

# Fluctuating Lipid Bilayer in an Arbitrary Potential: Theory and Experimental Determination of Bending Rigidity

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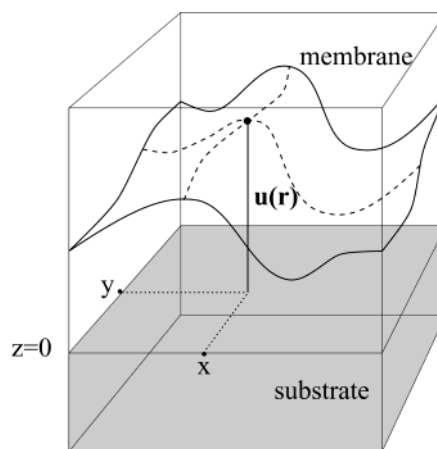
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We consider the position fluctuations of a membrane close to a substrate in an external potential and at finite temperature. We derive self-consistent equations for the average membrane position and its root mean square fluctuation amplitude in the range of moderate fluctuations much below the unbinding transition. These self-consistent equations can be solved for arbitrary potentials. We first check them for special known cases, then we apply our approach to lipid bilayers where van der Waals attractions are balanced by hydration repulsion associated to a hard wall. Finally, we compare these results with recent experiments on lipid bilayer.<sup>1</sup>

## I. Introduction

The purpose of the present paper is to develop a self-consistent theory for a fluid-free membrane floating near a solid substrate (Figure 1). The shape of the substrate potential determines the amplitude  $\sigma$  of thermal fluctuations which itself determines the effective potential. The average membrane position and its root mean square (rms) fluctuation amplitude are thus coupled and depend both on temperature and on bending energy of the membrane. This model situation is experimentally relevant as reported in a recent paper by Fragneto et al.<sup>1</sup>

We thus want to compute the equilibrium distance  $D$  between the bilayer and the substrate and the rms fluctuation amplitude  $\sigma$  for any given value of the bending modulus  $\kappa$ . However, although abundant,<sup>2</sup> the theoretical literature on fluctuations of a membrane submitted to an external potential does not address exactly this problem, to our knowledge. The difficulty is the following. According to Helfrich, the energy of a membrane is the sum of the external potential and the cost of bending.<sup>3</sup> At finite temperature, the potential determines the spectrum of fluctuations, according to the equipartition of energy. In turn, the entropy cost of fluctuations contributes to the free energy and determines the average position.<sup>3</sup> If the potential depends on the position, then the effective potential felt depends on how the membrane explores the space around its equilibrium position.<sup>4,5</sup> The effective



**Figure 1.** Schematic picture of thermal fluctuations of a membrane near a substrate, i.e., a flat hard wall at  $z = 0$ . The membrane does not contain overhangs or bubbles, so that the local position of an undulation can be described by a single-valued function  $u(\vec{r})$ , where  $\vec{r} = (x, y)$  denotes the lateral coordinates. The balance between the substrate interaction potential  $U(z)$  and the repulsion of entropic origin results in a mean interface position at  $D(T, \kappa) = \langle u(\vec{r}) \rangle$  which depends on temperature  $T$  and bending rigidity  $\kappa$  of the membrane.

potential, the average position, and the fluctuation amplitude are thus coupled quantities and must be determined self-consistently. This problem has already been identified and treated in a different context, namely multilamellar systems.<sup>6</sup>

Facing this task, three approaches seem possible. The first one is analytical: exact results can be obtained for some simple potential or well-defined limits,<sup>2,3</sup> as in the example of the renormalization treatment applied to the limit of large distance and large fluctuations (unbinding transition).<sup>4,5</sup> For moderate fluctuations in an arbitrary potential, this method fails, hence the second method: direct simulations.<sup>7–12</sup> We chose to develop a third method, using analytical equations, to gain a physical insight,

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especially on the effect of the potential on fluctuations, and on limiting cases. We build on the success of previous approaches for liquid surfaces using the solid-on-solid model.<sup>13–16</sup> We write implicit equations for arbitrary potential and finite distance  $D$ ; we then simplify them using a physical assumption, namely, the separation of small and large in-plane scales, and solve them numerically.

