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A molecular model for lipid-mediated interaction between proteins in membranes

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The loss of conformational freedom experienced by lipid chains in the vicinity of one, or two, impenetrable walls, representing the surfaces of hydrophobic transmembrane proteins, is calculated using a mean-field molecular-level chain packing theory. The hydrophobic thickness of the protein is set equal to that of the unperturbed lipid membrane (*i.e.*, no "hydrophobic mismatch"). The probability distributions of chain conformations, at all distances from the walls, are calculated by generating all conformations according to the rotational-isomeric-state model, and subjecting the system free energy to the requirement that the hydrophobic core of the membrane is liquid-like, and hence uniformly packed by chain segments. As long as the two protein surfaces are far apart, their interaction zones do not overlap, each extending over several molecular diameters. When the interaction zones begin to overlap, inter-protein repulsion sets in. At some intermediate distance the interaction turns strongly attractive, resulting from the depletion of (highly constrained) lipid tails from the volume separating the two surfaces. The chains confined between the hydrophobic surfaces are tilted away from the walls. Their tilt angle decreases monotonically with the distance from the walls, and with the distance between the walls. A nonmonotonic variation of the lipid-mediated interaction free energy between hydrophobic surfaces in membranes is also obtained using a simple, analytical, model in which chain conformations are grouped according to their director (end-to-end vector) orientations.

I. Introduction

The incorporation of an intrinsic protein into a lipid membrane is driven by the hydrophobic effect, namely, by the lower free energy of the system when the protein is surrounded by the hydrocarbon tails of the lipids rather than by water molecules. Nevertheless, the presence of the protein in the membrane generally involves a (tolerable but) significant perturbation in lipid chain organization, as compared to the protein-free membrane. Apart from specific lipid–protein interactions that depend on the chemical characteristics of the lipids and the proteins, several nonspecific mechanisms come into play when a "hydrophobic inclusion", such as an integral protein or peptide, is inserted into a lipid membrane. One important factor underlying these interactions is the fact that the lipid tails constituting the lipid membrane are flexible, possessing many possible chain conformations, whereas the intrinsic proteins are relatively stiff, exposing rigid impenetrable walls to the surrounding lipids. Consequently, those lipids in the immediate vicinity of the walls must adapt their conformational statistics to the presence of the incorporated inclusions, thereby experiencing elastic distortions which, sometimes, can induce long-range membrane deformations.

One of the most familiar characteristics of lipid–protein interaction, prevailing when a transmembrane protein of hydrophobic thickness h_P is inserted into a membrane of equilibrium thickness h_0, is the "hydrophobic mismatch" between the inclusion and the membrane, $\Delta h = h_P - h_0$. Attempting to bridge the hydrophobic mismatch, and thus avoid exposure of hydrophobic moieties to water, the flexible lipid tails will stretch (when $\Delta h > 0$) or compress (when $\Delta h < 0$), paying the necessary price of elastic deformation energy. The possible outcomes of the hydrophobic mismatch have been intensively studied both experimentally[1,2] and theoretically,[3–8] and include modifications in protein conformation,[9] lipid sorting in mixed membranes,[10] microdomain formation,[11] modulations in the lipid phase behavior[12] and peptide-mediated lipid phase transitions.[2,8]

Regardless of whether the hydrophobic mismatch is large, small or even identically zero, there is another source of conformational free energy loss which the lipids bordering a rigid protein surface must pay. This, much less studied, nevertheless ever-present and important, effect is associated with the obvious fact that once a lipid molecule is near an impenetrable wall it can no longer explore all the chain conformations available to it far away from the wall. The number of lipids experiencing this perturbation is proportional to the contact area between the protein surface and the lipid chains. Since the lipid–protein contact area diminishes as two (or more) proteins come into contact, this lipid-mediated interaction mechanism is naturally attractive, and thus at high concentrations of proteins in membranes may favor their aggregation into dense domains.

The lipid-mediated attraction between proteins was first pointed out and studied by Marčelja, using his mean-field theory of chain orientational order in lipid membranes.[13] It should be emphasized that Marčelja's theory was explicitly formulated for the case of zero hydrophobic mismatch. Subsequent, mean-field, treatments of lipid-mediated protein–protein interactions have also predicted purely attractive

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interaction potentials.[14,15] Note, however, that the thermodynamic order parameter in these theories is the hydrophobic mismatch, implying that the interaction potential is identically zero when the hydrophobic mismatch vanishes.

Recent Monte-Carlo studies[16,17] on a coarse-grained, membrane-like model system suggest a more complicated interaction potential between rigid membrane inclusions. These simulations reveal the existence of a short-ranged depletion-induced attraction, a fluctuation-mediated attraction at large distances between the inclusions and a repulsive barrier at intermediate separations. The long-range attraction found in these calculations is attributed to the overlap between the gradients of density and orientational fluctuations of the lipids around each protein.[16] A similar explanation for the attraction between membrane inclusions, reflecting the suppression of order parameter fluctuations in the protein vicinity, was earlier proposed by Schröder[18] using a mean-field theory of nematic liquid crystals. A nonmonotonic interaction potential between rigid membrane inclusions was also predicted by Lagüe *et al.*,[19] based on the hypernetted chain integral equation formalism for liquids. Here, the lateral density–density response function of the hydrocarbon core was extracted from a molecular dynamics simulation of a pure lipid bilayer and used as an input for the statistical thermodynamic theory. The interaction between two identical cylindrical inclusions of radius 5 Å was found attractive for separations smaller than 15 Å, and repulsive for larger distances. In both calculations, by Lagüe *et al.*[19] and Sintes and Baumgärtner,[16,17] the lipid-mediated interaction between inclusions was found to be nonmonotonic, exhibiting a repulsive barrier at some intermediate separation. This may be contrasted with the earlier, mean-field, treatments which predict a monotonically attractive interaction. In this paper we shall show that a nonmonotonic behavior of the lipid-mediated interaction energy between inclusions is also predicted by a mean-field, molecular-level, theory of chain packing in membranes. A highly simplified closed-form "director model", which will be presented in Section 4 of this paper, reproduces the same qualitative behavior.

The molecular-level chain packing theory alluded to above has previously been used to calculate the lipid perturbation free energy caused by the presence of a single transmembrane protein, as a function of the hydrophobic mismatch between the membrane and the inclusion.[7] It was found that the elastic perturbation free energy is nonzero even if $\Delta h \equiv 0$. (Depending on whether the lipid spontaneous curvature is positive or negative, the minimum in the perturbation free energy occurs at negative and positive values of Δh, respectively, and increases approximately quadratically around the minimum.)

Our main goal in the present work is to formulate a slightly different version of the molecular chain packing theory, and apply it to calculate the lipid-mediated interaction free energy between two hydrophobic inclusions, as a function of the distance between them. Our basic free energy expression contains explicitly only those free energy terms associated with the lipid tails. The interaction between the lipid headgroups (which are not involved in the elastic deformations associated with hydrophobic inclusions) are treated in the two limits of "strong" and "weak" correlations between headgroup positions. In the former case, the headgroups are uniformly distributed (equivalently, equidistant) on the hydrocarbon–water interface. In the opposite limit, they are allowed to relax, so as to minimize the packing free energy of the lipid tails. As we shall see, the interaction free energy is essentially the same in both limits. Focusing on the effects of conformational entropy loss inflicted by the presence of the impenetrable inclusion walls, we shall only consider the case $\Delta h = 0$. We will show that the lipid-mediated interaction potential is, indeed, nonmonotonic and derive numerical estimates for its strength. Later on in the paper we shall show that a qualitatively similar behavior is predicted by the simpler, physically more intuitive, director model.

