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ARTICLE

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Protein-induced bilayer deformations: the lipid tilt degree of freedom

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Abstract The theory of hydrophobic interaction between a transmembrane protein and a lipid bilayer is reinvestigated. The protein is modeled as a cylindrically symmetric rigid inclusion, residing in a symmetric, tension-free lipid bilayer. The hydrophobic coupling between the inclusion and the lipids may induce an elastic bilayer deformation, which is commonly described in terms of stretching (or compressing) the hydrocarbon chains of the lipids. In the present work, we additionally include the possibility of the average lipid director to tilt with respect to the normal direction of the hydrocarbon-water interface. The corresponding membrane deformation energy is expressed using both a phenomenological description of elastic lipid layer perturbations and employing a specific molecular lipid model. The molecular lipid model accounts for head group repulsions, interfacial tension, and the chain conformational free energy. Assuming incompressibility of the hydrocarbon chains, we estimate and compare typical membrane deformation energies induced by single gramicidin A channels, with and without the lipid tilt degree of freedom taken into account. The membrane deformation energies are conveniently expressed using a spring constant. We argue that the consideration of the lipid tilt degree of freedom leads to a severalfold reduction of the spring constant and should thus not be excluded from the description of protein-induced membrane deformations. Possible limits of membrane elasticity-based theories for lipid-protein interactions are discussed. Finally, we calculate inclusion-induced deformations of electrostatically charged bilayers, illuminating the coupling between electrostatic and elastic energies in charged membranes.

Key words Lipid-protein interaction · Membrane elasticity · Lipid tilt · Gramicidin A

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Introduction

Lipid membranes are self-assembled, soft materials, capable of incorporating various hydrophobic or amphipathic molecules like proteins, peptides, steroids, or cosurfactants. There are many examples of proteins that adopt a transmembrane orientation, thus spanning the whole lipid bilayer. In all these cases the protein can be characterized by a hydrophobic span which is comparable in length to the bilayer thickness. Even a certain degree of hydrophobic mismatch between the protein and lipid membrane can be tolerated. One among some other possible consequences of this mismatch (Killian 1998) is that the lipids, at least partially, adopt the hydrophobic thickness of the embedded protein to achieve an optimal shielding of the hydrophobic from the aqueous environment.

The hydrophobic mismatch hypothesis has attracted much interest, both theoretical and experimental. Hydrophobic coupling between lipids and proteins has been suggested to modify protein-protein interactions. For example, interactions between rhodopsin depends on the thickness of the host membrane (Ryba and Marsh 1992). Also, hydrophobic mismatch has been shown to induce a shift in the gel-to-liquid phase transition temperature (Piknova et al. 1993). Finally, the synthetic, α-helical WALP peptides induce a change in the mean hydrophobic thickness of a bilayer that correlates with the hydrophobic mismatch (de Planque et al. 1998). Some of these and other observations have been discussed in terms of theoretical approaches, including statistical lattice theories (Sperotto 1997), microscopic and phenomenological molecular models (Marčelja 1976; Mouritsen and Bloom 1984; Fattal and Ben-Shaul 1993), and molecular dynamics simulations (Chen et al. 1997).

At present, an important tool to study the energetics of membrane perturbations is gramicidin A (gA), a small uncharged peptide spanning a single leaflet of the bilayer as a $\beta^{6.3}$-helix. Upon N-N transmembrane dimerization a cation-selective channel is formed whose average

lifetime is well known to depend on the properties of the host membrane. In its conducting, dimerized state the hydrophobic thickness of gA has been estimated to be $2b_P = 22$ Å (Elliott et al. 1983) which, in general, may be different from the hydrophobic bilayer thickness. The formation of a single gA dimer will then induce a local elastic deformation of the lipid monolayers. The corresponding energy cost, ΔF, has been estimated based on a thermodynamic analysis of experimentally determined average channel lifetimes (Kolb and Bamberg 1977; Elliott et al. 1983) as a function of the lipid bilayer thickness. To this end, a linear spring constant, H, was defined via $\Delta F = 4H\,(b_P - b_0)^2$ where b_P is the gA-induced hydrophobic monolayer thickness in immediate vicinity to the channel, and b_0 is the relaxed, unperturbed hydrophobic monolayer thickness far away from the inclusion (Nielsen et al. 1998). A value of $H = 0.28\ k_B T$ Å^{-2} was estimated for a gA channel/monoacylglyceride bilayer system (Lundbæk and Andersen 1999) (k_B is the Boltzmann constant and T the absolute temperature).

In another study, the gA channel lifetime was measured in electrically charged membranes (Lundbæk et al. 1997). It was found that changes in the electrostatic bilayer energy influence the gA channel lifetime. This is remarkable because the gA channel itself is uncharged. The results have thus been interpreted as a coupling of electrostatic and elastic membrane interactions (Lundbæk et al. 1997).

Theoretical models to calculate inclusion-induced bilayer deformation energies have been based on phenomenological (liquid-crystal) elasticity theories that include chain compression, tension, and splay terms (Huang 1986; Dan et al. 1993, 1994; Aranda-Espinoza et al. 1996; Ring 1996; Nielsen et al. 1998; Harroun et al. 1999b). Minimization of the corresponding elastic energy allowed determination of the bilayer deformation profile and ΔF (and from this H). It has been noticed long ago that a more complete phenomenological description of ΔF should also contain an energy contribution related to the tilt of the lipid molecules (Helfrich 1973). However, the lipid tilt degree of freedom is usually not taken into account, perhaps partly because the corresponding lipid tilt modulus is not known from experiment.

Attempts to include the lipid tilt degree of freedom have been made based on a general phenomenological expression for the elastic free energy (Fournier 1998) and using a simple molecular lipid model (May and Ben-Shaul 1999). In these studies, interactions between membrane inclusions have been investigated. Correlations between the size and shape of inclusions and their ability to form ordered arrays in membranes or even to induce structural transitions of the lipid layers were derived. Yet, no systematic approach has been undertaken so far to calculate the spring constant, H, with special emphasis on the lipid tilt degree of freedom.

The theoretical calculations of ΔF are usually based on the hypothesis of strong hydrophobic coupling, which assumes perfect hydrophobic matching between the protein and bilayer. The exposure of nonpolar residues should only take place if the membrane deformation energy becomes larger than the energy cost of hydrophobic mismatch. It has been argued that this should start only once the hydrophobic mismatch is larger than about 5 Å (Harroun et al. 1999b). An experimental study, however, estimated only a partial matching between the synthetic WALP peptides and phosphatidylcholine bilayers of varying thicknesses (de Planque et al. 1998). Apart from the degree of the hydrophobic coupling, it is also uncertain what actually is the preferred hydrophobic thickness of a lipid bilayer at the contact region to a transmembrane protein. For example, it was found that gA in a dimyristoylphosphatidylcholine (DMPC) bilayer induces an increase in the hydrocarbon chain order, suggesting a gA-induced membrane thickening (de Planque et al. 1998). This finding is supported by a recent computer simulation of gA in DMPC (Chiu et al. 1999), where it was also concluded that essentially only one lipid shell surrounding the gA channel is perturbed compared to the bulk lipids. On the other hand, X-ray diffraction studies suggest that gA in DMPC membranes leads to a thinning of the average bilayer thickness (Kobayashi and Fukada 1998; Harroun et al. 1999a). Even though different studies use different techniques and gA concentrations, one may conclude that a clear understanding of what bilayer deformation gA induces has not yet emerged and that, perhaps, specific lipid-protein interactions (i.e. hydrogen bonds) may significantly modify the degree of hydrophobic coupling.