In section II, we derive the coupled self-consistent equations for the position and fluctuations in an arbitrary potential and test them theoretically. Section III addresses the interbilayer potential, solves it numerically, and discusses the results. Section IV discusses the agreement between the theory and the literature. Finally, we apply our theory to recent experiments<sup>1</sup> on phospholipid bilayers, leading to an estimation of  $\kappa$  at different temperatures.

## II. Self-Consistent Theory

In this section we first define the problem and the notations. We then examine the well-known cases of parabolic potentials and hard walls to suggest relevant approximations for the general case. We then treat separately the real space and the Fourier space, to derive the coupled self-consistent equations for the position and fluctuations in an arbitrary potential. We finally test them analytically on simple potentials for which the solution is already known.

**A. Position of the Problem. 1. Notations and Scale Separation.** We consider the (moderate) thermal fluctuations of a single flexible two-dimensional membrane without tension and close to a substrate. Notations are presented in Figure 1:  $\vec{r} = (x, y)$  and  $z$  the coordinates in and perpendicular to the plane of the substrate, respectively;  $z = 0$  the substrate position,  $z = u(\vec{r}) = u(x, y)$  the membrane position. We assume that the function  $u(\vec{r})$  is single-valued, neglecting overhangs of the membrane as well as separated vesicles. Two macroscopic, experimentally measurable quantities are the average position  $D$  and the rms fluctuation amplitude  $\sigma$ :

$$D = \langle u(\vec{r}) \rangle \quad (1)$$

$$\sigma^2 = \langle (u(\vec{r}) - D)^2 \rangle \quad (2)$$

We want to determine  $D$  and  $\sigma$  as a function of the temperature  $T$  and the membrane's bending rigidity modulus  $\kappa$ . The membrane fluctuates in a one-dimensional potential per unit surface  $U(z)$ ; for examples of substrate potentials see ref 17. Using standard approach and notations,<sup>3,18</sup> the Hamiltonian is the first-order expansion

of the energy in the membrane's curvature:

$$\mathcal{H}[u(\vec{r})] = \int_{\mathcal{A}} d^2\vec{r} \left[ U(u(\vec{r})) + \frac{1}{2} \kappa (\Delta u(\vec{r}))^2 \right] \quad (3)$$

It is difficult to calculate the free energy using this Hamiltonian. Previous approaches using the solid-on-solid model<sup>14–16</sup> separated length scales, to combine real space techniques at large scales and Fourier space techniques at small scales. The difficulty is of course to ensure the compatibility of both treatments. They successfully derived an effective theory for liquid surfaces, that is surfaces under tension, with an energy cost associated with area.

Here, we rather consider surfaces with a bending rigidity, the energy cost being associated with curvature. We follow this approach and treat in real space the term in  $U(u)$ , which mainly affects long-wavelength capillary waves. On the other hand, we treat in Fourier space the bending modes  $\sim \kappa q^4$  dominant at large wave vector for nanometer-scale fluctuations, which obey a Gaussian distribution. We derive simultaneously the partition sum  $\mathcal{Z}$  of the Hamiltonian (3),  $D$ ,  $\sigma$ , and the correlation lengths. Before considering the general case, we examine both well-known opposite, simple extremes: the parabolic potential and the hard wall steric hindrance.

