Domain-induced budding of fluid membranes

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ABSTRACT   Domains within fluid membranes grow by the aggregation of molecules which diffuse laterally within the membrane matrix. A simple theoretical model is introduced which predicts that a flat or weakly curved domain becomes unstable at a certain limiting size and then undergoes a budding or invagination process. This instability is driven by the competition between the bending energy of the domain and the line tension of the domain edge. For lipid bilayers, the budding domain can rupture the membrane and then it pinches off from the matrix. The same mechanism should also drive the budding of non-coated domains in biomembranes, and could even be effective when these domains are covered by a coat of clathrin molecules.

INTRODUCTION

Fluid membranes can easily change their shape. One particularly fascinating class of shape transformations are budding processes in which small vesicles bud off from a larger membrane surface. In biological cells, budding is a rather frequent event, because it represents the first step in the production of transport vesicles which shuttle between different compartments of the cell (1, 2). Two budding processes can be distinguished: (*a*) Endocytosis of the plasma membrane; and (*b*) Budding of the membranes bounding internal compartments such as the endoplasmic reticulum, the stack of Golgi cisternae, and the trans Golgi network. A highly schematic view of these phenomena is shown in Fig. 1.

Lipid bilayers provide the simplest model systems for biomembranes. Recently, budding has also been observed for such bilayers by phase contrast microscopy of giant vesicles (3–5). It was found that the experimentally observed shape transformations could be explained theoretically if one assumes that the lipid bilayer of the vesicle is laterally homogeneous (6–8).

Even though these shape transformations of lipid vesicles resemble the budding of biomembranes, the underlying mechanism must be quite different. Biomembranes are composed of many different lipids and proteins which can aggregate into clusters or domains. Indeed, the budding of biomembranes is preceded by the formation of such intramembrane domains (1, 2). One example is receptor-mediated endocytosis induced by clathrin-coated pits (9, 10).

The process of budding involves the selection of a certain length scale, namely the *size* of the budding vesicle. What is the mechanism underlying this selection? In the case of vesicles composed of a laterally homogeneous bilayer, this size is determined by *global* constraints on the vesicle. In contrast, domain-induced budding represents a *local* mechanism: as shown below, the size of the bud is now determined by the competition between the bending energy of the domain and the line tension of the domain edge.

Domain formation and domain growth. Bilayer membranes consisting of a lipid mixture which undergoes phase separation into two different phases represent simple model systems for the formation of intramembrane domains (11, 12). In most systems studied so far, one of the two phases was a gel or a polymerized state. In contrast, I will focus here on the case where *both* coexisting phases are *fluid*. One prominent example is a mixture of phospholipids and cholesterol which exhibits a broad coexistence region for a fluid "ordered" and a fluid "disordered" phase (13, 14). The possible influence of a polymerized coat of clathrin as found in biomembranes will be discussed at the end of the paper.

Within the coexistence region of the two fluid phases, small domains of the minority phase are nucleated within the matrix of the majority phase. After such a domain has been nucleated, its subsequent growth proceeds by the aggregation of molecules which diffuse within the matrix. If one can ignore interactions between different domains, the size L of a single domain grows as $L \sim (Dt)^{1/2}$ with time t where D is the diffusion coefficient of the molecules. It then takes the diffusion time $t_d \sim L^2/D$ until the domain has grown up to size L.

Spontaneous curvature and bending energy. Now, consider a mixture of two lipids which differ in their molecular shape: one lipid has an essentially cylindrical shape, while the other lipid has the shape of a truncated cone. A monolayer of this lipid mixture will exhibit a spontaneous curvature the size of which depends on the chemical composition. If the two adjacent monolayers within the bilayer have the *same* composition, the spontaneous curvatures of the two monolayers cancel and the bilayer has no such curvature. However, if the two adjacent monolayers of the bilayer have a *different* composition, the bilayer will typically exhibit a nonzero spontane-
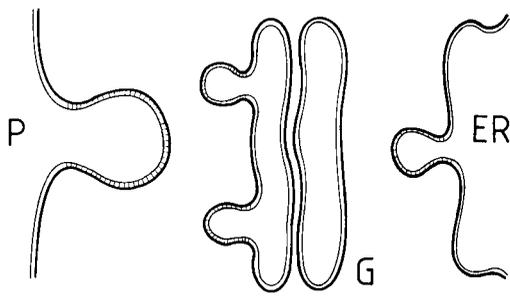
FIGURE 1 Highly schematic view of budding from the plasma membrane (*P*), from the Golgi apparatus (*G*), and from the endoplasmic reticulum (*ER*) of the cell.