II. Chain packing theory

Our model is described in Fig. 1. Two flat and parallel hydrophobic walls, representing the surfaces of the proteins, are perpendicularly embedded in a symmetric lipid bilayer; their height matches exactly the hydrophobic thickness of the unperturbed bilayer, h_0. The distance between the walls, measured along the x-axis of a Cartesian coordinate system, is d. The origin of the coordinate system is located in the middle of the left wall, with the z-axis oriented normal to the membrane midplane, which coincides with the xy-plane. We assume that the length of the walls along the y-axis, L, is much larger than the linear dimension of (the cross-section of) a lipid chain, implying that end effects are negligible and the system can be treated as translationally invariant along the y-axis. (Thus, our lipid perturbation energies are simply proportional to L. Nevertheless, for dimensional consistency we find it helpful to keep using this length in our formulation of the problem.) Note that this does not imply that chain conformations are restricted to the xz-plane but, rather, that there are no additional constraints on chain conformations in the y-direction except those imposed by the presence of other lipid chains. Strictly speaking, by treating the protein walls as planar (and hence ignoring curvature effects), our model applies for the interaction between two large proteins at relatively short separations, *e.g.*, two cylindrical proteins of large cross-section at distances smaller than their diameters. Yet, it should be noted that, as long as the radius of curvature of the inclusion is larger than the linear dimension of a lipid chain, the results derived from the model presented below can also be used to calculate interaction free energies between curved inclusions.

Even in cases where $h_P = h_0$ the membrane thickness in the vicinity of a hydrophobic inclusion, $h(x)$, is not necessarily constant. However, these thickness variations are small and we shall therefore assume $h(x) = h_0 = 2\bar{l}$; $\bar{l}$ denoting the average end-to-end length of an (unperturbed) lipid tail, being equal to the thickness of each monolayer. Thus, the hydrophobic volume available to the lipid chains between the two walls is $V_{bl} = Ldh_0 = 2V$ with $V = Ld\bar{l}$ denoting the volume per monolayer. The hydrocarbon–water surface area of the membrane section considered is $A_{bl} = 2Ld = 2A$, A denoting the surface area per monolayer. As usual, we treat the hydrophobic core of the membrane as a homogeneous incompressible liquid of lipid chains, each of volume v. Thus, the number of lipid chains, per monolayer, is $N_{bl}/2 = N = V/v$, and the

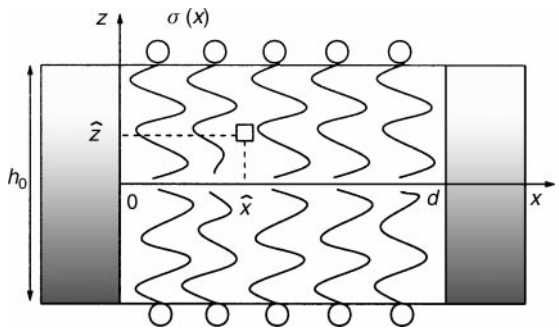

Fig. 1 Schematic illustration of a symmetric lipid bilayer bounded by two identical rigid protein walls at distance d. The hydrophobic thickness of the bilayer and the protein walls is h_0. The area density of headgroups on the hydrocarbon–water interface is $\sigma(x)$. Note that at any given point $\hat{x}$, $\hat{z}$ within the hydrophobic interior the average chain segment density is uniform.

average two-dimensional (2D) density of chain origins at the hydrocarbon–water interface is $\bar{\sigma} = N/A = 1/\bar{a} = \bar{l}/v$; $\bar{a}$ denoting the average cross-sectional area per chain, at the hydrocarbon–water interface. Note that for double-tail lipids the average area per lipid headgroup is $2\bar{a}$. In our chain packing theory outlined below, all the hydrocarbon chains constituting the hydrophobic core of the membrane are treated as identical. Furthermore, because their density is uniform, no distinction is made between neighboring chains originating from one or two adjacent headgroups. Thus, hereafter, we shall refer to $\bar{a}$ equivalently as the area per headgroup and area per chain.

In the formulation of the chain packing theory presented below, and the numerical results reported in the next section, we treat the headgroup position at the hydrocarbon–water interface as a continuous variable, with its x-coordinate, measuring the distance from the protein walls, varying between 0 and d. Note, however, that because of the nonzero (hard core) diameter, $d_{\min}$, of the hydrocarbon tail, the minimal distance of a chain origin from the surface of a hard wall is $d_{\min}/2$. Similarly, as long as there are lipid chains between the two walls, the minimal distance between their surfaces is $d_{\min}$. (Yet, the number of "trapped" chains at this separation is negligibly small.) We shall keep using $0 \leqslant x \leqslant d$ for the range of headgroup positions, but it should be understood that the impenetrable hydrophobic surfaces are actually positioned at $x = -d_{\min}/2$ and $x = d + d_{\min}/2$. For brevity, we shall keep referring to d (rather than $d + d_{\min}$) as the distance between the walls.

In many of their possible conformations, lipid chains originating in one of the two monolayers can reach (interdigitate) beyond the bilayer midplane. This fact is taken into account in our chain packing model (see below). Yet, because the lipid bilayer is symmetric with respect to its midplane, it is convenient to focus on one of its two constituent monolayers, say the "upper" one in Fig. 1. In the presence of the perturbing hydrophobic walls the headgroup density at the xy-plane (at $z = h_0/2$) is no longer necessarily uniform. Because the system is translationally invariant along y, the headgroup density is now a function of x, $\sigma = \sigma(x) = 1/a(x)$, with $a(x)$ denoting the locally varying area per headgroup. Because the membrane is incompressible,

$$\bar{\sigma} = \frac{1}{d}\int_0^d \mathrm{d}x\ \sigma(x) = \frac{N}{Ld} = \frac{h_0}{2v} \tag{1}$$

A lipid chain originating at point x, y of the interface can be found, with a certain (yet undetermined) probability $P(\alpha\,|\,x,y) = P(\alpha\,|\,x)$, in any one of a multitude of conformations $\{\alpha\}$, with α specified by the positions of all the atoms constituting the chain. [Our scheme for generating chain conformations is based on the rotational-isomeric-state (RIS) model,[20] see the next section.] This conditional probability satisfies, for all x, the normalization

$$\sum_\alpha P(\alpha\,|\,x) = 1 \tag{2}$$

The number of chains originating between x and $x + \mathrm{d}x$ (with y anywhere between 0 and L) is

$$\mathrm{d}N(x) = L\sigma(x)\ \mathrm{d}x = (N/\bar{\sigma}d)\sigma(x)\ \mathrm{d}x$$

Hence, on average, the number of chains in conformation α originating between x and $x + \mathrm{d}x$ is

$$\begin{aligned}\mathrm{d}N(x,\alpha) &= \mathrm{d}N(x)P(\alpha\,|\,x)\\ &= (N/\bar{\sigma}d)\sigma(x)P(\alpha\,|\,x)\ \mathrm{d}x \equiv NP(x,\alpha)\ \mathrm{d}x\end{aligned}$$

