Non-topological saddle-splay and curvature instabilities from anisotropic membrane inclusions

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(April 17, 1996)

Anisotropic inclusions are shown to induce spontaneous deviatoric bendings in lipidic membranes, by orienting at right angles across the bilayer. In the limit of strong membrane curvatures, a non-analytical bending energy term is generated that favors saddle-like and cylindrical shapes, without penalizing spherical ones. An "egg-carton" instability results in flat membranes as well as a wormlike shape instability for vesicles.

02.40-k, 68.10.Et, 82.70-y, 87.10+e

Fluid lipid bilayers are model systems of biological membranes [1]. Pure surfactant bilayers are rather well understood in terms of local hydrophilic and hydrophobic interactions, stress profiles and geometrical considerations [1–3]. Recent studies focus on *heterogeneities* as means of controlling membrane elastic moduli. The effect of cosurfactants [4,5] and anchored polymers [6–8], the behavior of two component membranes [9,10] and polymerized bilayers [11–13] have been theoretically and experimentally studied. Besides, models involving "hat" and "saddle" defects [14] or antagonistic membrane components [10] have been proposed to tentatively explain an apparent submicroscopic roughness in some lipid bilayers [15]. Isotropic membrane inclusions can yield spontaneous curvature effects [16] and corrugations [17], however only when present with different densities on both sides of the bilayer. In this Letter, we consider the effect of *anisotropic* membrane inclusions. Possible candidates are the dimeric surfactants called "gemini" [18,19] whose polar heads are linked by a spacer. We show that their orientation should couple with the membrane principal curvatures, reducing the bending rigidity κ and increasing $\bar{\kappa}$. Stronger effects might be expected from dimers of detergents with large polar heads, or from asymmetric integral proteins [20]. In the limit of large curvatures, we show that anisotropic inclusions yield a *non-analytical* saddle-splay contribution proportional to the modulus of the deviatoric bending. Above an inclusion concentration threshold, this term induces an "egg carton" instability in flat membranes and a vesicle instability yielding long wormlike shapes.

Let us consider a fluid lipidic bilayer containing a density n of anisotropic inclusions. For the sake of simplicity, we assume that n is constant throughout the membrane and that the inclusions are equally distributed on both sides of the bilayer. In this case, isotropic inclusions would yield no effect by exact compensation [17]. We consider a smoothly deformed membrane given by the equation $u(x,y)$ of its mid-surface. The orientation of a single inclusion can be described by a unit vector $\vec{a}$ lying in the plane (x,y). Its energy depends on the local curvature of the bilayer. To lowest order in the curvature tensor $u_{ij} = \partial^2 u/(\partial x_i \partial x_j)$, the hamiltonian of the inclusion can be written as

$$h(u_{ij}; a_k) = h_0 + T_{ij}(a_k)\, u_{ij} + \mathcal{O}(u_{ij}^2)\,, \qquad (1)$$

with summation over repeated indices implied. This equation describes the inclusion contribution to the spontaneous curvature of the membrane. The tensor T_{ij} can be decomposed in a term proportional to the identity tensor δ_{ij} and a term proportional to $a_i a_j$. Calling c_1 and c_2 the eigenvalues of u_{ij} (i.e., the principal curvatures) and calling ϑ the orientation of $\vec{a}$ with respect to, say, the axis of curvature c_1, we are led to

$$h(c_1, c_2, \vartheta) = h_0 + \alpha(c_1 + c_2) + \omega(c_1 - c_2)\cos 2\vartheta\,, \quad (2)$$

in which we can choose $\omega > 0$ without loss of generality. Hence, anisotropic inclusions behave as quadrupoles in the membrane curvature field. They will be disoriented by thermal fluctuations when $|c_1 - c_2| \lesssim c^\star(T)$, with

$$c^\star(T) \sim \frac{T}{\omega}\,, \qquad (3)$$

otherwise, they will tend to orient along the most favorable axis of principal curvature. Let us estimate $c^\star$ for various types of inclusions. For small geminated surfactants of size comparable to the monolayer thickness, we expect $1/c^\star$ to be a molecular size. Larger inclusions might yield larger effects. Let us imagine for instance an integral protein with a conical section of angle $+\theta_0$ in the xz plane and angle $-\theta_0$ in the yz plane. The lipids are tilted around it over some relaxation length ξ. This tilt and the corresponding energy can be relaxed by curving the membrane. To estimate the corresponding ω, we shall consider curving the membrane cylindricallly in the xz plane (Fig. 1). Calling $\vec{m}$ the projection of the lipid orientation onto the membrane plane, the distorsion energy density reads [21]

$$f_d = \frac{1}{2} B_\perp\, \vec{m}^2 + \frac{1}{2} K \left(\nabla_\perp \cdot \vec{m} - c\right)^2\,. \qquad (4)$$