ous curvature which will be denoted by C_{sp}. This happens, for example, if the domain within the bilayer does not extend across both monolayers but is restricted to one of them. Alternatively, the domain can acquire a spontaneous curvature if it contains membrane-spanning macromolecules which are inserted with a preferred orientation.

The shape of a lipid molecule depends on its environment, and it is difficult to give a general estimate for the spontaneous curvature C_{sp}. A rough idea about its magnitude can be obtained by comparison with surfactant mixtures in water which spontaneously form a dispersion of vesicles. For example, mixtures of two single-chained surfactants with oppositely charged head groups spontaneously form vesicles with a spontaneous curvature C_{sp} which varies from $1/80$ to $1/30$ nm^{-1} depending on the concentration of the surfactants (15).

Since the intramembrane domain is *fluid*, it does not build up any shear stress. The elastic energy for the curved domain is then given by its bending energy. This energy is minimal if the curvature of the domain is equal to the spontaneous curvature. In general, the scale of the bending energy is set by the bending rigidity which will be denoted by κ. The magnitude of κ can be deduced from experimental observations on the shape fluctuations (or flickering) of vesicles. For phospholipids, a typical value is $\kappa \simeq 10^{-19}$ J.

Edge energy and line tension. In general, the edge of an intramembrane domain will have an energy which is proportional to the length of the edge. Therefore, the domain has a tendency to attain a circular shape in order to minimize its edge energy.

The line tension, σ, is equal to the edge energy per unit length. Its magnitude can be estimated as follows. First, consider a domain in the lipid bilayer which extends across both monolayers. In this case, the edge of the domain represents a cut across the whole bilayer. The cross-section of such a cut consists of three distinct regions: two hydrophilic headgroup regions of combined thickness $\simeq 1$ nm and an intermediate hydrophobic tail region of thickness $\simeq 4$ nm. These two regions can have distinct interfacial free energies per unit area. For three-dimensional fluid phases, a typical value for the interfa-

cial free energy is $\simeq 10^{-2}$ J m^{-2}. If one assumes that this value is also applicable to the headgroup region and that the latter region gives the main contribution to the line tension, one obtains the crude estimate $\sigma \simeq 10^{-17}$ J μm^{-1}.

For a domain which extends only across one monolayer, the line tension is reduced by a factor $1/2$. In some systems, it can even be reduced by orders of magnitude. This happens if the lipid mixture exhibits a critical point at which the line tension goes to zero. Likewise, the line tension becomes small if the lipid bilayer contains edge-active molecules which preferentially adsorb at the domain edge; this is the two-dimensional analogue to the reduction of the interfacial free energy by surface-active molecules in three dimensions.

Edge energy versus bending energy. A *flat* domain will form a circular disk in order to attain a state with minimal edge length. However, as far as the edge energy is concerned, a *flat* circular disk does *not* represent the state of lowest energy, because the length of the edge can be further reduced if the domain forms a bud: the domain edge now forms the neck of the bud, and this neck narrows down during the budding process.

Budding involves an increase in the curvature and thus in the bending energy of the domain. Therefore, the budding process of fluid membranes is governed by the competition between the bending rigidity κ of the domain and the line tension σ of the domain edge. This competition leads to the characteristic *invagination length*, $\xi \equiv \kappa / \sigma$. Using the typical values $\kappa \simeq 10^{-19}$ J and $\sigma \simeq 10^{-17}$ J μm^{-1}, one obtains $\xi \simeq 10$ nm for domains across the bilayer and $\xi \simeq 20$ nm for domains which extend only across one of the monolayers. On the other hand, if σ has the relatively small value $\simeq 10^{-18}$ J μm^{-1}, these length scales are 100 nm and 200 nm, respectively. These values will be used below, compare Table I.

