and phase separation spontaneously occurs when the curvature of the double-well potential is negative, i.e., $\partial^2 f/\partial\phi_0^2 < 0$ [8]. The spinodal decomposition point is given by $\partial^2 f/\partial\phi_0^2 = 0$. For the double-well potential we used in this paper, the spinodal decomposition occurs when $0.21 < \phi_0 < 0.79$.

Go back to our original problem. For a membrane tube pulled out from a Lo domain ($\phi = 1$), the average value of the order parameter will decrease due to the curvature-driven diffusion. The lowest value of ϕ appears around the neck region since it's the nearest high curvature region connected to the lipid reservoir. When this lowest value of ϕ is smaller than 0.79, the spinodal decomposition occurs and Ld domains appear. This is clearly showed in Movie S5 and S6 when $\bar{v}_0 = 0.1$ and 0.2.

To summarize, the curvature-driven diffusion changes the lipid composition and finally the lipid mixture around the neck region becomes unstable, which leads to the spinodal decomposition and the appearance of alternate Ld and Lo domains. The pulling speed will determine the travelling speed and the number of Ld domains. There is only one Ld domain for $\bar{v}_0 = 0.1$, but multiple Ld domains for $\bar{v}_0 = 0.2$. If the pulling speed is very big, the lowest ϕ never falls below 0.79 so that spinodal decomposition doesn't occur at all.

IV. A SIMPLE THEORY ABOUT PULLING FORCE

To get more insight about pulling force, we can develop a simplified model. Assume the total length of the membrane tube is l and the total length of all Ld domains is $l_d = \sum_i l_{d,i}$, where $l_{d,i}$ is the domain size of the i-th Ld domain at a specific time (see Fig. 1C). Therefore, the length percentage or the normalized size of all Ld domains is $\beta = l_d/l$. To simplify the problem, we focus on the pulling process after the membrane tube is formed so that we don't have to consider the force barrier during tube formation and neglect the catenoid region. Furthermore, we assume the energy of phase boundaries are small compared with the energy of each phase. Therefore, the total energy of the system, $E_1 + E_2$ (see Eq. 1 and 2 in the main text), is simplified to

$$E = 2\pi r_d l\beta(\sigma_d + \frac{\kappa_d}{2r_d^2}) + 2\pi r_o l(1-\beta)(\sigma_o + \frac{\kappa_o}{2r_o^2}) \tag{S12}$$

where κ_d (κ_o), σ_d (σ_o) and r_d (r_o) are the bending stiffness, surface tension and radius of Ld (Lo) domains, respectively. The equations $\partial E/\partial r_d = 0$ and $\partial E/\partial r_o = 0$ yield the radius of each phase $r_d = \sqrt{\kappa_d/(2\sigma_d)}$ and $r_o = \sqrt{\kappa_o/(2\sigma_o)}$. The pulling force can be simply given by

$$F = \partial E/\partial l = F_o - \beta(F_o - F_d) \tag{S13}$$

where $F_d = 2\pi\sqrt{2\kappa_d\sigma_d}$ and $F_o = 2\pi\sqrt{2\kappa_o\sigma_o}$ are the pulling forces for pure Ld and Lo phase, respectively. So the pulling force is linear with β, which in turn is determined by diffusion of lipids and the lipid flow induced by pulling.

If the pulling speed is very small, the membrane tube is completely occupied by Ld phase. So $\beta = 1$ and the pulling force is F_d. If the pulling speed is very big, the whole tube is still in Lo phase. So $\beta = 0$ and the pulling force is F_o. The prediction in Eq. S13 agrees with simulation results for various pulling speeds very well (Fig. 5(b)). For intermediate pulling speeds ($\bar{v}_0 = 0.15$ and $\bar{v}_0 = 0.2$), the pulling force finally approaches a constant between F_d and F_o with small oscillations (Fig. 4(b) and Fig. 5(b)). It should be noted that Eq. S13 is based on the assumption that the energy is a single-valued continuous function of l. So this is not applicable to the experiment where step-wise total tube length extension was used [5].

In this simplified model, the driving force for the Ld domain growth, i.e., the energy decrease as β increases a unit value, can be defined as $q = -\partial E/\partial\beta = (F_o - F_d)l$. Only when $F_o = F_d$ or $\kappa_d\sigma_d = \kappa_o\sigma_o$, the driving force q is zero and the system is in equilibrium. F_o is usually bigger than F_d (see SI of Ref. [5]). Therefore, the driving force q will drive the formation of the lower energy Ld phase so that the total energy of the membrane tube is decreased.

The above method can also be used to explain the force decay when one Ld domain nucleates and grows in a Lo tube with fixed length (Fig. 4A). If the tube length l is a constant, the pulling force is introduced as a Lagrange multiplier to impose this constraint in the free energy as $E' = E - Fl$. The condition $\partial E'/\partial l = 0$ yields the same pulling force as Eq. S13.

It should be noted that the decreased energy is dissipated during the formation of Ld phase through irreversible diffusion process. For example, if the tip of the membrane tube is fixed as we discussed in the last section, the pulling force doesn't do any work to the system since the tube length l is a constant. The decreased energy is totally used to drive the formation of Ld phase. If the membrane tube is pulled at a constant speed v_0, there is an energy input by the pulling force at the rate Fv_0. If the rate of the energy input $Fv_0 \sim \kappa_0/\tau_2$ is comparable with the rate of the energy dissipation $M\kappa_0^2/R^4 \sim \kappa_0/\tau_1$ that drives the formation of Ld phase, the convection shouldn't be neglected. Therefore, the dimensionless pulling speed $\bar{v}_0$ can also be interpreted as the ratio of the above rates, which gives $\bar{v}_0$ another interpretation besides the Péclet number.

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