The second term arises from the splay of the lipids, which is coupled with the membrane bending elasticity. The

solution of the Euler-Lagrange equation associated with (4) is $m(s) = \theta \exp(-s/\xi)$, with s the curvilinear coordinate along the surface, θ the boundary tilt and $\xi = \sqrt{K/B_\perp}$. The resulting energy is $W = \frac{1}{2}\kappa\xi^{-1}\theta^2 - \kappa\theta c$ per unit length. With the boundary tilt given by $\theta(c) = \theta_0 - \frac{1}{2}Dc$ (the inclusion would fit perfectly in the membrane for $c = 2\theta_0/D$), we obtain $\omega = -D(\partial W/\partial c)|_{c=0}$, yielding after dividing by T

$$1/c^\star = \frac{K}{T}\theta_0 D\left(1 + \frac{D}{2\xi}\right). \tag{5}$$

With $K \sim \kappa \sim 20\,T$ for a phospholipid membrane, $D \sim 60\,\mathring{A}$, $\xi \sim 30\,\mathring{A}$ (a few lipid head sizes) and $\theta_0 \sim 45^\circ$, we obtain $1/c^\star \sim 2000\,\mathring{A}$. For large head detergent dimers, we might expect $1/c^\star \sim 300\,\mathring{A}$.

According to statistical mechanics, the free energy of a single inclusion derived from (2) is

$$\mu(c_1, c_2) = h_0 + \alpha(c_1 + c_2) - T\ln\int_0^{2\pi}\frac{d\vartheta}{\vartheta_0}\, e^{-\frac{\omega}{T}(c_1 - c_2)\cos 2\vartheta}, \tag{6}$$

where ϑ_0 is an irrelevant angle quantum. The integral in (6) is a Bessel function $2\pi\vartheta_0^{-1}\mathrm{I}_0[\omega(c_1 - c_2)/T]$. The total contribution of the inclusions to the membrane energy, $g(c_1, c_2)$, is given by $\frac{n}{2}\mu(c_1, c_2) + \frac{n}{2}\mu(-c_1, -c_2)$, since inclusions in opposite sides of the bilayer experience opposite curvatures. We obtain

$$g(c_1, c_2) = -nT\ln\,\mathrm{I}_0\left[\frac{c_1 - c_2}{c^\star(T)}\right], \tag{7}$$

in which we eliminated a constant contribution, namely $nh_0 - nT\ln(2\pi/\vartheta_0)$, that includes the rotational entropy of the inclusions. As expected in a symmetric bilayer, the term proportional to $(c_1 + c_2)$ disapears, however the contribution resulting from the anisotropy remains. A graph of g is shown in Fig. 2. The membrane bending energy is obtained by adding the contribution $g(c_1, c_2)$ to the Helfrich energy [22]

$$f(c_1, c_2) = \frac{1}{2}\kappa(c_1 + c_2)^2 + \bar{\kappa}c_1c_2\,, \tag{8}$$

where κ and $\bar{\kappa}$ are the bending modulus and the Gaussian modulus, respectively. We consider two limits:

a) $|c_1 - c_2| \lesssim c^\star$—If the deviatoric bending is smaller than the threshold $c^\star$, the inclusions contribution simply renormalizes the membrane bending moduli. From $g(c_1, c_2) \sim -\frac{1}{4}nT^{-1}\omega^2(c_1 - c_2)^2 + \mathcal{O}(4)$, we derive

$$\Delta\kappa = -\frac{n\omega^2}{2T} \tag{9a}$$

$$\Delta\bar{\kappa} = \frac{n\omega^2}{T}. \tag{9b}$$

Hence, the bending modulus κ is reduced, as is usually the case for cosurfactants [1]. Furthermore anisotropic inclusions favor *negative* Gaussian curvature (i.e., saddle shapes), as will be made clearer in the following.

