Domain-Induced Budding of Vesicles

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Phase separation within the fluid membrane of a vesicle can lead to domain-induced budding. This shape transformation should occur both for the spinodal decomposition and for the nucleation regime and should be observable in quench experiments on phospholipid-cholesterol vesicles. The budding process leads to limit shapes for which the bud is connected by an infinitesimal neck to the original vesicle. This neck can be characterized by a general relation for the principal curvatures of the adjacent membrane segments. The effect of the Gaussian curvature on the shape is also discussed.

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Vesicles of lipid bilayers exhibit an enormous variety of different shapes and shape transformations [1–7]. For example, they can exhibit the biconcave shapes of red blood cells (so-called discocytes), they can develop small satellites or buds, and they can form dumbbells, pears, or even tori [2,4,7]. More exotic shape transformations have also been observed in which a long necklace of small vesicles is formed [3].

Several attempts have been made to understand the physics behind these transformations. The main problem is to find appropriate control parameters which make it possible to compare the experimental observations with the results of theoretical models. Recently, it has been shown that temperature represents such a control parameter which can be used to change the vesicle shape. Since the thermal expansivity of the bilayer is large compared to that of water, the vesicle area changes more rapidly with temperature than the vesicle volume. This change in the area to volume ratio then leads to various shape transformations [2,5].

In this paper, we study the shape transformations of vesicles which are induced by another control parameter, namely, the composition of the lipid bilayer. More precisely, we consider vesicles which undergo phase separation into two types of domains which are both *fluid*. One important example are vesicles composed of mixtures of phospholipids and cholesterol [8] for which the coexistence of two fluid phases has been established very recently [9].

In general, a membrane domain embedded within the membrane matrix is bounded by a domain wall or edge with a finite *line tension* σ. Therefore, the edge energy of the domain grows linearly with the length of this edge. For a flat domain, this length is proportional to the linear size of the domain. The bending energy of the domain, on the other hand, is independent of the domain size. Furthermore, the bending of the domain reduces the length of its edge and thus lowers its edge energy. Indeed, if the domain forms a complete sphere, its edge energy is essentially zero. Therefore, the domain can lower its energy by forming a bud as soon as it has attained a certain size [10]. This critical size is reached when the bending energy of the bud, which is determined by the bending rigidity κ, is balanced by the line energy. The length scale where both energies are of the same order is set by the invagination length $\xi = \kappa/\sigma$.

This domain-induced budding process has to be distinguished from two other effects which have been recently proposed: (i) The phase separation within the membrane could lead to a striped phase and thus to a striped modulation of the membrane shape [11]; and (ii) the shape transformation of a homogeneous membrane could itself initiate phase segregation [12].

So far, domain-induced budding has only been studied for a simplified model in which the membrane domain was taken to form a spherical cap embedded in a flat membrane matrix [10]. In the present paper, we will determine the shape both of the domain and of the matrix by a systematic minimization procedure. In this way, we can incorporate the various constraints which arise for the closed membrane of real vesicles for which the surface area $A = 4\pi R_0^2$ is essentially constant (at constant temperature).

In general, a multicomponent membrane will exhibit a two-phase coexistence region as a function of temperature and composition. The two coexisting phases will be denoted by α and β. This $(\alpha\beta)$ two-phase region exhibits (i) a nucleation regime, in which one has to overcome an energy barrier in order to form a "critical" domain, and (ii) a regime of spinodal decomposition in which such a barrier is absent.

Now, consider a membrane which is initially prepared in a homogeneous state within the one-phase region and is then quenched into the $(\alpha\beta)$ two-phase region. In this Letter we will address two different cases: (i) The membrane is quenched deep into the spinodal decomposition regime. If the phase separation process is sufficiently fast, it will create vesicles consisting of an α and a β domain. As shown below, the *equilibrium* shape of such a vesicle is *not spherical* but exhibits a bud over a large range of the parameter values even if the difference P between the inside and the outside pressure vanishes. (ii) The membrane is quenched into the nucleation regime. If the activation energy for the "critical" domain is suffi-

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ciently large, only one domain will be nucleated initially and one may study the growth of such a domain. During the latter process, the enclosed volume V of the vesicle is essentially constant.