A SIMPLE MODEL

Now assume that the domain forms a spherical cap with curvature C. This cap is connected to the flat membrane matrix along a circular neck; the radius of this neck is denoted by N. This simple geometry is displayed in Fig.

TABLE 1 **Various length and time scales as appropriate for lipid bilayers characterized by spontaneous curvature C_{sp} and invagination length ξ**

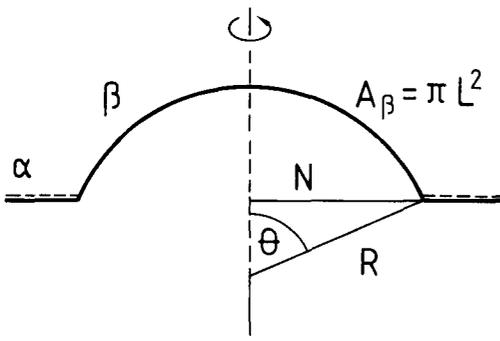| $|C_{sp}|$	[nm^{-1}]	0	1/80	1/30	0	1/80	1/30
ξ	[nm]	10	20	20	100	200	200
$4\xi|C_{sp}|$		0	1	2.7	0	10	27
L^0	[nm]	80	57	32	800	160	51
N_{ib}^0	[nm]	80	40	19	800	50	16
R_{cb}^0	[nm]	40	28	16	400	60	25
t_d	[sec]	10^{-2}	10^{-3}	10^{-3}	1	10^{-2}	10^{-3}
t_s	[sec]	10^{-8}	10^{-9}	10^{-10}	10^{-6}	10^{-9}	10^{-10}
t_f	[sec]	10^{-1}	10^{-2}	10^{-3}	10^2	10^{-2}	10^{-3}

FIGURE 2 Bud geometry for the simple model in which the β domain of area $A_\beta = \pi L^2$ forms a spherical cap with curvature C. This cap is connected along its neck with radius N to the flat α matrix.

2, where the phase of the membrane matrix is denoted by α and the phase of the membrane domain is denoted by β. The curvature C of the domain can be positive or negative, which allows to distinguish the two sides of the membrane.

The bending energy of the domain with surface area $A = \pi L^2$ is given by

$$E_{\text{bend}} = A\kappa(2C - 2C_{\text{sp}})^2/2 = 2\pi\kappa(LC - LC_{\text{sp}})^2. \quad (1)$$

The edge energy, on the other hand, is

$$E_{\text{edge}} = \sigma 2\pi N = \sigma 2\pi L\sqrt{1 - (LC/2)^2}. \quad (2)$$

It is convenient to introduce the dimensionless energy $\bar{E} \equiv (E_{\text{bend}} + E_{\text{edge}})/2\pi\kappa$, which has the form

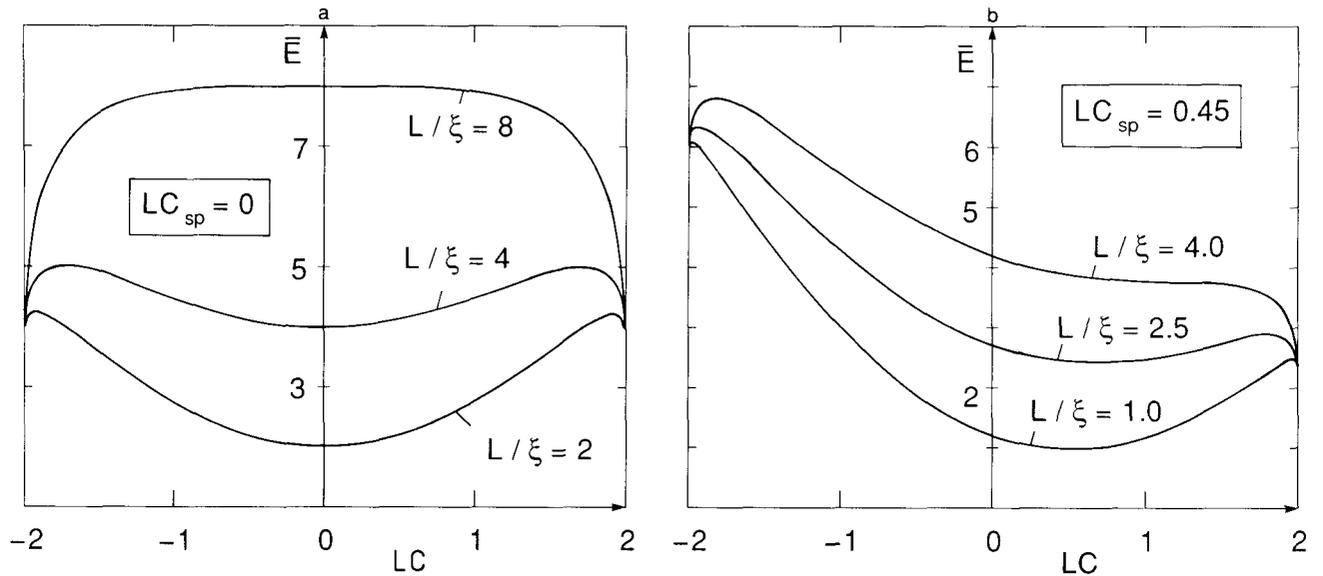$$\bar{E} = (LC - LC_{\text{sp}})^2 + (L/\xi)\sqrt{1 - (LC/2)^2}. \quad (3)$$