**2. Simplest Case: The Parabolic Potential.** In a parabolic potential  $U(z) = \frac{1}{2} E_0 z^2$ , the problem is linear and the most natural treatment is in the Fourier space. The 2D Fourier transform of the membrane position  $u(\vec{r})$  is

$$u(\vec{r}) = \frac{1}{(2\pi)^2} \int_{\mathcal{A}} d^2\vec{q} \tilde{u}(\vec{q}) \exp(i\vec{q} \cdot \vec{r})$$

$$\tilde{u}(\vec{q}) = \int d^2\vec{r} u(\vec{r}) \exp(-i\vec{q} \cdot \vec{r}) \quad (4)$$

Modes  $\tilde{u}(\vec{q})$  decouple and the Hamiltonian, eq 3, becomes

$$\mathcal{H}[\tilde{u}(\vec{q})] = \int_{\mathcal{A}} d^2\vec{q} \frac{1}{2} [\kappa \vec{q}^4 |\tilde{u}(\vec{q})|^2 + E_0 |\tilde{u}(\vec{q})|^2] \quad (5)$$

Equipartition of energy implies that the probability of a mode  $\tilde{u}(\vec{q})$  is

$$P[\tilde{u}(\vec{q}), \tilde{\sigma}(\vec{q})] = [2\pi \tilde{\sigma}_f^2(\vec{q})]^{-1/2} \exp\left(-\frac{|\tilde{u}(\vec{q})|^2}{2\tilde{\sigma}_f^2(\vec{q})}\right) \quad (6)$$

where the rms fluctuation amplitude  $\sigma_f$  of elastic modes is  $\tilde{\sigma}_f^2(\vec{q}) = k_B T (E_0 + \kappa q^4)$ . The correlation function  $g(R) = \langle u(\vec{r} + \vec{R}) u(\vec{r}) \rangle$  describes the influence of the membrane at  $u(\vec{r})$  on the fluctuating position at distance  $\vec{R}$  in terms of the bending energy  $\kappa$

$$g(R) = \int_0^\infty \frac{dq}{2\pi} \frac{q J_0(qR) k_B T}{E_0 + \kappa q^4} = -\frac{4}{\pi} \sigma^2 \text{kei}\left(\frac{2^{1/2} R}{\xi_{||}}\right) \quad (7)$$

where  $\text{kei}(x)$  is the Thomson function and the in-plane correlation length is  $\xi_{||} = (4\kappa/E_0)^{1/4}$ . The mean squared width of the fluctuations is

$$\sigma^2 = g(0) = \frac{1}{4\xi_{||}^2} \frac{k_B T}{E_0} = \frac{1}{8} \frac{k_B T}{(E_0 \kappa)^{1/2}} = \frac{k_B T}{16\kappa \xi_{||}^2} \quad (8)$$

The probability  $P[\delta u_R, \sigma(R)]$  of a difference  $\delta u_R = u(\vec{r} + \vec{R}) - u(\vec{r})$  of the  $R$  membrane positions at distance  $\vec{R}$  is given by eq 6 and the correlation function  $\sigma(R)^2 = 2(g(0)$

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–  $g(R)$ , so that one may define a perpendicular correlation length  $\xi_{\perp}$  for correlated patches of size  $\xi_{\parallel}$  by:  $\xi_{\perp} = 2g(\xi_{\parallel}) = \sigma(8 \operatorname{kei}(2^{1/2}/\pi))^{1/2} \approx 0.954\sigma$ . Note that the root-mean-squared width is almost equal to the perpendicular correlation length, i.e.,  $\xi_{\perp} \approx \sigma$ , and therefore  $\sigma(\xi_{\parallel})^2 = 2\sigma^2 - \xi_{\perp}^2 \approx \sigma^2$ . Thus, for a membrane fluctuating in a parabolic potential, one can relate measurable properties such as  $D$  and  $\sigma$  to physical properties of the membrane such as the bending modulus  $\kappa$ .

**3. Steric Hindrance by a Hard Wall.** In real space, hard walls are simply constraints on the integration range of the membrane position  $u(z)$ , which can be implemented straightforwardly. On the opposite, one cannot integrate exactly over the coupled bending modes of the interface. As mentioned above, the basic idea is to separate in-plane scales smaller and larger than  $\xi_{\parallel}$ . Long-wavelength bending modes are governed by the substrate potential  $U(z)$ ; microscopic fluctuations are influenced mostly by the bending rigidity  $\kappa$ .