Theoretical predictions for the behavior of two-component vesicles can be obtained from the minimization of the total energy of the vesicle. This energy consists (i) of the bending energies $E_b^{(\alpha)}$ and $E_b^{(\beta)}$ of the α and the β domain, respectively, and (ii) of the edge energy $E_l^{(\alpha\beta)}$ of the $(\alpha\beta)$ domain boundary:

$$E = E_b^{(\alpha)} + E_b^{(\beta)} + E_l^{(\alpha\beta)} . \tag{1}$$

The bending energies of the α and the β domains are taken to be [13]

$$E_b^{(i)} = \int dA^{(i)} \frac{\kappa^{(i)}}{2} (C_1 + C_2 - 2C_{sp}^{(i)})^2 , \tag{2}$$

with $i = \alpha,\beta$, bending rigidity $\kappa^{(i)}$ and spontaneous curvature $C_{sp}^{(i)}$. The variables C_1 and C_2 denote the principal curvatures of the membrane surface. The edge energy is given by the line integral

$$E_l^{(\alpha\beta)} = \oint dl\, \sigma \tag{3}$$

along the domain boundary.

The total energy E is minimized for given values of the domain areas A^α and A^β, of the pressure difference P or of the enclosed volume V, and of the line tension σ. The area ratio $x \equiv A^\beta/(A^\alpha + A^\beta)$ with $0 \le x \le 1$ plays the role of a control parameter for budding.

Shapes of minimal energy have been determined with the restriction to axisymmetry. Axisymmetric shapes are described by their contour line which can be parametrized by the arclength S in the interval $S_0 = 0 < S < S_2$, S_2 being the total length of the contour. The domain boundary is then located at a point S_1 on the contour.

The variation of the energy (1) leads to shape equations for the contour in the intervals $S_0 < S < S_1$ and $S_1 < S < S_2$ [14]. The minimization of the energy functional does not determine the boundary conditions of the shape equations at S_1 completely. This freedom corresponds to different ways to model the domain boundary. Two extreme cases are (i) that the domains α and β can meet in S_1 at any angle with no difference in energy, and (ii) that the surface has to be smooth at the domain boundary. The most physical assumption seems to be (ii), where both domains meet with the same angle. This boundary condition is used in the following.

First, consider a quench experiment into the spinodal decomposition regime of the multicomponent membrane. On long time scales, water can permeate the membrane which leads to zero pressure difference, $P = 0$. Let us first consider the simplest case for which the α and the β domain have identical bending rigidities, $\kappa^\alpha = \kappa^\beta$, and no spontaneous curvature, $C_{sp}^\alpha = C_{sp}^\beta = 0$.

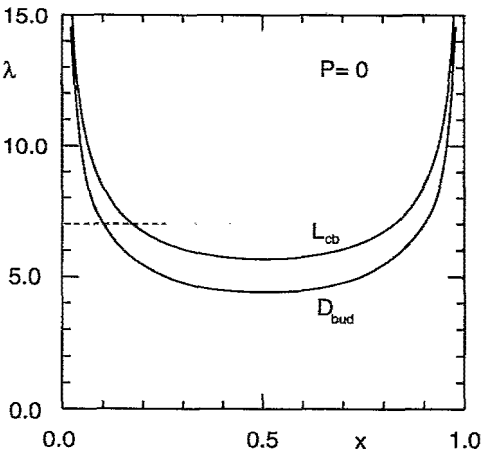

FIG. 1. Phase diagram for domain-induced budding of a vesicle for pressure $P = 0$ across the membrane as a function of the reduced line tension λ and the relative domain area x. The vesicle undergoes a discontinuous budding transition along the line $D_{\rm bud}$, and attains a limit shape at L_{cb} with an infinitesimal neck connecting the bud to the vesicle. The dashed trajectory corresponds to Fig. 2.

As shown in Fig. 1, the corresponding phase diagram exhibits a line $D_{\rm bud}$ of discontinuous budding transitions and a line L_{cb} of limit shapes with an infinitesimal neck. Since the domains α and β have the same elastic properties in this case, the energy E is invariant under the transformation $x \to -x + 1$ which corresponds to the exchange of the domains. The dashed line in Fig. 1 corresponds to a vesicle with reduced line tension $\lambda \equiv \sigma R_0/\kappa^\beta = R_0/\xi = 7$. The corresponding energy and equilibrium shapes are shown in Fig. 2 as a function of x and λ. In practice, the infinitesimal neck should have a diameter which is of the order of the membrane thickness, $a \simeq 5$ nm. The energy required to break such a neck is $2\pi a\sigma$ and the time for thermally activated fission is $\sim \exp(2\pi a\sigma/T)$.

If the vesicle membrane is composed of a phospholipid-cholesterol mixture, the bending rigidity $\kappa \simeq 4 \times 10^{-19}$ J as measured by optical microscopy [15] and the line tension $\sigma \simeq 10^{-12}$ J/m as measured by relaxation experiments in monolayers [16]. Thus, for these mixtures, the invagination length $\xi \simeq 400$ nm, and the sequence of shape transformations shown in Fig. 2 corresponds to the vesicle size $R_0 = 7\xi \simeq 2.8$ μm which is directly accessible to optical microscopy.

