Budded membrane microdomains as tension regulators

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We propose a mechanism by which changes of the mechanical tension of a composite lipid membrane are buffered by the invagination of membrane domains. We show that domain invagination, driven by differences in chemical composition, is a first-order transition controlled by membrane tension. The invaginated domains play the role of a membrane reservoir, exchanging area with the main membrane, and impose an equilibrium tension entirely controlled by their mechanical properties. The dynamical response of the reservoir reflects the tension-dependent kinetics of the domain shape transition, so that the tension of such a composite membrane is inherently transient and dynamical. The implications of this phenomenon for the mechanical properties of the membranes of living cells, where invaginated membrane domains are known to exist, are discussed.

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Endocytosis, exocytosis, cell motility and many other crucial cellular processes are known to be influenced by the tension of the cell membrane [1], and there is experimental evidence that surface area regulation within the cell is able to buffer variations of membrane tension [2,3]. The level of membrane tension in cells is thought to primarily reflect cytoskeleton anchoring to the membrane [4]; nevertheless, membrane invaginations such as the raftlike domains *caveolae* [5] are thought to be reactive to membrane stress [6,7]. Striking experiments showing caveolae flattening under tension [8] support the idea that the delivery of invaginated membrane area to the plasma membrane is controlled by tension [9]. In artificial systems such as giant vesicles, a composite membrane often phase separates into membrane domains, the shape of which is known to be dependent on the membrane tension [10], and domain budding has been observed upon the decrease of membrane stress [11].

The goal of this paper is to investigate the mechanical response of such a composite membrane to a dynamical perturbation. Experimentally, membrane tension can be very efficiently measured and altered by the extraction of a membrane tether from the vesicle or cell with an optical trap. In living cells, the variation of tension upon tether extraction involves cytoskeleton deformation, the breaking of membrane-cytoskeleton bonds [12], and changes in membrane morphology. Here, we focus on one possible membrane morphological change, namely, the flattening of invaginated membrane domains upon an increase of membrane tension. Assuming a direct relationship between the area and tension of the main membrane, we study the mechanical equilibrium between the main membrane and the reservoir, and the dynamic tension of the membrane under steady perturbation, controlled by the kinetics of membrane exchange with the reservoir.

We discuss in particular the response of a membrane reservoir made of domains that tend to be invaginated under low tension and flat under high tension. In order to assess the relaxation of tension by the reservoir, we assume that the tension of the membrane changes linearly with variation of its surface area, resembling an effective spring (Fig. 1). Extraction of membrane area in a tether removes area from the main membrane and increases its tension (the length of the spring in Fig. 1). The increase of tension results in an increased rate of domain flattening, which in turn releases some membrane area and decreases membrane tension. The variation of the cell tension with the tether area $\mathcal{A}_T$ reads

$$\gamma = \gamma_0 + K_s(\mathcal{A}_T + \mathcal{A}_{res} - \mathcal{A}_{res}^{(0)}) \tag{1}$$

where γ_0 and K_s are the membrane tension without tether and the stretching modulus, respectively of order $10^4 k_B T/\mu\text{m}^2$ and $10^4 k_B T/\mu\text{m}^4$ in cells [13]. The area of the reservoir $\mathcal{A}_{\text{res}}$ ($\mathcal{A}_{res}^0$ without tether) is the amount of area sequestered within the membrane invaginations. If membrane area is delivered to the main membrane, i.e., by vesicle fusion as during exocytosis, the "tether" area is negative. One can see that the membrane tension γ can be maintained constant upon tether pulling only if the decrease of reservoir area matches the increase of tether area.

The energy of one membrane domain includes the mem-

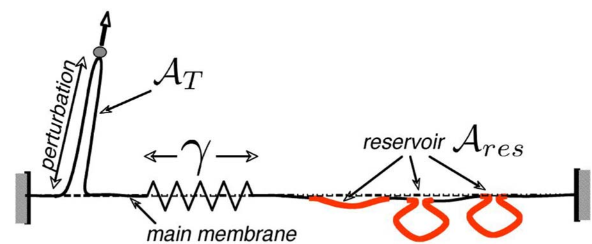

FIG. 1. (Color online) Sketch depicting the mechanical response of a membrane to the pulling of a membrane tether. Pulling an area $\mathcal{A}_T$ out of the cell either increases the membrane tension γ (the length of the spring), or triggers membrane exchange with a reservoir (flattening of invaginated membrane domains).

