Interaction between two cylindrical inclusions in a symmetric lipid bilayer

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We calculate the membrane-mediated interaction between two cylindrical inclusions in a symmetric lipid bilayer. Our theory takes two contributions to the free energy into account, the elastic behavior of the membrane and the conformational restrictions that the flexible hydrocarbon chains of the lipids experience in the vicinity of a rigid inclusion. The description of the elastic behavior is based on two order parameters, the hydrophobic thickness of the membrane and a director field that characterizes the average tilt of the lipid chains. Conformational restrictions of the lipid chains are taken into account by a simple director model. We show that the short-range interaction potential between two inclusions sensitively depends on the degree of hydrophobic mismatch and on the spontaneous curvature of the lipid layers. In particular, we find pronounced attraction if the hydrophobic mismatch is positive. For negative mismatch the attraction is much less pronounced and, additionally, an energetic barrier appears. The inclusions prefer a small but notable negative hydrophobic mismatch. Positive spontaneous curvature amplifies these behaviors.
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I. INTRODUCTION

Partitioning of transmembrane inclusions, like proteins or peptides, into a *fluid* lipid membrane and subsequent interaction between the inclusions is modulated by the physical state in which the membrane resides. A number of lipid properties, like the hydrocarbon chain length, phase state, head group structure and charge, as well as the composition of the membrane, affect the lateral organization of transmembrane inclusions.[1] Particular interest currently receives the question how transmembrane proteins (or peptides) adjust to changes in the membrane thickness.[2–4] Differences between the hydrophobic thicknesses of transmembrane protein segments and the host membrane, commonly referred to as *hydrophobic mismatch*,[5,6] can have various consequences for the proteins: failure of membrane insertion,[7–9] lateral oligomerization,[10,11] conformational changes,[12] and possibly also tilt of transmembrane helices.[13] Note that positive (negative) hydrophobic mismatch refers to a larger (smaller) thickness of the hydrophobic protein span compared to that of the host bilayer. Recently it has become possible to detect individual association processes among single transmembrane helices, and to estimate the corresponding interaction free energy.[14–16] In particular, it was shown that the free energy of dimerization between two transmembrane helices ranges from a few k_BT up to more than $10\,k_BT$ (where k_B is Boltzmann's constant and T is the absolute temperature), and depends on bilayer thickness. However, no clear picture so far has emerged regarding the energetic origin of the tendency to form dimers.

Interactions of various origin contribute to the dimerization energy. Some of them act *directly* between inclusions, like van der Waals, steric, or electrostatic interactions, and depend on the molecular details of the inclusion structure. Besides direct there are also *indirect*, membrane-mediated, interactions. One of these, nonspecific, interaction is caused by the hydrophobic mismatch and thus depends on the hydrophobic thickness of the bilayer. Another nonspecific membrane-mediated interaction arises from the decrease in motional freedom of the flexible hydrocarbon chains due to the rigid surfaces of the transmembrane helices.

Membrane-mediated interactions between inclusions have been studied in the past on the basis of different theoretical approaches. Perhaps the most simple method is membrane elasticity theory.[17–22] Here, the lipid bilayer is modeled as an elastic material that responds to the local inclusion-imposed perturbation. The perturbation typically decays over a distance of a few lipid molecules. Interference of the perturbed regions of two (or more) inclusions gives rise to an interaction. Note that membrane elasticity theory takes the material properties of the membrane only through (uniform) phenomenological constants into account; the material properties of the membrane are assumed to remain unaffected by the inclusions.

Besides membrane elasticity theory there are a number of *microscopic* models that describe membrane-inclusion interactions on a molecular level.[23–28] These models generally take the conformational freedom of individual lipid chains into account. For example, Fattal and Ben-Shaul[29] have used a chain packing theory to calculate the free energy of the interaction between a large inclusion (represented as a long wall of length L) and a lipid bilayer, as a function of the hydrophobic mismatch. They (i) obtained a lipid-protein in-

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teraction energy of about $0.37 k_B T\ L/\text{Å}$, (ii) showed that the bilayer prefers slightly negative hydrophobic mismatch, and (iii) found the lipid chains to be tilted—on average—away from the inclusion wall. However, the model did not allow to fully optimize the shape of the interfacial profile near the wall, and its application to more complex geometries is computationally impracticable.

The chain packing theory was also used to calculate the membrane-mediated interaction between *two* flat and parallel walls.[24] The interfacial profile between the walls was assumed to be flat (that is, no hydrophobic mismatch was considered). The free energy of the interaction between the walls as a function of their mutual distance was nonmonotonic; an energetic barrier separated short range attraction from longer range repulsion. This behavior can be rationalized on the basis of the conformational constraints that the inclusion walls impose on the flexible lipid chains. A simple phenomenological model, the so-called *director model* (which we shall also use in the present work), qualitatively accounts for this finding.[24]

It should be noted that a nonmonotonic interaction potential is not a surprising prediction. It is a common observation in dense fluids,[30] and mainly results from the granularity of the solvent. Also computational studies of lipid bilayerlike fluids predict nonmonotonic interaction potentials. Using Monte Carlo simulations Sintes and Baumgärtner[23] found two different attraction regimes between two proteins embedded in a membrane. At short distances the attraction was depletion induced. For longer distances it was ascribed to the gradients of density and orientational fluctuations of the lipids. In between, for protein–protein distances somewhat larger than the lipid diameter, a repulsive energy barrier occurred. In another series of studies Lagüe *et al.*[27,28] have applied hypernetted chain integral equation formalism to a lipid bilayerlike fluid medium. The lateral density–density response function of the hydrophobic core was extracted from MD simulations. It was found that, upon approaching, two proteins first experience a repulsive interaction which then transforms into short range attraction.

The combination of membrane elasticity theory with the director model can be expected to lead to similar results compared with a molecular-level chain packing calculation. Moreover, such a combined model can easily be applied to more complex geometries and allows a full optimization of the membrane shape. A first attempt to combine both models was recently made,[31] and used to calculate the free energy between a long wall and a lipid bilayer, as a function of the hydrophobic mismatch. Indeed, the results qualitatively reproduced the above-mentioned findings of Fattal and Ben-Shaul.[29] The present study again is based on the combination of membrane elasticity theory with the director model but this time we use it to calculate the interaction between two individual inclusions. Particularly, we consider two identical inclusions of cylindrical shape, embedded in a symmetric bilayer membrane. We analyze the membrane-mediated inclusion–inclusion interaction as a function of both the hydrophobic mismatch and the material properties of the membrane.

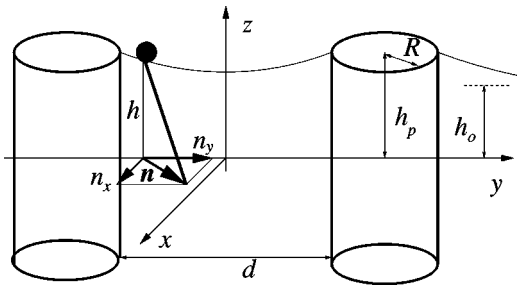

FIG. 1. Schematic representation of the inclusion-containing membrane.

