Theory of Fission for Two-Component Lipid Vesicles

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The coupling between Gaussian curvature and local lipid composition for two-component lipid vesicles can destabilize the narrow neck in a budding transition. Such a coupling reduces the Gaussian rigidity of a membrane and enhances the fission transition if the minor component lipids prefer to stay at regions with large positive Gaussian curvature. On the other hand, it increases the Gaussian rigidity and a fusion transition is enhanced if the minor component lipids are expelled from regions with large positive Gaussian curvature. [S0031-9007(97)03882-9]

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The study of shape transformations and topological changes such as budding, fission, and fusion of lipid vesicles has important physical and biological significance. In many ways, lipid vesicles provide excellent model systems to study the structure and function of cell membranes. The processes of budding, fission, and fusion of membranes are essential mechanisms for cells to transport nutrients and waste. Encapsulation of drugs and DNA by vesicles or liposomes also provides a powerful tool in controlled drug delivery. However, a fundamental understanding of essential aspects of release in general and fission and fusion in particular is still lacking.

Recent experiments have suggested that budding and fission of lipid vesicles can be explained by physical properties of lipid membranes alone [1]. Budding transitions and other shape transformations are quite common for single-component and multicomponent lipid vesicles [1–6]. It is well known that the shape of single-component lipid vesicles is largely determined by the minimization of bending energy of lipid membranes for a given area A and a given volume V [7]. For single-component lipid vesicles, varying temperature can control the shape transformation due to the resulting area change of the membrane [1,8]. Similar shape transformation by changing the ratio of surface area to volume can also be induced by applying osmotic pressure. For two-component lipid vesicles, shape transformations can also be controlled by the composition of the lipid bilayer [9,10]. In this case, the phase separation of two constituents is also subject to a line tension between two fluid domains. The shape of such a two-component lipid vesicle is therefore determined by the competition between bending energy and boundary energy for a given volume and domain area.

Fission and fusion are usually seen in multicomponent lipid vesicles [1,11]. Fission after budding can be induced in an artificial dimyristoyl phosphatidylcholine-cholesterol mixtures by applying osmotic pressure without changing temperature [1]. The experiment suggests that a coupling between curvature and local lipid composition might be important for such a fission process. However, the underlying mechanism of these topological changes is so far unclear.

In this paper, we study the effect of the coupling between Gaussian curvature and local lipid composition for two-component lipid vesicles after a budding transition. Here we first investigate the stability of a narrow neck connecting two buds in two-component lipid mixtures. Since fission involves topological change, the coupling of local lipid composition to Gaussian curvature is believed to be important for a fission transition. This coupling can be understood in a simple way as a result from molecular geometry to the curved surface where they stay. We show that this coupling can destabilize the narrow neck, which is stable in one-component lipid vesicles [3,4], and show that such a coupling can reduce the Gaussian rigidity, which in turn can enhance fission if the minor component lipids prefer to stay at regions with large positive Gaussian curvature. On the other hand, Gaussian rigidity is enhanced if the minor component lipids prefer to stay at regions with large negative Gaussian curvature.

The free energy functional in our model can be expressed as

$$\Phi[\Omega] = E[\Omega] + \sigma A[\Omega] - pV[\Omega] + \mu \int_\Omega dA\, \phi(r), \tag{1}$$

where Ω represents the whole surface of the vesicle, $E[\Omega]$ is the free energy of the vesicle, $A[\Omega]$ is the surface area, $V[\Omega]$ is the enclosed volume, and $\phi(r)$ is the local area fraction of the minor component lipid. σ, p, and μ are the Lagrange multipliers which can be adjusted to achieve the desired A, V, and lipid composition. For two-component lipid vesicles, we express $E[\Omega]$ as

$$E[\Omega] = \frac{\kappa}{2} \int_\Omega dA\, [c_1(\mathbf{r}) + c_2(\mathbf{r}) - c_0]^2 + \kappa_G \int_\Omega dA\, c_1(\mathbf{r}) c_2(\mathbf{r}) + \lambda \int_\Omega dA\, c_1(\mathbf{r}) c_2(\mathbf{r}) \phi(\mathbf{r}) + \frac{t}{2} \int_\Omega dA\, \phi^2(\mathbf{r}), \tag{2}$$