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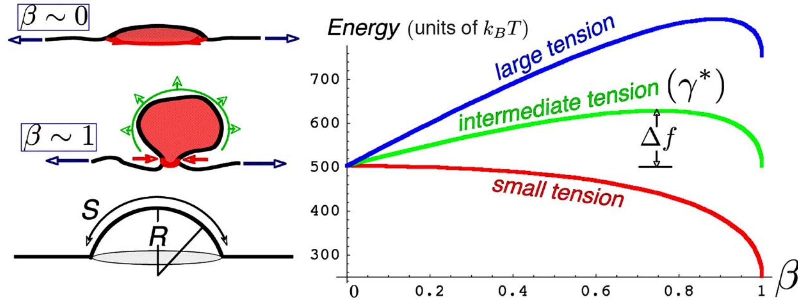

FIG. 2. (Color online) Left: A sketch of a flat and an invaginated domain, above our idealization for the domain shape; a spherical cap of area S, radius R (curvature $C=2/R$). The shape is defined by a single parameter $0<\beta=SC^2/16\pi<1$. Right: energy of a domain for increasing value of the membrane tension. Small tensions favor budded shape ($\beta=1$), and large tensions favor flat shape ($\beta=0$). At coexistence [tension γ^*, Eq. (3)], flat and budded domains have the same energy, and the tension is given by Eq. (3). The budded state remains a local minimum for high membrane tension and there exists an energy barrier Δf to domain flattening.

brane bending rigidity, which disfavors the budded state, and its composition difference from the rest of the membrane, which promotes domain invagination in order to reduce the length of the domain periphery (Fig. 2). Domains are treated as spherical caps of fixed area S and adjustable curvature C. Their shape is uniquely characterized by the parameter β [Fig. 2 and Eq. (2)], equal to unity for a fully budded domain (a sphere), and which vanishes for a flat domain. The energy f of a domain contains a surface-tension-independent part $f_{\sigma,\kappa}$. This itself involves a term arising from the line tension σ [10], proportional to the length of the cap edge (neck), and a term giving the bending energy of the cap, proportional to the bending rigidity κ and the squared curvature [14]. Simple geometry then gives

$$f_{\sigma,\kappa}[\beta] = \sqrt{\pi S}\sigma\sqrt{1-\beta} + 8\pi\kappa\beta, \quad \beta = SC^2/16\pi. \tag{2}$$

If the domain size exceeds a critical value $S_c=\pi(4\kappa/\sigma)^2$, an invaginated sphere ($\beta=1$) has a lower energy than a flat domain ($\beta=0$) and budding is expected [15]. We assume that $S>S_c$ in what follows. Typical values of the parameters $\sigma \sim k_BT/\text{nm}$ and $\kappa=20k_BT$ correspond to a critical size $S_c=(100\ \text{nm})^2$, similar to the size of caveolae. In practice invaginated domains remain attached to the mother membrane by a small neck. Here, this is controlled phenomenologically by assigning the value α_{bud} ($\ll 1$) to the ratio of the neck size to the radius of the fully budded invagination, giving an upper bound $\beta_{bud}=1-(\alpha_{bud}/2)^2$ to the shape parameter.

Including the membrane tension γ, the domain energy reads $f[\beta]=f_{\sigma,\kappa}[\beta]+\gamma S\beta$. Increasing membrane tension increases the energy of curved states $\beta>0$ and promotes the flat state (see Fig. 2). The flat states eventually become stable for a critical tension γ^*:

$$S\gamma^* = -(f_{\sigma,\kappa}[1] - f_{\sigma,\kappa}[0]) = 2\sqrt{\pi S}\sigma - 8\pi\kappa. \tag{3}$$

As can be seen in Fig. 2, the budded and flat domain shapes are separated by an energy barrier for intermediate tension. The existence of this barrier is crucial to their function as tension regulators, as it allows the coexistence of flat and invaginated domains. Variation of the membrane strain (the tether length) may occur without change of tension by adjusting the fraction of budded domains.