The aim of the present work is twofold. First, we present a phenomenological theory for single inclusion-induced deformations of symmetric membranes that takes into account the lipid tilt degree of freedom. Here, the elastic energy of a lipid molecule is expressed in terms of elastic moduli. The possibility of the lipids to tilt away from the normal direction of the hydrocarbon-water interface gives rise to the appearance of one additional elastic modulus, namely the tilt modulus. We show that in the limit of an infinitely high tilt modulus the theory reduces to earlier elasticity theories in which the lipid tilt was not included. Second, we use a specific molecular lipid model to calculate the elastic moduli (and thus ΔF). Since we are interested in a more realistic description of the membrane energetics, we have slightly modified our molecular model compared to an earlier version (May and Ben-Shaul 1999). In particular, we choose an elastic energy contribution for the lipid chains which is based on statistical thermodynamic mean-field calculations of the chain conformational free energy. The head group and interfacial contributions to the molecular free energy are approximated by the common opposing-forces model (Israelachvili 1992). Using this molecular lipid model, we show that H is severalfold larger if the lipid tilt degree of freedom is not taken into account. Based on this finding we suggest that lipid tilt should not be neglected when analyzing inclusion-induced bilayer deformations.

Motivated by experiments on gA lifetime in charged bilayers (Lundbæk et al. 1997), we finally use the molecular model to calculate the change in ΔF upon an electrical charging of the lipids. This demonstrates the interdependence of electrostatic and nonelectrostatic energies in charged bilayers.

Theory

Consider a single inclusion in a one-component, symmetric, tension-free lipid bilayer which is well above its main transition temperature. We shall treat the inclusion as a cylindrically symmetric rigid body with its axis of symmetry perpendicular to the bilayer midplane. Further, we assume the inclusion to be symmetric with respect to reflection through the bilayer midplane. Strong hydrophobic coupling between the inclusion and the bilayer induces a local deformation of the two monolayers. This monolayer deformation depends only on the distance, r, to the inclusion midaxis (measured, say, at the bilayer midplane), but not on the azimuthal angle, ψ (see Fig. 1).

We characterize the lipid molecules by two degrees of freedom. One is the tilt angle, $\theta(r)$, of the average chain director with respect to the normal direction of the bilayer midplane, and the other one is the effective chain length, $b(r)$, as schematically depicted in Fig. 1. Note that neither the effective lipid chain length nor the tilt angle are static quantities for a lipid layer in the liquid, disordered, state. Rather, they result from an averaging over many different chain conformations. Knowing the functions $\theta(r)$ and $b(r)$ for $r_P \le r < \infty$ and $0 \le \psi < 2\pi$ uniquely defines the lipid packing in a membrane that contains a single inclusion of radius r_P at its waist. For example, the hydrophobic thickness at position $\bar{r} = r - b(r)\sin\theta(r)$ is given by $h(\bar{r}) = b(r)\cos\theta(r)$. Note that $\theta(r) \equiv 0$ describes a planar monolayer. The functions $\theta(r)$ and $b(r)$ also determine the tilt angle, $\phi(r)$, of the lipid chain director with the normal of the hydrocarbon-water interface; this angle is given by:

$$\phi = \theta - \arctan\left(\frac{b'\cos\theta - b\theta'\sin\theta}{1 - b'\sin\theta - b\theta'\cos\theta}\right) \tag{1}$$

where here and in the following the prime denotes the derivative with respect to r. In the special case $\phi \equiv 0$ the chains point normal to the hydrocarbon-water interface and the two degrees of freedom, b and θ, are no longer independent of each other. From Eq. (1) we find for $\phi = 0$:

$$b' = \sin\theta \tag{2}$$

This exactly is the condition under which inclusion-induced membrane deformation energies are usually derived. Allowing for $\phi \neq 0$ is the subject of the present work.

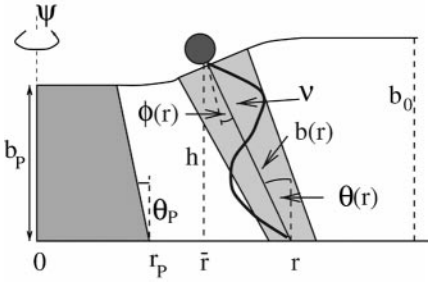

Fig. 1 Local characteristics of a lipid molecule of chain volume v residing in the upper monolayer of a symmetric bilayer: $b(r)$ is the average chain length, $\theta(r)$ is the tilt angle with respect to the normal of the bilayer midplane, and $\phi(r)$ is the angle between the local chain director and the normal of the hydrocarbon-water interface. The hydrophobic thickness of the relaxed monolayer far away from the inclusion is b_0. The inclusion is characterized by its height, $2b_P$, by the radius of its waist, r_P, and by its shape, θ_P

Phenomenological free energy

In this section we present a phenomenological description for inclusion-induced deformations of laterally isotropic membranes. Consider the change in free energy per molecule, $\Delta f = f - f_0$, with respect to the unperturbed, planar lipid layer, which is characterized by an equilibrium hydrophobic thickness b_0, that is $f_0 = f(b \equiv b_0, \theta \equiv 0)$. Throughout this work we shall assume incompressibility of the chain's hydrophobic volume, v, implying a molecular cross sectional area $a_0 = v/b_0$ for the unperturbed, planar membrane.

We may write f as a function of the (cylindrically symmetric) deformation profile around a single membrane inclusion up to quadratic order in b and θ as well as their first derivatives, b' and θ':

$$\frac{f}{a_0} = \frac{K}{2}\left(\frac{b}{b_0} - 1\right)^2 + \frac{\kappa}{2}\left(\theta' + \frac{\theta}{r} + \tilde{c}_0\right)^2 + \frac{\bar{k}}{r}\theta\theta' + \rho\left(\theta' + \frac{\theta}{r}\right)\left(\frac{b}{b_0} - 1\right) + \frac{k_t}{2}(\theta - b')^2 \tag{3}$$

The first term in Eq. (3) describes a compression-expansion of the planar lipid layer; the corresponding elastic modulus is K. The second and third terms in Eq. (3) account for the elastic energy of splay $\theta' + \theta/r$ and saddle-splay $\theta\theta'/r$ (Frank 1958), κ and $\bar{k}$ are the corresponding moduli, and $\tilde{c}_0$ is the optimal splay. Note that $\theta' + \theta/r$ and $\theta\theta'/r$ are respectively the trace and determinant of the tilt tensor (Hamm and Kozlov 1998) for a cylindrically symmetric lipid layer deformation.

The lipid splay, $\theta' + \theta/r$, and the relative chain compression-expansion, $b/b_0 - 1$, need not be independent from each other, which gives rise to the appearance of a coupling term in Eq. (3) and an elastic modulus, ρ. Finally, the last term in Eq. (3) describes the energy cost of tilting the lipid director with respect to the normal of the hydrocarbon-water interface; the corresponding tilt

modulus is k_t. Here, we have used the relation $\phi = (\theta - b')$ which follows from Eq. (1) and is valid up to first order in b, θ, b', and θ'. The presence of membrane tension would create an additional term $\sim b/b_0 - 1$; however, we do not include such a contribution because lipid membranes are usually free to optimize their lateral extension.

The expression for f in Eq. (3) is, of course, not the most general way to write down the elastic free energy of a lipid molecule. Rather it is appropriate, given that the bilayer can be treated as an incompressible elastic medium with certain common properties typical for lipids in the liquid-disordered state.