The function $P(x,\alpha)\ \mathrm{d}x = \mathrm{d}N(x,\alpha)/N$, denoting the fraction of chains in conformation α originating between x and $x + \mathrm{d}x$, is normalized according to

$$\int_0^d \mathrm{d}x \sum_\alpha P(x,\alpha) = 1 \tag{3}$$

and can be interpreted as the *joint* probability of finding an "α-chain" between x and $x + \mathrm{d}x$. The quantity

$$P(x) = P(x,\alpha)/P(\alpha\,|\,x) = [\mathrm{d}N(x)/\mathrm{d}x]/N$$

i.e.,

$$P(x) = \frac{1}{d}\frac{\sigma(x)}{\bar{\sigma}} \tag{4}$$

is the probability density of finding a chain (in any conformation) originating at x. It satisfies the normalization condition

$$\int_0^d \mathrm{d}x\ P(x) = 1$$

As is well known, the entropy associated with a discrete probability distribution, P_i, over a set of states $\{i\}$, is given by

$$S = -k_\mathrm{B}\sum_i P_i \ln P_i$$

with k_B denoting Boltzmann's constant.[21] For a continuous probability density, $P(x)$ [$P(x)\ \mathrm{d}x$ denoting the probability of finding the system between x and $x + \mathrm{d}x$], the entropy can only be defined with respect to some reference system characterized by the distribution $P_0(x)$, namely,[22]

$$S = -k_\mathrm{B}\int \mathrm{d}x\ P(x)\ \ln[P(x)/P_0(x)]$$

In our problem, where $P(x,\alpha)$ involves both continuous (x) and discrete (α) variables, the entropy of the system is given by

$$S = -Nk_\mathrm{B}\int_0^d \mathrm{d}x \sum_\alpha P(x,\alpha)\ln\left(\frac{P(x,\alpha)}{P_0(x)}\right) \tag{5}$$

measuring the joint (conformational and positional) entropy of one monolayer, relative to a monolayer with positional headgroup distribution $P_0(x)$. The factor N appears on the right-hand side of eqn. (5) because $P(x,\alpha)$ is a singlet distribution, *i.e.*, $S/N = \langle s\rangle$ is the average entropy per chain in the monolayer. In this connection it should be noted that because $P(x,\alpha)$ is a singlet distribution (rather than the many-chain distribution), S/N is a mean-field approximation to the monolayer entropy. [It should also be noted that, in fact, a given chain conformation, α, is defined by both discrete and continuous variables. However, we need not introduce an additional reference probability distribution $P_0(\alpha\,|\,x)$, as we shall only be interested in entropy differences that do not involve this distribution.]

Our reference state for the probability distribution of headgroup positions is the uniform distribution $P_0(x) = 1/d$, corresponding to $\sigma(x) = \bar{\sigma}$. To calculate the free energy of the monolayer, $F = E - TS$, T denoting the absolute temperature, we calculate the energetic contribution using

$$\begin{aligned}E/N = \langle\varepsilon\rangle &= \int_0^d \mathrm{d}x \sum_\alpha P(x,\alpha)\varepsilon(\alpha)\\ &= \int_0^d \mathrm{d}x\ P(x)\sum_\alpha P(\alpha\,|\,x)\varepsilon(\alpha)\\ &= \int_0^d \mathrm{d}x\ P(x)\langle\varepsilon\rangle_x\end{aligned}$$

with $\varepsilon(\alpha)$ denoting the internal (*trans*/*gauche*) energy of a chain in conformation α, and $\langle\varepsilon\rangle_x$ is the average internal energy of chains originating at x. [The internal energy per chain depends only on α; $\langle\varepsilon\rangle_x$ depends on x through $P(\alpha\,|\,x)$.]

With $P(x,\ \alpha) = P(x)P(\alpha\,|\,x) = [\sigma(x)/\bar{\sigma}d]P(\alpha\,|\,x)$, $P_0(x) = 1/d$, and the expressions above for the monolayer energy and entropy, the free energy per monolayer with two embedded inclusions at distance d from each other is given by

$$\begin{aligned} F &= N\langle f\rangle \\ &= N\int_0^d \mathrm{d}x \sum_\alpha P(x,\ \alpha)\left[\varepsilon(\alpha) + k_\mathrm{B}T\ \ln\left(\frac{P(x,\ \alpha)}{P_0(x)}\right)\right] \\ &= L\left[\int_0^d \mathrm{d}x\ \sigma(x) \sum_\alpha P(\alpha\,|\,x)[\varepsilon(\alpha) + k_\mathrm{B}T\ \ln\ P(\alpha\,|\,x)]\right. \\ &\quad \left. + k_\mathrm{B}T\int_0^d \mathrm{d}x\ \sigma(x)\ln\left(\frac{\sigma(x)}{\bar{\sigma}}\right)\right] \end{aligned} \tag{6}$$

The sum

$$f(x) = \sum_\alpha P(\alpha\,|\,x)[\varepsilon(\alpha) + k_\mathrm{B}T\ \ln\ P(\alpha\,|\,x)] \tag{7}$$

appearing on the right-hand side of eqn. (6) is the conformational free energy per chain, for those chains whose headgroups are anchored at x. The second term in eqn. (6) represents the excess entropy of the headgroup distribution, relative to the uniform distribution [where $\sigma(x) = \bar{\sigma}$]. In the protein-free membrane $P(\alpha\,|\,x) = P(\alpha)$ is independent of x, and hence $f(x) = f_0$ is, simply, the conformational free energy per chain in the membrane. Note also that in this limit $\sigma(x) = \bar{\sigma}$, and eqn. (6) yields $F = f_0\,\bar{\sigma}Ld = Nf_0$, *i.e.*, $\langle f\rangle = f_0$.

The actual probability distribution of the membrane–protein system $P(x,\ \alpha)$ [and hence $\sigma(x)$ and $P(\alpha\,|\,x)$] is the distribution function which minimizes F, subject to all the relevant constraints on the system. Apart from the trivial normalization condition, eqn. (3), we impose only one physical—packing—constraint on $P(x,\ \alpha)$. Namely, we require that the density of lipid chain segments in the hydrophobic core of the membrane is uniform (*i.e.*, the core is an incompressible, liquid-like, medium), as attested by numerous experiments. Note that this assumption is equally valid for protein-free and protein-containing membranes. Differences between the probability distributions corresponding to these systems appear because the lipid chains [and hence their $P(x,\ \alpha)$] must satisfy different boundary conditions, yet their packing constraints are identical. The boundary conditions, namely, the presence of two hydrophobic walls at distance d apart, will also dictate the dependence of F on d.

For the mathematical formulation of the uniform packing constraint, let $\mathrm{d}\hat{\boldsymbol{r}} = \mathrm{d}\hat{x}\ \mathrm{d}\hat{y}\ \mathrm{d}\hat{z}$ denote a small volume element around an arbitrary point $\hat{\boldsymbol{r}}$ within the hydrophobic core. Also, let $\langle\varphi(\hat{\boldsymbol{r}})\rangle$ denote the average density of chain segments at $\hat{\boldsymbol{r}}$; *i.e.*, $\langle\varphi(\hat{\boldsymbol{r}})\rangle\ \mathrm{d}\hat{\boldsymbol{r}}$ is the average number of chain segments in $\mathrm{d}\hat{\boldsymbol{r}}$. Our assumption that the hydrophobic core is uniformly packed by chain segments implies $\langle\varphi(\hat{\boldsymbol{r}})\rangle = \bar{\varphi} = \text{constant}$; $\bar{\varphi}$ denoting the uniform segment density in the hydrophobic core.

