Free Energy and Thermal Fluctuations of Neutral Lipid Bilayers

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A new method is proposed to calculate the free energy of lamellar liquid crystals lipid bilayers/water. The root-mean-square fluctuation of the distance between two neighboring bilayers is calculated for a number of distributions by minimizing the total free energy. Analytical solutions for the free energy are derived for a Gaussian distribution of distances, which are compared with numerical solutions obtained for more realistic distributions, which account for the correlation between the fluctuations of neighboring bilayers. Calculations are performed for typical values of the interaction parameters, and the comparison with experiment provides a more than qualitative agreement. The calculations also show that at infinite separation distance there is a minimum of zero free energy and that a local minimum can occur at a finite distance, which can be stable or unstable. The two minima are separated by a potential barrier. An unbinding transition occurs when the free energies of the two minima are equal to zero.

I. Introduction

It is well-known that the lipid molecules placed in water form multilamellar vesicles, which can be regarded locally as smectic liquid crystals, with stacks of bilayers aligned at constant separation, along a director axis. An important problem is to calculate the free energy of the system, from which one could derive the equilibrium separation distance and the corresponding binding free energy. It was recently noted[1,2] that the experimental data for both the interaction force per unit area (pressure) and the root-mean-square fluctuation of the distance between two neighboring bilayers as functions of the average separation between them are not satisfactorily explained by the existing theories.[3−5]

For neutral bilayers, there are no long-range double-layer forces which, coupled with the van der Waals attraction, could explain the stability of the lamellar structure. At small separations, the required repulsion is provided by the hydration force, which was investigated both experimentally[6−8] and theoretically.[9,10] However, it was experimentally observed that the lipid bilayers could be swollen in water up to very large interlayer distances,[11] where the short-range exponential hydration repulsion becomes negligible.

Helfrich was the first to suggest[3] that the entropic confinement (due to neighboring layers) of thermally excited out-of-plane undulations of the interfaces of the lipid bilayers (lamellae) leads to a long-range repulsion force, inverse proportional to the third power of the distance, which could be responsible for the hyperswelling of the bilayers. His original method was to assume a linear interaction between lamellae, dependent on an unknown elastic constant B. Because of the linearity, the oscillation modes are not coupled, and this allowed him to compute the free energy as a sum over the free energies of the individual modes. He showed that the unknown elastic constant could be obtained in a self-consistent manner, either from the bulk properties of the multilayer system or from the root-mean-square fluctuation σ_b of the bilayer positions.[3]

Since at large distances the van der Waals forces are inversely proportional to the fifth power of the distance, Helfrich showed that the entropic confinement repulsion would always dominate at large enough separations[3] and, consequently, that the separation between the bilayers can extend at infinity. Experiments appeared to confirm this conclusion.[11]

Some other experiments, however, indicated that the separation between membranes remained finite even in the presence of excess water.[1,12] A possible explanation could be that the Helfrich theory, which involves in the calculation of the entropic confinement only a hard-wall (steric) repulsion, does not account for other forces between membranes.

The role of undulation on the equilibrium of lipid bilayers was also examined by Lipowsky and Leibler,[13] who used a nonlinear functional renormalization group approach, and by Sornette,[14] who employed a linear functional renormalization approach. It was theoretically predicted that a critical unbinding transition (corresponding to a transition from a finite to an infinite swelling) can occur by varying either the temperature or the Hamaker constant. However, the renormalization group procedures do not offer quantitative information about the systems, when they are not in the close vicinity of this critical point.

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The effect of the nonlinear interaction forces between the lipid bilayers on the undulation was investigated by several groups.[4,15] An important advance was made in the perturbative theory of "soft confinement" of Podgornik and Parsegian.[5] In their work, the nonlinear interactions between bilayers (due to DLVO forces plus hydration repulsion) was considered as a small perturbation to a hard wall (steric) confinement. Using a variational method due to Feynman,[16] they obtained an equation for the unknown interaction constant B of an hypothetical harmonic potential, as a function of the separation distance a, by minimizing the free energy with respect to both σ_b and B. The quantity σ_b represents the root-mean-square fluctuation of the distribution of the positions of membranes, $\rho(u)$, u being the displacement of a point of the membrane surface from the average position, located at half-distance between its two neighboring bilayers, assumed rigid. A Gaussian distribution of membrane positions was employed for averaging the interaction energy in the partition function, and then σ_b^2 was calculated by assuming that $\rho(u)$ should verify a diffusion-type equation, with the boundary conditions $\rho(u) = 0$ at $u = \pm a$. The latter distribution was employed because the long tails of the Gaussian will lead to a divergent energy for the hard (rigid wall) confinement, which is their unperturbed state.

The purpose of this article is to present a variational analysis which no longer considers that the fluctuating bilayer is confined by two rigid walls. A Gaussian, a truncated Gaussian, or an asymmetrical Gaussian distribution is assumed for the fluctuating distances between two neighboring bilayers.

In the case of a linear interaction between neighboring lipid bilayers, Helfrich has demonstrated that the repulsive free energy due to confinement is inversely proportional to σ_b^2. While this result is strictly valid for a harmonic interaction potential (linear force), we assume that it can be extended to any interaction. We will examine later under what conditions this approximation is accurate.

The free energy of a bilayer in a given potential is composed of the energy of interaction with the other membranes and an entropic term, due to the bilayer confinement, which is inverse proportional to σ_b^2. The energy is increased by a distribution which has a large value of σ_b, because then a large part of the bilayer is in a region of stronger repulsive interactions; on the other hand the entropic term decreases with increasing σ_b. Consequently, there is an optimum σ_b for which the free energy is minimum.

Instead of computing via optimization the hypothetical elastic constant B corresponding to the interaction potential of the problem, as in the work of Podgornik and Parsegian,[5] we express B in terms of σ_b via the equipartition principle and calculate σ_b which minimizes the free energy. In addition, whereas in the Podgornik and Parsegian work, the calculation involved a small perturbation of a rigid wall confinement, in the present approach the hard wall confinement is not used as a base state. However, while the confining bilayers are not considered rigid, the exclusion of permeation between two neighboring membranes provides indirectly such a confinement. Most importantly, the present approach can employ an asymmetrical distance distribution for the fluctuating distances between two neighboring bilayers,[17] which cannot be used when one assumes that a bilayer is confined by two rigid walls. The former distribution is expected, because the fluctuations of neighboring membranes are increasingly correlated, as the distance between them becomes smaller.