b) $|c_1 - c_2| \gtrsim c^\star$—If the deviatoric bending is larger than $c^\star$, which is possible for large inclusions, g becomes approximatively

$$g(c_1, c_2) \sim nA - n\omega|c_1 - c_2|\,, \tag{10}$$

where $A = \frac{1}{2}T\ln[2\pi|c_1 - c_2|/c^\star]$ can be treated as a constant thanks to its weak logarithmic dependance. This linear behavior can be easily understood from a mean field picture. According to Eq. (2), the orientation of minimum energy for a single inclusion corresponds to $\vartheta = 0$ if $c_1 - c_2 < 0$ and to $\vartheta = \pi/2$ if $c_1 - c_2 > 0$. In both cases, the anisotropic energy contribution is exactly $\omega|c_1 - c_2|$. Since $c_1 - c_2$ changes sign from one monolayer of the membrane to the other, anisotropic inclusions will orient at right angles when the membrane is shaped as a saddle. This corresponds to the most favorable situation (Fig. 3). Conversely, if the membrane is shaped as a sphere, there is exact compensation on the two sides of the bilayer. Thus, in contrast with the usual Gaussian curvature term $\bar{\kappa}c_1c_2$, the presence of anisotropic inclusions provides an energy gain within the saddles that is *not* compensated by a corresponding energy loss in surrounding "hats". In other words, the Gauss-Bonet theorem does not apply to Eq. (10). This property was first understood by T. M. Fischer, who postulated by analogy with the spontaneous mean curvature a term proportional to $|c_1 - c_2|$ for pure membranes, but estimated it to be negligible [23,24].

Egg carton instability—The saddle-splay favored by anisotropic inclusions yields an instability. Let us compare the energy of a corrugated membrane,

$$u(x, y) = U\sin qx\ \sin qy\,, \tag{11}$$

with that of a flat one. Since the integral over the membrane of the Gaussian term $\bar{\kappa}c_1c_2$ is a topological invariant, the instability must arise from the non-analytical form (10). From the standard relations $c_1 + c_2 = \mathrm{Tr}\,u_{ij}$ and $c_1c_2 = \mathrm{Det}\,u_{ij}$, we infer $c_1 + c_2 = u_{xx} + u_{yy}$ and $|c_1 - c_2| = [(u_{xx} - u_{yy})^2 + 4u_{xy}^2]^{1/2}$. Hence, for the egg carton (11), $c_1 + c_2 = -2q^2U\sin qx\ \sin qy$ and $|c_1 - c_2| = 2q^2U|\cos qx\ \cos qy|$. Balancing the inclusion gain (10) in the saddles, of order $n\omega q^2U$, with the average bending cost $E_b = \frac{1}{2}\kappa(c_1 + c_2)^2$ of order κq^4U^2, we arrive at the instability criterion

$$\frac{n}{q^2U} \gtrsim \frac{\kappa}{\omega}. \tag{12}$$

Since the smallest acceptable value of q^2U that allows to use the linear form (10) is precisely $c^\star$, the egg carton instability occurs when the inclusion density overcomes $\kappa c^\star/\omega$, i.e., above the threshold

$$n_c \sim \frac{\kappa}{T}{c^\star}^2. \tag{13}$$

The curvature modulation is then of order $c^\star$; however a more complete calculation would be required to determine the corrugation's amplitude and wavevector. The inclusion coverage at threshold, $d^2\, n_c \sim (T/\kappa)\theta_0^{-2}[1 + D/(2\xi)]^{-2}$ according to our estimation of $c^\star$ for conical objects, can be very small for large inclusions with $\theta_0 \sim 1$.

Wormlike vesicle instability—The energy term (10) favors not only saddles but also *cylinders*, for which $|c_1 - c_2|$ is nonzero as well. This might cause an instability in the shape of vesicles, such as that recently reported in the presence of geraniol, a biological cosurfactant [25]. Let us compare the energy of a spherical vesicle $\mathcal{S}$, of radius R, with that of a cylindrical one $\mathcal{C}$, of radius r and length L, terminated by spherical end-caps. The constraint of constant membrane area implies $2\pi r L = 4\pi(R^2 - r^2)$. The two vesicles possess the same Gaussian energy and furthermore the bending energy $8\pi\kappa$ of $\mathcal{S}$ equals that of the end-caps of $\mathcal{C}$. Thus the energy excess ΔE of $\mathcal{C}$ with respect to $\mathcal{S}$ consists only in the energy of the cylindrical region of $\mathcal{C}$, namely

$$\Delta E(r) = 4\pi(R^2 - r^2)\left[\frac{\kappa}{2r^2} - nT \ln \mathrm{I}_0\left(\frac{1}{rc^\star}\right)\right] . \quad (14)$$