Several chains, originating at different points of the hydrocarbon–water interface, can contribute to the average chain segment density at $\hat{\boldsymbol{r}}$. The contribution of each of these chains to $\langle\varphi(\hat{\boldsymbol{r}})\rangle$ is an average over its many conformational states, α. Recalling that $P(x,\ \alpha)\ \mathrm{d}x$ is the fraction of chains in conformation α, anchored to the interface (anywhere along $0 \leqslant y \leqslant L$ and) between x and $x + \mathrm{d}x$, and using $\varphi(\hat{\boldsymbol{r}};\ x,\ \alpha)$ to denote their contribution to the segment density at $\hat{\boldsymbol{r}}$, we have

$$\langle\varphi(\hat{\boldsymbol{r}})\rangle = \int_0^d \mathrm{d}x \sum_\alpha P(x,\ \alpha)\varphi(\hat{\boldsymbol{r}};\ x,\ \alpha)$$

Since our system is translationally invariant along y, it follows that $\varphi(\hat{\boldsymbol{r}};\ x,\ \alpha) = \varphi(\hat{x},\ \hat{z};\ x,\ \alpha)$ is independent of $\hat{y}$. Thus

$$\langle\varphi(\hat{x},\ \hat{z})\rangle = \int_0^d \mathrm{d}x \sum_\alpha P(x,\ \alpha)\varphi(\hat{x},\ \hat{z};\ x,\ \alpha) = \bar{\varphi} \tag{8}$$

with the constant $\bar{\varphi}$ denoting the segment density in the liquid-like hydrophobic core.

The second equality in eqn. (8) is the mathematical expression of the (uniform density) packing constraint that $P(x,\ \alpha)$ must fulfill. It should be noted that this packing constraint applies to *all points* $\hat{x}$, $\hat{z}$ ($0 \leqslant \hat{x} \leqslant d$ and $-h_0/2 \leqslant \hat{z} \leqslant h_0/2$) within the hydrophobic core, and thus represents a (continuous) set of constraints on $P(x,\ \alpha)$. Note also that, because of chain interdigitation across the bilayer midplane, contributions to the segment density around the midplane arise from chains belonging to both monolayers. Thus, the integration over x in eqn. (8) should be regarded as extending over both interfaces of the bilayer. In our numerical calculations we account for this fact by treating $\varphi(\hat{x},\ \hat{z};\ x,\ \alpha)$ as the sum of the contributions corresponding to the conformation α originating at the upper interface and its mirror image at the lower interface.

In the present work, the lipid tails are modeled as fully saturated hydrocarbon chains, of the form $-(\mathrm{CH_2})_{n-1}-\mathrm{CH_3}$. The volume occupied by such a C-n chain corresponds to about $v = (n + 1)v$ where $v \approx 27$ Å^3 is the specific volume per $\mathrm{CH_2}$ segment in bulk liquid alkane phase.[23] (The specific volume of the terminal, $\mathrm{CH_3}$, segment is approximately twice as large.) From this we obtain the average chain segment density in the bilayer $\bar{\varphi} = (n + 1)/v$.

Minimizing the free energy functional, eqn. (6), with respect to $P(x,\ \alpha) = P(x)P(\alpha\,|\,x)$ [recall $P(x) = \sigma(x)/\bar{\sigma}d$], subject to the normalization condition, eqn. (3), and the packing constraint, eqn. (8), we finally obtain

$$P(x,\ \alpha) = \frac{1}{d}\frac{\chi(x,\ \alpha)}{q} \tag{9}$$

with

$$\chi(x,\ \alpha) = \exp\left[-\frac{\varepsilon(\alpha)}{k_\mathrm{B}T} - \int_0^d \mathrm{d}\hat{x}\int_0^{h_0/2} \mathrm{d}\hat{z}\ \lambda(\hat{x},\ \hat{z})\varphi(\hat{x},\ \hat{z};\ x,\ \alpha)\right] \tag{10}$$

denoting a generalized Boltzmann factor which includes two terms in its exponent. The first term is the energetic contribution [$\varepsilon(\alpha)$], which favors chain conformations of low internal energy (low *gauche* content). More important is the second (pressure × volume) term, which represents the effects of chain packing constraints on the statistical weights of the various conformations. The $\lambda(\hat{x},\ \hat{z})$ are the Lagrange multipliers conjugate to the packing constraints, eqn. (8). Note that each point within the hydrophobic core is associated with an independent Lagrange multiplier. Of course, for the numerical evaluation of λ, the hydrophobic core is discretized into small cells ($\Delta x\Delta z$, of size smaller than one chain segment) with a corresponding number of Lagrange multipliers λ.

The normalization factor q in eqn. (9) is the (positionally averaged) conformational partition function,

$$q = \frac{1}{d}\int_0^d \mathrm{d}x\ q(x), \quad q(x) = \sum_\alpha \chi(x,\ \alpha) \tag{11}$$

ensuring that $P(\alpha,\ x)$ fulfills the normalization condition, eqn. (3). For $P(x)$ and $P(\alpha\,|\,x)$ we have

$$P(x) = \frac{1}{d}\frac{\sigma(x)}{\bar{\sigma}} = \frac{1}{d}\frac{q(x)}{q}, \quad P(\alpha\,|\,x) = \frac{\chi(x,\ \alpha)}{q(x)} \tag{12}$$

The set of Lagrange multipliers, *i.e.*, the function $\lambda(\hat{x}\ \hat{z},)$ appearing in eqn. (10), can be determined (numerically) by substituting $P(x,\ \alpha)$, as given by eqn. (9), into the packing constraints given in eqn. (8). This results in the self-consistency equations

$$\int_0^d \mathrm{d}x \sum_\alpha \chi(x,\ \alpha)[\varphi(\hat{x},\ \hat{z};\ x,\ \alpha) - \bar{\varphi}] = 0 \tag{13}$$

which must be solved, simultaneously, at all positions $\hat{x}$, $\hat{z}$.

Once the function $\lambda(\hat{x}, \hat{z})$ is known, one can calculate all local properties (*e.g.*, chain conformational characteristics) of the lipid bilayer. For instance, the headgroup density profile is given by

$$\sigma(x) = P(x)\bar{\sigma}d = \bar{\sigma}q(x)/q$$

Local conformational chain properties are determined by the conditional probability

$$P(\alpha\,|\,x) = P(x, \alpha)/P(x) = \chi(x, \alpha)/q(x)$$

giving the probability of a chain anchored at point x of the interface to be found in conformation α. For instance, the average end-to-end vector, $\boldsymbol{r}_e(x)$, of chains originating at x, is given by

$$\boldsymbol{r}_e(x) = \sum_{\alpha} P(\alpha\,|\,x)[\boldsymbol{r}_t(x, \alpha) - \boldsymbol{r}_h(x)] \qquad (14)$$

with $\boldsymbol{r}_h(x)$ denoting the exact position of the headgroup at the interface [actually, the x and z coordinates of $\boldsymbol{r}_h(x)$ are $x_h = x$ and $z_h = \pm h_0/2$]; $\boldsymbol{r}_t(x, \alpha)$ denotes the coordinates of the terminal chain segment (CH_3).

