Interaction of conical membrane inclusions: Effect of lateral tension

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Considering two rigid conical inclusions embedded in a membrane subject to lateral tension, we study the membrane-mediated interaction between these inclusions that originates from the hat-shaped membrane deformations associated with the cones. At nonvanishing lateral tensions, the interaction is found to depend on the orientation of the cones with respect to the membrane plane. The interaction of inclusions of equal orientation is repulsive at all distances between them, while the inclusions of opposite orientation repel each other at small separations, but attract each other at larger ones. Both the repulsive and attractive forces become stronger with increasing lateral tension. This is different from what has been predicted on the basis of the same static model for the case of vanishing lateral tension. Without tension, the inclusions repel each other at all distances independently of their relative orientation. We conclude that lateral tension may induce the aggregation of conical membrane inclusions. [S1063-651X(98)04706-0]

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I. INTRODUCTION

Biological membranes consist of a fluid lipid bilayer with embedded amphiphilic macromolecules such as integral proteins [1]. Integral proteins are expected to be much less flexible than the lipid matrix. In a general sense, any molecule embedded in the membrane and differing in shape or elastic properties from the surrounding lipid molecules can be viewed as an inclusion. The phase behavior of inclusions in the plane of the membrane is determined by interactions between them. If the interaction energy is sufficiently large to compete with translational entropy, it can lead to lateral self-assembly of the inclusions. Attractive forces may result in a lateral aggregation of the inclusions, while repulsion can give rise to a regular array with maximal spacing.

Forces between membrane inclusions can be divided into two classes. The first class consists of the well-known direct interactions, namely electrostatic (for charged inclusions) and van der Waals forces. The second class comprises indirect interactions mediated by some kind of membrane deformation [2–8]. These interactions are determined by the shapes of the inclusions and the elastic parameters of the inclusions and the lipid bilayer. They can be static or dynamic, in one case being due to equilibrium deformations and in the other to shape fluctuations of the membrane.

Both types of indirect interactions have been theoretically studied for the case of zero lateral tension. The static interactions of inclusions affecting the membrane thickness [3] and conical deformations affecting the membrane shape [2] have been dealt with. Dynamic interactions were treated for inclusions modifying the local bending moduli [2,4,6,7], including the case of rigid disks [2,5,6].

In the following we consider the static interaction between conical inclusions in the presence of lateral tension. Two sketches of a truncated cone embedded in the membrane are given in Figs. 1 and 2. The cone is assumed to be rigid and to impose a uniform slope on the surrounding membrane, which returns asymptotically to the flat state at large distances.

We find a repulsive interaction at all values of the lateral tension if the two conical inclusions have equal orientation with respect to the membrane plane. By increasing the lateral tension, the interaction is weakened at larger but enhanced at smaller inclusion distances. In contrast, for opposite orientations of the inclusions in a membrane with nonvanishing lateral tension the sign of the interaction depends on the distance between the inclusions. At small separations the inclusions repel each other, while at large separations the interaction is attractive. With rising tension the attractive potential well deepens and moves towards smaller distances between the inclusions.

II. SHAPE AND ENERGY OF MEMBRANES WITH CONICAL INCLUSIONS

We consider a membrane with two embedded conical inclusions. The cross sections of the inclusions in the midplane of the membrane are circles of radius a. The centers of the two circles are separated by the distance R (see Fig. 3).

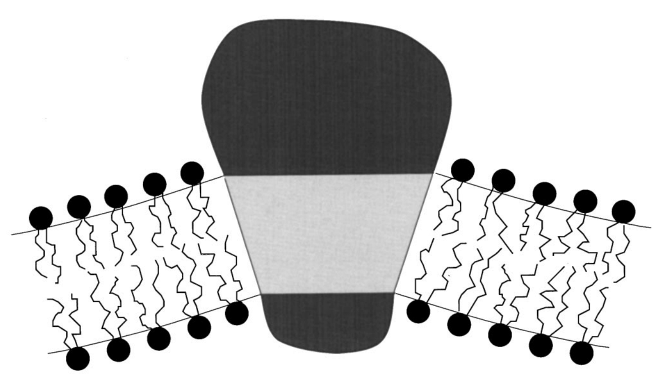

FIG. 1. Conical inclusion in a bilayer membrane.

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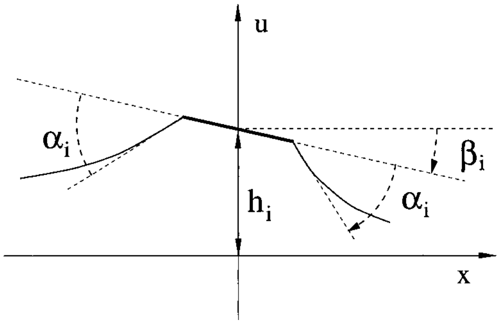

FIG. 2. Idealization of a conical inclusion as a rigid disk of height h_i and tilt angle β_i making a uniform contact angle α_i with the surrounding membrane. The cross section contains the axis of the cone.

In the absence of inclusions the membrane is assumed to be flat and to lie in the x-y plane of the Cartesian system of coordinates. We describe the membrane equilibrium shape produced by the inclusions by a function $u(x,y)$, which determines the displacement of the membrane from the x-y plane in the z direction (see Fig. 2).

