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Fission of a multiphase membrane tube

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A common mechanism for intracellular transport is the use of controlled deformations of the membrane to create spherical or tubular buds. While the basic physical properties of homogeneous membranes are relatively well-known, the effects of inhomogeneities within membranes are very much an active field of study. Membrane domains enriched in certain lipids in particular are attracting much attention, and in this Letter we investigate the effect of such domains on the shape and fate of membrane tubes. Recent experiments have demonstrated that forced lipid phase separation can trigger tube fission, and we demonstrate how this can be understood purely from the difference in elastic constants between the domains. Moreover, the proposed model predicts timescales for fission that agree well with experimental findings.

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Internal organization is one of the most intriguing aspects of the cell. Living cells have to actively maintain gradients of all sorts. Compartmentalisation and trafficking aid it in doing so, and both processes extensively use membranes. Not only are the various organelles in eukaryotic cells surrounded by membranes, but the basic intermediates in the intracellular transport pathways as well are membrane structures such as tubes and vesicles [1]. The generation and properties of these structures have been extensively studied, and much is already known about their biology, biochemistry [2] and their biophysics [3, 4]. The emerging view is that the shape of the bilayer membrane *in vivo* is controlled not only by embedded and associated proteins [5] but also to a large extent by the mechanical properties of the bilayer itself [3, 6]. For tubular structures in particular, mechanical effects play a major role: recent biomimetic experiments [4] have shown that kinesin motors walking on microtubules can exert pulling forces on the membrane and prompt the formation of membrane tubes that resemble tubules identified in living cells.

The existence of small membrane domains with a lipid composition that is markedly different from that of the rest of the membrane (sometimes referred to as "rafts" although considerable debate remains as to their precise interpretation) appears to be another key element of intracellular vesicular traffic [7], and also seems to be implicated in a multitude of cellular processes [8]. The heterogeneity in membrane composition can be attributed to a phase transition leading to a local segregation between the various lipids constituting the membrane [9]. Sphingolipid domains in particular have been shown to be more structured than a classical liquid membrane due to specific interactions between their constituents [1]. Under appropriate conditions they tend to aggregate into so-called *liquid-ordered* domains which are mechanically stiffer than the rest of the bilayer. Recently, an experi-
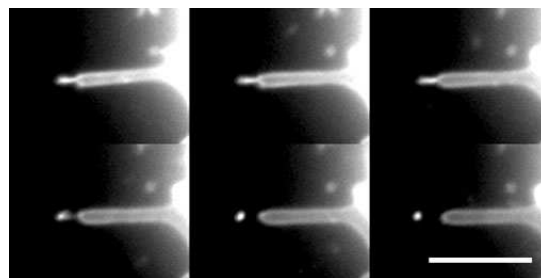

FIG. 1: Breakage of a heterogeneous membrane tube [16]. The brighter (and thinner) section at the tip on the left is a liquid-disordered DOPC domain. Fission events occur at the sites of formation of small domains resulting from phase separation. The time between two consecutive pictures is one second. Scale bar, $10\,\mu$m.

mental model system of vesicles including "raft-like domains" has been developed [10]; it provides an elegant and efficient tool to study their properties in a more controlled way than *in vivo*. This procedure allows for systematic studies of the effects of membrane composition [12], temperature changes [9] and protein absorption on the domain [13].

The physics of membrane tube formation from homogeneous vesicles has been studied both theoretically [14, 17] and experimentally [15]. Recent experiments involving one of us [16] study the interplay between lipid domains and the behavior of tubes, by pulling tubes from model membranes. Fig. 1 illustrates one of the suprising conclusions of these experiments - a sequence of snapshots taken at regular intervals (one second between two pictures) show an initially homogeneous tube that first undergoes phase separation (triggered experimentally by photoinduced oxidization of cholesterol), and, after about one second, ruptures precisely at the phase boundary and disconnects. The two lipid phases are easily distinguished, once separation has occurred, by the use of a

fluorescent marker that preferentially sits in the liquid-disordered domains. Furthermore, the same experiments show that fission events such as these happen *only* in the phase separated tubes - tubes in which the lipids are mixed are essentially stable indefinitely.