II. Free Energy of Entropic Confinement for a Linear Interaction

Following Helfrich,[3,18] one can compute the free energy of confinement, starting from the Hamiltonian of the confined bilayer

$$H = \int \mathrm{d}x\, \mathrm{d}y \left(\frac{1}{2} K_c \left(\frac{\partial^2 u(x,y)}{\partial x^2} + \frac{\partial^2 u(x,y)}{\partial y^2} \right)^2 + \frac{1}{2} B u^2(x,y) \right) \tag{1}$$

where K_c is the bending modulus of a bilayer, B is an unknown constant of the assumed linear interaction force between bilayers, and $u(x,y)$ denotes the displacement along z from the average position $z = 0$ of a point of a bilayer of coordinates x and y. Denoting by $\tilde{u}(q_x,q_y)$ the Fourier transform of $u(x,y)$, the partition function acquires the form

$$Z = \int \prod \mathrm{d}\tilde{u} \left(\exp\left(-\beta \sum_{q_x,q_y} \frac{1}{2} \tilde{u}^2 (K_c \vec{q}^4 + B) \right) \right) = \prod_{q_x,q_y} \left(\frac{2\pi k T}{K_c q^4 + B} \right)^{1/2} \tag{2}$$

where $q^2 = q_x^2 + q_y^2$.

The difference in the free energies of the free bilayer (which formally corresponds to $B \to 0$) and the confined one, per unit area, can be computed by replacing the summation over q_x,q_y with an integral

$$\Delta f = kT \frac{1}{(2\pi)^2} \int_0^\infty \mathrm{d}q 2\pi q \ln \left(\frac{K_c q^4 + B}{K_c q^4} \right)^{1/2} = \frac{kT}{8} \left(\frac{B}{K_c} \right)^{1/2} \tag{3}$$

The free energy due to the entropic confinement alone is obtained from eq 3 by subtracting the interaction energy per unit area

$$f_C = \frac{kT}{8} \left(\frac{B}{K_C} \right)^{1/2} - \frac{1}{2} B \langle u^2 \rangle \tag{4}$$

The unknown coupling constant B can be related to the mean-square fluctuation of the bilayer position $\sigma_b^2 = \langle u(x,y)^2 \rangle$ by using the equipartition principle

$$\tilde{u}^2 \left(\frac{1}{2} K_c q^4 + \frac{1}{2} B \right) = \frac{1}{2} kT \tag{5}$$

which leads to the following relation between σ_b^2 and B:

$$\sigma_b^2 = \langle u^2 \rangle = \sum_{q_x,q_y} \tilde{u}^2 = \frac{kT}{(2\pi)^2} \int_0^\infty \frac{2\pi q\, \mathrm{d}q}{K_c q^4 + B} = \frac{kT}{8(K_c B)^{1/2}} \tag{6}$$

Introducing eq 6 in eq 4, yields:[5,18]

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$$f_c = \frac{(kT)^2}{128K_c\sigma_b^2} \tag{7}$$

a result which is essentially due to Helfrich.[3]

The Helfrich formula for the free energy (eq 7) is strictly valid only for a linear interaction. However, eq 7 constitutes a good approximation, even for nonlinear interactions, as long as the interaction energy between layers is much smaller than the elastic energy of the modes corresponding to bending, hence if $B \ll K_c q^4$. In this case, the coupling between modes is minimal and eq 5 is approximately valid. Using the expression of B from eq 6, the above condition reduces to an inequality for the wavelength λ_{xy} in the xy plane

$$\frac{\lambda_{xy}}{\sigma} \ll 2\pi\left(\frac{8K_c}{kT}\right)^{1/2} \qquad \left(\lambda_{xy} = \frac{2\pi}{q}\right) \tag{8}$$

which indicates that eq 7 can be used for bilayers that possess a high bending modulus. For lipid bilayers, a typical value of the bending modulus is $K_c = 1.0 \times 10^{-19}$ J,[1] and the right-hand quantity becomes $\approx 10^2$.

III. Thermal Fluctuations for Soft Confinement

As already stated, the free energy due to the entropic confinement remains of the form of eq 7 even for nonlinear interactions, if the inequality (8) is satisfied. To compute the total free energy, one should calculate the pair distribution function of the distances between neighboring fluctuating bilayers. If the distribution of bilayer positions $\rho(u)$ is Gaussian with the root-mean-square σ_b and the undulations of neighboring bilayers are totally uncorrelated, then the distribution of distances z between two neighboring bilayers (see Figure 1) is the convolution of two Gaussians, which is again a Gaussian

$$P(z) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} du_1\, du_2\, \frac{1}{2\pi\sigma_b^2}\, e^{-u_1^2/2\sigma_b^2}\, e^{-u_2^2/2\sigma_b^2} \times \delta(z - (a - (u_1 - u_2))) = \frac{1}{(2\pi)^{1/2}\sigma}\, e^{-(z-a)^2/2\sigma^2} \tag{9}$$

where $\sigma = 2^{1/2}\sigma_b$.

Let us denote by V the interaction potential for planar bilayers. Assuming that the fluctuations of adjacent bilayers are totally uncorrelated, and using locally the planar approximation, the free energy per unit area can be written in terms of the distance distribution function $P(z)$

$$f(a,\sigma) = \int dz\, P(z)V(z) + \frac{(kT)^2}{64K_c\sigma^2} \tag{10}$$

where $a = \langle z\rangle$ is the average separation between neighboring bilayers and $\sigma^2 \equiv \langle (z - \langle z\rangle)^2\rangle$ is the root-mean-square fluctuation of the distribution $P(z)$.

The minimum of the free energy with respect to σ for any interaction potential $V(z)$ and all separation distances

$$\partial f(a,\sigma)/\partial\sigma = 0 \tag{11}$$

provides an expression for σ.

However, the assumption that the fluctuations of neighboring bilayers are totally uncorrelated leads to the unphysical result that they can permeate each other ($P(z) > 0$ for $z < 0$). One can approximately account for the correlation of the motions of the neighboring bilayers as follows.