At the boundaries of the conical inclusions the displacement u is assumed to fulfill the conditions (cf. [2])

$$u|_{r_i=a}=h_i+a\beta_i\cos\phi_i, \tag{1a}$$

$$\left.\frac{\partial u}{\partial r_i}\right|_{r_i=a}=\alpha_i+\beta_i\cos\phi_i,\quad i=1,2 \tag{1b}$$

where the subscript i takes the value 1 or 2 for the first and the second inclusion, respectively. By r_i and ϕ_i we denote the polar coordinates related to the center of projection E_i of the respective inclusion on the x-y plane (see Fig. 3). Equation (1a) describes the boundary of each inclusion as a circle of radius a whose center is at height h_i above the x-y plane and that is tilted with respect to the z axes by an angle β_i in the x direction. Equation (1b) takes into account that due to the conical shape of the inclusion the membrane is attached to the circumference of the tilted circle with a constant angle α_i. It is assumed in Eqs. (1) that the contact angle α_i and tilt angle β_i are small, $\alpha_i \ll 1$ and $\beta_i \ll 1$, so we set tan $\alpha_i=\alpha_i$ and tan $\beta_i=\beta_i$ and neglect contributions of the order of magnitude of β_i^2 determining the deviation of the inclusion projection from the circular shape. At large distances from the inclusions, $r_i \gg R$, the membrane remains flat, so $\nabla u \to 0$ for $r_i \to \infty$.

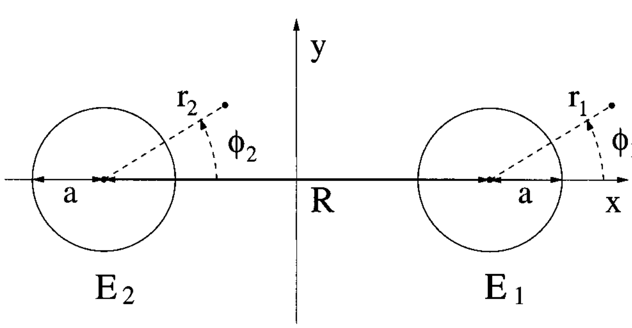

FIG. 3. x-y plane with inclusion projections E_1 and E_2.

The inclusions characterized by small α_i and β_i can produce only a weak deformation of the initially flat membrane, which means that the gradient of the function $u(x,y)$ remains small, $|\nabla u| \ll 1$, everywhere along the membrane. The membrane energy [9] can then be written in the approximate form

$$G=\int\left(\frac{\kappa}{2}(\Delta u)^2+\bar{\kappa}K+\frac{\gamma}{2}(\nabla u)^2\right)d^2r, \tag{2}$$

where κ denotes the bending rigidity, γ the lateral tension, K the Gaussian curvature, and $\bar{\kappa}$ the modulus of the Gaussian curvature. In our approximation, the Laplacian Δu equals the sum of the principal curvatures of the membrane J, while $\frac{1}{2}(\nabla u)^2$ gives the increase of membrane area per unit projected area due to membrane tilt ∇u. The integration of Eq. (2) is performed over the projected area. The membrane shape is determined by the Euler-Lagrange equation following from Eq. (2),

$$\Delta\Delta u=\frac{\gamma}{\kappa}\Delta u. \tag{3}$$

We derive the interaction energy of two conical inclusions in two steps. First, we solve the shape equation (3) accounting for the boundary conditions (1) and the asymptotic boundary condition $\nabla u \to 0$ for $r_i \to \infty$. Second, inserting the obtained function $u(x,y)$ into Eq. (2), we determine the membrane energy. Throughout this calculation we assume the inclusion distance R to be large compared to the radius of inclusion a and retain only the leading terms in a/R.

It is important to note that the interaction energy cannot depend on the modulus of Gaussian curvature $\bar{\kappa}$. According to the theorem of Gauss and Bonnet, an integral of the Gaussian curvature K over a surface is equal to the negative sum of the line integrals of the geodetic curvature k_g over the surface boundaries (apart from a constant that depends only on the genus of the surface). The value of the geodetic curvature at the inclusion boundaries is completely determined by the radius a and contact angles α_i ($|k_g|=1/a$ cos α_i) and does not depend on the distance R between the inclusions. Therefore, the integral of the Gaussian curvature K over the membrane must be independent of the distance R and does not contribute to the interaction potential.

For any given distance R between the inclusions the energy has to be minimized with respect to the heights h_i and tilt angles β_i. This results in conditions of zero vertical force and zero torque acting on each inclusion. The two conditions are expressed by the equations (see Appendix A)

$$\int_0^{2\pi}\left[a\frac{\partial}{\partial r_i}(\gamma u-\kappa\Delta u)\right]_{r_i=a}d\phi_i=0,$$
$$\int_0^{2\pi}\cos\phi_i\left[a^2\frac{\partial}{\partial r_i}(\gamma u-\kappa\Delta u)+\kappa a\Delta u\right]_{r_i=a}d\phi_i=0, \tag{4}$$

respectively. The integration in Eqs. (4) is performed over the boundary of each inclusion.