Of particular interest in this work is the variation of the membrane free energy

$$F(d) = N\langle f(d)\rangle = F_{bl}(d)/2$$

with the separation between the two hydrophobic protein walls, d. To this end, we obtain $\langle f\rangle$ by substituting $P(x, \alpha)$ as given by eqn. (9), together with eqn. (10), into eqn. (6), and obtain

$$\frac{\langle f\rangle}{k_B T} = -\ln q - \bar{\varphi}\int_0^d d\hat{x}\int_0^{h_0/2} d\hat{z}\;\lambda(\hat{x}, \hat{z}) \qquad (15)$$

In deriving eqn. (9) for $P(x, \alpha)$ we have minimized our free energy functional, eqn. (6), with respect to both $P(\alpha\,|\,x)$ and $\sigma(x) \propto P(x)$. Note, however, that F involves only the free energy of the chains constituting the hydrophobic core and does not account for direct interactions between the lipid headgroups. In other words, if the headgroup distribution $\sigma(x)$ deviates from the uniform distribution $\bar{\sigma}$, this deviation is due entirely to the preferred packing of the tails. This is the expected behavior when inter-chain (packing) repulsion is stronger than headgroup interaction. In the opposite limit, of strong headgroup interactions, we expect $\sigma(x) \approx \bar{\sigma}$. In the next section we shall see that the membrane free energy is nearly the same, regardless of whether $\sigma(x)$ is allowed to optimize F or is constrained to be fixed, namely, $\sigma(x) = \bar{\sigma}$. Yet, the local headgroup density in the vicinity of the protein walls can be quite different. In order to compare the two limiting cases (of "weak" *vs.* "strong" correlations between headgroup positions), let us briefly outline the derivation of

$$P(\alpha\,|\,x) = P(x, \alpha)/P(x) = dP(x, \alpha)$$

for the case of a uniform distribution of headgroup positions.

In the limit $\sigma(x) = \bar{\sigma}$, implying $P(x) = P_0(x) = 1/d$, the last term in eqn. (6) vanishes and F becomes a functional of the conditional probabilities $P(\alpha\,|\,x)$. The $P(\alpha\,|\,x)$ values corresponding to different headgroup positions, x, are coupled through the packing constraints, eqn. (8). The minimization of F now yields

$$P(\alpha\,|\,x) = \frac{\chi(x, \alpha)}{q(x)} \qquad (16)$$

with $q(x)$ and $\chi(x, \alpha)$ as given in eqn. (11) and (10), respectively. For the Lagrange multipliers $\lambda(\hat{x}, \hat{z})$ appearing in the expression for $\chi(x, \alpha)$, we find the self-consistency equations

$$\int_0^d dx\,\frac{1}{q(x)}\sum_{\alpha}\chi(x, \alpha)[\varphi(\hat{x}, \hat{z}; x, \alpha) - \bar{\varphi}] = 0 \qquad (17)$$

as obtained by substituting $P(x, \alpha) = P(\alpha\,|\,x)/d$ from eqn. (16) into the packing constraint, eqn. (8). Again, once we know $\lambda(\hat{x}, \hat{z})$, the free energy per molecule can be calculated, resulting in

$$\frac{\langle f\rangle}{k_B T} = -\frac{1}{d}\int_0^d dx\,\ln q(x) - \bar{\varphi}\int_0^d d\hat{x}\int_0^{h_0/2} d\hat{z}\;\lambda(\hat{x}, \hat{z}) \qquad (18)$$

III. Results and discussion

In this section we present numerical results for the lipid-mediated interaction free energy between two hydrophobic protein surfaces, as well as several lipid chain properties, based on the chain packing theory described in the previous section. In these calculations the lipid tails constituting the bilayer hydrophobic core are modeled as $-(CH_2)_{11}-CH_3$ chains. The volume of each such chain is $v = 13 \times 27$ Å^3 = 351 Å^3. For the hydrophobic thickness of the lipid bilayer and the protein walls we have used $h_P = h_0 = 22.0$ Å, implying $a_0 = 2v/h_0 = 1/\bar{\sigma} = 31.9$ Å^2 for the average cross-sectional area per chain. For double-tail lipids this corresponds to an area per headgroup of $2a_0 = 63.8$ Å^2.

For any given value of d we have solved a discretized version of the self-consistency equation [either eqn. (13) or eqn. (17) depending on whether the lipid headgroups are allowed to relax freely or not]. All possible chain conformations were generated based on the rotational-isomeric-state (RIS) model,[20] and used to calculate the chain segment densities $\varphi(\hat{x}, \hat{z}; x, \alpha)$. The RIS model is also used to calculate the internal chain energies, namely, $\varepsilon(\alpha) = n(\alpha)\varepsilon_g$; where $n(\alpha)$ is the number of *gauche* bonds along a chain in conformation α, and $\varepsilon_g = 1.175k_BT$ is the *gauche* bond energy. Further numerical details of the discretization and the calculation of $P(x, \alpha)$ are outlined in the Appendix.

The quantity of greatest interest in our calculations is $F(d)$, the lipid-mediated interaction free energy between two protein surfaces at distance d from each other. When $d \to \infty$ the membrane contains two isolated, noninteracting, surfaces, *i.e.*, $F(d \to \infty)$ becomes twice the protein-induced lipid perturbation free energy. As a reference system for calculating the protein-induced lipid perturbation, and the lipid mediated inter-protein interaction, we use the protein-free lipid bilayer, where all chains share exactly the same conformational properties. Using f_0 to denote the free energy per lipid chain in the unperturbed membrane, the protein–lipid–protein interaction free energy is given by

$$\Delta F(d) = F(d) - Nf_0 \qquad (19)$$

which, at $d \to \infty$, reduces to twice the perturbation energy associated with a single wall, per monolayer. [Equivalently, $\Delta F(d \to \infty)$ is the perturbation free energy of a bilayer by an isolated inclusion.] This quantity has previously been calculated as a function of the hydrophobic mismatch $\Delta h = h_P - h_0$ (for a membrane composed of C-14 chains) in the limit of strong headgroup interactions.[7] In the present work we use a similar approach to calculate the lipid-mediated inter-surface interaction potential, $\Delta F(d)$, for both "frozen" [$\sigma(x) = \bar{\sigma}$] and annealed lipid headgroup distributions.

In Fig. 2 we show $\Delta F(d)$ for the two limiting cases of weak and strong headgroup interactions, as calculated based on the chain packing theory described in the previous section. Also shown is the prediction of the simple director model discussed in Section 4.