If the bending rigidity κ^β of the growing domain is smaller than κ^α, the budding transition will occur for smaller buds. On the other hand, larger buds can be obtained if the growing domain is more rigid. Likewise, if $C_{sp}^\beta > C_{sp}^\alpha$, budding happens for smaller values of x and thus leads to smaller buds. For large C_{sp}^β, one can enter a regime where the spontaneous curvature of the growing domain alone causes budding even if the line tension $\sigma = 0$ [14].

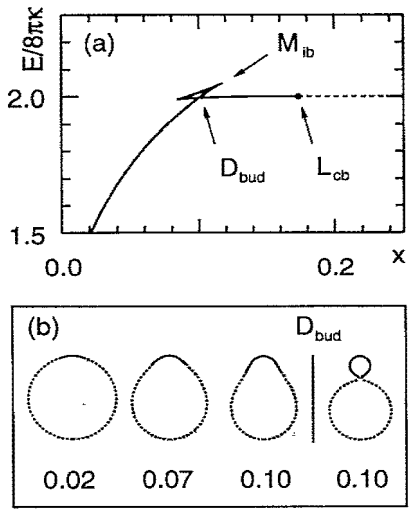

FIG. 2. (a) Energy E of the vesicle as a function of the relative domain area x for reduced line tension $\lambda = 7$, and (b) some of the corresponding vesicle shapes. The incomplete bud is stable up to $D_{\rm bud}$ with $x = 0.10$, metastable beyond $D_{\rm bud}$, and becomes unstable at M_{ib}. The complete bud coexists with the incomplete one at $D_{\rm bud}$ and attains a limit shape at L_{cb}. The shapes are axisymmetric; the α and the β domain correspond to the broken and the full contour, respectively.

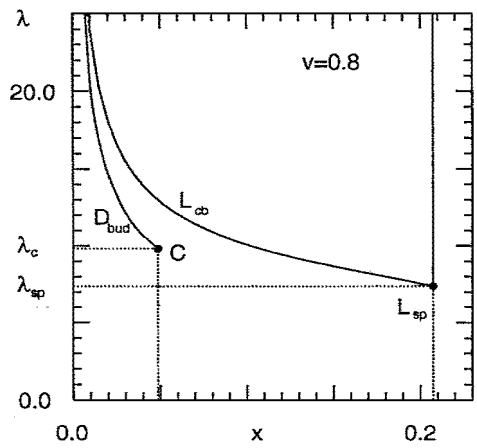

FIG. 3. Phase diagram of domain-induced budding of a prolate vesicle with constant enclosed volume $v = 3V/(4\pi R_0^3) = 0.8$ as a function of the reduced line tension λ and the relative domain area x. The line $D_{\rm bud}$ of discontinuous budding transitions ends in the critical point C.

As an example for the second type of experiment, consider a prolate vesicle with reduced volume $v = 3V/4\pi R_0^3 = 0.8$ which has been prepared by a quench from the one-phase region of the bilayer into the nucleation regime of its two-phase coexistence region. After such a quench, the bilayer remains in its homogeneous phase, say α, until a domain of the minority phase, say β, has been nucleated. The β domain will then grow by diffusion-limited aggregation within the α matrix and the relative surface area $x = A^\beta/A$ will increase in time.

For each value of x and of the elastic parameters of the membrane, one may determine the shape of lowest energy. In Fig. 3, the phase diagram for these shapes is shown as a function of x and of the reduced line tension λ for $\kappa^\alpha = \kappa^\beta$ and $C_{sp}^\alpha = C_{sp}^\beta = 0$.

As x is increased, the prolate vesicle undergoes a discontinuous budding transition denoted by $D_{\rm bud}$ provided $\lambda > \lambda_c \simeq 9.7$. As x is further increased, the bud closes its neck and forms a limit shape at L_{cb}. Comparison of Fig. 3 and Fig. 1 shows that the volume constraint truncates the line of discontinuous budding transitions at a critical point $(x, \lambda) = (x_c, \lambda_c) \simeq (0.5, 9.7)$ at which the budding transition is continuous. For $\lambda < \lambda_c$, there is no sharp budding transition. For $\lambda < \lambda_{sp} \simeq 7.4$, no limit shape with infinitesimal neck can be attained and no budding occurs. The shape transformation of the prolate vesicle with $\lambda = R_0/\xi = 12$ is shown in Fig. 4. The qualitative features of the phase diagram in Fig. 3 apply for all values of v with $1/\sqrt{2} < v < 1$. Close to $v = 1$, budding occurs only for large λ with $\lambda \sim 1/\sqrt{v-1}$.