For $C_{\text{sp}} = 0$, such a model has been previously used for vesicles generated by sonification. (16)

Complete and incomplete buds. As can be seen by inspection of Fig. 3, the reduced energy $\bar{E}$ has several minima and maxima as a function of the reduced curvature LC. There are always two boundary minima at $LC = \pm 2$ corresponding to complete spheres on both sides of the membrane. The complete sphere with the lower energy will be called the *complete bud*. For zero spontaneous curvature, $C_{\text{sp}} = 0$, both complete spheres have the same energy, see Fig. 3 a, and the complete bud can develop equally well on both sides. A finite value of C_{sp} breaks this symmetry, see Fig. 3 b, and budding occurs preferentially on one side of the membrane.

The curvature radius R_{cb} has the absolute value $|R_{\text{cb}}| = L/2$. Within the elastic model considered here, the complete bud is a limiting shape with zero neck radius. In practice, this neck will have a radius of the order of the membrane thickness, as long as it does not break off from the matrix.

For small values of L/ξ, the energy $\bar{E}$ exhibits another minimum at intermediate values of LC, (see Fig. 3). This minimum corresponds to the *incomplete bud* with curvature radius R_{ib} and neck radius N_{ib}. For $C_{\text{sp}} = 0$, this minimum is at $LC = 0$, see Fig. 3 a, and the incomplete bud is flat.

Instability of incomplete bud. In the following discussion, the domain will be characterized by fixed spontaneous curvature C_{sp} and fixed invagination length $\xi = \kappa/\sigma$. The domain size L, on the other hand, changes with time and thus plays the role of a control parameter for the budding process.

For small L, the energy $\bar{E}$ has the functional forms as

FIGURE 3 Reduced energy $\bar{E}$ of the budding domain as a function of the reduced curvature LC: (a) for zero spontaneous curvature, $C_{\text{sp}} = 0$, and (b) for nonzero spontaneous curvature with $LC_{\text{sp}} = 0.45$. The length scale L denotes the linear size of the domain; the invagination length $\xi = \kappa/\sigma$, where κ and σ are the bending rigidity and the line tension of the domain edge. The uppermost curve corresponds to the size $L = L^0$ at which the incomplete bud becomes unstable.

given by the bottom curves in Fig. 3. In this case, the domain forms an incomplete bud corresponding to the minimum of $\bar{E}$ at intermediate LC-values. As L grows, the edge of the domain becomes longer, and the energy of the incomplete bud is increased.

At a certain critical size, $L = L^*$, the incomplete and the complete bud have the same energy but are separated by an energy barrier. (The details of this analysis will be presented elsewhere (17)). This situation corresponds to the middle curves in Fig. 3. For the parameter values considered here, the energy barrier is typically large compared to the thermal energy $\simeq k_B T$ (where k_B is the Botzmann constant and T is the temperature). Therefore, the domain continues to grow in the incomplete bud state. For $L > L^*$, the incomplete bud is metastable up to the limiting size $L = L^0$ at which the energy barrier disappears and the incomplete bud becomes *unstable*. This corresponds to the top curves in Fig. 3.