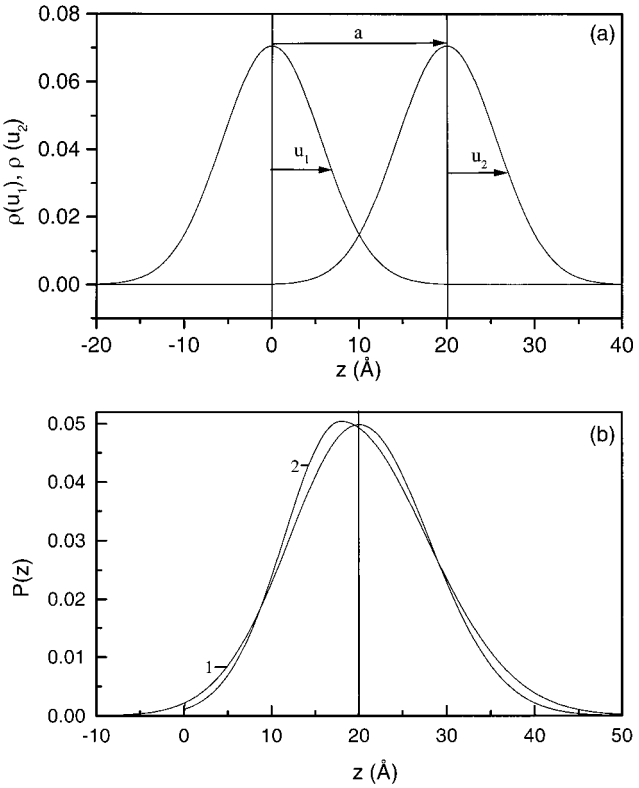

Figure 1. (a) Gaussian distributions of displacements from equilibrium positions, $\rho(u_1)$ and $\rho(u_2)$, for two neighboring bilayers, situated at the average distance a apart. (b) Distributions of distances between bilayers. (1) a Gaussian and a truncated Gaussian (the same distribution as Gaussian, except $P(z) = 0$ for $z < 0$) and (2) an asymmetric distribution ($\alpha = 1.4$).

First, we will assume that the fluctuations of neighboring bilayers are independent of each other, as long as the distance between them is positive, but forbid those fluctuations which involve their interpenetration. In this case, the distance distribution function is a truncated Gaussian (see Figure 1b). This is equivalent to superimposing an additional hard-wall potential to the interaction potential, and we will show that, in the asymptotic limit, the results are in qualitative agreement with those of Podgornik and Parsegian.[5]

Second, we will consider that the degree of correlation between the fluctuating bilayers depends on their separation distance, with total correlation as $z \to 0$ and no correlation as $z \to \infty$. This provides an asymmetric distribution (Figure 1b), which will be described using an asymmetry coefficient α in a truncated Gaussian distribution

$$P(z) = \frac{1}{C}\, e^{-(z-a')^2/2\sigma'^2}, \qquad 0 < z \le a'$$

$$P(z) = \frac{1}{C}\, e^{-(z-a')^2/2(\alpha\sigma')^2}, \qquad a' < z < \infty \tag{12}$$

where a' is the position of the maximum and the constant C is determined through normalization. The average separation distance $a = \langle z\rangle$ and the variance of the distribution (the mean-square fluctuation) $\sigma^2 = \langle z^2\rangle - \langle z\rangle^2$ can be computed as functions of the parameters a', α, and σ'.

Recent Monte Carlo simulations of bilayer fluctuations[17] revealed that the distance distribution function is asymmetric. While this result is expected, since it implies that the correlation between fluctuating bilayers decreases

with their separation, there are no quantitative analytic theories to predict the degree of asymmetry from the bilayer interaction parameters. However, the simulations show that the asymmetry is stronger only when a few bilayers are present. For a large number of bilayers, the distribution function tends to a limiting distribution which can be approximated by eq 12, with $\alpha \approx 1.4$.

Let us now present explicit calculations for each distribution.

III.1. Gaussian Distribution. The Gaussian distribution allows one to obtain analytical expressions for σ and for the free energy, when only the hydration force is present. It will be shown later that, for small distances, these expressions provide also good approximations for the truncated and asymmetric Gaussian distributions and that the van der Waals attraction does not modify substantially the expression of σ.

The hydration interaction energy between two neighboring planar bilayers,[6−10] separated by a distance z, is given by

$$V_H(z) = A_H e^{-z/\lambda} \tag{13}$$

The free energy per unit area $f(a,\sigma)$ acquires the form

$$f(a,\sigma) = \int_{-\infty}^{\infty} \frac{dz}{(2\pi)^{1/2}\sigma} e^{-(a-z)^2/2\sigma^2} A_H e^{-z/\lambda} + \frac{(kT)^2}{64K_c\sigma^2} = A_H e^{-a/\lambda} e^{\sigma^2/2\lambda^2} + \frac{(kT)^2}{64K_c\sigma^2} \tag{14}$$

and the minimum with respect to σ leads to

$$4 \ln\left(\frac{\sigma}{\lambda}\right) + \frac{\sigma^2}{2\lambda^2} - \ln\left(\frac{(kT)^2}{32A_H K_c \lambda^2}\right) = \frac{a}{\lambda} \tag{15}$$

The solution of the above equation is plotted in Figure 2, for $T = 300$ K and the following typical values of the parameters:[1,8,19] $K_c = 1 \times 10^{-19}$ J, $A_H = 0.035$ J/m^2, and $\lambda = 1.5$ Å. One can see from Figure 2a that σ remains finite even when $a = 0$ (i.e., the neighboring bilayers touch each other). In previous theories, which include the hard wall confinement,[3,5] the free energy diverges for $a \to 0$; in the present case, it remains finite because the repulsion is provided by the hydration force, which is finite at any distance.

One can see from Figure 2a that for separation distances less than 10 Å, the quadratic term can be neglected compared to the logarithms, and eq 15 becomes

$$\sigma^2 = \frac{\lambda kT}{4(2A_H K_c)^{1/2}} e^{a/2\lambda} \tag{16}$$

A recent improvement of the Caille theory of X-ray scattering by smectic liquid crystals[20,21] opens the possibility to obtain $\sigma = \sigma(a)$ from the diffraction spectrum. Extensive experimental measurements on lipid bilayers[1] indicated that, at low separations, there is an exponential dependence as predicted by eq 16. However, to obtain reliable interaction parameters from the fit of the experimental data, one should identify the validity domain of eq 16. As shown in Figure 2a, eq 16 is valid only for small values of a.

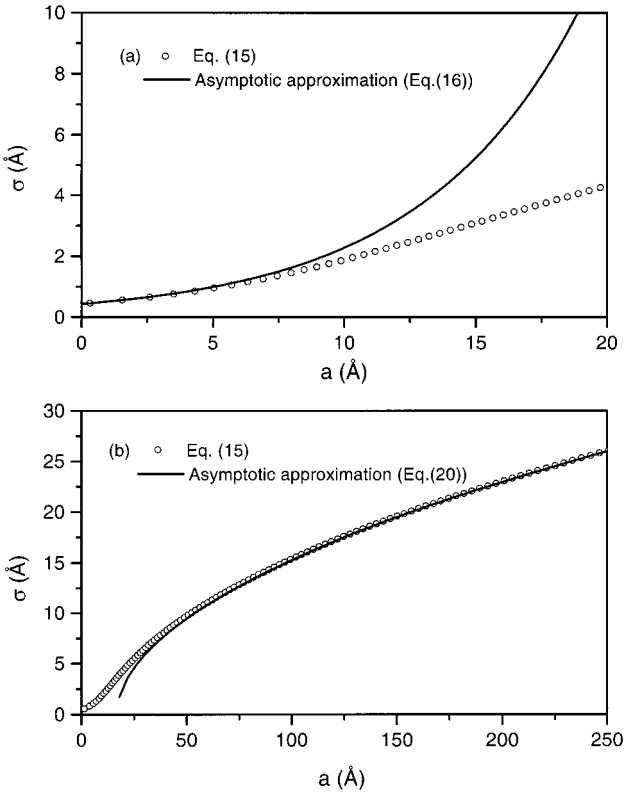

Figure 2. Equation 15 (circles): (a) asymptotic approximation for small values of a (eq 16), continuous line; (b) asymptotic approximation for large values of a (eq 20), continuous line.