The complete bud can have a neck with a finite diameter. This neck diameter decreases further with the growth of the domain until the neck closes completely as the limit shape L_{cb} has been attained. In the absence of the volume constraint, this limit shape consists of two spheres, one α and one β sphere, separated by an infinitesimal neck which contains the domain boundary. These limit shapes can be characterized by a simple relation. Such a neck relation was first proposed for *homogeneous* vesicles [5,6], where an infinitesimal neck can only exist for $C_{sp} \neq 0$. For limit shapes of *inhomogeneous* vesicles considered here, the neck condition has to be generalized. Without any constraint on V, we find that all numerically determined limit shapes are consistent with the generalized neck condition

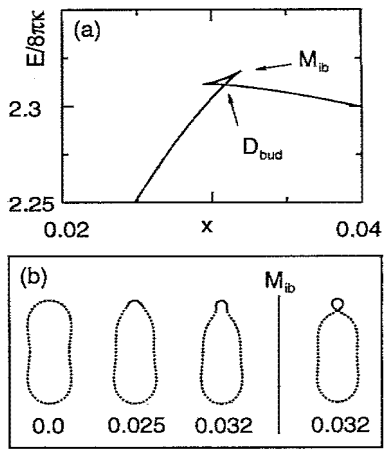

FIG. 4. (a) Energy E as a function of the relative domain area x for reduced line tension $\lambda = 12$. The symbols are the same as in Fig. 3. (b) Examples of shapes which correspond to points on the energy diagram (a) with different values of x.

$$\kappa^\alpha C^\alpha + \kappa^\beta C^\beta = \kappa^\alpha C^\alpha_{sp} + \kappa^\beta C^\beta_{sp} + \tfrac{1}{2}\sigma \ . \qquad (4)$$

Here, C^α and C^β are the curvatures of the two domains at the point where they meet to form the ideal neck. If $C^\alpha_{sp} = C^\beta_{sp}$, $\kappa^\alpha = \kappa^\beta$ and $\sigma = 0$, one recovers the relation $C^\alpha + C^\beta = 2C_{sp}$ for the homogeneous case. For identical domains with zero spontaneous curvature, the neck condition $C^\alpha + C^\beta = \sigma/2\kappa$ shows that the spontaneous curvature plays a role very similar to the line tension σ. The neck condition (4) completely determines the lines L_{cb} of limit shapes in the phase diagram. For the case shown in Fig. 1, the neck condition (4) leads to the line L_{cb} given by $\lambda = \lambda_L(x) = 2/\sqrt{x} + 2/\sqrt{1-x}$ in the (x, λ) plane.

In general, the bending energy for vesicle membranes also includes the Gaussian curvature term

$$E_G = \kappa_G \int dA \ C_1 C_2 \ , \qquad (5)$$

with Gaussian bending rigidity κ_G [13]. The Gauss-Bonnet theorem states that this integral over a closed surface is a topological invariant. It therefore has no effect on the shape of a homogeneous vesicle.

However, if the membrane is inhomogeneous, this is no longer true. In the case of a vesicle with two domains with $\kappa^\alpha_G \neq \kappa^\beta_G$, the Gaussian bending energy depends in fact on the vesicle shape and the budding transition is influenced by the Gaussian bending rigidities. While experiments with homogeneous membranes do not give any information about κ_G (assuming that the topology of the vesicle does not change), the Gaussian curvature energy has observable effects for domain-induced budding.

Thus, let us add two Gaussian curvature terms $E^\alpha_G + E^\beta_G$ to the energy E in (1). The additional contribution can be simplified according to [17]

$$E^{(\alpha)}_G + E^{(\beta)}_G = -(\kappa^\alpha_G - \kappa^\beta_G) \oint C_g dl + 2\pi(\kappa^\alpha_G + \kappa^\beta_G) \ . \qquad (6)$$

The first term is a contribution from the domain boundary which depends on the geodesic curvature C_g of this line. The second term gives a constant contribution and can be omitted.

For axisymmetric shapes, the geodesic curvature $C_g = -\cos\psi(S)/R(S)$ where $\psi(S)$ and $R(S)$ are the tilt angle of the contour and the distance of the contour from the symmetry axis, respectively. For the domain boundary with $S = S_1$, the integral in (6) thus leads to $E^{(\alpha)}_G + E^{(\beta)}_G = 2\pi(\kappa^\alpha_G - \kappa^\beta_G)\cos\psi(S_1)$ ($\psi = \pi/2$ corresponds to a direction parallel to the axis of rotational symmetry). This additional term acts to shift the domain boundary away from the neck [14].

In summary, we have shown that vesicles composed of two fluid domains undergo domain-induced budding. For phospholipid-cholesterol mixtures, these budded states should be observable by optical microscopy (i) as equilibrium states for vesicles which have been quenched into the spinodal decomposition regime (see Figs. 1 and 2) and (ii) if a vesicle is quenched into the nucleation regime and one follows the growth of the largest domain (see Figs. 3 and 4).

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