All three curves in Fig. 2 exhibit the same qualitative, non-monotonic, behavior of the lipid-mediated interaction potential. Namely, steep depletion attraction at very short separation, preceded by a repulsive barrier at intermediate separations between the protein surfaces. This behavior can be explained as follows. At large separations each surface perturbs the lipid chains in its vicinity, resulting in a constant asymptotic value of $\Delta F(d \to \infty)$. As the two surfaces approach

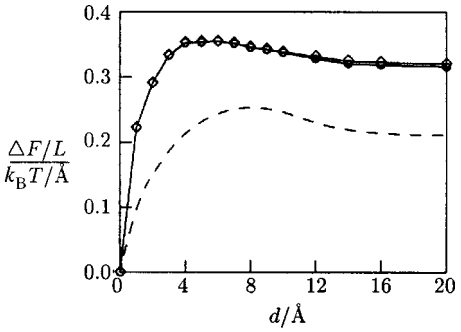

Fig. 2 The interaction free energy, $\Delta F/L$, between two parallel protein walls as a function of their separation d (per monolayer and per unit length of the wall). Two of the three curves shown were calculated using the detailed molecular chain packing theory, and represent the interaction potentials corresponding to the limits of weak (○) and strong (◇) headgroup interactions; *i.e.*, annealed headgroup density $\sigma(x)$ *vs.* uniform ("frozen") lateral density $\sigma(x) = \bar{\sigma}$, respectively. The dashed curve is the prediction of the simple director model presented in Section 4.

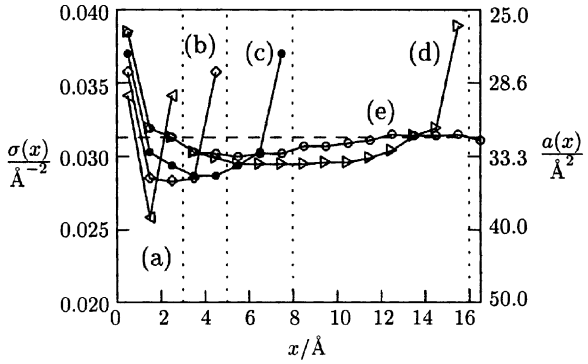

Fig. 3 Density profiles of headgroup positions at the hydrocarbon–water interface, $\sigma(x)$, in the limit of weak headgroup interactions. Profiles are shown for $d = 3$ Å (a), $d = 5$ Å (b), $d = 8$ Å (c), $d = 16$ Å (d), and $d = 28$ Å (e). In the limit of strong headgroup interactions the density is constant, $\sigma = \bar{\sigma} = 1/a_0 = 0.0313$ Å^{-2} (broken line).

each other the chains in the intervening region begin interacting with *both* surfaces, thus experiencing a larger conformational entropy loss. This is the origin of the repulsive barrier which becomes noticeable at separations d (on the order of 10 Å for C-12 chains) that slightly exceed the lateral dimension of the chains in the lipid core, *i.e.*, somewhat above $d \sim \sqrt{a}$ where a is the cross-sectional area per chain. As d decreases further, the interaction volume decreases and hence also N, the number of chains experiencing the confining walls. Eventually all chains are depleted from the interaction zone and $\Delta F(d \to 0) \to 0$. A similar behavior was found in the Monte-Carlo calculations by Sintes and Baumgärtner.[16,17] These simulations also reveal an additional long-ranged, fluctuation-mediated, attraction between the rigid proteins. Due to its mean-field nature our approach cannot account for this long-range effect.

Another conclusion from Fig. 2 is that $\Delta F(d)$ is rather independent of the constraints on the headgroup distribution $\sigma(x)$, as reflected by the fact that the curves corresponding to the frozen and the annealed distributions are essentially superimposed on each other. Actually, this result is not too surprising in view of the fact that the conformational packing statistics of the lipid tails is mainly dictated by the volumes available to the chains within the hydrophobic core, rather than by the exact position of the headgroup (*i.e.*, the chain origin) at the hydrocarbon–water interface. Consequently, whether additionally constrained or not, the local variations in headgroup densities cannot be too large. Moreover, owing to their conformational flexibility, once the chains enter the hydrophobic interior of the membrane (*i.e.*, one or two segments away from the headgroups) they can optimize their packing density even in cases where $\sigma(x)$ is not the headgroup density that the chains prefer most. In other words, the headgroup and chain free energies are nearly independent of each other. Recall, however, that our $F(d)$ accounts only for the chain contribution to the membrane free energy. The direct interaction between headgroups (which we have treated here as a constant contribution depending only on $\bar{\sigma}$) depends of course on $\sigma(x)$, preferring generally $\sigma(x) = \bar{\sigma}$.

The extent by which $\sigma(x)$ deviates from its uniform average ($\bar{\sigma}$) in the limit of no headgroup interactions is shown in Fig. 3. The calculated results reveal an increase in the density of headgroups in the immediate vicinity of the protein surface. This may appear surprising, considering that the loss of chain conformational freedom is expected to increase as the distance between the chain and the wall decreases. This apparent contradiction can only be resolved if those chains originating very near the wall begin diverging away from it once they enter the hydrophobic core, thereby reducing the number of forbidden ("wall crossing") conformations. In fact, because of their nonzero volume and because they must conform to the requirement of uniform chain segment density within the hydrophobic core, the tails are forced to divert from the wall, somewhere along the chain. Since the length of a fully stretched chain, l_s, exceeds the average thickness of one monolayer ($h_0/2 = \bar{l}$, the average end-to-end length of a chain in the unperturbed membrane), a chain originating near the wall must, on average, tilt away from it.

In Fig. 4 we show how the end-to-end vector, $\boldsymbol{r}_e(x)$, varies along the normal direction to the impenetrable walls. We note that, near the wall, the endgroup of the tail is repelled from the wall, relative to the position of the headgroup. (Inter-headgroup repulsion pushes the "first shell" headgroups towards the wall.) In addition to that, the chains are more stretched compared to those further away from the wall. The apparent tilting of the end-to-end vector is a direct consequence of the tail flexibility (*i.e.*, chain conformational freedom). Namely, the presence of the impenetrable wall excludes all chain conformations whose contour line crosses the wall. (Of course, these conformations are allowed in the protein-free membrane. A simple model featuring these effects is described in the next section.) The first few chain segments emanating from the headgroup are oriented approximately parallel to the wall, but the segments further down the chain are progressively pushed away from the wall. Pictorially, and very crudely, the average contour line of those chains originating near the (left) wall is "L-shaped". The extent of this chain tilting effect relaxes with the distance from the wall, as illustrated in Fig. 4. It should be emphasized that if the hydrophobic tails were regarded as

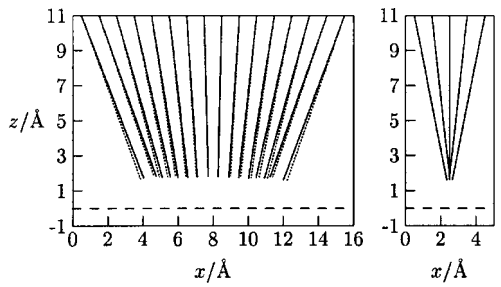

Fig. 4 Lipid chain tilting profiles induced by the impenetrable hydrophobic surfaces. The figure shows the variations in the average end-to-end vector, $\boldsymbol{r}_e(x)$, as a function of the chain position, x, between the two protein walls; for $d = 16$ Å (left) and $d = 5$ Å (right). Solid and dotted director lines correspond to the limits of weak and strong headgroup interactions, respectively. (The spacing between the lines is arbitrary and does not correspond to the average separation between neighboring chains.) The horizontal dashed line marks the bilayer midplane.

rigid rods (rather than flexible chains) no tail tilting could take place, because this would imply the existence of a void within the hydrophobic core, which we assume to be uniformly packed with chain segments.[5]

Returning to Fig. 4 we note that the average tilt angles are larger in the case of weak headgroup repulsion, consistent with the fact that in this limit the headgroups are more densely crowded near the hydrophobic walls. As anticipated, the extent of chain tilting decreases as the distance between the two surfaces decreases, owing to the opposing "repulsion" from the two apposed surfaces. To summarize these findings qualitatively: the larger the distance of a given chain segment along the chain from the headgroup, the larger is its maximal lateral span. Chain tilting provides a compromise way of minimizing the loss of this span by the walls.