The inclusion-induced elastic deformation energy of the bilayer membrane is the sum over all molecular contributions from both lipid monolayers:

$$\begin{aligned}\Delta F &= 2\int \Delta f(r,\psi)\mathrm{d}n \\ &= \frac{4\pi}{v}\int_{r_P}^{\infty}\Delta f(r) g_V(r) r\,\mathrm{d}r = 4\pi\int_{r_P}^{\infty}\bar{f} r\,\mathrm{d}r\end{aligned} \tag{4}$$

where, $\mathrm{d}n = g_V(r) r \mathrm{d}r\mathrm{d}\psi/v$ denotes the number of lipids whose chain ends are located within the area element $r\mathrm{d}r\mathrm{d}\psi$. The function g_V denotes the average chain length of the $\mathrm{d}n$ lipids. Note that for $\theta \equiv 0$ it is simply $g_V = b$. For an arbitrary choice of $b(r)$ and $\theta(r)$ one finds (May and Ben-Shaul 1999):

$$\begin{aligned}g_V(r) = b\cos\theta - \frac{b^2}{2}\theta' + \frac{b^3\theta'}{2r}\sin\theta\left(1 - \frac{1}{2}\sin^2\theta\right) \\ - \frac{b^2}{2r}\sin\theta\cos\theta\left(1 - \frac{b'}{2}\sin\theta\right)\end{aligned} \tag{5}$$

Defining the relative chain compression $s = (b/b_0 - 1)$ and expanding the local free energy density per unit area, $\bar{f} = \Delta f g_V/v$, in Eq. (4) up to second order in s, s', θ, and θ' we obtain:

$$\begin{aligned}\bar{f} = \frac{K}{2}s^2 + \tilde{c}_0\kappa\left(\theta' + \frac{\theta}{r}\right) \\ + \frac{\kappa}{2}(1 - b_0\tilde{c}_0)\left(\theta' + \frac{\theta}{r}\right)^2 + \frac{\bar{k}}{r}\theta\theta' \\ + (\rho + \tilde{c}_0\kappa)s\left(\theta' + \frac{\theta}{r}\right) + \frac{k_t}{2}(\theta - b_0 s')^2\end{aligned} \tag{6}$$

From Eqs. (4) and (6) one may derive the Euler-Lagrange equations governing the equilibrium deformation profile of the bilayer membrane in the vicinity of the inclusion. The result is two coupled differential equations for the unknown functions $s(r)$ and $\theta(r)$:

$$\begin{aligned}s'' + \frac{s'}{r} = \frac{K}{b_0^2 k_t}s + \frac{\rho + \tilde{c}_0\kappa + b_0 k_t}{b_0^2 k_t}\left(\theta' + \frac{\theta}{r}\right) \\ \left(\theta' + \frac{\theta}{r}\right)' = -\frac{\rho + \tilde{c}_0\kappa + b_0 k_t}{\kappa(1 - b_0\tilde{c}_0)}s' + \frac{k_t}{\kappa(1 - b_0\tilde{c}_0)}\theta\end{aligned} \tag{7}$$

Obviously, the saddle-splay modulus, $\bar{k}$, is irrelevant for the lipid layer deformation profile around a given rigid inclusion. The contribution of the saddle-splay term to ΔF is only a constant, namely $-2\pi\bar{k}\theta_P^2$, where θ_P characterizes the shape of the inclusion (see Fig. 1). The saddle-splay modulus becomes important if the inclusion would have internal degrees of freedom that allow its shape to adjust (Kim et al. 1998).

Equations (7) have to be solved subject to appropriate boundary conditions. Far away from the inclusion the membrane is unperturbed, implying $s'(\infty) = 0$ and $\theta(\infty) = 0$. Close to the inclusion, our assumption of strong hydrophobic coupling determines the remaining boundary conditions. That is, the lipids must adopt the size and shape of the hydrophobic core of the inclusion and thus:

$$s(r_P) = \frac{b_P}{b_0\cos\theta_P} - 1 \qquad \theta(r_P) = \theta_P \tag{8}$$

Here, the angle θ_P determines the shape of the inclusion (see Fig. 1). This introduces in a natural way a shape classification according to which an inclusion may be barrel-like ($\theta_P > 0$), vase-like ($\theta_P < 0$), or cylindric ($\theta_P = 0$).

Let us now consider the situation that the lipid tilt degree of freedom is not taken into account. We obtain this case in the limit $k_t \to \infty$, that is for an infinitely high tilt modulus. Then, we obtain from the first equation of Eqs. (7) the condition $\theta = b_0 s'$. That means there is no tilt between the lipid director and the normal direction of the hydrocarbon-water interface. In other words, the condition $\phi = 0$ [see Eqs. (1) and (2)] is fulfilled everywhere. After inserting $\theta = b_0 s'$ into Eq. (6) we obtain

$$\begin{aligned}\bar{f} = \frac{K}{2}s^2 + b_0\left(s'' + \frac{s'}{r}\right)[(\kappa\tilde{c}_0 + \rho)s + \tilde{c}_0\kappa] \\ + \frac{b_0^2}{2}\kappa(1 - \tilde{c}_0 b_0)\left(s'' + \frac{s'}{r}\right)^2 + b_0^2\bar{k}\,\frac{s's''}{r}\end{aligned} \tag{9}$$

and the corresponding Euler-Lagrange equation for the relative chain compression, $s(r)$, is:

$$\nabla^4 s = -\frac{1}{b_0\kappa(1 - \tilde{c}_0 b_0)}\left[\frac{K}{b_0}s + 2(\tilde{c}_0\kappa + \rho)\left(s'' + \frac{s'}{r}\right)\right] \tag{10}$$

where $\nabla^4 s = s'''' + 2s'''/r - s''/r^2 + s'/r^3$ is the radial component of the biharmonic operator. Equations (9) and (10) (with $\tilde{c}_0 b_0 \ll 1$) have been used in a number of approaches to calculate membrane deformation energies (Dan et al. 1993, 1994; Aranda-Espinoza et al. 1996). In some cases $\rho = 0$ was investigated, which corresponds to the description of the monolayer with respect to the so called neutral surface (see below), where bending and stretching deformations decouple (Huang 1986; Helfrich and Jakobsson 1990; Ring 1996; Nielsen et al. 1998; Harroun et al. 1999b).

The boundary conditions for Eq. (10) are determined by our requirement that the lipid chains facing the

inclusion not only match its hydrophobic length but also adopt its shape. Using $\theta = b'$, the boundary conditions in Eq. (8) read $s(r_P) = s_P + \theta_P^2/2$ and $s'(r_P) = \theta_P/b_0$. In the Results and discussion section we will compare membrane deformation energies for free lipid tilt relaxation [based on solutions of Eqs. (7)] and for suppressed tilt [solving Eq. (10)].

To work directly with the phenomenological free energy given in Eq. (3) requires us to know all material constants. Since not all of them are easily determined experimentally, it is more convenient to use a simple model for the molecular free energy, f, and to express the required material constants in terms of typical molecular interaction parameters. This is the subject of the next section.