where κ is the bending rigidity, κ_G is the Gaussian rigidity, λ is the coupling constant between Gaussian curvature and local lipid composition, and t is the temperature. The coupling constant λ can be expressed as $\lambda = \kappa_G' - \kappa_G$ in a two-component theory, where κ_G' is the Gaussian rigidity of the minor component lipids. For $\lambda < 0$, the minor component lipids prefer to stay at regions with large positive Gaussian curvature. Here, we have assumed a stable, homogeneous lipid mixture (i.e., $t > 0$).

Since fission occurs after the pinch off the narrow neck (of size a) of a budded state [as shown in Fig. 1(a)], we first investigate the neck stability in a two-component lipid vesicle. Here, axial symmetry is assumed. Minimizing the free energy functional $\Phi[\Omega]$ in Eq. (1) with respect to $\phi(r)$, the free energy of a budded state can be expressed as

$$\Phi[\Omega_1] = 4\pi\kappa_G' + \sigma' A[\Omega_1] - pV[\Omega_1] + \frac{\kappa}{2}\int_{\Omega_1} dA\,[c_1(\mathbf{r}) + c_2(\mathbf{r}) - c_0]^2 - \frac{\lambda^2}{2t}\int_{\Omega_1} dA\, c_1^2(\mathbf{r})c_2^2(\mathbf{r}) + \frac{1}{2t}\int_{\Omega_1''} dA\,[\mu + \lambda c_1(r)c_2(r)]^2, \quad (3)$$

and the composition profile is given by

$$\phi(\mathbf{r}) = \begin{cases} -[\lambda c_1(\mathbf{r})c_2(\mathbf{r}) + \mu]/t, & \mathbf{r} \in \Omega_1', \\ 0, & \mathbf{r} \in \Omega_1''. \end{cases} \quad (4)$$

Here, $\kappa_G' = \kappa_G - \mu\lambda/t$, $\sigma' = \sigma - \mu^2/2t$, and Ω_1 is shown in Fig. 1(a). Ω_1' represents the region where $c_1(\mathbf{r})c_2(\mathbf{r}) \geq -\mu/\lambda$, and Ω_1'' represents the region where $c_1(\mathbf{r})c_2(\mathbf{r}) < -\mu/\lambda$ and the local area fraction $\phi(\mathbf{r})$ vanishes. For $\lambda/\mu \ll R^2$, Ω_1'' vanishes as the neck size $a > \sqrt{\lambda/\mu}$. As shown in Ref. [4], the first four terms in Eq. (3) describe a budded state with a stable neck when the Lagrange multipliers σ, p, μ are chosen to be appropriate values. This neck is not stable for two-component lipid vesicles due to the fifth term in Eq. (3) which contributes a term proportional to $-1/a^2$. This term tends to destabilize the narrow neck of a budded state and leads the neck size to a smaller value until the neck is stabilized again by steric interaction among lipids, or by dehydration forces. However, the budded state is not necessarily the equilibrium state for $\kappa_G' < 0$. Fission can occur if a two-vesicle state [as shown in Fig. 1(b)] has a lower free energy. Furthermore, phase separation occurs in the neck region for $a < \sqrt{\lambda/\mu}$. The area fraction of minor component lipids vanishes in the Ω_1'' region since the molecular shape does not fit the large negative Gaussian curvature. If the radii of the two buds are different from each other, the distribution of minor component lipids will be enriched on the smaller bud due to the shape transformation. This effect should be distinguished from the domain-induced budding process in Ref. [10].