The energy scales in this system ($S=0.1\ \mu\text{m}^2$) are

$$\bar{\sigma} \equiv \sqrt{\pi S}\sigma \simeq \bar{\kappa} \equiv 8\pi\kappa \simeq 500k_BT, \quad \bar{\gamma} \equiv \gamma S. \tag{4}$$

The energy of surface tension competes with the line and bending energies for $\bar{\gamma}\simeq 500k_BT$, or $\gamma=2\times 10^{-5}\ \text{J/m}^2$ (corresponding to tether forces of order 10 pN). This is precisely in the range of mechanical tension recorded for cellular membranes [4], which is very encouraging for the biological relevance of our model. One notes that the energy scale is very large compared to the thermal energy k_BT, or to the energy of any "active temperature" present in biological systems [16]. This has two important physical consequences: (i) the shape transition of a domain is very discontinuous; a domain snaps open rather than continuously flattening upon tension increase; and (ii) the budding and flattening transitions should actually occur at different tensions, for which the respective energy barriers are of order k_BT. In biological systems, the "temperature" T might be seen as a parameter reflecting cellular activity, such as the polymerization of the actin cortex near the membrane, and the activity of membrane pumps. For simple cells such as red blood cells, it is typically a few times the thermodynamic temperature [16].

The bottleneck for the shape transition is the maximum of energy, which corresponds to a shape parameter $\beta_{max}=1-[\bar{\sigma}/(\bar{\kappa}+\bar{\gamma})]^2$. The budding and flattening tensions ($\gamma^{(1)}$ and $\gamma^{(0)}$, respectively) at which the corresponding energy barrier vanishes are

$$\bar{\gamma}^{(1)} = \bar{\sigma} - \bar{\kappa} < \bar{\gamma}^* = 2\bar{\sigma} - \bar{\kappa} < \bar{\gamma}^{(0)} = \frac{2}{\alpha_{bud}}\bar{\sigma} - \bar{\kappa} \tag{5}$$

where α_{bud}($\ll 1$) characterizes the finite neck size in the budded state (see above).

We investigate the tension regulation performed by a collection of $\mathcal{N}$ domains, of total area $\mathcal{N}S\beta_{bud}$ [where $\beta_{bud}=1-(\alpha_{bud}/2)^2$ is the value of the shape parameter in the budded state]. When flat and budded domains coexist, a fraction ϵ of domains are invaginated, and the reservoir area is $\mathcal{A}_{res}=S\mathcal{N}\epsilon\beta_{bud}$. The total membrane energy, including the contribution $f_{\sigma,\kappa}$ of each of the $\mathcal{N}$ membrane domains [Eq. (2)], and the total work done against membrane tension can be written

$$\mathcal{F} = \mathcal{N}\{\epsilon f_{\sigma,\kappa}[\beta_{bud}] + (1-\epsilon) f_{\sigma,\kappa}[0]\} + \int d\mathcal{A}\, \gamma[\mathcal{A}]. \quad (6)$$

Optimizing the energy for the fraction of invaginated domains $\partial \mathcal{F}/\partial \epsilon = 0$ leads directly to the regulation of membrane tension, when flat and budded domains coexist $(0 < \epsilon < 1)$. Substituting in Eq. (6) the expression for the surface tension Eq. (1) (with $\beta_{bud} \lesssim 1$), we find that the tension is set to the value γ^* of Eq. (3), which depends on the characteristics of the membrane reservoir (σ, κ, and S), but not on the tether area. Regulation is achieved by adjusting the fraction of budded domains to

$$\epsilon^* = \epsilon_0 - \frac{(\bar{\gamma}_0 + \bar{K}_s \mathcal{A}_T/S) - \bar{\gamma}^*}{\bar{K}_s \mathcal{N}}, \quad \bar{K}_s \equiv K_s S^2, \quad (7)$$

where ϵ_0 is the fraction of budded domains corresponding to the tension at rest γ_0, and where a normalized stretching coefficient $\bar{K}_s$ ($\sim 0.1\bar{\sigma}$), with dimension of energy, is introduced for convenience.

If the reservoir is given time to equilibrate, regulation starts for a level of perturbation corresponding to a tether area $\mathcal{A}_T^{(1)}$ (at which all domains are budded, $\epsilon^* = 1$), and ends at a tether area $\mathcal{A}_T^{(0)}$ (at which all domains are flat, $\epsilon^* = 0$). The tension of the cell membrane is then set to the value γ^*, for any perturbation within the range $\mathcal{A}_T^{(1)} < \mathcal{A}_T < \mathcal{A}_T^{(0)}$. If the perturbation is very fast, one expect a large difference between the regulated tension upon tether pulling and tether retraction, in agreement with Eq. (5).