Let us now calculate the free energy for small values of a. Equations 14 and 16 lead to

$$f(a) = A_H e^{-a/\lambda} e^{\sigma^2/2\lambda^2} + \frac{kT}{16(2^{1/2})}\left(\frac{A_H}{K_c\lambda^2}\right)^{1/2} e^{-a/2\lambda} \tag{17}$$

an equation which contains two repulsive contributions to the free energy. The first is due to hydration, which is enhanced by an exponential factor $e^{\sigma^2/2\lambda^2}$ due to undulation; this enhancement was also suggested by Sornette and Ostrovsky.[15] The other term is due to the entropic confinement. Both terms have exponential decays, but with decay lengths of λ and 2λ, respectively. The exponential decay in the second term is due to the exponential dependence of σ on the separation a.

A similar functional form for the free energy, with two exponential decays, was proposed by Evans and Parsegian[4,22]

$$f_{EP}(a) = A_H e^{-a/\lambda} + \frac{\pi kT}{16}\left(\frac{A_H}{K_c\lambda^2}\right)^{1/2} e^{-a/2\lambda} \tag{18}$$

The differences consist in the absence of the undulation enhancement in the first term of eq 18 and the numerical constant in the second term.

The variational treatment of hydration confinement by Podgornik and Parsegian[5] provided an expression similar to eq 18 in the limit of small a; however the coefficient multiplying the exponential of the second term was dependent on a.

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(22) Evans, E.; Needham, D. *J. Phys. Chem.* **1987**, *91*, 4219.

For large values of a, the quadratic term dominates the left-hand term of eq 15; neglecting the other terms, one can write

$$\sigma = (2a\lambda)^{1/2} \tag{19}$$

A better approximation is obtained by evaluating the neglected terms using eq 19. This leads to

$$\sigma = \left(2a\lambda + 2\lambda^2 \ln\left(\frac{(kT)^2}{128A_H K_c a^2}\right)\right)^{1/2} \tag{20}$$

Figure 2b shows that the asymptotic value of σ provided by eq 20 is in very good agreement with eq 15 for $a > 30$ Å.

Using eqs 14 and 20, the free energy acquires the form

$$f(a) = \frac{(kT)^2}{128K_c a^2} + \frac{(kT)^2}{64K_c\left(2a\lambda + 2\lambda^2 \ln\frac{(kT)^2}{128A_H K_c a^2}\right)} \tag{21}$$

For large values of a, the logarithm in the second term becomes negligible, and the free energy becomes proportional to a^{-1}

$$f(a) \approx \frac{(kT)^2}{128K_c a\lambda} \tag{22}$$

The derived equations involve the assumption that the bilayers can permeate each other. It will be shown later that, when the permeation is forbidden, σ becomes proportional to a for large values of a, as predicted by the existing hard wall confinement theories.[3,5] However, for small values of a, eq 15 constitutes a very good approximation.

III.2. Truncated Gaussian Distribution. As already noted, to avoid permeation, the integration range should start from zero and, when only the hydration forces are taken into account, eq 10 becomes

$$f(a,\sigma) = \int_0^\infty \frac{dz}{C} e^{-(a-z)^2/2\sigma'^2} A_H e^{-z/\lambda} + \frac{(kT)^2}{64K_c\sigma^2} = A_H e^{-a/\lambda} e^{\sigma'^2/2\lambda^2} \int_0^\infty \frac{dz}{C} e^{-(a'-z)^2/2\sigma'^2} + \frac{(kT)^2}{64K_c\sigma^2} \tag{23}$$

with $a' = a - (\sigma'^2/\lambda)$, where σ' is the root-mean-square fluctuation of the Gaussian distribution, which is different from σ, the root-mean-square fluctuation of the truncated Gaussian distribution However, when σ' is at least a few times smaller than a

$$\int_{-\infty}^0 dz\, e^{-(a-z)^2/2\sigma'^2} \ll \int_0^\infty dz\, e^{-(a-z)^2/2\sigma'^2}$$

and one can write that $\sigma \cong \sigma'$ and $C \cong \sigma(2\pi)^{1/2}$. When, in addition, σ' is a few times smaller than a', the integral of the first term of eq 23 is close to unity, and the results are well approximated by eq 15. However, σ' is not always smaller than a', and then numerical methods are necessary to obtain the values of σ which minimize the free energy in eq 23. This happens either when $a \to 0$ or, at large separations, when σ^2/λ becomes comparable or even larger than a. In these cases, the results differ from those obtained in the previous section.