Molecular free energy

We suggest the following expression for the free energy of a lipid molecule

$$f = \gamma a_i + \frac{B}{a_h} + \tau(b - l_c)^2 \tag{11}$$

Here, a_i and a_h are respectively the molecular cross sectional areas at the hydrocarbon-water interface and at the head group region. Our model is based on the assumption that the head groups interact only within a given surface located at fixed distance l_h above the hydrocarbon-water interface. In Eq. (11), $\gamma = 0.12\ k_BT$ Å^{-2} is the surface tension exerted at the hydrocarbon-water interface and B is a head group interaction parameter. The first two terms in Eq. (11) are well known as the opposing forces model (Israelachvili 1992). The third term in Eq. (11) is a phenomenological expression characterizing the chain conformational free energy, f_C. Here, it is assumed that this energy depends only on the effective chain length b. In the Results and discussion section we show that the proposed quadratic dependence is a good approximation for a planar lipid layer and relatively small changes of its thickness.

For non-planar lipid monolayer deformation profiles the chain conformational free energy in Eq. (11) may depend not only on the local chain length, b, but also on the aggregation geometry (i.e. its curvature). This dependence is neglected in the present approach.

Note that τ in Eq. (11) is the chain interaction strength and l_c is the optimal (effective) chain length for a planar bilayer. The numerical values for τ and l_c can be estimated from statistical mean-field calculations of chain packing based on a realistic acyl chain model (see Results and discussion section). For $l_c = 0$ the present model for f_C reduces to the simple compression model, which has been used recently to derive inclusion-induced deformation energies in lipid membranes (May and Ben-Shaul 1999). However, we will argue below that choosing a nonvanishing value for l_c is appropriate to approach a more realistic description of inclusion-induced membrane deformations.

Equation (11) provides a simple scheme to predict lipid aggregation geometries based on molecular interaction parameters. Note that the molecular model takes into account characteristic interactions in lipid bilayers, namely head group and chain repulsions and attractive interfacial tension. However, the model is not specific to a certain given lipid as it is not designed to account for lipid specific details like chain double bonds, or the ability of the head groups to form hydrogen bonds among each other.

Let us now investigate the relation between the molecular parameters of f in Eq. (11) and the material constants introduced in Eq. (3). It is convenient to introduce the dimensionless molecular parameters

$$\bar{B} = \frac{Bb_0^2}{\gamma v^2},\ \bar{\tau} = \frac{b_0^3\tau}{\gamma v},\ \bar{l}_h = \frac{l_h}{b_0},\ \bar{l}_c = \frac{l_c}{b_0} \tag{12}$$

The molecular area a of a planar lipid layer is characterized by $a = a_h = a_i = v/b$. A simple calculation shows that the relation

$$\bar{\tau} = \frac{1 - \bar{B}}{2(1 - \bar{l}_c)} \tag{13}$$

ensures that $b_0 = v/a_0$ is the hydrophobic thickness of a planar lipid layer in equilibrium. We thus define the molecular free energy with respect to the planar, unperturbed state, $\Delta f = f - f_0$ with $f_0 = f(b = b_0, \theta = 0) = \gamma v(3 + \bar{B} + \bar{l}_c\ (\bar{B} - 1))/(2b_0)$.

To relate the molecular areas a_i and a_h to the lipid layer deformation profile $b(r)$ and $\theta(r)$ we employ our assumption of a uniform (liquid-like) chain segment density throughout the hydrophobic region. Then v is constant and independent of $b(r)$ and $\theta(r)$. Recall the function g_V in Eq. (5) which determines the number $dn = g_V dA/v$ of lipids whose chain ends (on average) fall within the area element $dA = r dr d\psi$, measured at the midplane. In a similar way one may introduce the function g_A which relates the interfacial area $dA_i = g_A(b,\theta)dA$ of the dn lipids to dA. Analogously, $dA_h = g_A(b + l_h,\theta)dA$ is the cross-sectional area of the dn lipids measured at the head group interaction surface. Usage of g_V and g_A allows us to calculate the molecular cross-sectional areas $a_i(r) = dA_i/dn = v g_A(b,\theta)/g_V(b,\theta)$ and $a_h(r) = dA_h/dn = v g_A(b + l_h,\theta)/g_V(b,\theta)$. For the function $g_A(r)$ one finds (May and Ben-Shaul 1999)

$$g_A(r) = \left(1 - \frac{b}{r}\sin\theta\right) \times \sqrt{1 + b'^2 + b^2\theta'^2 - 2(b'\sin\theta + b\theta'\cos\theta)} \tag{14}$$

Using the expressions for g_V and g_A it is possible to expand f in Eq. (11) in terms of b, b', θ, and θ' up to quadratic order around the equilibrium $b = b_0$ and $\theta = 0$. Comparison of the resulting expression to the phenomenological free energy in Eq. (3) allows us to

express the material parameters in terms of the molecular interaction constants. The result is

$$
\begin{aligned}
&k_t = \gamma(1-\bar{B}) \quad \rho = \gamma b_0 \bar{B}(1+\bar{l}_h)\\
&\bar{k} = \gamma\frac{b_0^2}{2}\left\{1-\bar{B}\left[1+2\bar{l}_h(2+\bar{l}_h)\right]\right\}\\
&K = \gamma\frac{3-\bar{B}-2\bar{l}_c}{1-\bar{l}_c}\\
&\kappa\tilde{c}_0 = -\gamma\frac{b_0}{2}\left[1-\bar{B}\ (1+2\bar{l}_h)\right]\\
&\kappa = \gamma\frac{b_0^2}{2}\left[2\bar{B}(1+\bar{l}_h)\ (1+2\bar{l}_h)-1\right]
\end{aligned}
\tag{15}
$$

Inserting these expressions into Eqs. (7) determines the lipid layer equilibrium conformation [defined by the functions $s(r)$ and $\theta(r)$] in the vicinity of a single inclusion in terms of molecular interaction parameters. Once $s(r)$ and $\theta(r)$ are found, they may be used in Eqs. (4) and (6) to calculate the inclusion-induced membrane perturbation energy ΔF.

Let us briefly discuss two special cases. The limit of the simple opposing forces model (where lipid chain interactions are not taken into account) is obtained for $l_c = b_0$ or for $\tau = 0$. Then, because of Eq. (13) it must be $\bar{B} = 1$, or equivalently $a_0 = v/b_0 = (B/\gamma)^{1/2}$. Since chain interactions are ignored, there is no resistance against tilt of the lipid director and thus $k_t = 0$.

Another limit is, perhaps, more interesting. The two monolayers of lipid membranes may have an intrinsic tendency to curve owing to a nonvanishing spontaneous curvature. A planar bilayer is then frustrated. It has been discussed that, in certain cases, this frustration can be relieved by the presence of certain peptides (Epand 1998). Note that a membrane frustration relief simply means that the membrane deformation energy, ΔF, has negative sign. The limit of vanishing head group repulsions, that is $B = 0$, provides a simple illustration of the possibility of how inclusions may stabilize frustrated lipid membranes. In view of bilayer stability, the limit $B = 0$ is, of course, not appropriate. That is, the bilayer would be unstable with respect to the formation of highly curved structures, such as the inverse hexagonal, H_{II}, phase. However, the effect we see for $B = 0$ will also be present for somewhat larger B where the bilayer would still be stable (see below). If we use $B = 0$ for the elastic constants in Eqs. (15) and insert them into the Euler-Lagrange equations, Eqs. (7), we find $s'' + s'/r = \xi^2 s$ and $\theta = b_0 s'/2$ where $\xi = 2[(1-2\bar{l}_c/3)/(1-\bar{l}_c)]^{1/2}/b_0$ is a characteristic length for the decay of the membrane perturbation. The membrane profile is now given by

$$
s(r) = s_P\frac{K_0(\xi r)}{K_0(\xi r_P)}, \quad \theta(r) = -\frac{b_0}{2}s_P\xi\frac{K_1(\xi r)}{K_0(\xi r_P)} \tag{16}
$$