For a two-vesicle state, the free energy is given by

$$\Phi[\Omega_2] = 8\pi\kappa_G' + 4\pi\sigma'(R_1^2 + R_2^2) - \frac{4}{3}\pi p(R_1^3 + R_2^3) + 2\pi\kappa[(2 - c_0R_1)^2 + (2 - c_0R_2)^2] - 2\pi\frac{\lambda^2}{t}(R_1^{-2} + R_2^{-2}), \quad (5)$$

and the area fraction of minor component lipids is

$$\phi(\mathbf{r}) = \frac{-1}{t}\left(\frac{\lambda}{R_i^2} + \mu\right), \qquad i = 1, 2, \quad (6)$$

where R_1 and R_2 are the radii of the two vesicles. The first term in Eq. (5) is the renormalized integrated Gaussian curvature energy. For λ and μ both negative, the two-vesicle state has a reduced Gaussian rigidity κ_G' and fission might occur for negative values of κ_G'.

To investigate the feasibility of a fission transition, we compare the free energy of the two-vesicle state to that of the budded state in which two buds are connected by a narrow neck. Here, for simplicity, the approximate solutions to the variational problem in Eq. (1) is judiciously chosen by parametrizing Ω in the proximity of two spherical vesicles of radius R_1 and R_2. For small λ, the equilibrium radius can be one of $R_\pm = [(2\sigma' + 1) \pm \sqrt{(2\sigma' + 1)^2 - 8p}\,]/2p$ for given values of σ, p, and μ. Moreover, we adopt a trial shape Ω_1 in which the two end caps are spherical but the inner regions are described

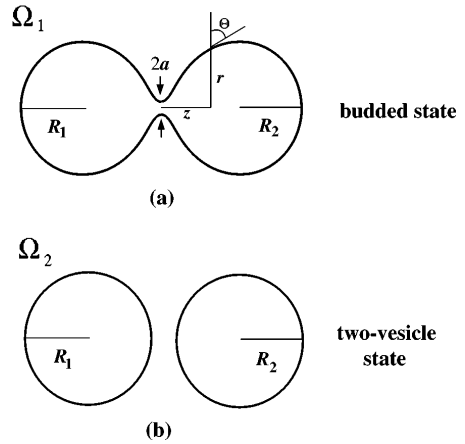

FIG. 1. Geometry of lipid vesicles in a budded state (a) and in a two-vesicle state (b). Axial symmetry of lipid vesicles about a horizontal axis is assumed. In the budded state, two end caps are spherical and the inner region has a neck of size a which connects those two buds. In general, R_1 and R_2 could be different.

by

$$\sin\Theta = \frac{r}{R_i} + a\left(\frac{1}{r} - \frac{1}{R_i}\right), \qquad i = 1, 2, \qquad (7)$$

which is chosen to fit the caps at the equators and to meet at the center in a neck of radius a. This shape is shown in Fig. 1(a). The local lipid composition profile is shown in Fig. 2 by taking $\lambda = -10$, $\mu = -10^4$, $t = 10^5$, and $R = R_+$ (these values are consistent with $c_0 = 1$ and thermal energy comparable with $\kappa = 1$). The local area fraction of the minor component lipids decreases dramatically near the neck region, and phase separation occurs when neck size $a < \sqrt{\lambda/\mu}$.

In Fig. 3, we show the free energy difference between the budded state and the two-vesicle state ($\Delta\Phi \equiv \Phi[\Omega_1] - \Phi[\Omega_2]$). For $\Delta\Phi > 0$, the two-vesicle state is favored over the budded state and fission can occur. Here, we take $\kappa = \kappa_G = 1$, $c_0 = 1$, $p = 1.1$, $\sigma' = 1$, $t = 10^5$, $\lambda = -10$, and $R_1 = R_2 = R_+$. As the neck size decreases, the free energy of the budded state decreases which indicates the narrow neck is not stable. Increasing the percentage of minor component lipids, or equivalently decreasing μ, tends to stabilize the two-vesicle state over the budded state. Other effects that are not included in our model, such as dehydration force and steric interaction, will increase the free energy of the budded state. Since a continuous model breaks down when the neck size is comparable to molecular size, we then take a cut off at the lower bound $\epsilon = a/R = 0.01$ by considering vesicle size about 1 μm and bilayer thickness about 10 nm. For $\Delta\Phi > 0$ at $\epsilon = 0.01$, such as those curves of $\mu \leq -20\,000$ in Fig. 3, the two-vesicle state has a lower free energy than the budded state. Under these conditions, we expect that the neck size of the budded state decreases and fission can occur spontaneously at the molecular level.