II. PHENOMENOLOGICAL THEORY

Consider a symmetric one-component lipid bilayer in which two rigid transmembrane inclusions reside at distance d from each other. The shape of the two inclusions is cylindrical, each with fixed radius R and length $2h_P$. The surface that separates the two monolayer leaflets from each other, also referred to as the midplane of the bilayer, is a flat surface because of the mirror symmetry. We identify the plane $z \equiv 0$ of a Cartesian coordinate system $\{x,y,z\}$ with the midplane. The midaxis of each inclusion, one intersecting the x,y-plane at $x=0$, $y=-(d/2+R)$ and the other at $x=0$, $y=d/2+R$, is parallel to the z-axis, as schematically displayed in Fig. 1.

Generally, the two inclusions experience a potential $F(d)$ as a function of their mutual distance, d. This potential can be caused by direct (for example electrostatic or van der Waals[16]) and indirect (membrane-mediated) interactions. We shall not consider direct interactions in the present work and only focus on the *indirect* contribution to $F(d)$.

Our model for $F(d)$ takes two characteristic properties of the inclusion-containing membrane into account. First, the inclusions are coupled to the membrane host through the hydrophobic effect.[32] If a *rigid* inclusion does not fit the thickness of the membrane host, then the fluidlike (and hence much *softer*) bilayer must adjust its local thickness accordingly. We describe the corresponding energy penalty, F_{el}, by membrane elasticity theory. Second, and equally important, the rigid structure of the inclusions directly affects the neighboring lipid chains by reducing their conformational freedom. That is, in the hydrophobic core of an unperturbed lipid membrane the hydrocarbon chains explore a large number of conformational states. This number is only limited through interactions of the lipid chains with other—*equally flexible*—chains. However, the presence of a *rigid* inclusion excludes all chain conformations that would penetrate into its interior. Hence, the lipids in the vicinity of a stiff inclusion are conformationally more restricted than those far away from it. We shall denote the corresponding contribution to the free energy by F_c.

The two free energies, F_{el} and F_c, result from the sum over the individual contributions of all perturbed lipids. Adopting a continuum description we express F_{el} and F_c as an integral of the respective area densities, f_{el} and f_c, over the entire area $A=\int da$ of the midplane,

$$F=F_{\text{el}}+F_c=2\int_A da(f_{\text{el}}+f_c), \tag{1}$$

where the factor of 2 accounts for the two equivalent monolayers of the bilayer. Note that Eq. (1) assumes additivity of $F_{\rm el}$ and F_c; this assumption will be further discussed in Sec. V E. In the following, we present our models for $f_{\rm el}$ and f_c.

A. Elastic free energy

Due to the mirror symmetry with respect to the bilayer midplane we only need to consider, say, the upper monolayer. At any position $\mathbf{r}=\{x,y\}$ within the midplane we describe the (upper) monolayer by *two order parameters*. The first is the relative change in hydrophobic thickness $u(\mathbf{r})=h(\mathbf{r})/h_0-1$, where $h=h(\mathbf{r})$ is the local hydrophobic monolayer thickness and h_0 the corresponding equilibrium value of an unperturbed membrane. Note that membrane elasticity theory is commonly based on only one order parameter, namely $u(\mathbf{r})$. The choice of this particular order parameter expresses the preconceived believe that stretching and splay deformations of the lipid tails are crucial determinants of the membrane energetics. Currently we are not aware of any experimental evidence that would suggest a different choice. Yet, our aim is to also take into account the influence of inclusion-induced conformational restrictions of the lipid tails. The corresponding energetic contribution can be expected to diminish upon the aggregation of inclusions. To account for conformational restrictions it is convenient to introduce a second functional degree of freedom of the membrane. This second order parameter is the director field $\mathbf{n}(\mathbf{r})=\{n_x,n_y\}$ that characterizes the *tilt* of a given lipid chain at position $\mathbf{r}$. The lipid tilt provides the link between elasticity theory and the director model. The director field $\mathbf{n}(\mathbf{r})$ is defined within the midplane of the bilayer; it points along the normalized projection of the average lipid's head-to-tail vector. In particular, $n_x=\sin\overline{\theta}\cos\overline{\phi}$ and $n_y=\sin\overline{\theta}\sin\overline{\phi}$ are directly related to the average tilt angle, $\overline{\theta}$, with respect to the z-axis, and to the azimuthal angle, $\overline{\phi}$, that specifies the tilt direction. For small deformations the elastic free energy density, $f_{\rm el}$, is obtained as an expansion with respect to u and $\mathbf{n}$ and their first derivatives up to quadratic order,

$$f_{\rm el}=\frac{K}{2}u^2+\frac{\kappa}{2}(\nabla\cdot\mathbf{n})^2+\kappa c_0(\nabla\cdot\mathbf{n})+\frac{\kappa_t}{2}(\mathbf{n}-h_0\nabla u)^2+\frac{K'}{2}(\nabla\times\mathbf{n})^2. \quad (2)$$

Note that $f_{\rm el}$ in Eq. (2) is an excess free energy density with respect to an unperturbed membrane where $u\equiv 0$ and $\mathbf{n}\equiv 0$. The first term describes the chain stretching contribution in which K is the corresponding chain stretching modulus. Because the hydrophobic volume of a lipid membrane is commonly considered to be conserved during a deformation, we can identify $2K\approx 0.4\,k_BT/\text{Å}^2$ with the experimentally accessible area stretching modulus of a lipid bilayer.[33]

The second and third terms in Eq. (2) account for the splay energy of the lipid chains. It was recently shown[34] that κ and c_0 correspond to the well-known bending stiffness and spontaneous curvature of a lipid monolayer, respectively.[35] The positive sign in front of the term $\kappa c_0(\nabla\cdot\mathbf{n})$ reflects a convention: the spontaneous curvature of a lipid monolayer increases with the bulkiness of the lipid's head groups. Note that c_0 refers to a single lipid monolayer; the spontaneous curvature of a symmetric bilayer, $c_0^{\rm bl}$, necessarily vanishes; $c_0^{\rm bl}=0$. In case of $c_0\neq 0$ the corresponding bilayer resides in an energetically *frustrated* state.[36] Typically, for lipid membranes $\kappa\approx 10k_BT$ and $-0.03\leqslant c_0\text{Å}\leqslant 0.03$.[37]