Our final remark in this section concerns the numerical value of $\Delta F(d \to \infty)$. In a previous study[7] the perturbation free energy of a membrane composed of C-14 chains by a single hydrophobic wall was found to be $\Delta F(d \to \infty) = 0.37 k_B T$ Å^{-1}. This figure was obtained for zero hydrophobic mismatch and an average cross-sectional area per chain of $a_0 = 32$ Å^2. Under similar conditions, but for C-12 chains, we found here $\Delta F(d \to \infty) = 0.32 k_B T$ Å^{-1} (see Fig. 2). Judging by these two numbers, the perturbation free energy (for the same a_0) scales nearly linearly with the chain length n.

IV. A simple director model

In this section we present a very simple, approximate, physical model, capable of explaining the qualitative behavior of $\Delta F(d)$, which in the previous section was derived based on the detailed molecular theory of Section 2. This model does not involve an explicit enumeration of all chain conformations, nor does it take into account the requirement of uniform chain packing within the hydrocarbon core. Yet, it yields a simple closed-form expression for $\Delta F(d)$, capturing the essential physical mechanism underlying the perturbation in lipid order by integral hydrophobic inclusions and the lipid-mediated interaction between such inclusions.

Let $\boldsymbol{n} = \boldsymbol{r}_e/|\boldsymbol{r}_e|$, hereafter referred to as the "director", denote the unit vector along the end-to-end chain vector. Many chain conformations (as specified by the *trans/gauche* sequence along the chain and the length of the chain $|\boldsymbol{r}_e|$) are associated with any given orientation of $\boldsymbol{n}$. For chains in an isotropic liquid, the number of conformations corresponding to a given director is, by symmetry, independent of its orientation. The first assumption in our simplified model is that this is also true for the lipid chains constituting the hydrophobic core of a membrane. Clearly, in a (protein-free) lipid bilayer the chain director is restricted to one (the hydrophobic) side of the hydrocarbon–water interface; *i.e.*, the tip of $\boldsymbol{n}$ can only be found on one half of the surface of the unit sphere prescribed by the director. Furthermore, within the bilayer, the probabilities of finding $\boldsymbol{n}$ along different directions can be markedly different. (By symmetry, the membrane normal is the preferred direction.) In principle, this fact can be taken into account in our model by assigning different probabilities $P(\boldsymbol{n})$ to different chain orientations. Yet, to keep the model as simple as possible, we shall assume that, in the protein-free bilayer, all director orientations within the hydrophobic hemisphere are equally probable. We shall also assume that $|\boldsymbol{r}_e| = \bar{l} = h_0/2 = v/a_0$, the average chain length in the unperturbed membrane. Based on this simple picture, the loss of chain conformational freedom inflicted by one or two impenetrable surfaces is determined by the fraction of forbidden director orientations, *i.e.*, those which intersect these surfaces, as illustrated by the shaded area in Fig. 5.

Consider first the lipid perturbation associated with a single wall. Using $q = q(1) = 2\pi$ (the area of a unit hemisphere) to denote the configurational space of the chain director in the protein-free membrane, and $q(\bar{x})$ the configurational space of a chain (director) originating at distance $\bar{x}$ from the wall, the (purely entropic) conformational free energy penalty experienced by this chain is $\Delta f(\bar{x}) = -k_B T \ln[q(\bar{x})/q]$. Clearly, $\Delta f(\bar{x})$ decreases with $\bar{x}$: from $\Delta f(\bar{x})/k_B T = \ln 2$ at $\bar{x} = 0$, to 0 for all $\bar{x} \geqslant 1$; see Fig. 5. The total perturbation energy associated with a single wall is obtained by adding the contributions $\Delta f(\bar{x})$ from all chain directors in the range $\bar{x} = 0, \ldots, 1$. Note that, for a wall of length L (along the y-axis), the number of chains originating in the interval x, $x + \mathrm{d}x$ of the hydrocarbon–water interface is $L\,\mathrm{d}x/a_0 = L\bar{l}\,\mathrm{d}\bar{x}/a_0$.

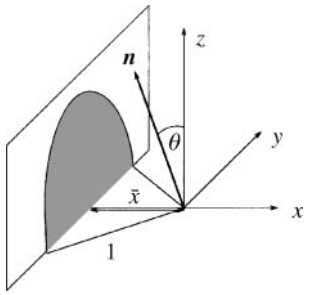

Fig. 5 Schematic illustration of a lipid chain and a single wall at distance $\bar{x}$. The lipid chain is characterized by a director $\boldsymbol{n}$, attached at position $x = y = 0$ at the interfacial plane, $z \equiv 0$. The angle between the z-axis and the director is θ. The wall is impenetrable for the director (relevant for the shaded region for which the distance to the director origin is less than $|\boldsymbol{n}| = 1$).

The director partition integral, $q(\bar{x})$, *i.e.*, the surface area of the truncated unit hemisphere, is easily calculated using x, $y = r(x)\sin\phi$, $z = r(x)\cos\phi$, with $r(x) = \sqrt{1 - x^2}$, to parameterize the points $\{x, y, z\}$ on the surface of the unit sphere; $r(x)$ denoting the distance of a surface point from the x-axis, and ϕ its azimuthal angle (with respect to z) in the yz-plane. For a director originating at distance $\bar{x}$ from the wall, the available configurational space (sphere surface) involves all points in the range $x = -\bar{x}, \ldots, 1$ and $\phi = -\pi/2, \ldots, \pi/2$. The limits on ϕ ensure $z \geqslant 0$. The configurational partition function for a chain anchored at $\bar{x}$ is thus

$$q(\bar{x}) = \int_{-\bar{x}}^{1} \mathrm{d}x \int_{-\pi/2}^{\pi/2} \mathrm{d}\phi\; r(x)\sqrt{1 + r'(x)^2} = \pi(1 + \bar{x}) \qquad (20)$$

with $r'(x) = \mathrm{d}r/\mathrm{d}x$. [Note that $r(x)\sqrt{1 + r'(x)^2} = 1$.]

The excess free energy per chain at $\bar{x}$, relative to the unperturbed membrane, is

$$\Delta f(\bar{x}) = -k_B T \ln[q(\bar{x})/q(1)] = -k_B T \ln[(1 + \bar{x})/2]$$

For the total lipid perturbation energy of a bilayer in contact with a single wall of length L we get

$$\frac{a_0 \Delta F}{2\bar{l}L} = \int_0^1 \mathrm{d}\bar{x}\; \Delta f(\bar{x}) = k_B T(1 - \ln 2) \qquad (21)$$

For C-12 chains packed with $a_0 = 32$ Å^2 we find that $\bar{l} \approx 11$ Å is on the order of the hydrophobic monolayer thickness. This leads to $\Delta F/L \approx 0.21 k_B T$ Å^{-1}, which may be compared to the value $\Delta F(d \to \infty)/L \approx 0.3 k_B T$ Å^{-1} that we have calculated based on the detailed chain packing theory; see Fig. 2. Considering its extreme simplicity, the prediction of the simple director model is quite satisfactory. The fact that the simple model yields a smaller value for the perturbation free energy can be attributed to the fact that the director is treated as a line, rather than, say, a cylindrical (or, more realistically, a "turnip-like") object of nonzero thickness. The number of conformations discarded by the presence of an impenetrable wall will be larger in the latter case.

