Composition Variation and Underdamped Mechanics near Membrane Proteins and Coats

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(Dated: July 8, 2014)

We study the effect of membrane proteins on the shape, composition and thermodynamic stability of the surrounding membrane. When the coupling between membrane composition and curvature is strong enough the nearby composition and shape both undergo a transition from over-damped to under-damped spatial variation, well before the membrane becomes unstable in the bulk. This transition is associated with a change in the sign of the thermodynamic energy and hence has the unusual features that it can favour the early stages of coat assembly necessary for vesiculation (budding), while suppressing the activity of mechanosensitive membrane channels and transporters. Our results also suggest an approach to obtain physical parameters that are otherwise difficult to measure.

Biological membranes are crucial to the structure and function of living cells [1]. Transmembrane proteins that play important roles in transport, adhesion and signalling are embedded in membranes [2, 3] that themselves consist of a mixture of lipids and other amphipathic components. Several experimental studies have shown that the interaction with the adjacent lipid molecules can regulate the function of membrane proteins [4–7]. Here, we are primarily interested in the non-specific lipid-protein interactions that arise from the coupling of their hydrophobic regions [8–14], although we can also allow for selective enrichment of membrane component(s) near the protein. We employ a continuum theory in which small deformations of the lipid environment near a rigid inclusion can be described by a number of local field variables, such as the profile of the mid-plane of the bilayer, its composition and membrane thickness [15–34]. Furthermore, the free-energy cost associated with thickness deformation is completely decoupled at lowest order [19], and it can be independently analyzed although we do not do so here.

We allow for selective enrichment/depletion of curvature sensitive inclusions in the vicinity of a membrane protein or, equivalently, lipid asymmetry between leaflets that is characterized by a local spontaneous curvature [34–39], the preferred mean curvature in the absence of any mechanical stresses on the membrane [36]. This variation may be relatively large near a membrane protein if its geometry is such that it bends or otherwise deforms the surrounding membrane, see Fig 1. Our approach leads to a general, real-space description of the membrane around an inclusion of arbitrary (or no) symmetry (see SI for full details). It can also be used to treat chemical constraints corresponding, e.g. to the preferential binding of one component to the protein face.

We consider a two-component membrane in which the local compositional asymmetry between the different layers and/or the density of curvature-sensitive inclusions is phenomenologically coupled to the local mean curvature of the membrane [31, 32]. When the compositional variation is weak and the membrane displacement is small, the free-energy can be written as a Landau-Ginzburg expansion [31–33, 40, 41],

\[
\mathcal{F}_\varphi = \frac{1}{2} \int_\mathcal{M} \left[ a \varphi^2 + b (\nabla \varphi)^2 + 2 c \varphi (\nabla^2 u) \right] d^2 r, \quad (1)
\]
where only the lowest-order terms are retained and $a$, $b$ and $c$ are phenomenological constants. Here, the scalar fields $\varphi(\mathbf{r})$ and $u(\mathbf{r})$ represent the local composition difference and bilayer mid-plane height, respectively, see Fig 1. Both deformation fields are described within a Monge representation, which allows us to write the free-energy associated with mid-plane deformation as

$$ F_u = \frac{1}{2} \int_{\mathcal{M}} \left[ \sigma (\nabla u)^2 + \kappa (\nabla^2 u)^2 \right] d^2 \mathbf{r}, \quad (2) $$

where $\sigma$ and $\kappa$ are the surface tension and bending rigidity of the membrane, respectively [42]. Here, the Gaussian curvature is neglected, and the global spontaneous curvature (if any) can be absorbed into $\varphi$, generating only unimportant additive constants to the free energy.

We now seek the ground state of the membrane and neglect fluctuations throughout. The membrane configuration $u(\mathbf{r})$ and its compositional asymmetry field $\varphi(\mathbf{r})$ can then be computed exactly by minimizing the free-energy functional, $F = F_u + F_{\varphi}$, leading to the following Euler-Lagrange equations:

$$ \nabla^2 u = (\nabla^2 - \beta^2) \phi, \quad (3) $$

$$ \nabla^2 (\nabla^2 - \alpha^2) u + \gamma^2 \nabla^2 \phi = 0, \quad (4) $$

where $\phi(\mathbf{r}) = (b/c) \varphi(\mathbf{r})$ and the coefficients $\alpha = \sqrt{\sigma/\kappa}$, $\beta = \sqrt{a/b}$ and $\gamma = c/\sqrt{\kappa b}$ represent the relevant inverse length scales of the model. By combining (3) and (4), a single differential equation for $\phi(\mathbf{r})$ can be obtained:

$$ (\nabla^2 - k_-^2)(\nabla^2 - k_+^2) \phi = 0, \quad (5) $$

where the form of $k_{\pm}$ is given by

$$ k_{\pm} = \frac{1}{2} \left[ \sqrt{(\alpha + \beta)^2 - \gamma^2} \pm \sqrt{(\alpha - \beta)^2 - \gamma^2} \right]. \quad (6) $$