Statement of problem and summary. In this Letter, we address the dramatic loss of stability following phase separation from a mechanical point of view. We extend the theoretical models developed for homogeneous tubes [17] to study the junction between two distinct phases, each of which far away from the junction has a tubular shape. Experiments suggest that phase separation occurs on a much faster timescale than fission, and that the nucleation of the two phases leads to the formation of cylindrical domains between a more rigid and a less rigid phase. We therefore choose not to model the dynamic of the phase separation process [18]. The tube radii and the junction length are generally small compared to the length of each phase domain. In order to minimize the interfacial energy between adjacent domains, the interface rapidly becomes a circle perpendicular to the tube direction. The coarsening stage of the phase separation process proceeds very slowly to eventually form two homogeneous phases in equilibrium, but this slow relaxation is always preempted by tube fission.

We assume here that the tube and junctions are axisymmetric with respect to the direction along which the tube is pulled (the z axis). We consider one junction between two semi-infinite tubes each consisting exclusively of one of the phases. Finite-size effects associated with the limited size of individual domains, while possibly relevant, fall outside the scope of the present paper. The small radius of the tubes (about 40 nm) does not allow a quantitative determination of the shape of the junctions [16], and for this reason we restrict ourselves to a minimal model which emphasizes the roles of the most relevant physical parameters. We show that tube fission can be driven either by the line tension or by the jump of the elastic coefficients at the interface between the two phases, and we compare the two processes that undoubtedly both contribute in the experiments.

Model. We use an elastic membrane free energy as introduced by Canham and Helfrich [19], and numerically determine equilibrium junction shapes. Fig. 2 gives a schematic representation of the tube and the coordinate system used in the following. Our axisymmetric surface is parametrised by the arc length s along the contour and described by the local tube radius $r(s)$ and the angle $\psi(s)$. They are related by the geometric relations $\dot{r} = \cos\psi$ (dots denote derivatives with respect to s). The interface is located at $z = s = 0$.

The free energy of the system is obtained by extending the description of tubular membranes [17] to the specific
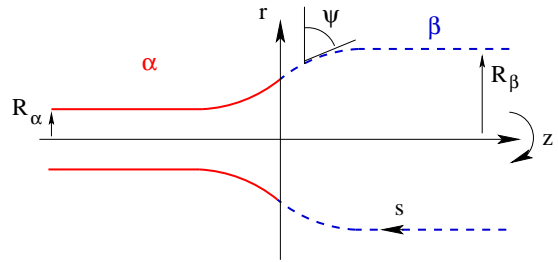

FIG. 2: Schematic representation of the junction.

case of a biphasic tube [20, 21] as follows:

$$\mathcal{F} = \sum_{i=\alpha,\beta} \int_{\Omega_i} \left[2\kappa_i \mathsf{H}^2 + \kappa_\mathrm{G}^{(i)} \mathsf{K} + \sigma_i \right] \mathrm{d}S + \oint_{\partial\Omega} \tau \, \mathrm{d}\ell - \int f \, \mathrm{d}z. \quad (1)$$

The two phases are denoted by α and β, and for each phase i the free energy is integrated over its membrane area Ω_i. The κ_i and $\kappa_\mathrm{G}^{(i)}$ are the bending- and Gaussian rigidities of the respective phases. This free energy includes the bending energy to lowest order in the principal curvatures, where H is the mean curvature and K the Gaussian curvature. The two layers of the membrane are assumed to be symmetric - both phases contain cholesterol molecules which have a high flip-flop rate. Any stress due to area differences between the leaflets or to an asymmetry of the layers is thus quickly relaxed. Finally, Lagrange multipliers σ_i are introduced to ensure a constant surface in each phase. These σ_i are interpreted as surface tensions. We take our tube to be infinite, and assume the presence of a lipid reservoir. In the experiments, such a reservoir is provided by the large mother vesicles from which the tubes are drawn. Provided the area per lipid remains constant during the process this implies a constant surface tension in each of the phases.