Let us follow a heuristic argument derived by Helfrich in 1978 and consider an undulated membrane as a surface with random humps of typical height  $\sigma = \xi_{\perp}$  extending over an area  $\sim \xi_{\parallel}^2$ .<sup>3</sup> We interpret these humps as independent particles that exert an ideal gas pressure,  $P_s = k_B T(2DL_{\parallel})^2$ . The typical length  $L_{\parallel} = c_{\xi} \xi_{\parallel}$  is of the order of the parallel correlation length, so that one may choose  $c_{\xi} \approx 1$ . Using  $\xi_{\parallel} = (4\kappa/E_0)^{1/4}$  for the parallel correlation length and assuming a linear relationship

$$\sigma^2 = \mu D^2 \quad (9)$$

between the roughness  $\sigma$  and the separation distance  $D$  to the wall, one finds the steric interaction energy per area

$$U_s(D) = c_H \frac{(k_B T)^2}{\kappa D^2} \quad (10)$$

Here  $c_H = 1/(64c_{\xi}\mu)$  denotes a dimensionless prefactor which determines the strength of the fluctuation-induced repulsion  $U_s(D)$  of a membrane at mean distance  $D$  to the substrate. This steric repulsion potential with the dimensionless prefactor  $c_H = (3\pi^2/128) \approx 0.231$  was Helfrich's result in 1978. But computer simulations yield smaller prefactors, namely,  $c_H = 0.116$ ,<sup>9</sup>  $c_H = 0.1$ ,<sup>11</sup>  $c_H = 0.074$ ,<sup>7</sup> and  $c_H = 0.0798$ ,<sup>8</sup> so that the precise value of the prefactor  $c_H$  remains an open debate. We'll return to this point in section II.C.2. Although a residual tension can affect the undulation interaction,<sup>15,19</sup> we assume in the following a vanishing tension of the membrane.

**B. General Case: Arbitrary Substrate Potential.** For practical applications, the substrate potential  $U(z)$  is usually more complicated than a simple hard wall steric hindrance. In general, it admits a single minimum at position  $z = z_{\min}$ , where it has a second derivative  $E_0$

$$E_0 \equiv \left[ \frac{d^2 U(z)}{dz^2} \right]_{z=z_{\min}} \quad (11)$$

It is not possible to simply add to  $U(z)$  the above steric repulsion potential  $U_s(z)$ .<sup>4</sup> We must entirely recompute the free energy from the beginning. For that purpose, we evaluate the partition function in three steps: Fourier space, real space, and compatibility condition.

**1. Fourier Space Treatment.** In Fourier space the rms fluctuation amplitude  $\sigma$  is given by the integral over all modes

$$\sigma^2 = \int_0^{q_{\max}} \frac{d^2 \bar{q}}{(2\pi)^2} \bar{\sigma}^2(q) \quad (12)$$

where  $\bar{\sigma}^2(q)$  denotes the (yet unknown) mean squared width of a single mode of wavevector  $\bar{q}$ . Two energy terms can be introduced: first, an elastic contribution from bending modes, eq 5:  $\bar{\sigma}_f^2(q) = k_B T(\kappa q^4)$ . Second, an excluded-volume effect due to the steric interaction  $\sigma_s^2$ , from which we construct dimensionally the quantity:  $E_0^s = k_B T(\xi_{\parallel} \sigma_s)^2$ , where the factor  $\xi_{\parallel}^{-2}$  counts the number of hindered large-scale modes with wavevector  $0 \leq q \leq q_{\parallel}$  smaller than the inverse length  $q_{\parallel} = (2\pi/L_{\parallel})$  of a typical patch. This intrinsic width  $\sigma_s^2$  is mainly governed by the steric interactions with the hard walls and does not depend on the wavevector  $q$ . Since both contributions have different physical origins, one may treat them as independent and add them to obtain the mean square of a mode  $q$

$$\frac{1}{\bar{\sigma}^2(q)} = \frac{1}{\bar{\sigma}_f^2(q)} + \frac{1}{\xi_{\parallel}^2 \sigma_s^2} = \frac{E_0^s + \kappa q^4}{k_B T} \quad (13)$$