where K_0 and K_1 are the modified Bessel functions of zeroth and first order, respectively. This result for $s(r)$ and $\theta(r)$ might be regarded as surprising since the tilt angle $\theta(r_P)$ can no longer be chosen independently but is coupled to the initial chain perturbation, s_P. So, what conformation does the bilayer adopt close to an inclusion that imposes a certain s_P and θ_P, which are not coupled according to Eq. (16)? The answer lies in the formation of a "micellar" region in which (for a given azimuthal angle ψ) all chain directors cross in one single point located either in the bilayer midplane (for positive mismatch) or in the hydrocarbon-water interface (for negative mismatch), as schematically illustrated in Fig. 2. It is beyond the scope of the present work to analyze ΔF in the general case for which micellar regions may occur. Our aim here is only to show that ΔF can be negative for an appropriate choice of s_P and θ_P. Let us therefore assume $\theta_P = \theta(r_P)$ according to Eq. (16). That is, negative (positive) mismatch is coupled to a barrel-like (vase-like) inclusion shape. (With this choice for s_P and θ_P, the inclusion shape corresponds to the sum of the black and shaded regions in Fig. 2. Our choice thus excludes the formation of a "micellar" lipid region.) We have calculated ΔF in the limit of a large inclusion circumference ($r_p \gg 1/\xi$). The result is

$$
\Delta F = -\gamma\pi r_P b_0^2 s_P\xi\left(1-\frac{s_P}{2}\right) \tag{17}
$$

Thus, ΔF is negative only for vase-like inclusions of positive mismatch (left illustration in Fig. 2). This result is intuitively clear since large vase-like inclusions support the preferred shape of lipids that have a tendency to form inverted phases. The numerical values for $-\Delta F$ in Eq. (17) can easily exceed $10\,k_BT$, even for $b_P - b_0 < 1$ Å. However, the stability of a bilayer membrane requires B not being too small (certainly not to vanish), and any numerical estimate based on Eq. (17) can thus only serve as an upper bound.

To sum up, the limit of vanishing head group repulsion allows a clear molecular understanding of how inclusions may act to relieve the stress of frustrated membranes and of what the optimal inclusion shape is.

Monolayer bending rigidity

We can write the molecular free energy in Eq. (3) for a cylindrically bent monolayer of main curvatures $c_1 = \tilde{c}$

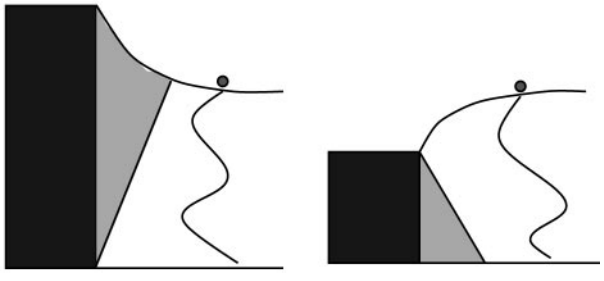

Fig. 2 In the limit of vanishing head group repulsion our model predicts the occurrence of micellar regions (*shaded regions*) if s_P and θ_P as imposed by the inclusion (*black region*) are not coupled through Eq. (16). This is illustrated here for a cylindrical inclusion of positive (*left*) and negative (*right*) hydrophobic mismatch

and $c_2 = 0$, whose lipids do not exhibit a tilt ($\phi = 0$). We obtain this case by inserting $\theta' = -\tilde{c}$ and $\theta = b'$ into Eq. (3) and by going to the limit r $\to \infty$:

$$\frac{\Delta f}{a_0} = \frac{K}{2}\left(\frac{b}{b_0} - 1\right)^2 + \frac{\kappa}{2}(\tilde{c} - \tilde{c}_0)^2 - \rho\tilde{c}\left(\frac{b}{b_0} - 1\right) \qquad (18)$$

It is well known that elastic moduli for lipid layers refer to a certain describing surface. In Eq. (18) this surface is the midplane of a symmetric bilayer. It is more common, however, to express the elastic moduli with respect to the neutral surface. For this special choice the coupling term ρ in Eq. (18) vanishes. According to Helfrich we write the elastic monolayer energy in terms of the molecular cross-sectional area a and curvature c, both measured at a distance δ from the hydrocarbon-water surface as illustrated in Fig. 3:

$$\frac{\Delta f}{a_0} = \frac{\tilde{K}}{2}\left(\frac{a}{a_0} - 1\right)^2 + \frac{k}{2}(c - c_0)^2 \qquad (19)$$

where a_0 is the equilibrium cross sectional area for a planar monolayer (Helfrich 1973). In Eq. (19), k is the Helfrich bending rigidity, c_0 is the spontaneous curvature, and $\tilde{K}$ is the area compressibility modulus. We now express b and $\tilde{c}$ as a function of c and a under the condition that the chain volume v is conserved; the result is:

$$b = \frac{v}{a}\left\{1 + c\left(\frac{v}{2a} - \delta\right) + \frac{c^2}{2}\left[\left(\frac{v}{a}\right)^2 - 3\frac{v}{a}\delta + 2\delta^2\right]\right\} \qquad (20)$$

and $\tilde{c} = c/(1-(v/a-\delta)c)$. Inserting these expressions into Eq. (18) and expanding up to quadratic order in a/a_0-1 and c we find $\tilde{K} = K$, $kc_0 = \kappa\tilde{c}_0$ and

$$\delta = \frac{b_0}{2} - \frac{\rho}{K}$$
$$k = \kappa - \frac{\rho^2}{K} - 2\tilde{c}_0\kappa\left(\frac{b_0}{2} + \frac{\rho}{K}\right) \qquad (21)$$

These relations together with Eqs. (15), allow us to calculate k and c_0 in terms of the molecular constants appearing in Eq. (11).

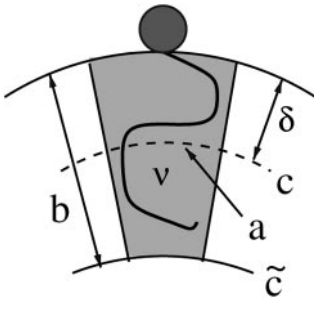

Fig. 3 A segment of a cylindrically bent monolayer. The *shaded region* corresponds to a single lipid of fixed chain volume v. The neutral surface has a molecular cross sectional area a, curvature c, and a distance δ to the hydrocarbon-water surface. Furthermore, $\tilde{c}$ is the curvature at the chain end region and b is the hydrophobic monolayer thickness

Results and discussion

The chain contribution, $f_C(b) = \tau(b-l_c)^2$, to the molecular free energy in Eq. (11) depends on the two parameters τ and l_c. We can estimate these parameters based on a statistical thermodynamic mean-field theory of chain packing in a planar, symmetric bilayer which has previously been applied to a variety of other systems and phenomena (Ben-Shaul and Gelbart 1994; Ben-Shaul 1995). In short, this theory is based on the determination of the probabilities for all possible lipid chain conformations under the constraint of a uniform (liquid-like) average chain segment density throughout the hydrophobic core. To this end, many alkyl-chain conformations are generated on a computer using the rotational isomeric state (RIS) model (Flory 1969). The constraint of a uniform chain segment density gives rise to the appearance of a lateral pressure profile. The smaller the available cross sectional area, $a = v/b$, per molecule in a planar bilayer, the larger is the lateral pressure and the higher the chain conformational energy. The minimal value of a is given by $a = v/b^*$ where b^* is the length of a fully stretched, all-*trans*, chain. Approaching $b = b^*$ leads to a steep increase in f_C as is shown in Fig. 4 for a double-chained -$(CH_2)_{13}$-CH_3 (C-14) lipid of chain volume $v = 2 \times 405$ Å^3. Our molecular model introduced in Eq. (11) assumes a quadratic dependence of f_C on b. It is indeed reasonable to fit the result of the numerical calculation in Fig. 4 by $f_C = \text{const} + \tau(b-l_c)^2$. Confining the fit to the region 10 Å $< b <$ 15 Å (solid line in Fig. 4), we obtain $\tau = 0.089\ k_BT$ Å^{-2} and $l_c = 10.3$ Å.