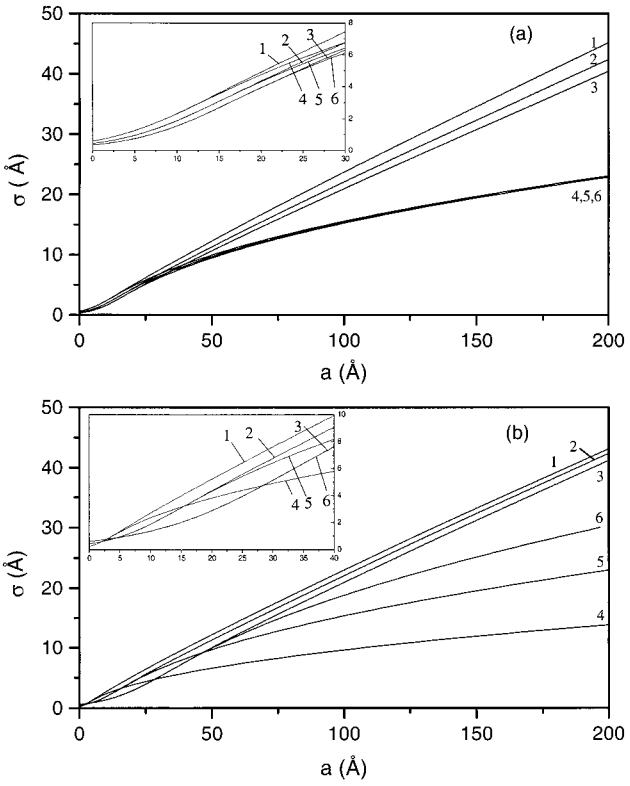

Figure 3. Root-mean-square fluctuation σ vs average separation distance a, for a Gaussian and a truncated Gaussian distribution. (a) $K_c = 1 \times 10^{-19}$ J and $\lambda = 1.5$ Å: truncated Gaussian, (1) $A_H = 0.01$ J/m^2, (2) $A_H = 0.035$ J/m^2, (3) $A_H = 0.1$ J/m^2; Gaussian, (4) $A_H = 0.01$ J/m^2, (5) $A_H = 0.035$ J/m^2, (6) $A_H = 0.1$ J/m^2. (b) $K_c = 1 \times 10^{-19}$ J and $A_H = 0.035$ J/m^2: truncated Gaussian, (1) $\lambda = 0.5$ Å, (2) $\lambda = 1.5$ Å, (3) $\lambda = 3$ Å; Gaussian (4) $\lambda = 0.5$ Å, (5) $\lambda = 1.5$ Å, (6) $\lambda = 3$ Å. In the inserts, the same functions are presented for small values of a at a larger scale.

The results of the numerical computations are compared with the analytical ones provided by eq 15 in Figure 3, for various values of the hydration interaction constant A_H (Figure 3a) and of the decay length λ. In all the cases investigated, a linear asymptotic behavior was obtained for σ as a function of a, as suggested by Helfrich. As already noted, this occurs because the truncation is equivalent to a hard wall repulsion, which limits the fluctuations of the bilayers. However, the proportionality coefficient $\mu = \sigma^2/a^2$ differs from those obtained from the theories for hard wall confinement[3,5] and is dependent on the parameter A_H of the hydration force. It is of interest to note that the asymptotic behavior of $\sigma(a)$ is almost independent of λ for the truncated Gaussian distribution, but almost independent of A_H for the Gaussian distribution (see eq 20).

III.3. Asymmetric Distribution. When in eq 12 σ' is at least a few times smaller than a', one can neglect the integral from $-\infty$ to 0, and the following expressions are obtained for C, a', and $\sigma^2 = \langle z^2\rangle - \langle z\rangle^2$:

$$C = \left(\frac{\pi}{2}\right)^{1/2} \sigma'(\alpha + 1)$$

$$a' = a - \sigma'\left(\frac{2}{\pi}\right)^{1/2} (\alpha - 1) \tag{24}$$

$$\sigma^2 = \sigma'^2\left(\alpha^2 - \alpha + 1 - \frac{2}{\pi}(\alpha - 1)^2\right)$$

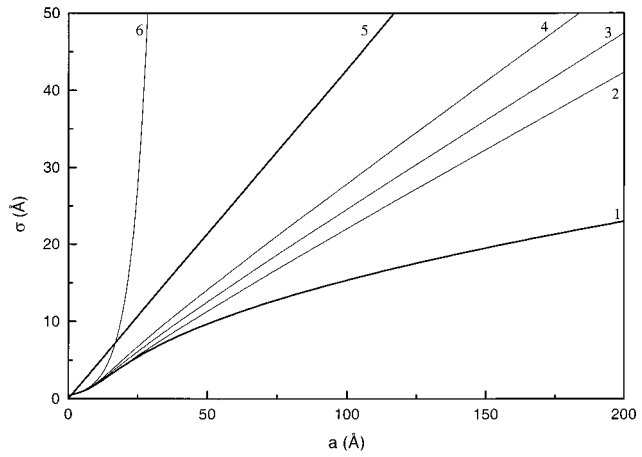

Figure 4. Root-mean-square fluctuation σ vs the separation distance a, for (1) eq 15 and (2−4) σ calculated for truncated Gaussian distribution with various asymmetry coefficients: (2) $\alpha = 1$; (3) $\alpha = 1.4$; (4) $\alpha = 2.0$; (5) Helfrich proportionality relation, $\sigma^2 = \mu a^2$ ($\mu = 0.183$); (6) approximate solution for small distances, eq 16.

The following approximate expression can be written for the free energy:

$$f(a,\sigma') = \int_0^\infty dz\, P(z) A_H e^{-z/\lambda} + \frac{(kT)^2}{64K_c\left(\alpha^2 - \alpha + 1 - \frac{2}{\pi}(\alpha-1)^2\right)\sigma'^2} \approx \frac{2A_H e^{-a/\lambda} e^{\sigma'^2/2\lambda^2}}{(\alpha+1)} \int_0^\infty \frac{dz}{(2\pi)^{1/2}\sigma'} e^{-(a''-z)^2/2\sigma'^2} + \frac{(kT)^2}{64K_c\left(\alpha^2 - \alpha + 1 - \frac{2}{\pi}(\alpha-1)^2\right)\sigma'^2} \quad (25)$$

where

$$a'' = a - \sigma'\left(\frac{2}{\pi}\right)^{1/2}(\alpha - 1) - \frac{\sigma'^2}{\lambda}$$

It should be emphasized that eqs 24 and 25 are only approximate. In the calculation, we used eqs 10 and 12 for the numerical minimization of $f(a,\sigma')$, with respect to σ', for various values of the asymmetry coefficient α. The results are presented in Figure 4. For a smaller than about 20 Å, eq 15, and for a smaller than about 10 Å, eq 16, constitute accurate approximations. The figure indicates that again σ is proportional to a in the asymptotic limit $a \to \infty$, with a proportionality coefficient dependent on the degree of asymmetry α. The value $\mu = 0.183$ was derived for the hard wall confinement.[5] Recent Monte Carlo simulations of soft confinement of bilayers (interacting via van der Waals and hydration forces) provided lower values for the proportionality coefficient $\mu = 0.06-0.12$.[17] The results in Figure 3 show that, for a truncated Gaussian distributions, involving only hydration interactions, even lower values are obtained. However, using asymmetric Gaussian distributions, as in Figure 4, the values of μ, which are in the range 0.06−0.08, becomes compatible with those obtained from Monte Carlo simulations.