To obtain the full kinetic response of the membrane to strain, we describe the transition as a classical Kramers' process [17], where the transition time between two states is exponential with the energy barrier Δf that has to be overcome in the process: $\tau = \tau_0 \exp(\Delta f / k_B T)$. Here, τ_0 is the characteristic fluctuation time of the domain shape, assumed to be the same for both domain flattening and budding. The transition time is very much dependent upon the membrane tension. Assuming that the transition of a single domain occurs with negligible change of tension (this implies $\mathcal{N} \gg 1$), the transition is fully described by the energy $f[\beta] = f_{\sigma,\kappa}[\beta] + \bar{\gamma}\beta$, with γ given by Eq. (1). The kinetic evolution of the fraction ϵ is given by

$$\tau_0 \frac{d\epsilon}{dt} = -\epsilon e^{-(f_{max} - f[\beta_{bud}])/k_B T} + (1-\epsilon) e^{-(f_{max} - f[0])/k_B T} \quad (8)$$

where the maximum of energy f_{max} corresponds to the least favorable domain shape $\beta_{max} = 1 - \bar{\sigma}^2/(\bar{\kappa} + \bar{\gamma})^2$.

In order to mimic a tether pulling experiment, where the tether is typically extracted at constant speed ($\sim \mu$m/s), we consider the reservoir response to a perturbation applied with a given rate $\dot{\mathcal{A}}_T$: $\mathcal{A}_T = \mathcal{A}_T^{(0)} + \dot{\mathcal{A}}_T t$. The force of such a dynamical perturbation is influenced by the viscous dissipation (e.g., around the cytoskeleton anchors) and by the kinetic response of the reservoir. Here, we account only for the latter effect, for which the rate of the dynamical perturbation basically sets a time scale for the evolution of the membrane morphology. This time scale in turn corresponds to a particular height of the barrier of energy between the two domain shapes, and thus to a membrane tension at which the transition can occur.

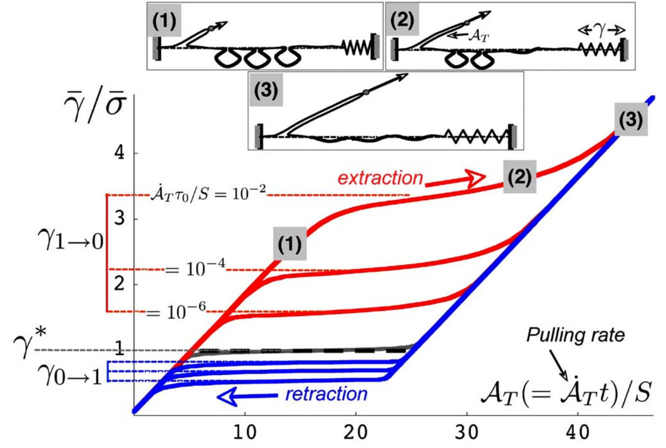

FIG. 3. (Color online) Sketch of the change in membrane morphology upon tether extraction. At low strain (1) all membrane domains are budded, and the membrane tension γ increases linearly with the membrane area $\mathcal{A}_T$. For large perturbation (3) all domains are flat and a similar linear increase is observed. In between, (2), the membrane tension is maintained at a plateau value while flat and budded domains coexist. The equilibrium reservoir response (γ^*, dashed gray line) corresponds to a quasistatic perturbation. The dynamical responses at constant rate $\dot{\mathcal{A}}_T$ is shown upon extraction [(red) right arrow] and retraction [(blue) left arrow] for various rates. The hysteresis, which increases with the perturbation rate, illustrates the kinetic nature of the domain shape transformation.

If the perturbation is applied slowly ($\dot{\mathcal{A}}_T \tau_0 \ll \mathcal{A}_{res}$), the reservoir has time to equilibrate ($d\epsilon/dt = 0$), and the fraction ϵ_T^* of budded domain is found from Eq. (8) to be given by $\epsilon_T^*/(1-\epsilon_T^*) = e^{(f[0]-f_{bud})/(k_B T)}$, with $f[0] - f_{bud} \simeq 2\bar{\sigma} - [\bar{\gamma}(\epsilon) + \bar{\kappa}]$. The fraction ϵ_T is the equivalent of the equilibrium fraction ϵ^* [Eq. (7)], which takes thermal fluctuations into account ($\epsilon_T^* \simeq \epsilon^*$ if $\bar{\sigma} \gg k_B T$). Thermal fluctuations smooth the transition between budded and flat domains by allowing states of nonminimal energy to be populated. As a consequence, the tension is not perfectly constant during the transition, and the slope at mid plateau is of order $\partial \gamma^* / \partial \mathcal{A}_T|_{plat} \simeq 4 k_B T / (S \mathcal{A}_{res})$. If, on the other hand, the perturbation is applied very fast, the shape transition requires small energy barriers, which means high tension for bud flattening, and low tension for domain budding, close to $\gamma^{(0)}$ and $\gamma^{(1)}$ of Eq. (5), respectively.