For a planar bilayer, the quadratic form for f_C is a good approximation in the considered b region. It should be noted, however, that our quadratic model for f_C implies another approximation because close to an

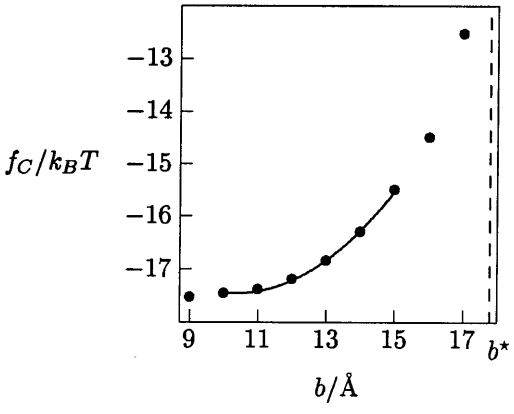

Fig. 4 The molecular chain free energy, f_C, derived from statistical mean-field calculations based on the RIS model for a saturated, double-chained C-14 chain (*solid circles*) as a function of the hydrophobic half thickness, b, of a symmetric planar bilayer. The *broken line* marks the value $b = b^*$, the length of a fully stretched chain. The *solid line* represents the quadratic fit $f_C = \text{const} + \tau(b-l_c)^2$ within the region 10 Å < b < 15 Å. We find $\tau = 0.089\ k_BT$ Å^{-2} and $l_c = 10.3$ Å

inclusion the membrane will not be perfectly planar. We thus neglect a possible dependence of τ and l_c on the local geometry.

The remaining parameters of our molecular model, B and l_h, are both lipid head group specific. However, the results of ΔF show only a weak l_h dependence. Therefore, we shall consider only one fixed value, namely $l_h = 1.7$ Å.

As we vary B the elastic properties of the monolayer will adjust. For a given B the elastic properties can be calculated through Eqs. (15), (12), and (21). If B is small the spontaneous curvature, c_0, will have a negative sign; large values of B imply positive c_0. It is well known that the stability of a lipid layer requires the relation $k > -2\bar{k} > 0$ to be fulfilled. This turns out to be the case for $344 < B/k_BT$ Å$^2 < 586$, which corresponds to $-1/51$ Å $< c_0 < 1/65$ Å. That is, $c_0 < -1/51$ Å and $c_0 > 1/65$ Å imply a lipid monolayer instability with respect to a saddle and spherical deformation, respectively.

The membrane deformation energy with and without lipid tilt relaxation

A frustration free bilayer ($c_0 = 0$) is obtained for $B = 469\ k_BT$ Å^2, further leading to $k = 7.5\ k_BT$ and $\bar{k} = -2\ k_BT$. For this special choice we display in Fig. 5

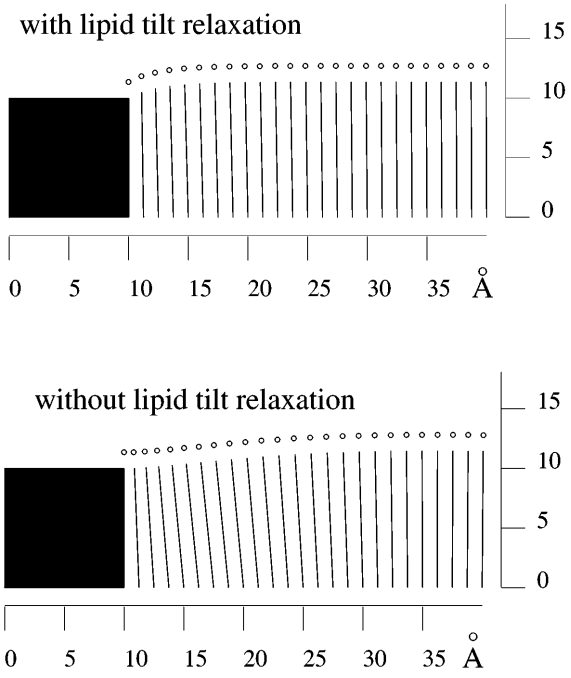

Fig. 5 The membrane deformation profile in the vicinity of a cylindrical membrane inclusion for $\tau = 0.089\ k_BT$ Å^{-2}, $l_c = 10.3$ Å, $l_h = 1.7$ Å, and $B = 469\ k_BT$ Å^2, which implies a vanishing spontaneous curvature, c_0. The *black region* marks the inclusion, whereas the *solid lines* represent the chain directors at arbitrary positions. The *circles* mark the corresponding head group positions. The *top figure* is derived for free lipid tilt relaxation, and the *bottom figure* under the constraint $\phi = 0$ (lipid tilt with respect to the interface is suppressed)

the inclusion-induced membrane deformation profile, calculated with free lipid tilt relaxation (top) and with suppressed lipid tilt (bottom), that is $\phi = 0$. The inclusion was chosen to be cylindrical ($\theta_P = 0$) with $r_P = 10$ Å and $b_P = 11$ Å, as is supposed to be appropriate for gA. We note, however, that the location of the tryptophans close to the hydrocarbon-water interface may give the gA a somewhat vase-like shape ($\theta_P < 0$). From the lipid deformation profile in Fig. 5 it is clear that owing to the cooperative action of lipid tilt and chain extension the monolayer perturbation is less pronounced compared to the case of a suppressed tilt degree of freedom.

Let us now consider the inclusion-induced membrane deformation energy, ΔF, as a function of the hydrophobic inclusion half thickness, b_P. Figure 6 shows $\Delta F(b_P)$ for several different choices of B. The solid lines are derived for cylindrical inclusions ($\theta_P = 0$), and correspond to $B = 374\ k_BT$ Å^2, $B = 469\ k_BT$ Å^2, and $B = 578\ k_BT$ Å^2, respectively. The broken line shows ΔF for $B = 374\ k_BT$ Å^2 and a vase-like inclusion with $\theta_P = -0.1$. For the left diagram in Fig. 6, the lipid tilt with respect to the hydrocarbon-water interface, ϕ, is allowed to relax whereas in the right diagram it is fixed at $\phi = 0$. For all cases displayed in Fig. 6 the deformation energies are quadratic, suggesting the use of the spring constant description (as introduced by Nielsen et al.) also for relaxed lipid tilt. For cylindrical inclusions, we always find $\Delta F(b_P = b_0) = 0$ even if the lipid layers have a high positive or negative spontaneous curvature, c_0. However, if the inclusion shape is not cylindrical the optimal mismatch may be a nonvanishing one. The appropriate spring constant description is then:

$$\Delta F = \Delta F_0 + 4H(b_P - b_P^0)^2 \qquad (22)$$

where $b^0{}_P$ is the optimal mismatch and $\Delta F_0 = \Delta F(b^0{}_P)$ the corresponding energy. In the Theory section we have seen that certain vase-like inclusions may imply $\Delta F < 0$