IV. The Inclusion of the van der Waals Interaction

Let us now complete the calculation by adding the van der Waals interaction potential to the hydration interaction. The bilayers are composed of hydrophilic headgroups, which are hydrated by water, and of hydrophobic hydrocarbon chains. A complete theory should take into account that their dielectric properties are different, by using different Hamaker constants.[22] The problem can be simplified by considering that, for the purpose of van der Waals interaction, the polar headgroups, being hydrophilic, are part of the water lamellae. Denoting by t_h the total thickness of the headgroups in the lamellae and by t_c the thickness of the layer of hydrocarbon chains, the van der Waals attraction between two bilayers separated by a distance z, measured in water, becomes (we include only the interactions between the nearest neighbors)

$$V_{vdW}(z) = -\frac{H}{12\pi}\left(\frac{1}{(z+t_h)^2} + \frac{1}{(z+t_h+2t_c)^2} - \frac{2}{(z+t_h+t_c)^2}\right) \quad (26)$$

where H is the Hamaker constant.

Typical values for the Hamaker constant were estimated to range from 10^{-21} to 10^{-20} J.[1,19,22,23] The values of the bending modulus are either not known for some lipids or uncertain by a factor of 4, around 10^{-19} J, for other lipids.[1] The values of the parameters of the hydration force were estimated from the fit of experimental data[1,19] with a formula derived by Evans and Parsegian[4] (reproduced here as eq 18). The values lie in the rather large range of $\lambda = 1.3-2.1$ Å and $A_H = 6.0 \times 10^{-3}$ to 2.5×10^{-2} J/m^2. In addition to depending upon the expression employed in the fitting of the data, large differences in A_H, by orders of magnitude, can be obtained whether the distance z in the hydration force is measured from the boundary between the hydrocarbon chains and headgroups or between the headgroups and water.[1]

In this section we present the results of the calculations performed for σ and the free energy for $T = 300$ K and the following values of the parameters: $H = 1.0 \times 10^{-20}$ J, $t_c = 37.8$ Å, $t_h = 7.6$ Å, $K_c = 1.0 \times 10^{-19}$ J, $\lambda = 1.5$ Å, and $A_H = 0.035$ J/m^2.

The root-mean-square fluctuation of the distance distributions, σ, will be computed for the following cases: (1) using the analytical formula (15), which involves only the hydration force ($H = 0$); (2) for a truncated Gaussian distribution, with only the hydration force present ($H = 0$); (3) for a truncated Gaussian distribution, with hydration force and van der Waals interactions with a Hamaker constant $H = 1.0 \times 10^{-20}$ J; (4) for an asymmetric Gaussian distribution with $\alpha = 1.4$ and $H = 0$; (5) for an asymmetric Gaussian distribution with $\alpha = 1.4$ and $H = 1.0 \times 10^{-20}$ J; (6) for an asymmetric Gaussian distribution with $\alpha = 2.0$ and $H = 0$; (7) for an asymmetric Gaussian distributions with $\alpha = 2.0$ and $H = 1.0 \times 10^{-20}$ J; (8) using Helfrich's proportionality relation, with $\mu = 0.183$.

The results of these computations are presented in Figure 5, which shows that (i) σ is only slightly affected by the van der Waals interactions, but depends on the distribution considered, and (ii) for $a < 20$ Å, the results for all the distributions employed are well approximated by the simple expression, eq 15, which can be used to extract some of the interaction parameters from the experimental values of $\sigma(a)$ for small values of a.

The free energy calculations are presented in Figure 6 as a function of the average distance a, for the following cases:

(23) Petrache, H. I.; Tristram-Nagle, S.; Nagle, J. F. *Chem. Phys. Lipids* **1998**, *95*, 83.

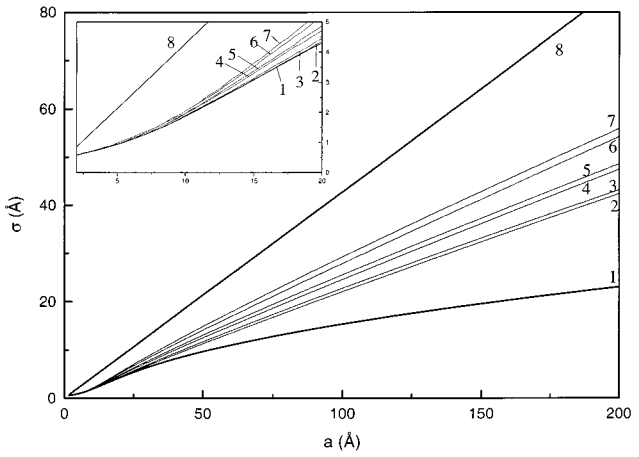

Figure 5. Root-mean-square fluctuation σ vs separation distance a, for $K_c = 1 \times 10^{-19}$ J, $t_h = 7.6$ Å, $t_c = 37.8$ Å, $A_H = 0.035$ J/m^2, $\lambda = 1.5$ Å for: (1) eq 15 ($H = 0$); (2) truncated Gaussian distribution, $H = 0$; (3) truncated Gaussian distribution, $H = 1.0 \times 10^{-20}$ J; (4) asymmetric Gaussian distribution, $\alpha = 1.4$, $H = 0$; (5) asymmetric Gaussian distribution, $\alpha = 1.4$, $H = 1.0 \times 10^{-20}$ J; (6) asymmetric Gaussian distribution, $\alpha = 2.0$, $H = 0$; (7) asymmetric Gaussian distribution, $\alpha = 2.0$, $H = 1.0 \times 10^{-20}$ J (8) Helfrich proportionality relation ($\mu = 0.183$). In the inserts, the same functions are presented for small values of a at a larger scale.

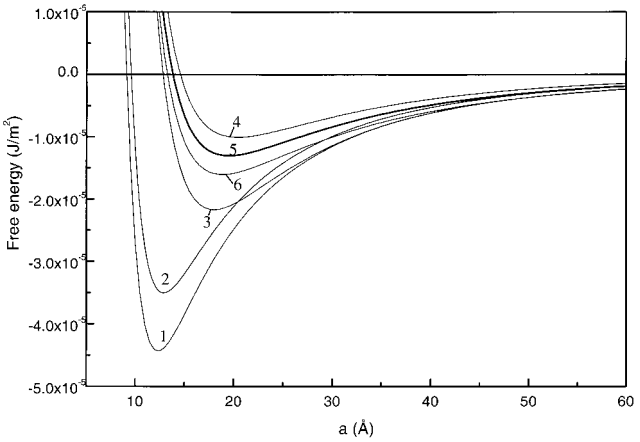

Figure 6. Free energy per unit area of bilayer, for $H = 1.0 \times 10^{-20}$ J, $K_c = 1 \times 10^{-19}$ J, $t_h = 7.6$ Å, $t_c = 37.8$ Å, $A_H = 0.035$ J/m^2, $\lambda = 1.5$ Å: (1) $K_c \rightarrow \infty$ (energy of planar bilayers); (2) calculated by adding the entropic term (eq 7 with $\sigma^2 = \mu a^2$) to the energy of planar bilayers; (3) eq 18; (4) truncated Gaussian distribution; (5, 6) asymmetric Gaussian distribution with (5) $\alpha = 1.4$ and (6) 2.0.