III. INTERACTION IN THE ABSENCE OF LATERAL TENSION

We first consider the important limiting case of zero lateral tension $\gamma=0$. The shape equation (3) then reads

$$\Delta\Delta u=0. \tag{5}$$

To solve this equation satisfying the boundary conditions (1) and the conditions of equilibrium (4) we use the following ansatz. We consider the function $u(x,y)$ describing the shape of the membrane in the form

$$u=u_1(r_1,\phi_1)+u_2(r_2,\phi_2), \tag{6}$$

where r_i,ϕ_i denote polar coordinates with respect to the center of inclusion i. The relationships between the polar coordinates related to the first and the second inclusion are (see Fig. 3)

$$r_1=\sqrt{R^2+r_2^2-2Rr_2\cos\phi_2}, \tag{7}$$

$$\cos\phi_1=\frac{r_2\cos\phi_2-R}{\sqrt{R^2+r_2^2-2Rr_2\cos\phi_2}}. \tag{8}$$

The functions u_1 and u_2 in Eq. (6) are general solutions of the shape equation (5) in polar coordinates. They are obtained from Eq. (B2) derived in Appendix B and have the form

$$\begin{aligned}u_i(r_i,\phi_i)=&\text{const}+c_0^{(i)}\ln r_i+c_1^{(i)}r_i\cos\phi_i\\&+c_2^{(i)}r_i\ln r_i\cos\phi_i+c_3^{(i)}\frac{\cos\phi_i}{r_i}\\&+c_4^{(i)}\cos 2\phi_i+c_5^{(i)}\frac{\cos 2\phi_i}{r_i^2}+\cdots\\&+c_{2n}^{(i)}\frac{\cos n\phi_i}{r_i^{n-2}}+c_{2n+1}^{(i)}\frac{\cos n\phi_i}{r_i^n}+\cdots.\end{aligned} \tag{9}$$

Terms of Eq. (B2) proportional to $\sin n\phi$ are omitted in Eq. (9) because of the mirror symmetry of the system with respect to the x-z plane (see Fig. 3) and terms exhibiting higher than logarithmic divergence for $r_i\rightarrow\infty$ are left out since they violate the boundary condition of an asymptotically flat membrane $\nabla u\rightarrow 0$ for $r_i\rightarrow\infty$. The only exceptions are $r_i\ln r_i\cos\phi_i$ and $r_i\cos\phi_i$. From the boundary condition of an asymptotically flat membrane it can be concluded immediately that the coefficient $c_2^{(1)}$ must be equal to $-c_2^{(2)}$. The sum of the corresponding terms then diverges only logarithmically for $r_i\rightarrow\infty$. The terms $r_i\cos\phi_i$ are proportional to the Cartesian coordinate x and thus describe rotations of the membrane as a whole [10]. Any such rotations must be equal but opposite, i.e., $c_1^{(1)}=-c_1^{(2)}$, to satisfy the boundary conditions at infinity, so that we can drop these terms as well.

The coefficients $c_j^{(i)}$ in Eq. (9) are determined from the boundary conditions (1) and equilibrium conditions (4). Consider these conditions at the circumference of inclusion 2. To apply them we have to express the membrane shape (6) in the vicinity of the inclusion. The function u_2 is simply given by Eq. (9) with $i=2$. To present the function u_1 in a convenient form we take Eq. (9) with $i=1$ and insert Eqs. (7) and (8) into it. In the vicinity of the second inclusion the value of r_2 is close to the inclusion radius $r_2\approx a$. Using the assumption $a\ll R$ and, consequently, $r_2\ll R$ we perform a Taylor expansion about the center of the inclusion projection E_2,

$$\begin{aligned}u_1|_{r_2\ll R}=&\text{const}+c_0^{(1)}\left(\ln R-\frac{r_2}{R}\cos\phi_2-\frac{r_2^2}{2R^2}\cos 2\phi_2\right)\\&-c_3^{(1)}\left(\frac{1}{R}+\frac{r_2}{R^2}\cos\phi_2\right)\\&+c_2^{(1)}\bigg(-R\ln R+(1+\ln R)r_2\cos\phi_2\\&-\frac{r_2^2}{2R}+\frac{r_2^3}{12R^2}(\cos 3\phi_2-3\cos\phi_2)\bigg)\\&+c_4^{(1)}\left(1-\frac{r_2^2}{R^2}(1-\cos 2\phi_2)\right)+\frac{c_5^{(1)}}{R^2}\\&-c_6^{(1)}\left(\frac{1}{R}+\frac{r_2}{R^2}\cos\phi_2\right)+\frac{c_8^{(1)}}{R^2}+O\left(\frac{r_2^3}{R^3}\right).\end{aligned} \tag{10}$$

The resulting expression for the membrane shape $u=u_1+u_2$ is

$$\begin{aligned}u|_{r_2\ll R}=&f_0^{(2)}(r_2)+f_1^{(2)}(r_2)\cos\phi_2+f_2^{(2)}(r_2)\cos 2\phi_2\\&+f_3^{(2)}(r_2)\cos 3\phi_2+\cdots,\end{aligned} \tag{11}$$

where

$$f_0^{(2)}=\text{const}+c_0^{(2)}\ln r_2-r_2^2\left(\frac{c_2^{(1)}}{2R}+\frac{c_4^{(1)}}{R^2}\right),$$

$$\begin{aligned}f_1^{(2)}=&c_2^{(2)}r_2\ln r_2+\frac{c_3^{(2)}}{r_2}+r_2\bigg[-\frac{c_0^{(1)}}{R}+c_2^{(1)}\left(1+\ln R-\frac{r_2^2}{4R^2}\right)\\&-\frac{c_3^{(1)}+c_6^{(1)}}{R^2}\bigg],\end{aligned} \tag{12}$$

$$f_2^{(2)}=c_4^{(2)}+\frac{c_5^{(2)}}{r_2^2}+(-c_0^{(1)}+2c_4^{(1)})\frac{r_2^2}{2R^2},$$

$$f_3^{(2)}=\frac{c_6^{(2)}}{r_2}+\frac{c_7^{(2)}}{r_2^3}+\frac{c_2^{(1)}r_2^3}{12R^2}.$$

Inserting Eq. (11) into the boundary conditions (1) and equilibrium conditions (4) at the inclusion 2 we obtain a series of equations for the coefficients $c_j^{(i)}$. To account for the boundary and equilibrium conditions at the inclusion 1, we perform the same procedure as described above to obtain identical equations in which the index 2 is replaced by 1 and vice versa.