The fourth term in Eq. (2) is the tilt energy of the monolayer where κ_t denotes the tilt modulus.[21,38] Note that $|\mathbf{n}-h_0\nabla u|$ specifies the average tilt angle $\tilde{\theta}=\overline{\theta}-h_0|\nabla u|$ of the lipid chain *with respect to the normal direction of the monolayer's height profile*, $h(\mathbf{r})$. Specifically, in the limit $\kappa_t\to\infty$ it is $\mathbf{n}=h_0\nabla u$ and the lipid chain at $\mathbf{r}$ points normal to the surface $h(\mathbf{r})$. Note that in writing Eq. (2) it is *assumed* that $f_{\rm el}$ depends only on $\tilde{\theta}$, but it does not depend directly on $\overline{\theta}$. The most general expression of the (quadratic order) tilt energy would involve three individual terms $\sim\mathbf{n}^2$, $\sim(\nabla u)^2$, and $\sim\mathbf{n}\cdot\nabla u$, each with its own prefactor.[39] However, for a lipid membrane the main contribution to $f_{\rm el}$ results from the head groups and the part of the hydrocarbon chain region near the head groups.[36,40] Close to the bilayer midplane the hydrocarbon chains are much more disordered than close to the head groups.[41] Therefore, the spatial orientation, $\overline{\theta}$, of the lipid chains with respect to the bilayer midplane does not directly affect $f_{\rm el}$. We note that the tilt modulus κ_t has never been determined by experiment, but it was estimated theoretically that it is approximately $0.1<\kappa_t\text{Å}^2/k_BT<0.2$.[31,34]

The last term in Eq. (2) describes the twist of the lipid molecules within the lipid layer. The corresponding coefficient K' is unknown. Yet, it was argued previously that K' is considerably smaller than the bending modulus κ.[42]

B. Conformational chain restrictions

The hydrophobic core of a fluid membrane consists of flexible hydrocarbon chains that rapidly change their conformations. The director field $\mathbf{n}(\mathbf{r})$ [see Eq. (2)] thus represents *average* orientations of the corresponding lipid chains. In the vicinity of a rigid inclusion the number of available conformations is reduced because the lipid chains are not able to penetrate into the inclusion interior. The closer the (average) distance of a lipid chain to the inclusion, the larger the number of inaccessible chain conformations. The free energy $f_c=f_c(\mathbf{n},\mathbf{r})$, expressing the conformational chain restrictions in Eq. (1), must therefore be an explicit function of $\mathbf{r}$. It also depends on the director field, $\mathbf{n}$; however to keep our model simple we shall not consider any direct dependence of f_c on u or ∇u (see also the discussion in Sec. V E). For an inclusion-containing membrane there will be a particular director field $\mathbf{n}(\mathbf{r})=\mathbf{n}_0(\mathbf{r})$ which yields the minimal conformational chain energy, $f_c^0=f_c(\mathbf{n}_0,\mathbf{r})$. We refer to $\mathbf{n}_0=\mathbf{n}_0(\mathbf{r})$ as the *spontaneous director field*. For an inclusion-free membrane the spontaneous director field vanishes identically ($\mathbf{n}_0(\mathbf{r})\equiv 0$) and so do the inclusion-induced conformational restrictions, implying $F_c^0=2\int_A da\, f_c^0=0$. On the other hand, in the absence of elastic interactions, $\mathbf{n}(\mathbf{r})=\mathbf{n}_0(\mathbf{r})$ at all positions $\mathbf{r}$, and $F=F_c=F_c^0$. In general however, the elastic energy competes with the inclusion-induced conformational energy cost. Then, the optimal director field, $\mathbf{n}$, must be calculated by a minimization of the full free energy $F=F_{\rm el}$

$+F_c$, and no longer coincides with $\mathbf{n}_0$. For sufficiently small deviations of $\mathbf{n}$ from $\mathbf{n}_0$ we can expand f_c up to quadratic order

$$f_c = f_c^0 + \triangle f_c \tag{3}$$

with

$$\Delta f_c = \tfrac{1}{2}(\mathbf{n}-\mathbf{n}_0)\underline{\kappa}_t^c(\mathbf{n}-\mathbf{n}_0), \tag{4}$$

where $\underline{\kappa}_t^c$ is a modulus that describes an additional resistance of the lipid layer with respect to tilt. [Recall that a tilt modulus κ_t was already introduced in Eq. (2). This tilt modulus has its origin in the tilt-induced stretching of the hydrocarbon chains.] The tilt modulus $\underline{\kappa}_t^c$ has its origin in the conformational chain restrictions. Generally $\underline{\kappa}_t^c$, is a tensor because near the inclusion the lipid layer is no longer isotropic. Only sufficiently far away from the inclusion the membrane is laterally isotropic, implying $\mathbf{n}_0=0$, $f_c^0=0$ and $\underline{\kappa}_t^c=\kappa_t^c\,\underline{1}$ where $\underline{1}$ is the unit tensor. The chain conformational free energy $f_c=\kappa_t^c\mathbf{n}^2/2=\kappa_t^c\overline{\theta^2}/2$ then provides a contribution to the free energy which directly depends on $\overline{\theta}$.

III. MICROSCOPIC MODEL

In the following we employ the *director model*[24] to describe the inclusion-induced conformational restrictions of the lipid chains. This model enables us to calculate $\mathbf{n}_0$, f_c^0, and $\underline{\kappa}_t^c$. The underlying idea of the director model is rather simple but its consequences compare well with much more involved mean-field, molecular-level, chain packing calculations.[24,29]

In the director model, any given lipid chain is represented by a fluctuating director $\mathbf{h}=h_0\{\cos\phi\sin\theta,\sin\phi\sin\theta,\cos\theta\}$ of length h_0 and orientation $\omega=\{\theta,\phi\}$. All chain conformations that point into the same spatial direction ω are represented by a particular $\mathbf{h}(\omega)$. Our main assumption is to assign the same internal energy to any possible $\mathbf{h}(\omega)$ (this energy can then be set to zero). Physically it seems plausible to introduce an orientation dependent internal energy, say, $w(\omega)=\kappa_t^0(\mathbf{h}/h_0)^2$ with a microscopic tilt modulus κ_t^0. However, it was recently shown that even for $\kappa_t^0=0$ the predictions of the director model agree qualitatively with the results of the molecular-level chain packing calculations. (As mentioned in the Introduction, both approaches predict very similar interaction behavior between two large rigid membrane-matching inclusions.[24]) We have therefore set $\kappa_t^0=0$ in the present work which also considerably simplifies the numerical procedure used to find the optimal membrane perturbation.

An unperturbed director is able to adopt all orientations $0\leqslant\phi\leqslant 2\pi$ and $0\leqslant\theta\leqslant\pi/2$ within the hydrocarbon core. The conformational space thus corresponds to the area $\int d\omega=2\pi h_0^2$ of a hemisphere with radius h_0. If the lipid chain resides close to a rigid inclusion it suffers from conformational restrictions. Within the director model we account for these restrictions by excluding all those orientations from the partition sum q for which the director would penetrate into the inclusion interior. The presence of one or more inclusions (of arbitrary shape and spatial orientation) is described by a function $\theta(\phi)$ that characterizes the reduction of the conformational space at given azimuthal angle ϕ to $0\leqslant\theta\leqslant\theta(\phi)$. In fact the function $\theta(\phi)$ describes a curve on the inclusion surface which is at distance h_0 away from the director origin. If at a particular ϕ no inclusion can be ''seen'' (because either there is no inclusion or the distance to the inclusion surface is larger than h_0 for all $0\leqslant\theta\leqslant\pi/2$), then $\theta(\phi)=\pi/2$. We see that within the director model only a corona of width h_0 around a given inclusion contributes to F_c. The chains of all lipids further away than h_0 do not ''see'' the inclusion surface and hence remain unperturbed. If the distance between the two inclusions is $d<2h_0$ then some directors will be perturbed simultaneously by both inclusions. This interference of the coronas of the two inclusions gives rise to an interaction.