By separation of variables, a solution of equation (5) that vanishes in the far-field limit can be found to be

$$ \phi(r, \theta) = \phi_+(r, \theta) + \phi_-(r, \theta), \quad (7) $$

where $r$ and $\theta$ are the usual polar coordinates, as illustrated in Fig 1, and $\phi_{\pm}$ is defined by

$$ \phi_{\pm}(r, \theta) = \frac{k_+^2 - k_-^2}{2k_+^2 - \beta^2} \sum_{n=0}^{\infty} \mathcal{V}^\pm_n(\theta) K_n(k_{\pm} r), \quad (8) $$

where $K_n$ are the modified Bessel functions of the second kind of order $n$, and $\mathcal{V}^\pm_n(\theta) = \mathcal{A}_n^\pm \cos(n\theta) + \mathcal{B}_n^\pm \sin(n\theta)$, with $\mathcal{A}_n^\pm$ and $\mathcal{B}_n^\pm$ as arbitrary constants. This solution can be now used to obtain the membrane configuration through equation (3), which yields

$$ u(r, \theta) = u_+(r, \theta) + u_-(r, \theta) + u_h(r, \theta), \quad (9) $$

where solutions that diverge at infinity are excluded, and $u_h(r, \theta)$ is the homogeneous solution of (3), namely

$$ u_h(r, \theta) = \sum_{n=0}^{\infty} \mathcal{W}_n(\theta) r^{-n}, \quad (10) $$

The functions $\mathcal{V}^\pm_n(\theta)$ and $\mathcal{W}_n(\theta)$ are determined by the boundary conditions at the interface $\partial \mathcal{M}$, located at a distance $r_0$ from the symmetry axis, see Fig 1. These are specified by the height $U(\theta)$ and contact angle $U'(\theta)$ at which the mid-plane of the bilayer meets the inclusion. This choice of boundary conditions is motivated by assuming a strong coupling between the transmembrane domain of the inclusion and the membrane hydrophobic core. Also, the normal derivative of $\phi$ is chosen to vanish everywhere on $\partial \mathcal{M}$, which is used to obtain an unique solution [43] (see SI for details).

This methodology allows us to compute exactly the lowest order estimates to the membrane profile, its local
phase behavior, and the total deformation energy, given an arbitrary model for the shape of the rigid inclusion, through $U(\gamma)$ and $U'(\gamma)$, i.e., a general solution to the problem. First, we consider a simple illustrative example in which the height, $U(\gamma)$, is chosen to be a constant $z_0$, while the contact angle has a non-zero value only within an angular interval $\omega$, see Fig 2. This corresponds to a rigid inclusion that induces a local mid-plane deformation only within a specific region along its hydrophobic belt, with the remaining part preferring a flat membrane. The Connolly surface of a leucine transporter, LeuT, exhibits similar features [44, 45]. The height $z_0$ is not entirely arbitrary, being set by the overall balance of normal forces (see SI). Similarly, the condition of torque balance leads to a tilt of the inclusion (see SI for details), as also illustrated in Fig 1. Typical solutions due to such an asymmetrically-shaped inclusion that exerts no net torque are shown in Fig 2 for physiologically reasonable values of $\alpha$, $\beta$, and $\gamma$. The induced $\phi(r)$ shows a rich variation as $w$ is varied between 0 and $2\pi$, which correspond to membrane inclusions with a cylindrical and a conical shape, respectively [16–22].

To better understand the role of the coupling term $\gamma$ we consider symmetric, conical inclusions ($w = 2\pi$) in what follows, noting that the transition from over- to under-damped variation also appears for inclusions with other (or no) symmetry. Although $\gamma$ can have either sign we restrict our attention $\gamma > 0$ without loss of generality by redefining $\phi \to -\phi$ when $\gamma$ would otherwise be negative.