The equations obtained for the coefficients $c_j^{(i)}$ can be solved order by order in the small parameter a/R. The solutions are

$$c_0^{(1)}=\alpha_1 a+O\left(\frac{1}{R^3}\right),\quad c_4^{(1)}=\frac{\alpha_2 a^3}{R^2}+O\left(\frac{1}{R^3}\right),$$

$$c_5^{(1)}=-\frac{1}{2}\frac{\alpha_2 a^5}{R^2}+O\left(\frac{1}{R^3}\right), \tag{13}$$

and equivalent results for $c_j^{(2)}$, the remaining coefficients being of third or higher order in a/R.

We are now in a position to compute the energy of the membrane. Omitting the contribution of the integral of the Gaussian curvature, which is independent of the distance R between the inclusions (see above), we obtain from Eq. (2)

$$G(R)=\int\frac{\kappa}{2}J^2d^2r, \tag{14}$$

where the curvature J is given by

$$J=\Delta u=-4c_4^{(1)}\frac{\cos 2\phi_1}{r_1^2}-4c_4^{(2)}\frac{\cos 2\phi_2}{r_2^2}+\cdots. \tag{15}$$

In the first nonvanishing order in a/R, the energy of interaction of the inclusions is

$$\boxed{G(R)=4\pi\kappa(\alpha_1^2+\alpha_2^2)\frac{a^4}{R^4}+O\left(\frac{1}{R^5}\right).} \tag{16}$$

According to Eq. (16), the energy is positive and decays monotonically at all values of the contact angles α_1,α_2 and all distances between the inclusions R. This means that in the case of zero lateral tension the interaction between the rigid conical inclusions is always repulsive. The result (16) is in agreement with an earlier calculation [2] which in addition predicts a contribution proportional to $\bar{\kappa}$, the modulus of Gaussian curvature. We think that there should be no $\bar{\kappa}$ term (see the end of Sec. II).

IV. INTERACTION IN THE PRESENCE OF LATERAL TENSION

We now extend the methods of the preceding section to analyze the interactions of inclusions embedded in a membrane subject to nonvanishing lateral tension γ. The shape equation (3) can be written as

$$\Delta\Delta u=\xi^2\Delta u, \tag{17}$$

where $\xi=\sqrt{\gamma/\kappa}$ has the dimension of a reciprocal length. To find a solution of the shape equation satisfying the boundary conditions (1) and equilibrium conditions (4) we use, as in the Sec. III, the ansatz (6) with the functions $u_i(r_i,\phi_i)$ being general solutions of the shape equation (17) in polar coordinates. These functions are taken from Eq. (B3) derived in Appendix B and have the form

$$u_i=\text{const}+c_0^{(i)}K_0(\xi r_i)+c_1^{(i)}r_i\cos\phi_i+c_2^{(i)}K_1(\xi r_i)\cos\phi_i$$
$$+c_3^{(i)}\frac{\cos\phi_i}{r_i}+c_4^{(i)}K_2(\xi r_i)\cos 2\phi_i+c_5^{(i)}\frac{\cos 2\phi_i}{r_i^2}+\cdots$$
$$+c_{2n}^{(i)}K_n(\xi r_i)\cos n\phi_i+c_{2n+1}^{(i)}\frac{\cos n\phi_i}{r_i^n}+\cdots. \tag{18}$$

The coefficients of all terms of Eq. (B3) proportional to $\sin n\phi$ are taken to be equal to zero because of the symmetry of the system. Also, the coefficients of terms violating the boundary condition of asymptotically vanishing gradient of the displacement $\nabla u\to 0$ for $r_i\to\infty$ must be zero. As we do not consider rotations of the system we set $c_1^{(i)}=0$. In addition, we omit in Eq. (18) the logarithmic term of Eq. (B3), as the related change of the area of the membrane would result in an infinite energy of the lateral tension γ.

Equation (18) transforms into Eq. (9) in the limit of vanishing lateral tension $\gamma\to 0$ (i.e., $\xi\to 0$). This can be shown by inserting into Eq. (18) the approximative expressions of the Bessel functions $K_n(x)$ for small arguments x,

$$K_0(x)\approx -\ln x,\quad K_1(x)\approx\frac{1}{x},\quad K_n(x)\approx\frac{(n-1)!}{2}\left(\frac{2}{x}\right)^n$$
$$-\frac{(n-2)!}{2}\left(\frac{2}{x}\right)^{n-2}\quad\text{for } n\geq 2. \tag{19}$$

The coefficients $c_j^{(i)}$ in Eq. (18) are determined by the boundary conditions (1) and equilibrium conditions (4) in a way similar to that described in Sec. III. For example, we present u_1 in the vicinity of the inclusion 2 by inserting Eqs. (7) and (8) and obtain after an expansion in the small parameter r_2/R,

$$u_1|_{r_2 \ll R} = c_0^{(1)}\left(K_0(\xi R) + \xi K_1(\xi R) r_2 \cos\phi_2 + \frac{1}{4}\xi^2 r_2^2 [K_0(\xi R) + K_2(\xi R)\cos 2\phi_2] \right)$$
$$+ c_2^{(1)}\left(-K_1(\xi R) - \frac{1}{2}\xi[K_0(\xi R)+K_2(\xi R)] r_2 \cos\phi_2 - \frac{1}{8}\xi^2 [K_1(\xi R)+K_3(\xi R)] r_2^2 \cos 2\phi_2 - \frac{1}{4}\xi^2 K_1(\xi R) r_2^2 \right)$$
$$+ c_4^{(1)}\left(K_2(\xi R) + \frac{1}{2}\xi[K_1(\xi R)+K_3(\xi R)] r_2 \cos\phi_2 + \frac{1}{8}\xi^2 [K_0(\xi R)+K_4(\xi R)] r_2^2 \cos 2\phi_2 + \frac{1}{4}\xi^2 K_2(\xi R) r_2^2 \right)$$
$$- c_3^{(1)}\left(\frac{1}{R} + \frac{r_2}{R^2}\cos\phi_2 \right) + \frac{c_5^{(1)}}{R^2} + \cdots . \qquad (20)$$