(1) $K_c \rightarrow \infty$. In this case, the entropic term vanishishes and the free energy is provided by the energy of planar (nonundulating) bilayers.

(2) The free energy is obtained by adding to the energy obtained under (1) the entropic term, using for σ the relation $\sigma^2 = \mu a^2$ suggested by Helfrich,[3] with the value $\mu = 0.183$.[5] This procedure neglects both the fluctuation enhancement of the energy and the more involved dependence of σ on a, obtained through the minimization of the free energy with respect to σ.

(3) The free energy was computed by adding to the Evans−Parsegian formula (eq 18) the van der Waals attraction between nonundulating bilayers. This kind of calculation, which was often used to fit the experimental data, offers results in qualitative agreement with our approach only for small values of a. However, even for small a, the fluctuation enhancement of the energy (as in eq 17) is neglected. It should be noted that the free energy computed in this manner would always have a stable minimum at finite distance, since the exponential has a shorter range than the van der Waals attraction.

(4) The free energy is computed via minimization, by using the truncated Gaussian distribution in eq 10.

(5, 6) The free energy is computed by using the asymmetric Gaussian distribution, with $\alpha = 1.4$ and 2.0, respectively, in eq 10.

The addition of the hard wall entropic confinement free energy to the interaction energy, as under (2), only raises somewhat the minimum, but almost does not modify its position. In contrast, in all the other cases, which take into account the other interactions in the calculation of entropy, considerable shifts of the equilibrium distance and of the value of the free energy at the minimum occur. This observation indicates that a common procedure, to add to the conventional interactions, calculated as for planar layers, the free energy due to the hard wall entropic confinement is inaccurate.

The asymptotic value of the free energy is zero for $a \rightarrow \infty$. Since the entropic free energy has a slower decay, for all distance distributions employed (4−6), than the van der Waals energy, the free energy will be positive at sufficiently large distances. This does not mean, however, that an unbinding of the bilayers will occur, since a negative free energy minimum (hence an absolute minimum) can exist at a finite distance. Reciprocally, the existence of a local minimum for a given set of parameters does not necessarily imply that the bilayers are bound, since the local minimum can be positive and hence unstable. However, this unstable minimum can be separated from the absolute minimum (which is zero and located at infinity) by a potential barrier and, if the latter is sufficiently high, the metastable state can survive for a long time. Similarly, if the minimum is negative but small in absolute value, a perturbation can cause the bilayers to extend to the unstable state at infinity. These results can explain the contradictory experimental observations[11,12] for the same phospholipid bilayers. The first authors[11] observed that the bilayers extend at very large separations, while the latter[12] authors observed that they swell up to small distances.

In Figure 7 we present the free energy for an asymmetric Gaussian distribution ($\alpha = 1.4$) as a function of distance for various values of the Hamaker constant (with all the other parameters unchanged). For $H > 3.825 \times 10^{-21}$ J, a stable minimum is obtained at a finite distance. For $H < 3.825 \times 10^{-21}$ J, the stable minimum is at infinite distance; however, for 3.825×10^{-21} $J > H > 3.45 \times 10^{-21}$ J, a local (unstable) minimum is still obtained at finite distance. For $H = 3.825 \times 10^{-21}$ J, a critical unbinding transition occurs, since the minima at finite and infinite distances become equal. However, these two minima are separated by a potential barrier, with a maximum height of 1.68×10^{-7} J/m^2, located at a separation distance of 90 Å. The results remained qualitatively the same for any combination of the interaction parameters.

V. Comparison with Experimental Data

A common procedure to determine the values of the interaction parameters between bilayers is to fit the experimental data with a functional form predicted by theory. It was shown[1,19] that the equilibrium separation distance between bilayers as a function of the osmotic pressure applied can be well fitted by expression (18). A recent improvement of Caille theory[20,21] of X-ray scattering by smectic liquid crystals allowed the simultaneous measurement of the average separation distance and the

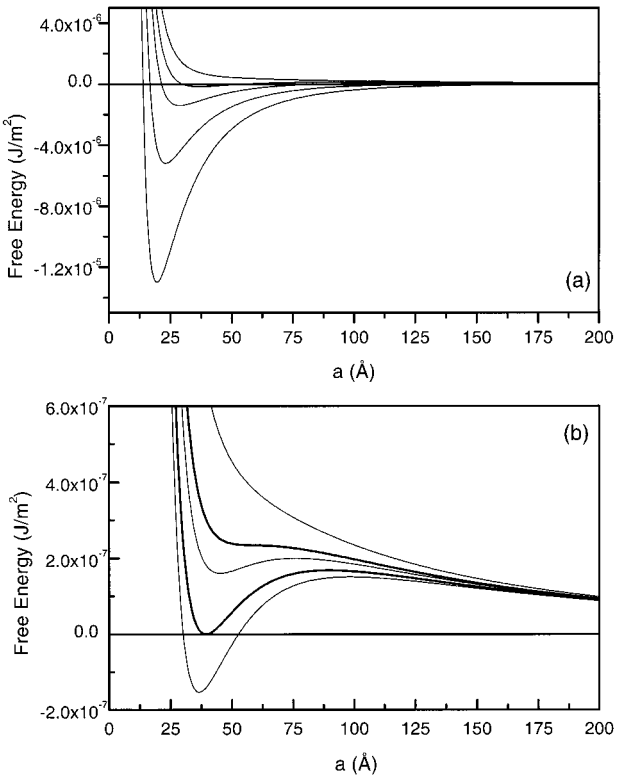

Figure 7. Free energy per unit area, computed for an asymmetric Gaussian distribution, for $K_c = 1 \times 10^{-19}$ J/m^2, $t_h = 7.6$ Å, $t_c = 37.8$ Å, $A_H = 0.035$ J/m^2, $\lambda = 1.5$ Å, $\alpha = 1.4$, and various values of H: (a) from bottom to top, $H = 10.0$, 7.0, 5.0, 4.0, and 3.0×10^{-21} J; (b) from bottom to top, $H = 4.0$, 3.825, 3.6, 3.45, and 3.0×10^{-21} J. For $H > 3.825 \times 10^{-21}$ J, the minimum at finite distance is stable. For $H < 3.825 \times 10^{-21}$ J, the minimum at infinity is the stable one. For $H < 3.45 \times 10^{-21}$, the minimum at infinity is the only one.