The phase boundary between the budded state and the two-vesicle state is shown in Fig. 4 for $\lambda = -5$ and -10. Here, we choose the following values for the other parameters: $\kappa = \kappa_G = 1$, $c_0 = 1$, $p = 1.1$, $\sigma' = 1$, and $R_1 = R_2 = R_+$. In Fig. 4, below the phase boundary curves, a budded state is the equilibrium phase and a budding transition occurs continuously by changing temperature, osmotic pressure, or lipid composition. In these cases the narrow neck is stabilized by the dehydration force or by the steric interaction. Above the phase boundary, the two-vesicle state is the equilibrium phase and a fission transition can occur. The phase boundary is approximately given by $\kappa_G' = \kappa_G - \lambda\mu/t < 0$. In other words, the condition for fission to occur for two-component lipid vesicles is that the renormalized Gaussian rigidity is negative, and the proposed coupling between local lipid composition and local Gaussian curvature can provide a driving force to destabilize the narrow neck of a budded vesicle which is stable in one-component lipid vesicles. This result applies to temperatures higher than the chain melting temperature of constituent lipids since our model assumes fluid lipid bilayer. In addition, we have assumed that, in a flat bilayer, a homogeneous fluid mixture is thermodynamically stable—i.e., $t > 0$. We note that near the critical temperature for phase separation, $t \simeq 0$, the effects described above are enhanced. On the other hand, at higher temperatures, the effect of the coupling term decreases as $1/t$, and fission is disfavored. For simplicity, we have ignored the coupling term between local lipid composition and local mean curvature $[\phi(c_1 + c_2)]$ in Eq. (2). Including this term will not change our results significantly

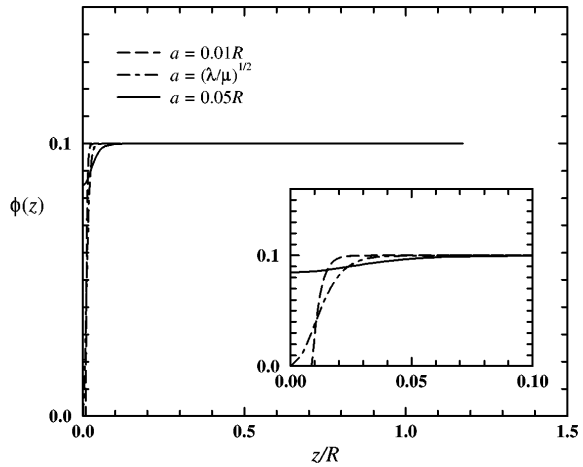

FIG. 2. The area fraction of the minor component lipids in the inner region, as described by Eq. (7). Inset is an enlargement of the neck region. The value of $\phi(z)$ decreases dramatically near the neck region. Here z is the axis of symmetry, and $z = 0$ at the center of the neck [as shown in Fig. 1(a)]. For the neck size $a > \sqrt{\lambda/\mu}$, $\phi(z)$ is nonzero on the whole surface of lipid vesicles. For a smaller neck size, $\phi(z)$ vanishes in the neck region.