We have to stress that in this case the expansions up to the second order in r_2/R are sufficient only if $\xi a < 1$. For $\xi a \gg 1$, which is equivalent to the condition of strong lateral tension, the series (20) converges too slowly to be approximated by the sum of just a few Taylor terms. This can be seen from the asymptotic expansion of the functions $K_n(x)$, which for large arguments are proportional to $\exp(-x)/\sqrt{x}$ irrespectively of n.

Inserting the sum (18) with $i=2$ and Eq. (20) into the boundary conditions (1) and the equilibrium conditions (4) at inclusion 2, we obtain for $a \ll R$ and $\xi a < 1$ a set of linear equations for the coefficients $c_j^{(i)}$. Applying the same procedure to satisfy the boundary and equilibrium conditions at the inclusion 1 leads to an analogous set of equations. Solving all these equations for $a \ll R$ and $\xi a < 1$ we obtain

$$c_0^{(1)} = -\alpha_1 a + \cdots, \quad c_2^{(1)} = -\frac{1}{2}\alpha_2 a (\xi a)^2 K_1(\xi R) + \cdots, \qquad (21)$$
$$c_4^{(1)} = -\alpha_2 a (\xi a)^2 K_2(\xi R) + \cdots$$

and corresponding results for $c_0^{(2)}$, $c_2^{(2)}$, and $c_4^{(2)}$. The coefficients $c_3^{(i)}$ and $c_5^{(i)}$ are given by the relations

$$c_3^{(i)} = -\frac{1}{2} c_2^{(i)} \xi a^2 K_2(\xi a), \quad c_5^{(i)} = -\frac{1}{4} c_4^{(i)} \xi a^3 K_3(\xi a). \qquad (22)$$

The interaction energy $G(R)$ of the inclusions is obtained by integration of $\frac{1}{2}\kappa(\Delta u)^2$ over the x-y plane. Transforming the area integrals into line integrals over the boundaries of the inclusions as shown in Appendix C, we find the dominant terms for small a/R and $\xi a < 1$,

$$\boxed{G(R) = 2\pi\kappa\alpha_1\alpha_2(\xi a)^2 K_0(\xi R) + \pi\kappa(\alpha_1^2+\alpha_2^2)(\xi a)^4 K_2^2(\xi R) + \cdots .} \qquad (23)$$

In the limit of vanishing tension $\gamma \to 0$ (i.e., $\xi \to 0$), this expression for the interaction energy coincides with Eq. (16), as can be seen by expanding the Bessel functions for small arguments according to Eqs. (19).

The interaction energy (23) depends on the relative orientation of the conical inclusions. If the cones are oriented in the same direction, their contact angles α_1, α_2 have the same sign. In this case the energy of interaction is always positive and decreases with increasing distance R. The repulsive potential is illustrated in Fig. 4 for two identical and equally oriented inclusions at different lateral tensions. By increasing the tension the interaction is weakened at large, but enhanced at small inclusion distances.

If the inclusions are oppositely oriented, the contact angles α_1 and α_2 have different signs and the energy of interaction behaves nonmonotonically, as illustrated in Fig. 5. The energy has a minimum at a finite separation R^* of the inclusions. This means that the forces between the inclusions change from repulsive to attractive depending on the distance R. For distances shorter than R^* the inclusions repel each other, while for separations $R > R^*$ the interaction is attractive. With rising lateral tension the separation of zero force R^* moves towards smaller values and the associated potential well deepens (see Fig. 5).

V. CONCLUSION

To summarize, we derived an interaction energy between two conical inclusions embedded in a fluid membrane sub-

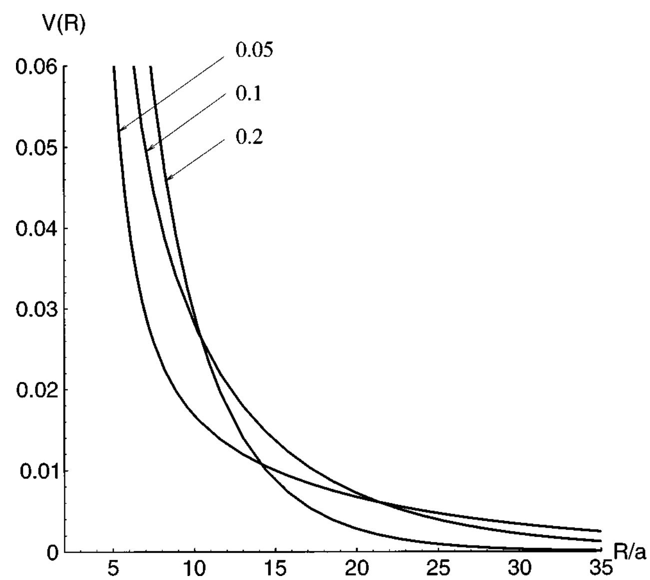

FIG. 4. Dimensionless interaction potential $V(R) = G(R)/\alpha_1^2\kappa$ of two equally oriented inclusions ($\alpha_1 = \alpha_2$) as a function of the dimensionless distance R/a for $\xi a = 0.05, 0.1, 0.2$.