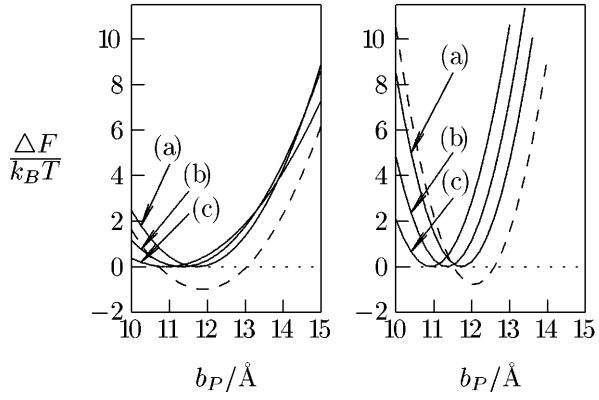

Fig. 6 Bilayer deformation energy for $B = 374\ k_BT$ Å^2 (*a*), $B = 469\ k_BT$ Å^2 (*b*), and $B = 578\ k_BT$ Å^2 (*c*) as a function of b_P. All *solid lines* correspond to $\theta_A = 0$. The *dashed line* was derived for $B = 374\ k_BT$ Å^2 and $\theta_A = -0.1$. In the *left diagram* the tilt degree of freedom is taken into account whereas in the *right diagram* any lipid tilt with respect to the interface is suppressed ($\phi = 0$)

in the limit of vanishing head group interaction. In Fig. 6 (broken lines) we see that this conclusion remains valid for frustrated but stable bilayer membranes. In fact, for our example ($\theta_P = -0.1$ and $c_0 = -1/70$ Å, see Table 1) not only a positive ($b_P > b_0$) but also a negative ($b_P < b_0$) mismatch may stabilize the bilayer if the mismatch is not too large. Note that we also found that vase-like inclusions will not stabilize lipid layers of vanishing or positive spontaneous curvature. Analogously, barrel-like inclusions of moderate mismatch may stabilize membranes whose lipid layers have positive c_0 (results not shown).

The comparison of the left and right diagrams in Fig. 6 suggests the lipid tilt degree of freedom to be important for the magnitude of H. With suppressed lipid tilt, H is generally several times larger than without. The numerical values for the spring constant from Fig. 6 are listed in Table 1 (where H denotes the spring constant for free tilt relaxation and $H_{\phi=0}$ is the spring constant for suppressed tilt). As was already mentioned, our molecular free energy model is not specific to a certain lipid. Thus, we do not intend to quantitatively compare the values for H to the experimental one [$H = 0.28\ k_BT$ Å^{-2} for the gA channel/monoacylglyceride bilayer system (Lundbæk and Andersen 1999)]. Yet, it is a satisfying result that the experimental value is somewhat larger than our estimate for free tilt relaxation and somewhat smaller for suppressed tilt relaxation.

A comparison that can be made is with the recent calculation of Fattal and Ben-Shaul (1993), which was based on a microscopic lipid model and a molecular theory of chain packing statistics. In their work, ΔF was calculated based on the same mean-field theory that is employed in the present work to calculate τ and l_c (see Fig. 4). However, in the work of Fattal and Ben-Shaul the dependence of the chain conformational energy on the presence of the inclusion wall as well as on the local geometry was taken into account (whereas both are neglected in the present work). On the other hand, ΔF was (1) not minimized with respect to the lipid head group distribution on the interface, (2) based on an exponential membrane shape relaxation, and (3) calculated in the limit that r_P is much larger than the typical membrane relaxation length (found to be ≈3–7 Å). Note that, despite the various approximations, both approaches predict very similar monolayer perturbation profiles, including the lipid tilt angle relaxation. Based on Fig. 8 of their paper (Fattal and Ben-Shaul 1993) one can estimate $H \approx 0.28\ k_BT$ Å^{-2} for a cylindrical inclusion of $r_P = 10$ Å in a membrane composed of C-14 lipids and a head group interaction parameter of $B = 120\ k_BT$ Å^{-2}. This should be contrasted to $H = 0.34\ k_BT$ Å^{-2} which we obtain using the present approach (H is virtually independent on whether we chose $l_c = 1.7$ Å or $l_c = 0$). Even though the membrane perturbation profile and the head group distribution on the interface is not exactly the same, we may conclude that both theories lead to similar spring constants. This has an important consequence. It suggests that that the confinement of the lipid chain configurational freedom owing to the presence of the rigid inclusion does not strongly affect the microelasticity close to the inclusion. This is thus an argument in favor of the applicability of simple phenomenological, liquid-crystal theory-based approaches (like the present one).

Let us briefly discuss why continuum theories like the present one may nevertheless fail to correctly describe the membrane deformation energy, ΔF. First of all, continuum theories tacitly assume that all characteristic lengths are large compared to typical molecular dimensions. However, our results imply that the membrane perturbation extends only over very few lipid layers.

Further, the molecular model according to Eq. (11), in particular the head group energy, may have shortcomings. For example, it does not assign any bending stiffness to the lipid head groups. In other words, if the neutral surface during bending should coincide with the head group interaction surface, then bending of the head group region would not cost any elastic energy. Further, there may be head group interactions, like the formation of hydrogen bonds, that are not likely to follow a simple $1/a_h$ dependence.

Usage of the phenomenological model according to Eq. (3) instead of a molecular model depends on the knowledge of the elastic constants. Some of the uncertainties to assign correct values to the elastic moduli for a given system (like the monoacylglyceride bilayer) have been discussed recently (Nielsen et al. 1998). The tilt modulus, k_t, has not yet been determined experimentally.

Finally, specific interactions between the inclusion and the lipid head groups may change the microelasticity close to the inclusion. For example, it was suggested that the presence of gA in membranes partly dehydrates the lipids and induces a concomitant local increase in the molecular cross sectional area (Ge and Freed 1999).

In view of all these sources of error it is remarkable that liquid-crystal theory-based predictions of ΔF have successfully been used to explain experimental results concerning the lifetimes of gA channels. The advantage of using a molecular model like the one in Eq. (11) is that simple modifications of lipid properties, like changing the chain length, modifying the electric charge of the head group (May 1996), or even using mixed membranes

Table 1 Head group repulsion parameter, B (in units of k_BT Å^2), inclusion tilt, θ_P, lipid layer equilibrium hydrophobic thickness, b_0, bending rigidity, k, spontaneous curvature, c_0, and the spring constants (in units of k_BT Å^{-2}) corresponding to the systems shown in Fig. 6. For free and for suppressed lipid tilt relaxation the spring constants are denoted by H and by $H_{\phi=0}$, respectively

B	θ_P	b_0 (Å)	c_0 (Å^{-1})	k/k_BT	H	$H_{\phi=0}$
374	0	11.7	−1/70	7.6	0.21	0.71
469	0	11.3	0	7.5	0.16	0.67
578	0	10.9	1/70	7.0	0.11	0.61
374	−0.1	11.7	−1/70	7.6	0.18	0.66

(May and Ben-Shaul 1995) can be studied systematically. Since measurements have been performed for charged membranes (Lundbæk et al. 1997) we shall investigate in the following how modifications of the electrostatic energy would be expected to change ΔF.