The partition sum associated with the director model is

$$q=\int d\omega = h_0^2\int_0^{2\pi}d\phi\int_0^{\theta(\phi)}d\theta\sin\theta. \tag{5}$$

The corresponding partition sum of an unperturbed director is $q_0=q[\theta(\phi)\equiv\pi/2]=2\pi h_0^2$ and thus

$$\frac{q}{q_0}=1-\langle\cos\theta(\phi)\rangle_\phi, \tag{6}$$

where $\langle\cos\theta(\phi)\rangle_\phi=1/(2\pi)\int_0^{2\pi}d\phi\cos\theta(\phi)$ denotes averaging over the azimuthal angle ϕ. The free energy $F_c^0=2\int_A da f_c^0$ associated with the inclusion-induced conformational restrictions results from an integration of the free energy density

$$f_c^0=-\frac{1}{a}\ln\frac{q}{q_0}, \tag{7}$$

where here and in the following we express all energies in units of k_BT. In Eq. (7) a denotes the cross-sectional area per lipid chain; typically $30\leqslant a\ \text{Å}^{-2}\leqslant 35$. The spontaneous director field $\mathbf{n}_0=\langle\mathbf{n}_P\rangle=\int d\omega\mathbf{n}_P/q$ is given by the midplane projection, $\mathbf{n}_P=\{h_x,h_y\}/h_0=\sin\theta\{\cos\phi,\sin\phi\}$, of the normalized director, $\mathbf{h}/h_0$, averaged over all accessible orientations. Thus

$$\mathbf{n}_0=\frac{q_0}{q}\left\langle\begin{Bmatrix}\cos\phi\\ \sin\phi\end{Bmatrix}\int_0^{\theta(\phi)}\sin^2\theta d\theta\right\rangle_\phi. \tag{8}$$

As outlined above, the function $\theta(\phi)$ is determined by the inclusion geometry and by the distance between the inclusion and the director origin. For an inclusion-free membrane $\theta(\phi)\equiv\pi/2$ and thus $\mathbf{n}_0=0$ everywhere. In the presence of one or more (arbitrarily shaped) inclusions, $\mathbf{n}_0$ can be computed numerically according to Eq. (8). Let us also calculate the tilt modulus $\underline{\kappa}_t^c=\underline{\chi}^{-1}$, given in Eq. (4). It is the inverse of the tilt susceptibility,

$$\underline{\chi}=a\langle(\mathbf{n}_P-\mathbf{n}_0)\circ(\mathbf{n}_P-\mathbf{n}_0)\rangle, \tag{9}$$

where $\circ$ denotes the outer product. In an unperturbed membrane patch the response with respect to tilt is laterally isotropic. Indeed, using $\theta(\phi)=\pi/2$ in Eq. (9) we obtain $\underline{\kappa}_t^c=\kappa_t^c\underline{1}$ with

$$\kappa_t^c=\frac{3}{a}. \tag{10}$$

In the present work we shall ignore the inclusion-induced modifications of $\underline{\kappa}_t^c$ and simply work with the scalar result indicated in Eq. (10) so that

$$\Delta f_c = \frac{\kappa_t^c}{2}(\mathbf{n}-\mathbf{n}_0)^2. \tag{11}$$

Let us shortly recall some explicit results for a few characteristic situations that have been discussed in previous works.[24,31] Consider the most simple case, namely a single sufficiently large inclusion that can be represented as a long planar wall (of length $L \gg h_0$), oriented normal to the membrane midplane and located along the y-axis. If the distance from the wall to the director origin is x, then $q/q_0 = (1+x/h_0)/2$ and $F_c^0 = N(1-\ln 2)$, where $N = 2Lh_0/a$ is the number of directors (that is, lipid chains) perturbed by the wall. Each director contributes, on average, only a fraction of k_BT. However, the number of directors scales with the length L, and can become large. For a director at distance $x < h_0$ away from a single straight wall we also find $\mathbf{n}_0 = \{(1-x/h_0)/2,0\}$, exemplifying the general result that the average director points *away* from the inclusion. The corresponding tilt modulus [see Eq. (4)] connected with conformational restrictions,

$$\underline{\kappa}_t^c = \frac{1}{a}\begin{Bmatrix} 12/(1+x/h_0) & 0 \\ 0 & 3 \end{Bmatrix} \tag{12}$$

(with $0 \leqslant x \leqslant h_0$) shows a rigidification of the lipid bilayer in the vicinity to the inclusion.

Another simple case is that of two parallel walls, located at distance d from each other. For $d > 2h_0$ the walls do not interfere with each other, and consequently the conformational free energy penalty is $F_c^0 = 2N(1-\ln 2)$ with N as given above. For $d < 2h_0$ the walls do interact with each other. Particularly, within the region $0 \leqslant d \leqslant h_0$ one obtains

$$F_c^0 = -N\frac{d}{h_0}\ln\frac{d}{2h_0} \tag{13}$$

which has a maximum of $F_c^0 = 2N/e$ at $d = 2h_0/e$. Hence the free energy of the interaction between two parallel walls, $\Delta F_c^0(d) = F_c^0(d) - F_c^0(2h_0)$, is nonmonotonic, exhibiting an energy barrier of height $\Delta F_c(2h_0/e) = 0.122 \times N$.

IV. EULER EQUATIONS, BOUNDARY CONDITIONS, AND NUMERICAL PROCEDURE

In order to find the optimal configuration of the perturbed membrane we functionally minimize F in Eq. (1). This requires us to solve the Euler equations that follow from Eqs. (2), (3), and (11),

$$\begin{aligned} &\kappa_t h_0^2 \Delta u = Ku + h_0\kappa_t(\nabla\cdot\mathbf{n}), \\ &\kappa\nabla(\nabla\cdot\mathbf{n}) = \kappa_t(\mathbf{n}-h_0\nabla u) + \kappa_t^c(\mathbf{n}-\mathbf{n}_0) + K'\nabla\times(\nabla\times\mathbf{n}). \end{aligned} \tag{14}$$

These equations need to be solved subject to three boundary conditions at the inclusion surfaces $\mathbf{r}_G$. The first one assumes hydrophobic matching between the inclusion and the host membrane, implying

$$u(\mathbf{r}_G) = u_0, \tag{15}$$

where $u_0 = h_P/h_0$ quantifies the extent of hydrophobic mismatch [recall $h_P = h(\mathbf{r}_G)$]. To obtain the remaining two boundary conditions at $\mathbf{r}_G$ we assume that the corresponding directors are free to optimize their spatial orientation. That is, they are allowed to reorient in order to optimally compromise between the elastic interactions and the conformational chain restrictions. This degree of freedom leads to the so-called natural boundary conditions,[43]

$$(\nabla\cdot\mathbf{n})|_{\mathbf{r}_G} = c_0, \quad (\nabla\times\mathbf{n})|_{\mathbf{r}_G} = 0, \tag{16}$$

that we shall employ in the present work. At this point we note that recent molecular-level chain packing calculations[24,29] clearly show that the lipid directors at the inclusion boundary can tilt away from the inclusion surface without leaving a void region inside the hydrocarbon core. We thus do not impose in the present work the commonly used boundary condition $\mathbf{n}|_{\mathbf{r}_G} = 0$ (Refs. 19, 21, 44, 45) which would imply angular matching between the surface of the cylindrical inclusion and the neighboring lipid directors.