Then with the exact expression (8) for Gaussian fluctuations we have:  $\sigma_s^2 = 4\sigma^2$ . Note that  $\sigma_s$  and therefore  $\sigma$  are still unknown quantities which have to be determined by an additional equation. To this end we introduce a similar argument in real space.

**2. Real Space Treatment.** In real space we treat the large-scale behavior of the membrane. The small-scale behavior appears only via the correlation function  $g(R)$ , hence the lateral correlation length  $\xi_{\parallel}$ . We decompose the membrane in patches of size  $\xi_{\parallel}^2$  which fluctuate freely in the substrate potential  $U(z)$ . Each patch contributes as  $U(z) \xi_{\parallel}^2$  to the energy of the system. We treat it as a Gaussian fluctuating membrane. The probability  $P[\delta u_{\xi_{\parallel}}]$  of a deviation  $\delta u_{\xi_{\parallel}} = z - D$  of the membrane position  $u(\vec{r})$  from its mean value  $D$  is a Gaussian distribution (eq 6) with the width  $\sigma(\xi_{\parallel}) = \sigma$  due to the bending modes at small scales. In the partition function, we use a conditional probability. Thus we normalize the Gaussian probability  $P$  by the probability of finding a patch at  $z$ :  $P_0[z] = \exp(-z^2/2\sigma_s^2)/(2\pi\sigma_s^2)$ , with the width  $\sigma_s$  of a single mode for  $q \rightarrow 0$ . The partition function of the system thus is

$$\begin{aligned} \mathcal{Z} &= \int dz \exp\left(-\frac{U(z)\xi_{\parallel}^2}{k_B T}\right) \frac{P[z-D]}{P_0[z-D]} \quad (14) \\ &= \int dz \left(\frac{\sigma_s}{\sigma}\right)^2 e^{-(U(z)\xi_{\parallel}^2/k_B T) - ((z-D)^2/2)((1/\sigma^2) - (1/\sigma_s^2))} \end{aligned}$$

We finally obtain an effective width  $\sigma_f^{-2} = \sigma^{-2} - \sigma_s^{-2}$ . The partition sum (14) could in principle determine explicitly  $D(\sigma_s) = \langle z \rangle$  and the standard deviation  $\sigma^2(\sigma_s) = \langle (z-D)^2 \rangle$  through simple integrals, but they depend on the unknown parameter  $\sigma_s$ . The missing information comes from the compatibility argument.

**3. Compatibility Argument.** Assuming that both above approaches,  $\sigma_s^2 = 4\sigma^2$  and  $\sigma_f^{-2} = \sigma^{-2} - \sigma_s^{-2}$ , lead to the same value of the total width  $\sigma$ , yields  $\sigma_f^2 = 4/3\sigma^2$ . For the in-plane correlation length  $\xi_{\parallel}$ , we assume that the

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relation  $\xi_{\parallel}^2 = 16\kappa\sigma^2/k_B T$  (eq 8) remains valid in general.<sup>4</sup> We finally obtain the following equations:

$$\begin{aligned} \zeta &= \int dz e^{-16(\kappa/(k_B T)^2)\sigma^2 U(z) - 3((z-D)^2/8\sigma^2)} \quad (15) \\ D &= \frac{1}{\zeta} \int dz z e^{-16(\kappa/(k_B T)^2)\sigma^2 U(z) - 3((z-D)^2/8\sigma^2)} \\ \sigma^2 &= \frac{1}{\zeta} \int dz (z-D)^2 e^{-16(\kappa/(k_B T)^2)\sigma^2 U(z) - 3((z-D)^2/8\sigma^2)} \end{aligned}$$