The interface between the two phases is described by a jump in the values of the bending rigidities κ_i, $\kappa_G^{(i)}$ and in the surface tension σ_i, and by a positive line tension τ at the interface $\partial\Omega$. The last term in the free energy is the work performed by the external force f needed to pull the tube. We neglect the small effect of pressure [14].

The variational derivation of the shape equations of the surface has been detailed elsewhere [23], and yields

$$\dddot{\psi} = -\frac{\dot{\psi}^3}{2} - \frac{2\cos\psi}{r}\ddot{\psi} + \frac{3\sin\psi}{2r}\dot{\psi}^2 + \frac{3\cos^2\psi - 1}{2r^2}\dot{\psi}$$
$$-\frac{\cos^2\psi + 1}{2r^3}\sin\psi + \frac{\sigma}{\kappa}\dot{\psi} + \frac{\sigma}{\kappa}\frac{\sin\psi}{r} \quad (2)$$

Far away from the junction, we recover homogeneous cylindrical tubes with $\psi = \pi/2$ and $R_i = (\kappa_i/2\sigma_i)^{1/2}$. Mechanical equilibrium implies that the forces at both extremities are equal and that $f = 2\pi(2\sigma_i\kappa_i)^{1/2}$, which imposes that $\sigma_\alpha/\sigma_\beta = \kappa_\beta/\kappa_\alpha$: The surface tension jumps discontinuously across the interface.
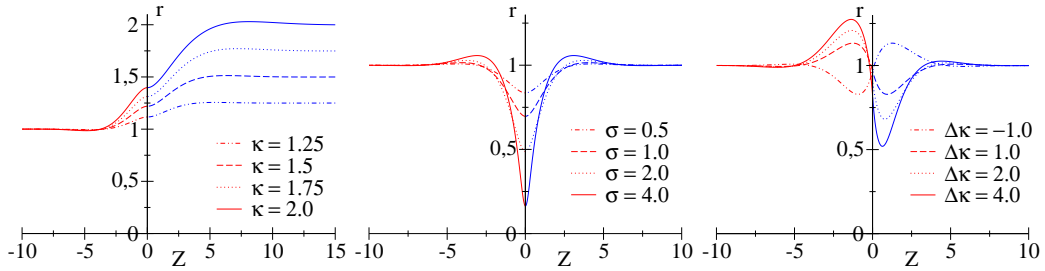
FIG. 3: Numerical shapes of the junction for various line tensions and differences of elastic rigidities in dimensionless units. The length scale is the radius of phase α ($R_\alpha = 1$), the energy scale is the bending rigidity of phase α so that $\kappa_\alpha = 1$. *(a)*: shapes for various ratios of bending rigidities. The line tension vanishes ($\tau = 0$) and the Gaussian rigidities are equal ($\Delta\kappa_G = 0$). The values of $\kappa_\beta/\kappa_\alpha$ are 1.25, 1.5, 1.75, and 2.0 *(b)*: shapes for various line tensions. The elastic rigidities are equal: $\Delta\kappa_G = 0$ and $\kappa_\alpha = \kappa_\beta$. The values of the line tension are $\tau = 0.5$, 1.0, 1.5 and 2.0. *(c)*: shapes for various differences in Gaussian rigidity. The line tension vanishes ($\tau = 0$) and the bending rigidities are equal ($\kappa_\alpha = \kappa_\beta$). The values of the difference in Gaussian rigidity are $\Delta\kappa_G = \kappa_G^\beta - \kappa_G^\alpha = -1.0$, 1.0, 2.0 and 4.0.