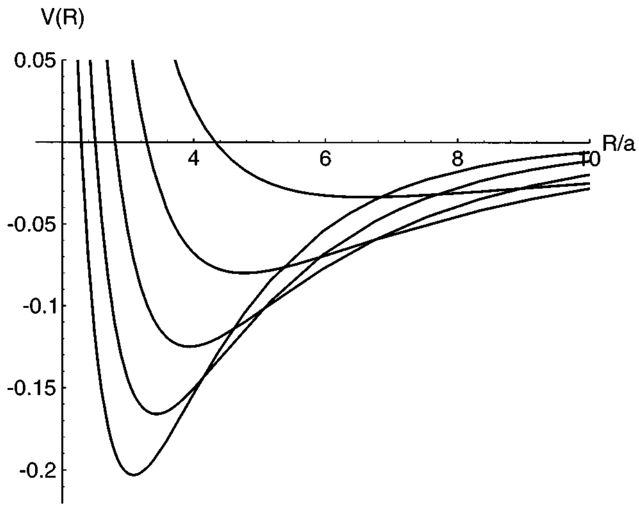

FIG. 5. Dimensionless interaction potential $V(R)=G(R)/\alpha_1^2\kappa$ of two oppositely oriented inclusions ($\alpha_1=-\alpha_2$) as a function of the dimensionless distance R/a. The potential well deepens with increasing $\xi a=0.1,0.2,0.3,0.4,0.5$. Note that $R/a=2$ means two disks in contact. Near this value the results can only be regarded as estimates.

ject to lateral tension. For this purpose, we calculated the equilibrium shape of an almost flat membrane and its bending energy in the presence of inclusions as a function of their distance. In contrast to the case of vanishing tension, this interaction depends on the orientations of the inclusions with respect to the membrane plane. For oppositely oriented inclusions the interaction changes from repulsive to attractive as the separation increases, while equally oriented inclusions repel each other at all distances. This is very different from the case of vanishing lateral tension where the interaction of conical inclusions is always repulsive, independently of relative orientation.

We did not consider in this study the contribution of thermal undulations of the membrane to the interaction between the inclusions [2,5,6]. In the case of nonvanishing lateral tension this may be partially justified by the fact that the undulations are diminished by the tension. Moreover, others have found for the case of zero tension that the static part of the interaction exceeds the dynamic one for $\kappa(\alpha_1^2+\alpha_2^2)>3kT$ [2] or $1.5kT$ [5,6] where k is Boltzmann's constant and T is temperature. If the tension-induced forces dominate, they should lead to interesting phase behavior of embedded inclusions. For example, the attractive interaction between pairs of oppositely oriented conical inclusions may favor the formation of clusters with a regular structure where inclusions with different signs of the contact angles alternate. For an estimate of the attractive interaction one may use Fig. 5. With $\kappa=1\times10^{-19}$ J (typical of lipid bilayers), $\alpha=0.5$ (26.8°), and $\xi a=0.4$, the minimum of the interaction energy $G(R)=V(R)\alpha_1^2\kappa$ is roughly -4×10^{-21} J ($\approx kT$ at room temperature). Because of $\xi=\sqrt{\gamma/\kappa}$, the lateral tension needed to produce $\xi a=0.4$ is given by $\gamma=0.16\kappa/a^2$. For $a=4$ nm and $\kappa=1\times10^{-19}$ J, one finds $\gamma=1$ mN/m, which is below the known tension of lipid bilayer rupture [11].

While our results are intuitively appealing and may be obtainable more directly, we performed a complete perturbation calculation to make sure that no terms are missed. The boundary and equilibrium conditions for the membrane with conical inclusions are central to our calculations. They resulted in a set of linear equations for the coefficients of two superimposed expansions, one for either inclusion. This method is extendable to a larger number of inclusions by using similar sets of boundary conditions. In computer-aided calculations the shape of a membrane could be determined with any desired precision and for any number of inclusions.

APPENDIX A: DERIVATION OF THE EQUILIBRIUM CONDITIONS (4)

To derive the equilibrium conditions (4) we study a variation

$$v(r,\phi,\epsilon)=u(r,\phi)+\epsilon\,\delta u(r,\phi) \tag{A1}$$

of the equilibrium membrane displacement $u(r,\phi)$ on a circular ring S: $a\leqslant r\leqslant b$, $0\leqslant\phi\leqslant 2\pi$ around a conical inclusion. To simplify the notation we leave out indices of the polar coordinates r,ϕ. The variation is restricted by the boundary conditions (1) of the inclusion. So

$$\delta u|_{r=a}=\delta c+\delta\beta\; a\,\cos\,\phi, \tag{A2}$$

$$\left.\frac{\partial\delta u}{\partial r}\right|_{r=a}=\delta\beta\,\cos\,\phi, \tag{A3}$$

where $\epsilon\delta c$ and $\epsilon\delta\beta$ denote the changes of the height of the inclusion center and the tilt angle, respectively. At $r=b$ we set

$$\delta u|_{r=b}=\left.\frac{\partial\delta u}{\partial r}\right|_{r=b}=0. \tag{A4}$$