It follows from the expression (3) for the energy $\bar{E}$ that the limiting domain size L^0 is given by (17)

$$L^0 = 8\xi/[1 + (4\xi|C_{sp}|)^{2/3}]^{3/2}, \qquad (4)$$

and that this domain forms an incomplete bud with neck radius

$$N_{ib}^0 = 8\xi/[1 + (4\xi|C_{sp}|)^{2/3}]^2. \qquad (5)$$

For lipid bilayers, these two length scales can be estimated using the appropriate values for the spontaneous curvature C_{sp} and for the invagination length ξ, (see Table 1). In the latter table, the time scales $t_d \simeq L^2/D$ for the growth of the domain up to size $L = L^0$ have also been included using the typical value $D \simeq 10^{-12}$ m^2 sec^{-1} for the diffusion coefficient in fluid bilayers.

Transformation from incomplete to complete bud. During the transformation from the incomplete to the complete bud, the neck becomes narrower and the domain has to pull in membrane area. In principle, a variety of area reservoirs could be accessible to the budding domain such as, e.g., adhering vesicles which fuse with the membrane. For simplicity, let us focus on the case where the membrane matrix surrounding the bud is essentially flat but exhibits thermally-excited undulations. In this situation, the transforming bud can pull in the excess area stored in the undulations or pull in area by stretching the matrix surrounding it.

For the bud geometry considered here, the bud has to pull in the area $\delta A_1 = \pi N^2$ where N is the neck radius of the incomplete bud. In order to pull out this area from the surrounding membrane matrix, the bud has to perform a certain amount of work, δF_1. The maximal work which the transforming bud with $L = L^0$ can do is given by the difference, $\delta \bar{E}^0$, between the energies of the incomplete and the complete bud, compare Fig. 3. This implies a certain *minimal* value for the size L_1 of the membrane matrix which is necessarily perturbed by the transforming bud. This minimal value follows from the two relations $\delta A_1 = \pi N^2$ with $N = N_{ib}^0$ and $\delta F_1 = 2\pi\kappa\delta\bar{E}^0$. (The details of this analysis will be presented elsewhere [17]).

The time scale t_s for stretching a membrane of size L_1 can be estimated from the sound velocity within the membrane; the time scale t_f for flattening the undulations of this membrane is determined by the coupling to overdamped surface waves (18) in the aqueous medium. Using the parameters for lipid bilayers, one finds the estimates displayed in Table 1. In all cases, the time scale t_s for stretching the matrix is small compared to the time scale t_f for flattening its undulations. Thus, the transformation from the incomplete to the complete bud will first lead to the stretching of the matrix. The resulting lateral tension will then flatten the membrane undulations.

Since the time scale t_s is also small compared to the diffusion time t_d, the domain size stays essentially constant during the transformation step. This implies that the complete bud has the radius $R_{cb}^0 \simeq L^0/2$, (see Table 1).

Detachment of budding domain. During the transformation towards the complete bud, the neck radius decreases, which implies that the lateral tension Σ exerted by the bud onto the surrounding matrix increases. Within the model considered here, this tension is given by

$$\Sigma = (1/2\pi N)(\partial E/\partial N) = (\sigma/N) - 8\kappa(1 - C_{sp}/C)/L^2. \quad (6)$$

By definition, this tension is positive if the surrounding matrix is pulled by the bud, and negative if the bud is pulled by the matrix.

For the parameter values in Table 1 as appropriate for lipid bilayers, the lateral tension Σ_{cb} of the complete bud with neck radius $N_{cb} \simeq 4$ nm is primarily determined by the first term in (6), i.e., $\Sigma_{cb} \simeq \sigma/N_{cb}$. Using the above estimates for the line tension, one finds that Σ_{cb} is of the order of 10^{-3} J m^{-2}. This tension is comparable to the tension of rupture, Σ_{max}, for lipid bilayers which typically lies in the range $(1-5) \times 10^{-3}$ J m^{-2} (Reference 19). Since the edge represents a linear defect, the membrane will rupture along this edge and the budding domain will become a budding vesicle for $\Sigma_{cb} \simeq \Sigma_{max}$.