The mismatch between constants such as the bending rigidities appears only in the boundary conditions and strongly affects the interface shape. At the interface ($s = 0$), four boundary conditions must be satisfied. Two conditions are the continuity of the radius $r(s)$ and the angle $\psi(s)$ [20]; two additional conditions stem from the variational procedure and relate the first and the second derivatives of the angle ψ on each side of the interface to the values of r, ψ, κ_α, κ_β, $\Delta\kappa_G = \kappa_G^\beta - \kappa_G^\alpha$ and τ.

Results. Fig. 3 illustrates the different effects that line tension and differences in elastic rigidities individually have on the two-phase tube. The first possible discontinuity at the junction is a jump in bending rigidities (Fig. 3(a)). The ratio of the bending rigidities in the two phases $\kappa = \kappa_\beta/\kappa_\alpha$ fixes the ratio of the radii away from the junction and of the surface tensions in the two phases. In the absence of both line tension and jump in Gaussian rigidity, the radius decreases smoothly from the values of the more rigid phase to the value in the less rigid phase, but with a remarkable structural feature - a small plateau (*i.e.* a membrane region with a horizontal tangent) occurs around the junction. This plateau is also given by an analytical linear calculation [24].

When line tension dominates (Fig. 3(b)), the radius at the interface decreases with increasing line tension. It vanishes for a huge line tension. Note that our description breaks down at scales comparable to the bilayer width. Despite the fact that the radius goes to zero the mean curvature remains finite; in the highly pinched limit a saddle point develops at the neck which keeps the total curvature energy finite.

When the discontinuity in Gaussian rigidities dominates (Fig. 3(c)), numerical evidence suggests that the neck radius does not decrease all the way down to zero. Moreover, stability arguments given below impose a bound on the maximum absolute value of $\Delta\kappa_G$. However, the presence of the neck favors the breaking process. In this case, fission does not occur exactly at the interface but at the neck. One thus expects to find, after fission, a small patch of one phase still attached to the other phase. Since details at the length scale of the neck itself cannot be resolved experimentally, this effect might be relevant to determine the dominant fission mechanism.

Discussion. For general experimental conditions, all three effects are superimposed at the junction. A quantitative analysis of the shape in order to extract the various parameters is then difficult, especially as little to nothing is experimentally known about the precise shape of the junction. Typical values of the bending rigidity of liquid bilayers are around $25\,k_BT$, and the rigidity of the liquid ordered phases can be up to several times higher. Recently, the bending modulus of a heterogeneous vesicle has been obtained by comparing the experimental shape to numerical solutions of the shape equations [25]. The Gaussian rigidity $\kappa_G^{(i)}$ is notoriously difficult to measure experimentally, but a recent study cites values of $\kappa_G^{(i)} = -0.83\kappa_i$ [26]. Stability arguments impose that $-2\kappa_i < \kappa_G^{(i)} < 0$.