The physical mechanism at the origin of tension regulation and the membrane hysteretic response to tether extraction and retraction are shown in Fig. 3. To obtain an analytical expression of the plateau height with the perturbation rate, we approximate that the tension is almost constant during regulation ($d\bar{\gamma}/dt \sim 0$) so that the energy barrier is of order $\Delta f/(k_B T) \sim \ln \mathcal{A}_{res}/(\dot{\mathcal{A}}_T \tau_0)$. The plateau tensions upon increase and decrease of the perturbation are then respectively given by $\bar{\gamma}_{1\to0} = \bar{\gamma}^{(0)} - 2/\alpha_{bud}^{3/2}\sqrt{\bar{\sigma} k_B T \ln}$ and $\bar{\gamma}_{0\to1} = \bar{\gamma}^{(1)} + \sqrt{\bar{\sigma} k_B T \ln}$, with $\ln \equiv \ln[\mathcal{A}_{res}/(2\dot{\mathcal{A}}_T \tau_0)]$. As expected for activated processes [18], the dependence of the tension at transition on the rate of perturbation $\dot{\mathcal{A}}_T$ is logarithmic. The same is true for the slope of the plateau, which can be estimated by identifying the plateau inflection point. The condition

$d^2\bar{\gamma}/dt^2=0$ imposes $\dot{\Delta f}/(k_BT)\simeq\dot{\epsilon}/\epsilon$, corresponding to a plateau slope $\partial\bar{\gamma}_{1\to 0}/\partial\mathcal{A}_{T}|_{plat}\simeq\sqrt{2k_BT\bar{\sigma}/\alpha_{bud}^3\ln}/\mathcal{A}_{res}$. The study of both the height and slope of the tension plateau gives valuable information about the kinetics of area transfer from the reservoir and hence about such properties as their line tension and bending energy.

Regardless of the initial state of the membrane, the tension under dynamical tether extraction shows an initial increase due to the delay in the reservoir's response. The tension at rest of a membrane initially in equilibrium with a partially unfolded reservoir is γ^* of Eq. (5), fully controlled by the mechanical properties of the membrane domains forming the reservoir. In such a situation, a quasistatic perturbation leaves the membrane tension almost constant, but tether extraction at any finite speed leads to an initial increase of tension of purely kinetic origin prior to the tension plateau. The difference between the quasistatic and dynamic plateaus can be of order 10^{-4} J/m^2 (with $\sigma\sim k_BT/\text{nm}$). This corresponds to a difference in force of order 10 pN, the same scale as the forces measured upon tether extraction on cells [3].

In summary, we have derived the mechanical reactivity of a composite fluid membrane with domains. The domains may bud off, but remain connected to the main membrane, playing the role of a reservoir reactive to membrane tension. The budding and flattening transitions are first order, which means that under an increase of tension, the invaginations snap open above a critical strain rather than continuously flattening. The tension of the composite membrane shows a plateau during the transition, corresponding to the coexistence of flat and invaginated domains.

This study provides a framework to study the regulation of the tension of a cell membrane. Membrane pits coated with proteins (e.g., the caveolae) may act as a reservoir if they possess two well-defined shapes, separated by an energy barrier (a hypothesis consistent with experimental observations [9]). A ring of specialized membrane proteins such as dynamin [19] is often present at the neck of a membrane invagination. These proteins most probably influence the domain line energy, and might even dominate the energy required to flatten the domain. If anything, neck proteins can only increase the energy barrier to flattening, thereby reinforcing tension regulation. This work also opens the possibility of a quantitative "force spectroscopy" of the cell membrane. One could thereby obtain structural information on the membrane organization, in much the same way information on a protein structure can be gathered from force measurement upon protein unfolding [20]. As a first step, one may identify the fairly regular oscillation of the force during regulation in [3], with the flattening of single domains. Preliminary analysis [13] hints at domains of area $S\sim$ (400 nm)^2.

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