All remaining boundary conditions for the Euler equations [namely, $n_x|_{x=0} = 0$, $(\partial n_y/\partial x)_{x=0} = 0$, $(\partial u/\partial x)_{x=0} = 0$ and analogously along the x-axis] follow from the mirror symmetry with respect to the x- and y-axes. In addition to that, far away from the inclusions the membrane is unperturbed, implying $u(|\mathbf{r}|\to\infty) = 0$ and $\mathbf{n}(|\mathbf{r}|\to\infty) = 0$.

Obtaining the free energy, $F = F_{\rm el} + F_c^0 + \Delta F_c$, of an inclusion-containing membrane involves two steps. The first is the calculation of F_c^0 defined by Eqs. (6) and (7). The second step involves the computation of $F_{\rm el} + \Delta F_c$ which is based on the numerical calculation of the Euler equations; Eqs. (14). To solve the Euler equations for two cylindrical membrane inclusions we have used bipolar coordinates within the x,y-plane,

$$\begin{aligned} x(\varphi,v) &= b\,\frac{\sin\varphi}{\cosh v + \cos\varphi}, \\ y(\varphi,v) &= b\,\frac{\sinh v}{\cosh v + \cos\varphi}, \end{aligned} \tag{17}$$

with $b = d\sqrt{(1+4R/d)}/2$. Because of the symmetry along both the x and y axis, we only need to solve the Euler equations outside the cylinders in the region $0 < x < \infty$ and $0 < y < \infty$. The corresponding ranges of the variables, φ and v, in Eqs. (17) are $0 < \varphi < \pi$ and $0 < v < \operatorname{arcsinh}(b/R)$. The three coupled partial differential equations, Eqs. (14), were solved iteratively. Each iteration step consisted of solving either one of the equations for u, n_v, or n_φ while using the solution obtained in the previous iteration. After about 20 iterations we obtained a self-consistent solution.

V. RESULTS AND DISCUSSION

Our results are based on the following set of material parameters: $h_0 = 14$ Å, $\kappa = 10k_BT$, $K = 0.2k_BT/\text{Å}^2$, $K' = 5k_BT$, and $\kappa_t = 0.1k_BT/\text{Å}^2$. The choice of these values is discussed in the Theory. For the inclusion radius we use $R = 7$ Å. This value accounts for both the hard core radii of the inclusion and a lipid tail. The latter contributes about one half of the cross-sectional extension of a stretched hydrocar-

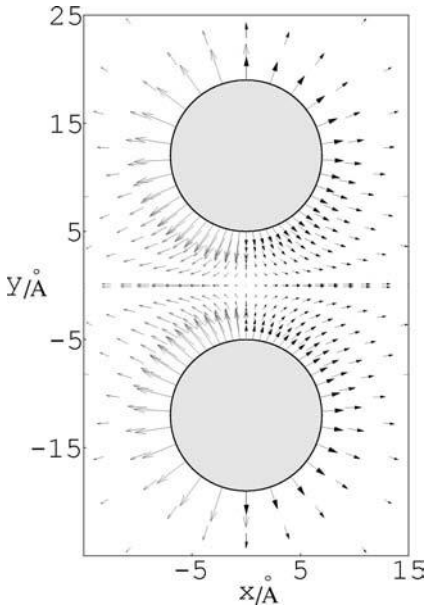

FIG. 2. The director fields, $\mathbf{n}(\mathbf{r})$ and $\mathbf{n}_0(\mathbf{r})$. The left-hand side ($x \leqslant 0$; unfilled arrowheads) displays $h_0\mathbf{n}_0(\mathbf{r})/2$; the right-hand side ($x \geqslant 0$, filled arrowheads) displays $h_0\mathbf{n}(\mathbf{r})$. The factors of $h_0/2$ and h_0, respectively, were added to improve the visual appearance of the directors. The calculation corresponds to $d=10$ Å and $u_0=0$.

bon chain (≈ 1.5 Å). A typical choice of the cross-sectional area per chain in a lipid membrane is $a=32.5$ Å^2;[46] it gives rise to $\kappa_t^c=0.1k_BT/$Å^2 [see Eq. (10)]. In the following we are interested in variations of the distance, d, between the inclusions, of the hydrophobic mismatch, u_0, and of the spontaneous curvature, c_0.

A. Membrane conformation for matching inclusions

We first present the membrane conformation for the particular case $d=10$ Å and $u_0=0$. That is, no hydrophobic mismatch is present. Figure 2 displays the numerically calculated director fields, $\mathbf{n}(\mathbf{r})$ and $\mathbf{n}_0(\mathbf{r})$ [the left-hand side where $x \leqslant 0$ shows $h_0\mathbf{n}_0(\mathbf{r})/2$, and the right-hand side where $x \geqslant 0$ shows $h_0\mathbf{n}(\mathbf{r})$. The factors of $h_0/2$ and h_0, respectively, were added to improve the visual appearance of the directors]. The relative change in hydrophobic thickness, $u(\mathbf{r})$, is displayed in Fig. 3.

The directors are generally tilted away from the inclusion, indicating their unfavorable interaction with the rigid inclusion surface. Whereas for a single inclusion cylinder all

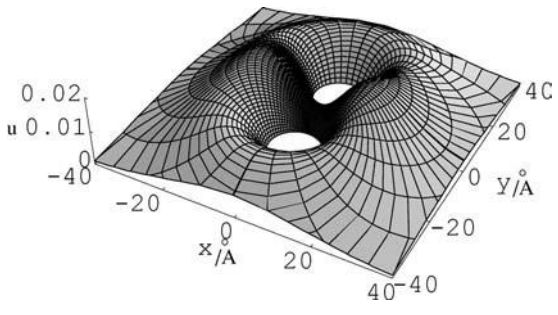

FIG. 3. The relative hydrophobic monolayer thickness, $u(\mathbf{r})$, corresponding to Fig. 2.