For values of $\gamma$ less than $\gamma_d = |\alpha - \beta|$, the distortion is found to be monotonically decreasing, see Fig 3(b). However, as $\gamma$ is increased above this point, the solutions show an underdamped behavior, with the membrane displacement decaying to zero for large distances. The magnitude of this amplitude becomes large as $\gamma$ approaches $\gamma_c = \alpha + \beta$, suggesting the presence of an instability. In fact $\gamma > \gamma_c$, where $k_\pm^2 < 0$ as shown in Fig 3(a), corresponds to Leibler’s criterion for curvature-induced instabilities in (bulk) membranes [31, 32]. The point $\gamma = \gamma_d$ instead corresponds to a critically damped system, separating the real and complex domain of $k_\pm$. The solutions are thermodynamically stable on either side of this boundary. When $\gamma_d > \gamma > \gamma_c$, the rate of decay $\lambda$ of the membrane undulations and its wave number $\omega$ can be obtained by approximating $K_n(\rho) \approx e^{-\rho} (\pi \rho / 2)^{-1/2}$ for $\rho \gg n$ [48] in Eq. (9) to obtain

$$u(r) \approx e^{-\lambda (r - r_0)} \frac{\sqrt{r/r_0}}{\sqrt{r/r_0}} \cos \left[ \omega (r - r_0) + \vartheta \right], \quad (12)$$

where $\vartheta$ is a phase angle that only depends on $\alpha$, $\beta$ and $\gamma$. Here, $\lambda$ and $\omega$ are given by the real and imaginary parts of (6), respectively.

We find that the wavelength of the pre-critical undulations diverges as we approach $\gamma = \gamma_d$, and the decay length diverges for $\gamma = \gamma_c$, which signals the presence of a bulk instability. While $\alpha$ can be measured through various experimental techniques [49–53], the parameters $\beta$ and $\gamma$ are more elusive [54]. Our analysis suggests a possible way to measure them, e.g., by tuning the system to lie near the instability threshold $\gamma_0 \lesssim \gamma_c$. Here the amplitude of the undulations are large and long-ranged and $\gamma$ and $\beta$ can be inferred by comparison with (9) or its approximation (12). This tuning might be achieved by controlling the surface tension, e.g., using a micropipette aspiration technique [49], so as to approach the critical tension $\sigma_c = \kappa (\gamma - \beta)^2$, although the presence of thermal fluctuations may mean that some averaging will be required to resolve the ground state, particularly far away from the inclusion.

Mechanosensitive membrane channels have been widely studied and reveal the interplay between the biological function of transmembrane proteins and the adjacent membrane structure and composition. Through
conformational changes from a closed to an open state that allows the passage of solvent through the membrane, they can equilibrate an osmotic imbalance between the interior and exterior of cells [55–57]. Although many examples of these channels are found in nature, the bacterial mechanosensitive channels of large (MscL) and small conductance (MscS) are prototypes of such proteins. Experimental studies have have shown that the channel opening probability is related to the membrane tension and the size of the open pore [46, 47, 58–61]. One possibility is that the channel simply dilates open at high tension but the transition between the closed and open states might also involve, e.g., a change in slope at the protein-membrane interface [62]. In a two-component membrane such a change in boundary conditions between the closed and open states couples to both the shape and asymmetry field in the nearby membrane and hence contributes to a change in the free energy of the channel-membrane system. Here, for simplicity, we take the angle at the channel wall to be non-zero in a conical closed state and zero in the open state. We explore the thermodynamic effect of this gating-by-tilt by comparing the deformation energy $\mathcal{F}$ of the membrane to the experimental estimate of the energy required to open the channels at zero tension, inferred by assuming purely dilational opening. Fig 4(a) shows that the even modest changes in the boundary angle at the face of the channel could give rise to a significant thermodynamic energy under gating-by-tilt. Moreover, an unexpected regime is identified in which the membrane can act to close, rather than open, the channel, characterized by a negative total energy $\mathcal{F}$ relative to the open state. Our results indicate that lipid composition variation, and its coupling to curvature, could play a role in regulating the function of membrane channels.

Finally, the presence of a regime of negative deformation energy when the distortion is underdamped motivated us to study the thermodynamics of protein coat formation on a membrane. Such coats are important in regulation, e.g., membrane trafficking using clathrin coats, or in infection, where viral coats assemble at the plasma membrane [1]. Fig 4(b) shows both the compositional variation near a coat of size $r_0 = 10 \, \text{nm}$ and the variation of the overall membrane energy $\mathcal{F}_c$ with coat radius $r_0$ (right). In both cases we assume a typical intrinsic coat curvature of $50 \, \text{nm}^{-1}$. The membrane energy can exhibit an initial decrease, driving coat assembly. A characteristic size can also appear corresponding to the minimum of this energy. In both figures (a) and (b) we use $\kappa = 20 \, k_B T$. See SI for details.

We acknowledge stimulating discussions with Dr P. Sens (Paris) and Prof M. Freissmuth (Vienna) and fund-
ing from EPSRC under grant EP/E501311/1 (a Leadership Fellowship to MST).

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