Equations 15 are the main result of the present paper, and what follows derive from them. They constitute a self-consistent set of three equations in the three unknown  $Z$ ,  $D$ ,  $\sigma$ , depending on the data  $\kappa$ ,  $T$ , and  $U(z)$ . They are valid for any potential  $U(z)$ . They rely on a physical hypothesis, namely, that we can treat scales larger and smaller than  $\xi_{\parallel}$  separately but exactly.<sup>15</sup> The test of this hypothesis will be the validity of the solutions of these equations. Let us first try to obtain exact results in simple cases (section II.C), then numerical solutions in less simple cases (section III), and finally compare them to experimental data (section IV.B).

**C. Analytic Results in Various Potentials.** We can solve analytically three cases: small fluctuations near the minimum of the potential, membrane between two parallel walls, linear potential near a wall. The three of them have already been studied analytically or numerically, allowing for tests of our equations.

**1. Small Fluctuation Amplitude.** Consider the limit of “small fluctuations” near the minimum  $z = z_{\min}$  of the potential  $U(z)$ . We will specify below the exact meaning of “small”. The membrane explores a potential (eq 11):

$$U(z) = \frac{1}{2} E_0 (z - z_{\min})^2 + O(z - z_{\min})^3 \quad (16)$$

We can solve the self-consistent equations (15) exactly, leading to the relations

$$\sigma^2 = \frac{k_B T}{8(E_0 \kappa)^{1/2}} \quad \text{and} \quad D = z_{\min} \quad (17)$$

This is of course the exact result (eq 8) derived from the Hamiltonian (3). We thus define “small” fluctuations in a self-consistent way:  $T/\kappa$  must be small enough that  $\sigma$  (eq 17b) is much smaller than the scale of variation of  $d^2 U/dz^2$  due to its higher order terms (eq 16).

**2. Two Hard Walls.** Helfrich<sup>3</sup> introduced the problem of a membrane confined between two hard walls,  $U(z) = 0$  for  $-D < z < +D$ , to compute steric interactions between membranes. Equation 15 yields immediately  $D = 0$  by symmetry. The integration of the partition sum yields  $\zeta = (8\pi/3)^{1/2} \text{erf}((3/8)^{1/2} D/\sigma)$ , where  $\text{erf}[x]$  is the error function. Finally, the last equation in eq 15 leads to a self-consistency constraint for Helfrich’s<sup>3</sup>  $\mu = \sigma^2/D^2$  (eq 9)

$$\left(\frac{24}{\pi\mu}\right)^{1/2} e^{-(3/8)\mu} = \text{erf}\left(\left(\frac{3}{8\mu}\right)^{1/2}\right) \quad (18)$$

Solving numerically this equation, we find  $\mu \approx 1/5$ , in fair agreement with Helfrich’s guess  $\mu \sim (1/6)$  ( $1/12 \leq \mu \leq 1/6$ ). Moreover, assuming  $c_{\xi} \approx 1$ , our value of  $\mu$  determines the prefactor of eq 10:  $c_H = 1/(64\mu) \approx 0.08$ . This self-consistent value equals exactly the value from Monte Carlo simulations.<sup>8</sup>

**3. Linear Potential.** Consider a membrane in the semispace  $z > 0$ , near a hard wall at  $z = 0$ , in a linear

potential  $U(z) = Pz$ . Here  $P$  has the dimension of a pressure. This case has been extensively studied by using effective theory, functional renormalization group approach, and numerical Monte Carlo simulation; for a review see ref 2. Solving our self-consistent equations with this linear potential leads to exact expressions for  $\sigma$  and  $D$  as a function of the pressure  $P$

$$\sigma = \left[\frac{(k_B T)^2}{64c_{\perp}\kappa}\right]^{1/3} P^{-1/3} \quad \text{and} \quad D = c_{\perp}\sigma \quad (19)$$