Omitting Gaussian curvature, the membrane energy (2) can be written as

$$\begin{aligned}G&=\int_S\left(\frac{\kappa}{2}(\Delta v)^2+\frac{\gamma}{2}(\nabla v)^2\right)d^2r=\int_0^{2\pi}\int_a^b\left\{\frac{\kappa}{2}\left(\frac{\partial^2 v}{\partial r^2}+\frac{1}{r}\frac{\partial v}{\partial r}\right.\right.\\&\quad\left.\left.+\frac{1}{r^2}\frac{\partial^2 v}{\partial\phi^2}\right)^2+\frac{\gamma}{2}\left[\left(\frac{\partial v}{\partial r}\right)^2+\frac{1}{r^2}\left(\frac{\partial v}{\partial\phi}\right)^2\right]\right\}r\,dr\,d\phi\\&=\int_0^{2\pi}\int_a^b f(v,v_r,v_\phi,v_{rr},v_{\phi\phi},r)dr\,d\phi.\end{aligned} \tag{A5}$$

In equilibrium the energy G is minimal. So

$$\begin{aligned}\left.\frac{dG}{d\epsilon}\right|_{\epsilon=0}&=\int_0^{2\pi}\int_a^b\left(\frac{\partial f}{\partial v}\frac{dv}{d\epsilon}+\frac{\partial f}{\partial v_r}\frac{dv_r}{d\epsilon}+\frac{\partial f}{\partial v_\phi}\frac{dv_\phi}{d\epsilon}\right.\\&\quad\left.+\frac{\partial f}{\partial v_{rr}}\frac{dv_{rr}}{d\epsilon}+\frac{\partial f}{\partial v_{\phi\phi}}\frac{dv_{\phi\phi}}{d\epsilon}\right)_{\epsilon=0}dr\,d\phi=0.\end{aligned} \tag{A6}$$

By partial integrations we obtain

$$\frac{dG}{d\epsilon}=\int_0^{2\pi}\int_a^b\left(\frac{\partial f}{\partial v}-\frac{\partial}{\partial r}\frac{\partial f}{\partial v_r}-\frac{\partial}{\partial\phi}\frac{\partial f}{\partial v_\phi}+\frac{\partial^2}{\partial r^2}\frac{\partial f}{\partial v_{rr}}+\frac{\partial^2}{\partial\phi^2}\frac{\partial f}{\partial v_{\phi\phi}}\right)\frac{dv}{d\epsilon}dr\ d\phi+\int_0^{2\pi}\left[\frac{\partial f}{\partial v_r}\frac{dv}{d\epsilon}-\left(\frac{\partial}{\partial r}\frac{\partial f}{\partial v_{rr}}\right)\frac{dv}{d\epsilon}+\frac{\partial f}{\partial v_{rr}}\frac{dv_r}{d\epsilon}\right]_a^b d\phi \tag{A7}$$

and, inserting $f(v,v_r,v_\phi,v_{rr},v_{\phi\phi},r)$ as defined in Eq. (A5), are led to

$$\frac{dG}{d\epsilon}=\int_0^{2\pi}\int_a^b[\kappa\Delta\Delta v-\gamma\Delta v]\frac{dv}{d\epsilon}r\ dr\ d\phi+\int_0^{2\pi}\left[r\frac{\partial}{\partial r}(\gamma v-\kappa\Delta v)\frac{dv}{d\epsilon}+\kappa r\Delta v\frac{dv_r}{d\epsilon}\right]_a^b d\phi. \tag{A8}$$

The equilibrium displacement $u(r,\phi)$ fulfills the shape equation (3) of a tense membrane. So the integrand of the area integral in Eq. (A8) is zero at $\epsilon=0$. Taking into account Eqs. (A2) and (A3), we conclude that

$$\delta G=\left.\frac{dG}{d\epsilon}\right|_{\epsilon=0}=\int_0^{2\pi}\left[r\frac{\partial}{\partial r}(\gamma u-\kappa\Delta u)(\delta c+\delta\beta\ a\ \cos\ \phi)+\kappa r\Delta u\,\delta\beta\ \cos\ \phi)\right]_{r=a}d\phi=0. \tag{A9}$$

Since $\delta\beta$ and δc are independent of each other, we arrive at the equations

$$\delta G(\delta c)=\delta c\int_0^{2\pi}\left[a\frac{\partial}{\partial r}(\gamma u-\kappa\Delta u)\right]_{r=a}d\phi=0, \tag{A10}$$

$$\delta G(\delta\beta)=\delta\beta\int_0^{2\pi}\cos\ \phi\left[a^2\frac{\partial}{\partial r}(\gamma u-\kappa\Delta u)+\kappa a\Delta u\right]_{r=a}d\phi=0, \tag{A11}$$

which state that the vertical force and the torque, respectively, acting on the inclusion must be zero in equilibrium.

APPENDIX B: GENERAL SOLUTIONS OF THE SHAPE EQUATIONS IN POLAR COORDINATES

In this appendix we derive the general solution of the shape equation $\Delta\Delta u=\xi^2\Delta u$ in polar coordinates. We perform the calculation in two steps. We first look for the solution $J(r,\phi)$ of an intermediate equation $\Delta J=\xi^2 J$ and then solve the equation $\Delta u=J(r,\phi)$. General solutions of the latter equation are also general solutions of the shape equations. Below we consider separately the case of $\xi=0$, corresponding to the vanishing lateral tension $\gamma=0$ and the case of nonvanishing ξ.