Typical graphs of $\Delta E(r)$ are shown in Fig. 4. The instability condition is $\Delta E < 0$ for $r \lesssim R$. In the limit of large vesicles $Rc^\star \gg 1$, expanding the Bessel function in power series yields $\Delta E \sim \pi(R^2 - r^2)[2\kappa - nT/c^{\star 2}](1/r^2)$. We thus obtain the same threshold as previously

$$n_c = 2\frac{\kappa}{T}c^{\star\, 2}. \quad (15)$$

For $n \lesssim n_c$, vesicles should exhibit strong shape fluctations. As soon as $n \gtrsim n_c$, they turn to cylinders with a small equilibrium radius r_e of order $1/c^\star$ (Fig. 4). To estimate it, we can set $r_e \ll R$ and use the asymptotic form (10) for the inclusions contribution. This gives $\Delta E \sim 4\pi R^2[\frac{1}{2}\kappa/r^2 - nTc^{\star -1}/r]$. Hence $r_e \sim \kappa c^\star/(nT)$, i.e.,

$$\frac{1}{r_e} \sim 2\frac{n}{n_c}c^\star . \quad (16)$$

This value is independent of R in the large vesicle limit. Long wormlike vesicles (Fig. 5) should behave as flexible polymers [25] and could undergo a "pearling" instability. Preliminary calculations show that such an instability is possible although not as favorable as the egg carton one in flat membranes. It would be also interesting to study whether an instability yielding *spikes* on the vesicle surface (like the blood cell echinocytosis) would not compete with the transformation to wormlike vesicles.

In summary, inclusions with an *anisotropic* spontaneous curvature could play an important role in the morphology of membranes. Whereas isotropic inclusions yield no spontaneous mean curvature when symmetrically absorbed in a bilayer, anisotropic inclusions yield a spontanous deviatoric bending $c_1 - c_2 \neq 0$ by orienting at right angles across the bilayer. This favors saddle-like or cylindrical membrane shapes. The effect of small anisotropic inclusions is mainly to reduce the bending modulus κ and to increase the Gaussian modulus $\bar{\kappa}$. Large inclusions, such as big dimeric detergents of proteins, yield in the limit of strong mesoscopic curvatures an energy term proportional to the *modulus* of the deviatoric bending. Such a non-analytical term favors saddles without penalizing spheres, contrary to the usual Gaussian curvature term. We showed that above an inclusion concentration threshold n_c (as small as the inclusions are large), flat membranes undergo an "egg-carton" instability with a mesoscopic roughness. This might be an alternative to the "hat model" to explain the roughness of real membranes [14]. Above n_c, spherical vesicles should transform to *wormlike* vesicles with a diameter also probably in the mesoscopic range. This might explain the recently observed "entangled tubular vesicle phase" [25], obtained in the presence of geraniol, a branched cosurfactant that could possibly yield anisotropic associations with the membrane lipids [26]. Extension of the present model should include inhomogenities in the distribution of the inclusions and the investigation of a spiked vesicle regime.

I wish to thank P. Galatola, C. Marques, D. Morse, P. Pincus and C. Safinya for useful discussions. This work was partially supported by the NSF under grant DMR-93-01199 and MRL Program under grant DMR-9123048, by the CNRS and a NATO fellowship.

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FIG. 1. Lipid tilt distorsion around a conical inclusion in a flat (right) or curved (left) membrane.

FIG. 2. Contribution of anisotropic inclusions to the bending energy of a membrane. For $|c_1 - c_2| \gtrsim c^\star$ the energy gain is almost linear.

FIG. 3. Anisotropic inclusions (arbitrary scale) in an membrane with deviatoric bending. The inclusions depicted are supposed to "prefer", besides a mean curvature of whatever sign, an excess of curvature around their short axis and a default of curvature around their long axis. By orienting at right angles across the bilayer, both inclusions can satisfy this differential preference. An additional mean curvature does not change the tendency of the inclusions to align.

FIG. 4. Energy excess of cylindrical vesicles of radius r with respect to spherical ones of radius R, for $n \sim 1.5\, n_c$. The minimum is obtained for $r_e \sim 1/c^\star$, independently of the vesicle total area.

FIG. 5. Wormlike vesicles induced by anisotropic inclusions.