BUDDING OF BIOMEMBRANES

In biological cells, budding of membranes represents the first step in the production of vesicles for intracellular transport. In these systems, vesicles which bud off from one "mother" membrane fuse again with other "target" membranes (1, 2). The mother and target membranes have different compositions of lipids and proteins. If all molecules were to enter the budding vesicles in a random fashion, the components of the different membranes would rapidly intermix. Since this does *not* happen, the budding vesicles *must* be formed from intramembrane domains!

Intermixing is prevented more effectively if the budding domain matches the composition of the target membrane more closely. Thus, one would expect that

the evolution of the cell has produced mechanisms to regulate the composition of the domains. Indeed, several structures have been identified which act towards such a specific aggregation (1, 2): (a) Aggregation of membrane-bound receptors which bind specifically to ligand molecules; (b) In many cases, the aggregated domains are covered by a coat of proteins; and (c) In some cases, the coat contains a network of clathrin molecules (9, 10).

However, as long as the biomembrane is fluid, the growing domain *must* bud as soon as it has grown up to a certain size, *irrespective* of the specific aggregation mechanism. If the coat led to a gel-like or polymerized state of the domain with bond energies, which are large compared to the thermal energy, $k_B T$, the shape of the bud would freeze in, unless the bonds are broken up again by enzymes. In principle, such an active process could be involved in the shape changes of the clathrin network. However, it will now be argued that the observations on clathrin-coated domains are also consistent with the universal budding mechanism proposed here.

It is believed that clathrin-coated domains provide the major pathway for endocytosis of the plasma membrane but that non-clathrin-coated domains are involved in most exocytic processes of internal membranes (10). In general, the main function for clathrin seems to be that it *facilitates* the uptake of receptors and ligands: there is "life without clathrin" even though it is less efficient. The building blocks of the clathrin coat are receptor molecules with clathrin attached to receptor tails via assembly polypeptides (9, 10). The clathrin molecule has three kinked legs extending from a central vertex. In aqueous solution, these trimers spontaneously assemble into polyhedral cages. Similar cages have been identified in various tissues: for example, clathrin cages with curvature $C_{cl} \simeq 1/60$, $1/45$, and $1/38$ nm^{-1} have been found in brain, liver, and fibroblast cells, respectively (20).

The binding energy of the clathrin molecules within the polymerized network can be estimated from the depolymerization process, which is regulated in the cell by special uncoating proteins. It seems that these proteins need to hydrolyze three ATP molecules in order to detach one clathrin trimer from the polymerized cage (21), which implies a binding energy $\simeq 24 \times 10^{-20}$ J $\simeq 60 k_B T$.

Originally, it was thought that the clathrin molecules adsorb onto the protein–lipid bilayer and first form a planar hexagonal network. However, because of the large binding energy involved in the polymerization, it would cost a lot of energy to disassemble and reassemble this network during the subsequent budding process. Thus, it seems plausible to assume that the molecules polymerize *only once* during the budding process and then form a network with curvature $C \simeq C_{cl}$.

If all building blocks of the coat had an appropriate conical shape which leads to a spontaneous curvature $C_{sp} \simeq C_{cl}$, the polymerization could proceed at the growing edge of the domain. In this case, each building block is incorporated into the polymerized network as it is attached to the growing domain. Since different receptors

are assembled within the same coated pit, such a spontaneous curvature would have to arise from the geometry of the receptor tails with the attached clathrin.

On the other hand, the aggregating molecules could also have a spontaneous curvature C_{sp} which is *small* compared to the curvature C_{cl} of the polymerized clathrin cage, provided the domain stays initially *fluid*. In the latter case, the budding of the domain would still be governed by the interplay of bending energy and line tension, and the polymerization would only set in during the late stages of the budding process, when the curvature of the budding domain becomes compatible with the curvature of the clathrin cage.