1. Vanishing lateral tension $\xi=0$

In this case the shape equation has the form $\Delta\Delta u=0$. A solution of the intermediate Laplace equation $\Delta J=0$ on a circular ring can be found by the method of separation of variables and reads [12,13]

$$J(r,\phi)=a_0+b_0\ \ln\ r+\sum_{n=1}^{\infty}(a_n\ \cos\ n\phi+b_n\ \sin\ n\phi)r^{-n}+\sum_{n=1}^{\infty}(c_n\ \cos\ n\phi+d_n\ \sin\ n\phi)r^n. \tag{B1}$$

The general solution of the linear inhomogeneous equation $\Delta u=J(r,\phi)$ is the sum of a special solution and the general solution of the homogeneous equation $\Delta u=0$, the latter having the form of Eq. (B1). We obtain

$$u(r,\phi)=A_0r^2+B_0r^2(\ln\ r-1)+(A_1\ \cos\ \phi+B_1\ \sin\ \phi)r\ \ln\ r+\sum_{n=2}^{\infty}(A_n\ \cos\ n\phi+B_n\ \sin\ n\phi)r^{-n+2}+\sum_{n=1}^{\infty}(C_n\ \cos\ n\phi+D_n\ \sin\ n\phi)r^{n+2}+\overline{A_0}+\overline{B_0}\ \ln\ r+\sum_{n=1}^{\infty}(\overline{A_n}\ \cos\ n\phi+\overline{B_n}\ \sin\ n\phi)r^{-n}+\sum_{n=1}^{\infty}(\overline{C_n}\ \cos\ n\phi+\overline{D_n}\ \sin\ n\phi)r^n. \tag{B2}$$

The terms with unbarred coefficients belong to the special solution, which can be directly checked by its insertion into $\Delta u=J$. The A_0 term of Eq. (B2) corresponds to the a_0 term of Eq. (B1), etc. The terms with barred coefficients give the general solution of $\Delta u=0$ in analogy to Eq. (B1).

2. Nonvanishing lateral tension $\xi\neq 0$

By applying the method of separation of variables described in [12,13] also to the case of nonvanishing lateral tension we find the general solution of the intermediate equation $\Delta J=\xi^2 J$,

$$J(r,\phi)=a_0K_0(\xi r)+\sum_{n=1}^{\infty}(a_n\ \cos\ n\phi+b_n\ \sin\ n\phi)K_n(\xi r)+\sum_{n=1}^{\infty}(c_n\ \cos\ n\phi+d_n\ \sin\ n\phi)I_n(\xi r),$$

where I_n and K_n denote modified Bessel functions. A general solution of the equation $\Delta u=J(r,\phi)$ again consists of the sum of a special solution and the general solution (B1) of the Laplace equation $\Delta u=0$. It can be written in the form

$$u(r,\phi)=A_0K_0(\xi r)+B_0I_0(\xi R)+\overline{A_0}+\overline{B_0}\ \ln r+\sum_{n=1}^{\infty}(A_n\cos n\phi+B_n\sin n\phi)K_n(\xi r)+\sum_{n=1}^{\infty}(C_n\cos n\phi+D_n\sin n\phi)I_n(\xi r)+\sum_{n=1}^{\infty}(\overline{A_n}\cos n\phi+\overline{B_n}\sin n\phi)r^{-n}+\sum_{n=1}^{\infty}(\overline{C_n}\cos n\phi+\overline{D_n}\sin n\phi)r^{n}, \tag{B3}$$

taking into account that $K_0(\xi r)$, $K_n(\xi r)\cos n\phi$, $I_0(\xi r)$, $I_n(\xi r)\cos n\phi$, and the corresponding terms containing $\sin n\phi$ are eigenfunctions of the Laplace operator.

APPENDIX C: REDUCING AREA INTEGRALS TO LINE INTEGRALS IN THE CALCULATION OF THE MEMBRANE ENERGY

In the calculation of the energy of the tense membrane

$$G=\int\left[\frac{\kappa}{2}(\Delta u)^2+\frac{\gamma}{2}(\nabla u)^2\right]d^2r \tag{C1}$$

we encounter, due to our ansatz (18), integrals of the form

$$I(f,g)=\int_{\mathbf{R}^2/E_1\cup E_2}\left[\frac{\kappa}{2}\Delta f\Delta g+\frac{\gamma}{2}\nabla f\cdot\nabla g\right]d^2r, \tag{C2}$$

where either f obeys $\Delta f=(\gamma/\kappa)f$, which is true for the terms of Eq. (18) containing a Bessel function, or g is a solution of $\Delta g=0$, or both (see Appendix B). E_i denotes the projection of the inclusion i into the x-y plane (see Fig. 3). Applying a theorem of Green we may write

$$\int_{\mathbf{R}^2/E_1\cup E_2}(\nabla f\cdot\nabla g)\ \ d^2r=-\int_{\mathbf{R}^2/E_1\cup E_2}f\Delta g\ d^2r-\int_0^{2\pi}f\frac{\partial g}{\partial r_1}\bigg|_{r_1=a}a\ d\phi_1-\int_0^{2\pi}f\frac{\partial g}{\partial r_2}\bigg|_{r_2=a}a\ d\phi_2 \tag{C3}$$

if $r_i f(\partial g/\partial r_i)$ goes to zero for $r_i\to\infty$. Since

$$\int_{\mathbf{R}^2/E_1\cup E_2}\left[\frac{\kappa}{2}\Delta f\Delta g-\frac{\gamma}{2}f\Delta g\right]d^2r=0 \tag{C4}$$

for $\Delta f=(\gamma/\kappa)f$ or $\Delta g=0$, we find

$$I(f,g)=-\frac{\gamma}{2}\int_0^{2\pi}f\frac{\partial g}{\partial r_1}\bigg|_{r_1=a}a\ d\phi_1-\frac{\gamma}{2}\int_0^{2\pi}f\frac{\partial g}{\partial r_2}\bigg|_{r_2=a}a\ d\phi_2. \tag{C5}$$

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