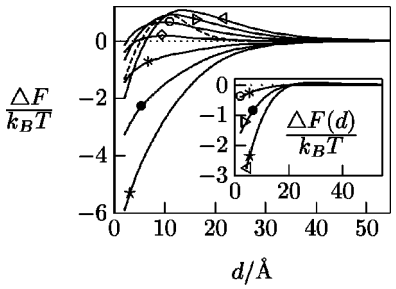

FIG. 4. Interaction free energy $\triangle F(d)$ for $u_0=-0.3$ (◁), $u_0=-0.2$ (▷), $u_0=-0.1$ (○), $u_0=0$ (◇), $u_0=0.1$ (*), $u_0=0.2$ (●), and $u_0=0.3$ (★). The broken line shows $\triangle F_c^0(d)=F_c^0(d)-F_c^0(2h_0)$. The spontaneous curvature of the lipid layers vanishes for all curves ($c_0=0$). The inset shows $\triangle F(d)$ for the case that inclusion-induced conformational chain restrictions are not taken into account ($\mathbf{n}_0 \equiv 0$). For all curves, the boundary conditions at the inclusion are the natural ones as given by Eq. (16).

directors point exactly in radial direction, the presence of *two* interacting inclusions gives rise to a more intricate spatial pattern of director orientations. Consider the spontaneous director tilt $\mathbf{n}_0(\mathbf{r})$ (Fig. 2, left-hand side). Here, the director orientations are no longer cylindrically symmetric for those directors that are closer than h_0 to both inclusion surfaces. For example, a director located at $x=8$, $y=13$ would "see" a small part of the second inclusion, and will contribute (even though to a very small extent) to the membrane-mediated inclusion–inclusion interaction.

In the presence of elastic interactions the optimal director orientations $\mathbf{n}(\mathbf{r})$ deviate in a characteristic fashion from $\mathbf{n}_0(\mathbf{r})$. Most notably, in the immediate vicinity to the inclusion surfaces $\mathbf{n}$ is much shorter than $\mathbf{n}_0(\mathbf{r})$. This directly reflects the competition between elastic interactions and inclusion-induced conformational restrictions of the flexible hydrocarbon tails: The tendency of $\mathbf{n}(\mathbf{r})$ to adopt the spontaneous tilt field, $\mathbf{n}_0(\mathbf{r})$, cannot be realized because this would induce a too high elastic free energy penalty. Closer inspection reveals another feature: The changes of $\mathbf{n}(\mathbf{r})$ are less pronounced compared to $\mathbf{n}_0(\mathbf{r})$ but, due to the elastic response of the bilayer, they extend over a larger spatial region. Recall that there is no hydrophobic mismatch between the inclusion and the membrane. Nevertheless, owing to the coupling between the elastic free energy and the conformational chain restrictions there is a small but notable membrane thickening in the vicinity of the inclusion (see Fig. 3). This thickening results from the fact that $\mathbf{n}_0(\mathbf{r})$ points away from the inclusion surface, thus inducing a monolayer bending towards the midplane.

B. Interaction between two inclusions

Of greatest interest in the present work is the interaction free energy,

$$\Delta F(d)=F(d)-F(\infty) \tag{18}$$

as a function of the distance, d, between the inclusions. Figure 4 shows $\Delta F(d)$ for various different values of the hydrophobic mismatch, u_0, and for vanishing spontaneous curvature of the two lipid monolayers ($c_0=0$). Figure 4 also displays the contribution $\Delta F_c^0(d)=F_c^0(d)-F_c^0(2h_0)$ (broken line) which is independent of u_0 and c_0. Similarly to the

results for two straight walls,[24] $\Delta F_c^0(d)$ is nonmonotonic: an energy barrier near $d=2h_0/e=10$ Å separates an attractive from a repulsive region. This similarity is not unexpected because the chain conformational confinement between two walls is qualitatively the same as for two rigid cylinderlike inclusions. For the present inclusion size ($R=7$ Å) the magnitude of the interaction free energy $|\Delta F_c^0(d)|$ is generally small; $|\Delta F_c^0(d)| \lesssim k_B T$.

The consequences of adding the elastic contribution to the free energy strongly depends on the extent of hydrophobic mismatch, u_0. Yet, even without any hydrophobic mismatch (where $u_0=0$) the elastic energy affects the interaction between the two inclusions. The changes with d of elastic and nonelastic interactions partially compensate each other, leading to a very weak dependence of ΔF on d. We thus do not expect a notable membrane-mediated association tendency for two matching inclusions in a lipid bilayer.

Let us now discuss $\Delta F(d)$ in the presence of hydrophobic mismatch (still with vanishing spontaneous curvature of the lipids; $c_0=0$). Figure 4 shows a pronounced asymmetry of ΔF with respect to the hydrophobic mismatch, u_0. In particular, positive mismatch gives rise to strong attraction between inclusions. Negative mismatch merely leads to an energy barrier with no appreciable gain in free energy upon close association. The asymmetry of $\Delta F(d)$ with respect to u_0 has its origin in the presence of the nonvanishing spontaneous director field, $\mathbf{n}_0$. Generally, the spatial pattern of $\mathbf{n}_0$ exhibits positive spontaneous splay (see Fig. 2). The actual director field, $\mathbf{n}$, tends to follow this pattern but the presence of elastic interactions leads to deviations. For example, the elastic interactions arising from positive hydrophobic mismatch favor negative splay. We then have two opposed tendencies (one promoting positive and the other promoting negative splay) that induce a high free energy penalty for a single isolated membrane inclusion. Consequently, if two inclusions dimerize, we expect a correspondingly high gain in free energy. For negative mismatch we encounter a different situation because $u_0<0$ enhances the tendency to adopt positive splay (as does $\mathbf{n}_0$). In this case, ΔF changes only moderately with d, as seen in Fig. 4.

The asymmetry of ΔF with respect to u_0 is no longer present if we neglect the inclusion-induced conformational restrictions of the lipid chains. To illustrate this, we display in the inset of Fig. 4 ΔF for $\mathbf{n}_0(\mathbf{r})\equiv 0$. Then, the free energy contains only the elastic part [see Eq. (2)] and the conformational chain restrictions for an inclusion-free membrane [see Eq. (11) with $\mathbf{n}_0=0$]. The boundary conditions for deriving the curves in the inset of Fig. 4 are, again, given by the natural ones [see Eq. (16)]. Recall that the natural boundary conditions allow optimal relaxation of the director field, $\mathbf{n}$, at the surface of the membrane inclusions. We note that another commonly used set of boundary conditions is based on $\mathbf{n}|_{\mathbf{r}_G}=0$ which imposes angular matching of the boundary directors to the shape of the cylindrical inclusions.[19,21,44] In any case, irrespective of the boundary conditions, $\mathbf{n}_0\equiv 0$ implies $F(d,u_0)=F(d,-u_0)$, and leads to attractive interactions between membrane inclusions for any nonvanishing hydrophobic mismatch.