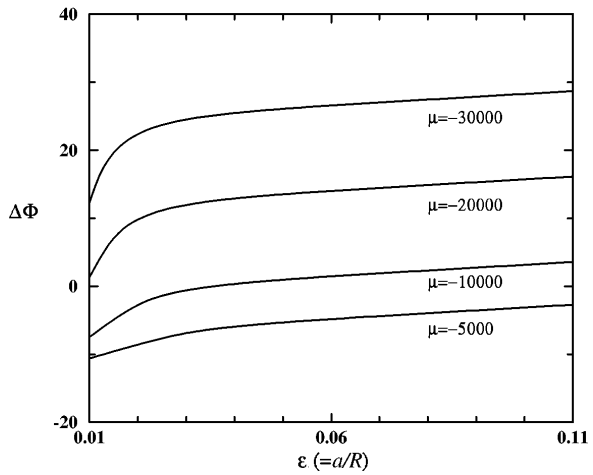

FIG. 3. Free energy difference ($\Delta\Phi$) between a budded state and a two-vesicle state for $\mu = -5000$, $-10\,000$, $-20\,000$, $-30\,000$. The other parameters are $\kappa = \kappa_G = 1$, $c_0 = 1$, $p = 1.1$, $\sigma' = 1$, $t = 10^5$, $\lambda = -10$, and $R_1 = R_2 = R_+$. As the neck size ($a = R\epsilon$) decreases, the free energy of the budded state decreases which indicates an unstable neck in our model. Here we choose the lower bound cutoff at $\epsilon = 0.01$ for vesicle size about 1 μm and bilayer thickness about 10 nm.

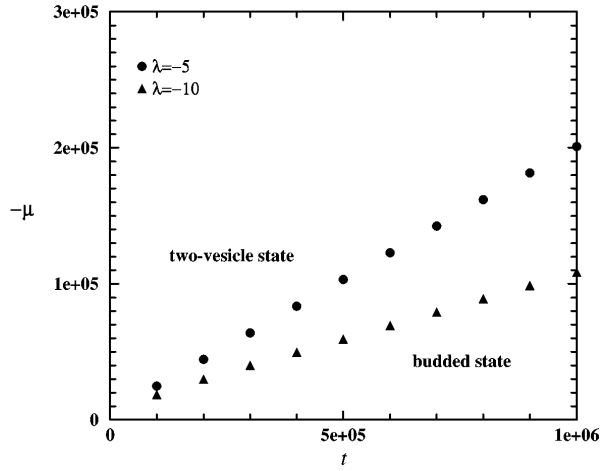

FIG. 4. The phase boundary between the two-vesicle state and the budded state as a function of t and $-\mu$ for $\lambda = -5$ and -10. The other parameters are $\kappa = \kappa_G = 1$, $c_0 = 1$, $p = 1.1$, $\sigma' = 1$, and $R_1 = R_2 = R_+$. Below the data points, the budded state is stable, while the two-vesicle state is the equilibrium state above the data points. At higher temperatures, the budded state is favored and fission is disfavored.

since its contribution to the free energy difference $\Delta\Phi$ vanishes for small neck size a.

Our prediction is consistent with the experimental results in Ref. [1] in which fission was not observed by thermally creating excess surface area. Furthermore, our theory shows that fission can be induced by applying osmotic pressure or increasing the percentage of minor component lipids. Choosing the shape of minor component lipids which fits regions with large positive Gaussian curvature can also enhance the fission transition. On the other hand, for $\lambda > 0$ in our model, the minor component lipid prefers to stay at regions with large negative Gaussian curvature. In this case, fusion instead of fission is enhanced. We note that our results are model dependent since the budded state is described by a variational trial solution, and the phase boundary between the two-vesicle state and the budded state might be shifted away from those curves in Fig. 4. However, the general trends of our predictions are believed to be true. To test our theory, one possible experiment is to see the predicted fission by applying optical tweezers on two-component lipid tubules. Similar experiments have been done for single-component lipid tubules [12] in which case the optical tweezers induce a surface tension on the lipid membranes and it changes the ratio of surface area to volume of the tubules [13]. The resulting pearled state consists of many vesicles connected by narrow necks. For two-component lipid tubules, we expect that the narrow necks are unstable and the lipid tubule may lead to many isolated vesicles after applying the optical tweezers, if appropriate minor component lipids are chosen.

In summary, we have shown that the coupling of local lipid composition to Gaussian curvature can destabilize the narrow neck in a budded state of lipid vesicles. Depending on the molecular shape factors of minor component lipids, this coupling can reduce (or increase) the Gaussian rigidity and fission (or fusion) can be enhanced.

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