The two possible modes of polymerization just described are qualitatively different. If the building blocks are polymerized at the edge of the growing domain, the curvature of the domain *stays constant* during the whole budding process. In contrast, if the polymerization is postponed until the curvature of the bud is compatible with the curvature of the clathrin cage, the domain curvature *increases continuously* during the budding process. For the endocytosis of large hen oocytes, electron microscopy seems to support the latter possibility, since it indicates such an increase of the domain curvature (1). Therefore, even the budding of clathrin-coated domains could be governed by the general budding mechanism proposed here.

In general, the elastic properties of a coated domain depend on the molecular structure of the coating. Both the bending rigidity κ and the line tension σ will *increase* with the thickness l of the coat. This implies that the coating increases the lateral tension (6) and thus *facilitates* the fission of the budding vesicle. The thickness l as observed by electron microscopy is typically $\simeq 20$ nm, which is five times the thickness of a bilayer. However, if κ and σ were roughly proportional to l, the invagination length $\xi = \kappa/\sigma$ would be roughly independent of l and the coat thickness l would have had only a small effect on the bud size. Indeed, the bud sizes of biomembranes as observed by electron microscopy are quite similar to the bud sizes as estimated above for lipid bilayers, see Table 1.

OUTLOOK

In summary, a simple theoretical model has been introduced for domain-induced budding of membranes. This model predicts that domains should always undergo budding as soon as they have grown up to a certain limiting size L^0 and that the bud size is $\simeq L^0/2$.

For bilayer membranes composed of lipid mixtures, the model gives rough estimates for this bud size and for the time scales involved in the budding process, (see Table 1). These theoretical predictions are accessible to experiments. For example, one could study vesicles or multilayer suspensions composed of phospholipids and cholesterol. Initially, the concentration and the temperature are chosen in such a way that the bilayer membranes are in one of the two fluid phases and thus are laterally homogeneous. Then, a temperature quench is per-

formed into the two-phase region where the two fluid phases can coexist. This will lead to the nucleation of intramembrane domains, which should then undergo the budding process described here.

So far, such experimental studies have not been performed in a systematic way. It has been recently observed that vesicles composed of phospholipid–sphingomyelin mixtures undergo budding phenomena. (Döbereiner, H. G., J. Käs, D. Noppl, and E. Sackmann, preprint, TU Munich). However, one expects that the bilayers of these vesicles undergo a phase transformation from a gel to a fluid phase.

The general instability mechanism for budding as proposed in this paper should also apply to the budding of non-coated domains in biomembranes. In fact, this mechanism could even be effective in the presence of coating proteins such as clathrin. In the latter case, the budding domain would stay unpolymerized until the curvature of the bud has attained a value which is compatible with the curvature of the polyhedral clathrin cage. Furthermore, within the context of biological evolution, the general budding mechanism discussed here seems to provide a plausible starting point for the subsequent evolution of more elaborate mechanisms. Such additional mechanisms could be used by the cell for "fine tuning", i.e., in order to enhance or to suppress the budding process arising from the basic instability of the growing domain. For example, the spontaneous curvature of the domain could be changed by enzymes or by the adsorption of specific molecules, or the budding process could be regulated by pulling "strings" such as actin filaments.

The simple model studied here has some obvious theoretical limitations. For example, it does not take into account any curvature energy for the neck itself. Therefore, the shape of the membrane makes a sharp bend along the domain edge, per Fig. 2. However, if the lipid molecules within the α and the β phase do not exhibit any preferred tilt of the hydrocarbon chains, the two membrane segments should join in a smooth way.

In order to overcome these limitations of the simple model, we have recently developed a systematic theory based on the minimization of curvature models. (Jülicher, F., and R. Lipowsky, manuscript in preparation.) In these models, the membrane surface is described by its two principal curvatures, C_1 and C_2, and its elastic energy is given by

$$\mathcal{H} = \oint dA_\alpha \kappa_\alpha (C_1 + C_2)^2 + \oint dA_\beta \kappa_\beta (C_1 + C_2)^2 + \oint dl\,\sigma$$

as appropriate for a surface consisting of α and β domains with bending rigidities κ_α and κ_β. One can also include the effect of two different spontaneous curvatures or of an overall constraint on the vesicle volume. The results of these systematic studies lead to more complex phase domains arising from the increased number of parameters, but the basic instability mechanism discussed here is again confirmed.

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