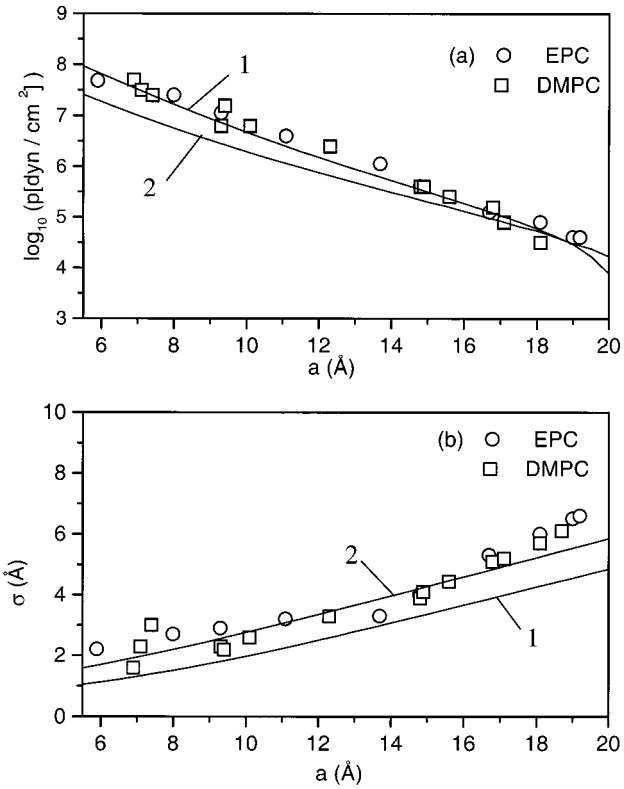

Figure 8. Experimental data from ref 1 for EPC (circles) and DMPC (squares) bilayers compared with predictions obtained using some typical values for the interaction parameters: (1) $K_c = 1.0 \times 10^{-19}$ J, $H = 1.0 \times 10^{-20}$, $t_h = 37.8$ Å, $t_c = 7.6$ Å, $\alpha = 1.4$, $A_H = 0.035$ J/m^2, $\lambda = 1.5$ Å; (2) $K_c = 0.5 \times 10^{-19}$ J, $A_H = 0.01$ J/m^2, and the other parameters as in (1).

root-mean-square fluctuation σ as a function of the applied osmotic pressure, and the authors[1,2] noted disagreement between the existing theories and experiment.

Figure 8 reproduces the experimental data of ref 1 for EPC (egg phosphatidylcholine) (circles) and DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine) (squares). Figure 8a presents $p = p(a)$ and Figure 8b presents $\sigma = \sigma(a)$. Petrache et al.[1] have shown that agreement can be obtained by fitting eq 18 to the pressure data, for various values of H ($(1.65-7.13) \times 10^{-21}$ J) and K_c ($(0.5-2.0) \times 10^{-19}$ J). In all the cases, the fitting provided $\lambda = 2.0 \pm 0.3$ Å. The experimental data for $\sigma = \sigma(a)$ are well described by the exponential function $\sigma^2 \propto e^{a/\lambda_{fl}}$ for distances between 5 and 20 Å, with $\lambda_{fl} \approx 6$ Å.[1] Combining the Helfrich expression (eq 7) with that of Evans and Parsegian (eq 18), one obtains $\sigma^2 \propto e^{a/2\lambda}$, which is in disagreement with experiment because $\lambda_{fl} \neq 2\lambda$.

Now σ and the pressure (computed as the derivative of the free energy per unit area) will be calculated, using the procedure outlined in this article. For the Hamaker constant and the bending modulus, typical values from literature, namely, 1.0×10^{-20} and 1.0×10^{-19} J, respectively, will be used. For t_h and t_c we employed the values obtained from X-ray data,[1] $t_h = 7.6$ Å and $t_c = 37.8$ Å for EPC and $t_h = 7.6$ Å and $t_c = 36.4$ Å for DMPC, respectively. Because the hydrocarbon thicknesses t_c of EPC and DMPC produced almost no difference (see eq 27), in the following only the results for the EPC are presented ($t_c = 37.8$ Å). For the degree of asymmetry α the value of 1.4, which is in agreement with the Monte Carlo simulations,[17] was selected.

It is clear that one cannot use the values of the parameters obtained through the fitting of a different expression to experimental data, to compare the present calculations to the same experimental data. One can proceed in two different ways: either to determine all the parameters from fitting the present equations to the experimental data or to use the experimental data for one of the physical quantities (p or σ) to determine some parameters and to verify if the second (σ or p) is in agreement.

Because the number of parameters is large, it is obvious that one can obtain "agreement" with experiment by fitting the present equations. It is, however, more meaningful to employ the second procedure.

The values of $\lambda = 1.5$ Å and $A_H = 0.035$ J/m^2 provided agreement with the $p = p(a)$ data (curve 1 of Figure 8a). Because for these values, the σ experiments were underestimated, a lower value for K_c was chosen. Selecting $K_c = 0.5 \times 10^{-19}$ J required a value of A_H of 0.01 J/m^2 to preserve the position of the minimum of the free energy. Under these conditions (all the other parameters remaining unchanged), curve 2 was obtained. While in better agreement with $\sigma(a)$, the agreement with $p(a)$ was somewhat deteriorated. There is no doubt that better agreement can be further achieved by fitting the parameters; however, even using some typical values from literature for the parameters, a more than qualitative agreement with experimental data was obtained.

VI. Conclusions

A new variational analysis of the interactions of fluctuating bilayers is proposed, in which the entropic

confinement energy is considered inverse proportional to the root-mean-square fluctuation of the distribution function of the distance between two successive bilayers. This approximation can be used, even for nonlinear interactions, when the bending elastic energy of the bilayer provides the dominating contribution to the mode energy. For a given functional form of the distribution, σ was obtained by minimizing the free energy. Analytical results were derived for a Gaussian distribution and compared with numerical results obtained for truncated and asymmetric Gaussian distributions.

It is shown that, in the asymptotic limit, the present approach recovers the results of other existing theories, but important differences occur at relatively short distances. As reported in the literature,[1,2] there are no theories or simulations, which are in agreement with the experimental data for both the pressure and the root-mean-square fluctuations of the distance between two neighboring bilayers, as a function of the average distance, for the same set of interaction parameters. Comparison with recent experimental data shows that, with the present approach, a more than qualitative agreement can be obtained.

The present approach recovers the well-known result that a transition from a bounded to an unbounded state can be achieved by varying the Hamaker constant. However, the stable and the unstable states (which can be either at infinity or at finite distance) are separated, in the vicinity of the transition point, by a potential barrier. This might explain the contradictory experimental observations on infinite or finite swelling of some lipid bilayers.[11,12]

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