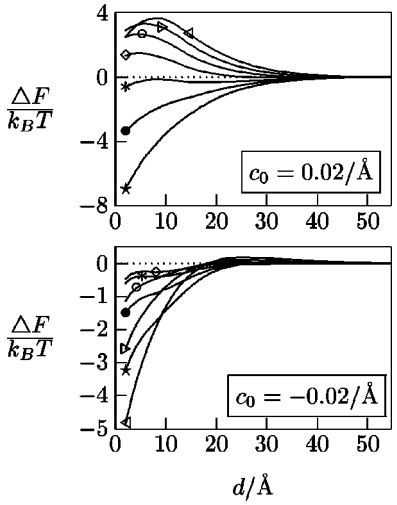

FIG. 5. Interaction free energy $\triangle F(d)$ of two rigid inclusions for $u_0=-0.3$ (◁), $u_0=-0.2$ (▷), $u_0=-0.1$ (○), $u_0=0$ (◇), $u_0=0.1$ (*), $u_0=0.2$ (●), and $u_0=0.3$ (★). The two diagrams correspond to different values of the spontaneous curvature: $c_0=0.02/\text{Å}$, $c_0=-0.02/\text{Å}$.

C. Influence of the spontaneous curvature

Figure 5 shows how the spontaneous curvature, c_0, affects $\Delta F(d)$. The two diagrams are derived for $c_0=\pm 0.02\ \text{Å}^{-1}$. Recall that both the presence of the spontaneous director field, $\mathbf{n}_0$, and a negative hydrophobic mismatch, $u_0<0$, favor the development of positive splay in the director field, $\mathbf{n}$. In this case, lipid layers with $c_0>0$ should benefit from the interaction with rigid inclusions. Indeed, the upper diagram in Fig. 5 shows that for $u_0<0$ two isolated inclusions are energetically preferred over a single dimer. In other words, repulsive interactions dominate. For positive mismatch ($u_0>0$) we recall that the elastic membrane interactions favor negative splay in the spatial pattern of $\mathbf{n}$. The opposed tendencies that originate from $u_0>0$ and $c_0>0$ explain the enhanced attraction between two inclusions as compared to $c_0=0$.

Figure 5 predicts lipid layers with negative spontaneous curvature ($c_0<0$) to generally induce attractive interactions for both positive and negative mismatch. The reason is the presence of the spontaneous director field, $\mathbf{n}_0$, which induces $\mathbf{n}$ to exhibit positive splay near the inclusions. The ensuing high free energy penalty of isolated inclusions is partially relieved upon inclusion–inclusion association.

D. Single isolated inclusion

Figure 6 displays the free energy, F, of a single isolated inclusion as a function of the hydrophobic mismatch for different values of the spontaneous curvature, c_0. We point at two important features of F that have previously been derived and analyzed for a single isolated wall on the basis of both molecular-level chain packing calculations[29] and within the approach used in this work.[31]

First, the minimum of F with respect to u_0 is typically found for negative u_0. Hence, membrane inclusions prefer negative hydrophobic mismatch. This property has its origin in the coupling between the elastic membrane interactions and the inclusion-induced conformational restrictions of the

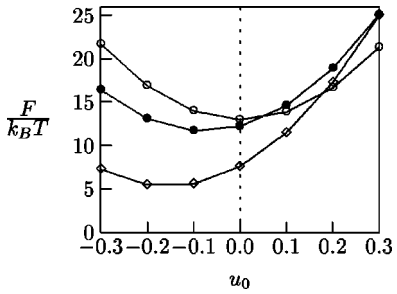

FIG. 6. Free energy F as a function the hydrophobic mismatch, u_0, for a single, isolated, inclusion. The three curves correspond to different spontaneous curvature of the lipid layer: $c_0=0$ (●); $c_0=0.02$ Å^{-1} (◇); $c_0=-0.02$ Å^{-1} (○).

lipid chains. That is, both the spontaneous director field, $\mathbf{n}_0$, and a moderate negative hydrophobic mismatch simultaneously favor a similar positive splay pattern of $\mathbf{n}$ near a rigid inclusion. This then creates the energetically most opportune situation as reflected in Fig. 6.

Second, positive spontaneous curvature ($c_0>0$) of the lipid layers drastically lowers F for negative hydrophobic mismatch. Again, the reason is the formation of the positive splay pattern for moderate negative hydrophobic mismatch. An intrinsic tendency of the lipid layers to adopt positive splay (that is $c_0>0$) should then further lower F as Fig. 6 indeed shows. In this case, the elastic free energy density near an inclusion is lower than far away from it. That is, cylindrical inclusions relieve part of the *frustration* that a planar membrane with positive spontaneous splay suffers from. However, the gain in elastic free energy does not compensate for the loss of chain conformational energy. In fact we always find $F>0$. Our results thus suggest that the specific structure of the lipid bilayer provides a positive contribution to the insertion free energy of a rigid inclusion (like a transmembrane protein or peptide). This contribution adds to the classic hydrophobic effect.[47,48]

E. Discussion of the simplifications, possible extensions, and experimental relevance

We have only focused on the interaction between two individual inclusions. This situation is appropriate for low inclusion densities. At high densities, where multibody interactions dominate, different approximations like the often employed cell model are more appropriate.[19–21,49]

We have neglected the possibility of the cylindrical inclusions to tilt. While tilt has been suggested to occur for positive mismatch[3] a recent study[50] showed that modifications in the degree of hydrophobic mismatch need not necessarily affect the tilt angle of a single transmembrane peptide. Hence, it is currently not clear whether experimentally observed tilt of membrane inclusions is induced by hydrophobic mismatch or whether it is an intrinsic property that arises from an asymmetry of the inclusion itself.

The present work employs a number of rather strong assumptions, like the neglect of an orientational energy dependence in the director model, or the spatial uniformity of the modulus, κ_t^c. However, our main goal to *qualitatively* illustrate the implications of conformational chain restrictions for the dimerization of membrane inclusions should not be affected by these assumptions. Moreover, we expect the two assumptions to partially neutralize each other. This is because, on the one hand, by the assumption of a spatially uniform κ_t^c we have neglected a localized stiffening of the lipid layers and thus we underestimate $F(d)$. On the other hand, an additional orientational energy dependence in the director model would weaken the inclusion–inclusion interactions predicted by the director model and thus we overestimate $F(d)$.

We have used a continuum approach, although the relative changes in hydrophobic thickness take place at distance over only a few molecular diameters. The use of continuum elasticity theory is certainly an approximation. However, the hydrocarbon tails constituting the core of a fluid membrane are extremely flexible and adopt a large number of different states. This conformational averaging weakens the correlations between individual lipid chains. Also experimental findings point at the applicability of continuum elasticity theory. For example, Harroun *et al.*[44] measured the thinning of lipid membranes upon insertion of short transmembrane peptides. Nielsen and Anderson[51] analyzed the mismatch dependent changes of gramicidin A channel lifetime. In both cases membrane elasticity theory—even in its most simple version—was found in agreement with experimental data. We can thus expect that even spatial changes over a small number of lipid tails can usefully be described by continuum elasticity theory.