The equilibrium free energy of the tube can be calculated from Eq.(1) and allows a discussion of the stability of the tube and of its fission. We show in Fig. 4 the free energy of a tube as a function of the dimensionless radius at the neck r_{neck}/R_0 in the specific case of $\kappa_\alpha = \kappa_\beta$ and $\kappa_G^\alpha = \kappa_G^\beta$. This energy is maximal for a vanishing radius: at this point, the membrane is maximally bent. Fission of the tube by pinching requires one to cross this energy barrier. The free energy of the ruptured tube is also shown on the figure. It is lower than the top of the barrier by the contribution of the Gaussian curvature due to the change in topology upon rupture, which equals $4\pi\kappa_G$. Notice that the bending energy does *not* change upon rupture: at vanishing radii, the neck is a saddle point with vanishing mean curvature [24]. The ruptured tube corresponds to a transient shape since in the absence of an applied force, the tubes retract to form two spheres. In the absence of line tension the tube is
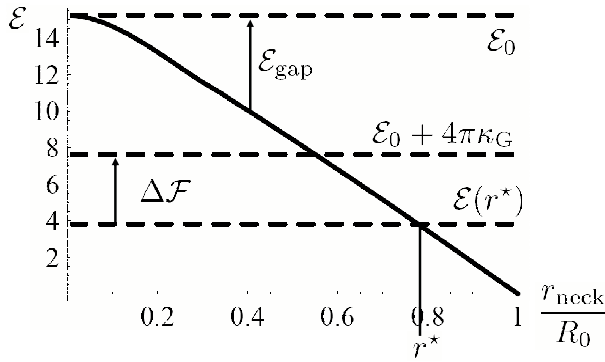
FIG. 4: Schematic energetics of fission. The solid curve plots the free energy of a tube pinched by line tension as a function of the dimensionless neck radius. Every equilibrium radius $r^\star$ has a corresponding energy $\mathcal{E}(r^\star) = \mathcal{E}_{\text{bend}} + \mathcal{E}_\tau$ which defines in turn an energy barrier for fission $\mathcal{E}_{\text{gap}}$ and a free energy gain upon fission $\Delta\mathcal{F}$. For clarity, this figure assumes identical elastic rigidities on both sides.

uniform $r(z) = R_0$ and its energy is zero, and a homogenous tube is thus thermodynamically stable only if the free energy of the ruptured tube is positive. Numerically, we have determined this stability limit as $\kappa_G > -1.29\kappa$.

The values of the parameters then fix the value of r_{neck}/R_0. We have also evaluated the energy barrier against fission by pinching from this macroscopic model. Note, however, that this is only a lower bound to the real energy barrier, as it ignores effects at the molecular lengthscale which certainly is attained when the neck becomes very thin. To compare our results to the experiments, we have computed the various energies at the following (measured or realistic) parameter values. With bending rigidities $\kappa_\alpha = 40\,k_BT = 1.6 \cdot 10^{-19}\,\text{J}$, $\kappa_\beta = 70\,k_BT = 2.9 \cdot 10^{-19}\,\text{J}$, Gaussian rigidities $\kappa_G^\alpha = -33.2\,k_BT = -1.38 \cdot 10^{-19}\,\text{J}$, $\kappa_G^\beta = -58\,k_BT = -2.3 \cdot 10^{-19}\,\text{J}$, surface tensions $1 \cdot 10^{-6}\,\text{N/m}$ in phase α and $5.7 \cdot 10^{-7}\,\text{N/m}$ in phase β, and a line tension $7 \cdot 10^{-12}\,\text{N}$, we have determined the height of the energy barrier to be $\mathcal{E}_{\text{gap}} = 7.8\,k_BT$. If we assume that fission is a thermally activated process [27], the average time until fission t_b occurs is $t_b = t_0 \exp \mathcal{E}_{\text{gap}}/k_BT$. Using a hydrodynamic argument, we estimate the basic time scale as $t_0 = \eta R_\alpha^3/\kappa_\alpha$, where η is the viscosity of water. For the parameter values cited above this yields a timescale $t_0 \approx 1.44 \cdot 10^{-4}\,\text{s}$. We thus expect the experimental time until fission to be approximately $350\,\text{ms}$. This is in good agreement with the experimentally observed typical time for fission, which is of order 1 s.

Conclusion. We have studied the behavior of a multiphase membrane tube using thermodynamic arguments. The shape of the junction between two domains depends on three quantities: the line tension of the interface and the jumps in the two elastic constants. While experimen-

tal precision is not yet at a level where these results can be compared directly to our calculated tube shapes, we have also considered the breaking time of a two-phase tube. Our modeling, based on an energetic approach, predicts a strong dependence of the fission dynamics on the elastic properties of the phases and yields results that are in good agreement with the experimental data.

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