Electrically charged bilayers

In Eq. (11) we have introduced a molecular model that we use to describe the energetics of a lipid molecule in a membrane. The head group contribution in this model is based on a $1/a_h$ dependence of the lipid head group energy on the molecular head group area and a parameter, B, describing the interaction strength. In general, there may be energies of quite different physical origin contributing to B, like steric repulsions, hydration forces, and also electrostatic interactions. Our aim in the following is to investigate inclusion-induced membrane deformation energies of electrically charged membranes. It is therefore convenient to separate the contribution of the lipids net charge, B_{et}, from all other head group contributions, B_0 (all nonelectrostatic as well as dipolar and higher order electrostatic ones). That is, we write $B = B_0 + B_{et}$, where B_{et} is the first-order coefficient of a series expansion of the electrostatic energy, f_{et}, with respect to $1/a_h$. Thus:

$$B_{et} = -a_0^2 \left(\frac{\partial f_{et}(a_h)}{\partial a_h} \right)_{a_h = a_0} \tag{23}$$

In the present work we will approximate f_{et} by the well-known expression for the electrostatic free energy of a perfectly flat, uniformly charged surface in contact with a 1:1 electrolyte based on Poisson-Boltzmann theory:

$$\frac{f_{et}}{k_B T} = 2\lambda \left[\frac{1-q}{p} + \ln(p+q) \right] \tag{24}$$

with $q = \sqrt{p^2+1}$ and $p = 2\pi\lambda l_B l_D / a_h$. Here, $l_B = 7.14$ Å is the Bjerrum length, l_D is the Debye length, and λ is the charge fraction per lipid. That is for $\lambda = 0$ the lipids are uncharged and for $\lambda = 1$ each lipid head group carries one, say, negative charge. One may calculate B_{et} and the result for B is then:

$$B = B_0 + \frac{a_0^2 (q-1)}{\pi l_B l_D} \tag{25}$$

The expression for f_{et} in Eq. (24) is valid only approximately. First of all, there are the usual approximations inherent in Poisson-Boltzmann theory, like its mean-field nature or the vanishingly small ionic radii in the electrolyte. Apart from this, the inclusion-containing membrane is neither perfectly flat nor is it uniformly charged. Both its flatness and its uniform charge density are perturbed by the presence of the inclusion and the corresponding membrane deformation. However, f_{et} in Eq. (24) still gives a reasonable approximation of the electrostatic energy within PB theory for Debye lengths that are not much smaller than the inclusion radius.

In general, B_{et} will depend on a_0 since f_{et} in Eq. (23) does not follow a $1/a_h$ dependence. Only in the Debye-Hückel limit (where $p \ll 1$) one finds $f_{et} = 2\pi\lambda^2 l_B l_D k_B T / a_h$ and, upon insertion of this expression into Eq. (23), $B_{et} = 2\pi\lambda^2 l_B l_D k_B T$ independent on a_0.

Let us investigate the change in the inclusion-induced membrane deformation energy due to a change of the lipid bilayer surface charge density. Start with a fully charged membrane ($\lambda = 1$) in a 1:1 electrolyte of Debye length $l_D = 10$ Å which is characterized by $B = B_0 + B_{et} = 374\ k_B T$ Å^2 + $113\ k_B T$ Å^2 = $487\ k_B T$ Å^2. This bilayer has a nearly vanishing spontaneous monolayer curvature of $c_0 = 1/400$ Å. The corresponding ΔF is shown in Fig. 7 (broken line) as a function of b_P. The minimum corresponds to $b_0 = 11.3$ Å and the spring constant is $H = 0.15\ k_B T$ Å^{-2}. Consider now that by some process (like the binding of multivalent counterions or a strong increase in the salt concentration, n_0) the bilayer becomes effectively uncharged ($\lambda = 0$), whereas all other nonelectrostatic interactions remain unchanged. This implies less head group repulsion, $B = B_0 = 374\ k_B T$ Å^2, and the new equilibrium monolayer thickness $b_0 = 11.7$ Å is about 0.4 Å larger than for the fully charged bilayer. Furthermore, the lipid layer now exhibits a rather large negative spontaneous curvature of $c_0 = -1/70$ Å, which also reflects the smaller head group repulsion. The corresponding ΔF is shown in Fig. 7 (solid line). The spring constant for the uncharged membrane is $H = 0.21\ k_B T$ Å^{-2}. From Fig. 7 it follows that for a negative hydrophobic mismatch of, say 2–3 Å, the electrostatic charging of the membrane can induce a change of several $k_B T$ for ΔF. Interestingly, a correspondingly strong change of ΔF is not found for positive hydrophobic mismatch. That is, the changes in H and b_0 nearly compensate the change in ΔF for positive mismatch. Our calculation may give a reasonable description for a recent measurement on a decane-con-

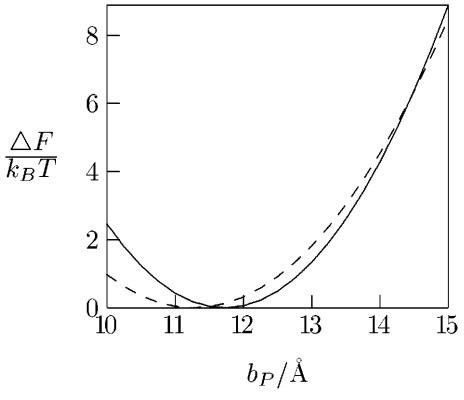

Fig. 7 The membrane deformation energy, ΔF, as a function of the hydrophobic inclusion half thickness b_P for an uncharged membrane (*solid line*, $\lambda = 0$) and for a fully charged membrane and a Debye length of $l_D = 10$ Å (*broken line*, $\lambda = 1$). In both cases, $B_0 = 374\ k_B T$ Å^2 (corresponding to $c_0 = -1/70$ Å for the uncharged membrane). The charged membrane is characterized by an effective head group interaction parameter $B = 487\ k_B T$ Å^2 implying a spontaneous curvature of $c_0 = 1/400$ Å

taining gA/dioleoylphosphatidylserine (DOPS) system for which a Ca^{2+}-induced screening of the lipid charges was estimated to induce a change in ΔF of about 4 k_BT (Lundbæk et al. 1997).

Conclusions

In this work we have investigated the influence of the lipid tilt degree of freedom on inclusion-induced lipid bilayer deformations. The spatial bilayer relaxation around a single inclusion is compared for lipids whose average chain director is constrained to point normal to the hydrocarbon-water interface and for lipids whose tilt angle is allowed to adjust. In the former case the theory reduces to earlier approaches that take into account only a lipid stretching degree of freedom. The latter case compares well with a recently investigated microscopic model (Fattal and Ben-Shaul 1993) in which the lipid tilt was not constrained. We find that the possibility of the lipids to tilt leads to a significant shortening of the typical membrane perturbation length [which is in accordance with a recent computer simulation of gA in DMPC (Chiu et al. 1999)] and to a severalfold lowering of the deformation energy. The present calculations thus suggest the need to take the lipid tilt degree of freedom into account in the analysis of protein-induced bilayer deformations, like the ones induced by gA.

The advantage to use a specific molecular model was exploited in the present work by investigating how electrostatic and nonelectrostatic energies interact with each other. Yet, other applications are suggested by the amount of available experimental results on gA membrane systems. One would be the calculation of inclusion-induced deformations of mixed bilayers (Lundbæk and Andersen 1994, Lundbæk et al. 1996). Another one is to estimate the bending rigidity for gA-containing membranes. This would allow us to test if the enhancement of undulation forces in DMPC stacks upon an increase in gA concentration can account for the experimentally determined increase in the average membrane-to-membrane distance (Kobayashi and Fukada 1998).

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