Let us discuss the additivity of F_{el} and F_c in Eq. (1). Generally, both F_{el} and F_c reflect conformational fluctuations of the fluidlike lipid tails in the lipid layer. F_{el} and F_c are only additive if there are two *statistically independent* mechanisms of which one mainly contributes to F_{el} and the other to F_c. (If so, the partition sum for F factorizes and F itself turns additive.) Our approach is indeed based on two such mechanisms. One corresponds to stretching of the lipid tails for fixed orientation. The other changes the average chain orientation and leaves the chain length unaffected. As a consequence, we have obtained two distinct tilt moduli, κ_t and κ_t^c, as appearing in Eqs. (2) and (11). The physical basis for the separability of F can be understood as follows: the contribution to the tilt deformation that involves stretching of the lipid tails (κ_t) mainly affects those lipid conformations that point into the stretching direction. On the other hand, a pure tilt deformation (with no concurrent stretching) depends mainly on the chain conformations that are close to the interfacial region of the lipid layer. In fact (so far unpublished) mean-field chain packing calculations support this notion.

Recall that we did not consider any direct dependence of f_c on the hydrophobic thickness u. This can be justified in terms of the director model. Assume we would have considered statistical averaging of the director projection along the z-direction [we did consider only averaging of the director projection onto the x,y-plane; see Eq. (8)]. The presence of a rigid body in the vicinity of a given director affects the averaging. However, averaging along the z direction is modified to a much lesser extent than averaging of the director projection onto the x,y-plane. This is seen easily for a planar wall where $\langle \mathbf{h}_z \rangle = h/2$ is the same for $x=0$ and $x=h_0$, and

only increases slightly in between (the same argument is true for the corresponding stretching modulus in z-direction which depends on $\langle \mathbf{h}_z^2 \rangle$). This is in strong contrast to the averaging normal to the wall as outlined in Sec. III. Thus, as a first approximation the conformational restrictions can be treated as independent of the hydrophobic mismatch.

We also emphasize again that our study only considers indirect (that is membrane-induced) interactions between two inclusions. However, interactions between inclusions can also arise from direct forces such as van der Waals and electrostatic forces. For uncharged inclusions (or in solutions with high salt concentrations where electrostatic interactions are screened) the short-ranged forces between inclusions would be determined by both, the van der Waals interactions, which are always attractive, and the short-ranged membrane-induced interactions, which can be either attractive or repulsive.

We have estimated the energy of the van der Waals interaction between two equal cylinders immersed in the membranous continuum of the same height as the cylinders.[46] The membranous continuum was composed of two regions: one corresponding to the headgroups and the other corresponding to the phospholipid tails. The cylinders, the headgroup region and the tail region were characterized by different dielectric constants (ε_c, ε_h, and ε_t, respectively). This interaction is always attractive. Taking $\varepsilon_c=4$, $\varepsilon_h=35$, and $\varepsilon_t=2$ we have obtained that for $d=10$ Å the values of this energy are smaller than $10^{-2}k_BT$, however, they may for example reach the values of $0.2k_BT$ for $d=2$ Å. As in some cases the lipid-mediated interaction between the cylinders is also of this order (see Figs. 4 and 5) the contribution of the van der Waals interaction may in these cases not be negligible. It can be noted that the energies of the single isolated inclusion are generally an order of magnitude higher than the energies of the lipid-mediated interaction between the inclusions even when the distance between the inclusions is only less than a nanometer (compare Fig. 6 and Figs. 4 and 5). Accordingly, the director field is of non-negligible strength only in a small region in the vicinity of the inclusions. These notions are important in validating the assumptions and estimating the constants within the theoretical description of the mutual dependence between the lateral distribution of the inclusions and the shape of a closed membrane. The single-inclusion energy can be taken as a starting point in the statistical mechanical derivation of the free energy of the pool of laterally mobile inclusions within the membrane.[52,53] If the lateral density of the inclusions is small enough, the single inclusion energy applied within the mean curvature field model[52] may be considered as a fair approximation. In this work we have considered cylindrical inclusions that are inserted in a flat membrane segment. In reality, inclusions may have different shapes while the closed membrane is generally curved. Therefore it would also be of importance to generalize the problem considered in this work as to include various shapes of inclusions and take into account a nonzero local membrane curvature.

Our study is motivated by a number of recent experiments in which association processes between two (and sometimes more) synthetically designed transmembrane peptides were observed.[10,54,55] For example, Yano *et al.*[14] find a completely hydrophobic ''inert'' model peptide to adopt transmembrane orientation in a particular lipid bilayer and to dimerize with a corresponding association free energy of about $5k_BT$ (unfortunately so far, no results are available for varying hydrophobic mismatch). The fact that the model peptide was lacking any specific interaction and nevertheless showed a tendency to self-aggregate promoted the authors to suggest some kind of ''basal'' driving force for helix association. Our present theoretical approach suggests the membrane as the actual driving force. Self-association of transmembrane helical peptides was also observed by Renthal and Velasquez[16] with the particular focus on the influence of smooth versus rough helix interface. Association energies were generally found somewhat larger than $10k_BT$ with higher values for smoother helices. Note that the lipids resided in a micellar environment which may influence the aggregation energetics and does not permit direct comparison with the present study. Still, it would be interesting to have results available as function of the lipid chain length. An experimental attempt to directly correlate transmembrane helix association with the thickness of the host bilayer was recently presented by Mall *et al.*[15] Peptides of two different hydrophobic lengths were incorporated into phosphatidylcholine bilayers of various lipid chain lengths. The experimental data obtained by a fluorescent quenching method could best be fitted by a model that assumes dimerization between helices. The corresponding association free energy was generally found to increase from about $2k_BT$ for thin membranes to $\approx 4k_BT$ for thick membranes; at the same time it was essentially independent on the peptide length. Thus, a mismatch hypothesis would not be in agreement with the particular system studied by Mall *et al.* Yet, for other systems the mismatch hypothesis has proven to be a useful concept. Among many available examples (for a recent review, see Lee[4]) we mention a study by Ren *et al.*[9] who find a membrane-matching poly-leucine peptide to reside in the host membrane in transmembrane orientation. For sufficiently large positive or negative mismatch the peptide was found to either oligomerize or to adopt a nontransmembrane orientation.

In summary, currently available experimental results on transmembrane helix association point at a rather complex, system dependent, energetics. Even though the predictions of our present study are not sufficient to explain the various experimental findings they can—due to their generic nature—be expected to contribute to the energetics of *all* events of helix dimerization in lipid membranes.

VI. CONCLUDING REMARKS

The present approach allow us to derive the membrane-mediated interaction between two cylindrical inclusions embedded in a lipid bilayer. This interaction was analyzed in terms of the bilayer properties and the hydrophobic mismatch. The new aspect in this work is the consideration of the inclusion-induced conformational confinement of the lipid chains. We have cast the underlying physics into a simple (approximate) theoretical description. Our analysis suggests that there is a direct (nonmonotonic) contribution to the inclusion–inclusion interaction arising from the confor-

mational confinement of the lipid chains. However, even more important is the influence of the conformational restrictions on the elastic membrane interactions. The coupling between both leads to a characteristic asymmetry of the inclusion-induced interactions with respect to the hydrophobic mismatch. That is, positive hydrophobic mismatch generally leads to strong attraction whereas negative mismatch merely gives rise to an energetic barrier.

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