The director model can also be used to derive the interaction free energy between two walls in the membrane, $\Delta F(d)$. As long as the distance between the walls, d, is larger than $2\bar{l}$,

the perturbation regions of the two walls do not interfere and $\Delta F(d) = \Delta F_\infty$. At smaller distances a certain fraction of the lipid chains will interact with both surfaces. As we found using the detailed chain packing theory, the fraction of perturbed directors as well as the extent of the perturbation increases as d decreases, implying an increase in $\Delta F(d)$. Concomitantly, the volume between the walls and hence the number of chains experiencing the perturbation diminishes with d. Thus we expect the appearance of a repulsive barrier at intermediate separations followed by a depletion attraction at very small separations, as explained in more detail below. This qualitative behavior is indeed corroborated by the director model, as shown in Fig. 6.

The curve describing $\Delta F(d)$ in Fig. 6 represents the results obtained by extending our simple director model to a membrane containing two parallel apposed planar walls. Explicitly, defining $\tilde{d} = d/\bar{l}$ we find that, for $1 \leqslant \tilde{d} \leqslant 2$,

$$\frac{a_0 \Delta F}{\bar{l} L k_B T} = 2\left[\tilde{d} - \left(1 + \frac{\tilde{d}}{2}\right)\ln\left(\frac{\tilde{d}}{2}\right) - (1 + \ln 2)\right] \qquad (22)$$

and for $0 \leqslant \tilde{d} < 1$ the result is

$$\frac{a_0 \Delta F}{\bar{l} L k_B T} = -\tilde{d}\ln\left(\frac{\tilde{d}}{2}\right) \qquad (23)$$

Recall that $\Delta F(\tilde{d} \geqslant 2) \equiv 0$.

The behavior of the interaction potential between the two walls is in qualitative agreement with the chain packing theory. In addition, the director model provides a simple interpretation for the nonmonotonic dependence of $\Delta F(d)$ on the distance between the two surfaces. The average conformational free energy loss, per director, resulting from the confinement by the two walls increases logarithmically with d, namely, $\Delta F(d)/N(d) \sim \ln(\tilde{d}/2)$. On the other hand, for the number of chains confined between the two walls we have $N(d) \sim d$, and hence $\Delta F(d) \sim \tilde{d}\ln(\tilde{d}/2)$, explaining the appearance of a barrier at some intermediate d and the vanishing of $\Delta F(d)$ at $d \to 0$. From eqn. (22) and (23) we find that the repulsive barrier peaks at $\tilde{d} = 2/e \approx 0.74$, at which point many chains strongly interact with both surfaces.

To compare the director model prediction for $\Delta F(d)$ with the results obtained from the chain packing theory, we again choose $a_0 = 32$ Å^2 and $\bar{l} = 11$ Å. The result is shown by the dashed line in Fig. 2. The nonmonotonic behavior of $\Delta F(d)$ is similarly reproduced by both models. We have already commented on the different asymptotic values obtained for the interaction free energy in the limit of isolated inclusions ($d \to \infty$). The appearance of the repulsive barrier at a larger value of d predicted by the director model as compared to the chain packing theory can be explained as follows. The requirement of uniform chain segment density imposed in the chain packing theory strongly prefers those conformations whose average orientation does not deviate considerably from the membrane normal; *i.e.*, those conformations characterized by a small tilt angle. In contrast, equal probabilities have been assumed for all tilt angles in the director model. The average lateral extensions of these chains are therefore relatively large. Hence, they begin interacting with both walls at larger interwall separations, thereby shifting the repulsive barrier to a higher value of d.

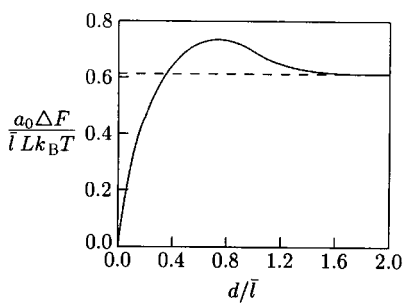

Fig. 6 The lipid-mediated interaction free energy, $\Delta F(d)$, per monolayer between two apposed planar surfaces at distance d, according to the simple director model, as given by eqn. (22) and (23).

V. Concluding remarks

Using a molecular-level theory for the lipid chain packing in a bilayer membrane, appropriately extended to account for the effects of impenetrable protein walls on chain conformational statistics, we have calculated the lipid-mediated interaction potential between the walls. Focusing on the case of zero hydrophobic mismatch, we found that the interaction between the surfaces becomes repulsive as the inclusions approach each other and their respective perturbation zones begin to overlap, peaking at distances corresponding to just one or two molecular diameters. The same qualitative behavior is predicted by the simple, analytical, director model. Our results are in qualitative agreement with the more detailed simulation studies of Sintes and Baumgärtner.[16] Our mean-field theory cannot account for the long-ranged, fluctuation-mediated, attraction found in these simulations.

In addition to interaction free energies our model yields relevant information on the conformational properties of the lipid chains between the hydrophobic walls. In particular, we found that to satisfy better the requirement of uniform segment density within the hydrophobic core, the chain directors are tilted away from the walls.

Our results apply to all integral membrane proteins that have a sufficiently large diameter of their hydrophobic core. Yet, they are also expected to hold, at least qualitatively, for single membrane-spanning helices. Taking into account the finite radius of the helix would be a straightforward extension of the present calculations. Another, perhaps more interesting, application of the present formalism would be the investigation of inclusion shapes that differ from simple membrane-spanning cylinders. Of particular interest would be the calculation of the interaction potential between amphipathic α-helical peptides that only partially penetrate the hydrophobic membrane interior.

Acknowledgements

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Appendix

Numerical details

To calculate $P(x, \alpha)$ we have used a discretized version of eqn. (13) [and analogously for eqn. (17)]. To this end, the region defined by $0 \leqslant x \leqslant d$ and $0 \leqslant z \leqslant h_0$ of the xz-plane was divided into $n_x n_z$ small elements of area $\Delta x \Delta z$, with $\Delta x = \Delta z = 1$ Å. The distance d between the walls was chosen such that $d = n_x \Delta x$. All possible chain conformations (*trans*/*gauche* bond sequences) were generated according to the rotational-isomeric-state model.[20] For each bond sequence the segment positions were calculated for n_x uniformly distributed headgroup positions in the region $0 \leqslant x \leqslant d$ and 120 different (uniformly distributed) chain orientations. All those chain conformations that penetrate into either the aqueous phase or the interior of the walls were assigned zero probability. The symmetry of the bilayer was taken into account by

"mirror imaging" of all chain segments that cross the bilayer midplane, *i.e.*, those located in the range $z < 0$.

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