They involve the dimensionless variable  $c_{\perp} = (D/\sigma)$ , solution of the implicit equation

$$c_{\perp} = \left(\frac{\pi}{24}\right)^{1/2} e^{((1/(24^{1/2}c_{\perp}) - (3/8)^{1/2}c_{\perp})^2)} \text{erf}\left(\frac{1}{24^{1/2}c_{\perp}} - \left(\frac{3}{8}\right)^{1/2}c_{\perp}\right) \quad (20)$$

The numerical solution of eq 20 is  $c_{\perp} = 1.81$ . This value must be compared with the simulation result  $c_{\perp} = 5^{1/2} \approx 2.2$ ,<sup>10</sup> which was in excellent agreement for a membrane between two hard walls discussed in the previous section. However, the exponent  $-1/3$  agrees with the expected behavior known from numerical simulations and scaling arguments.

### III. Numerical Calculations for a Lipid Bilayer

In this section, we want to study a bilayer–bilayer interaction potential. In the experimental setup reported in refs 1 and 20, a lipid bilayer is floating close to a wall covered with an identical, but immobile, bilayer. To derive analytical results, even approximate, is possible only within a rough, “effective” approach. We then suggest a more realistic expression for  $U(z)$ , show how to solve numerically our eqs 15, and discuss the results.

**A. Effective Potential.** To serve as a comparison basis for our results, we want to first approximate analytical results for the bilayer–bilayer interaction potential. We thus crudely model the total free energy of the membrane by an effective potential, the sum of two contributions

$$U_{\text{eff}}(z) = -\frac{A}{12\pi} \frac{1}{z^2} + c_H \frac{T^2}{\kappa} \frac{1}{(z - z_0)^2} \quad (21)$$

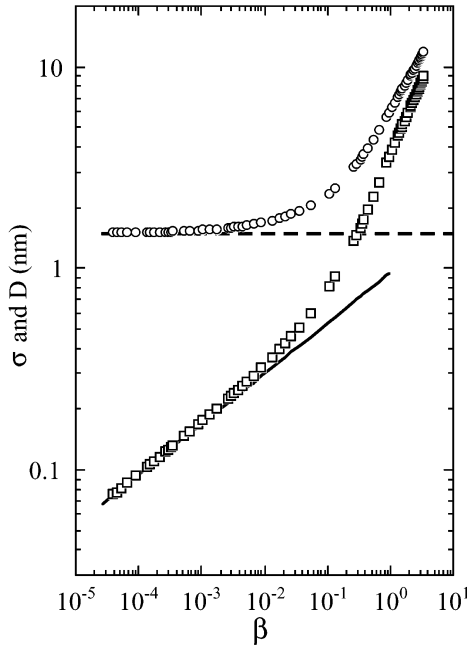
The first term is the bare attractive potential energy, approximated by its  $z^{-2}$  term, dominant at short distance. The second term is the entropic repulsion, approximated in the spirit of Helfrich, but where the hard wall is apparently shifted to the position  $z = z_0$  to account for hydration repulsion. Membrane position  $D_{\text{eff}}$  and mean fluctuation  $\sigma_{\text{eff}}$  are increasing functions of the relevant dimensionless parameter  $\beta$

$$\beta = \frac{(k_B T)^2}{A\kappa} \quad (22)$$

Minimizing the effective free energy (21) yields

$$D_{\text{eff}} = z_0 \frac{\beta_u^{1/3}}{\beta_u^{1/3} - \beta^{1/3}} \quad (23)$$

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**Figure 2.** Fluctuation amplitude  $\sigma$  (squares) and mean interbilayer distance  $D$  (circles) plotted versus the dimensionless parameter  $\beta = (k_B T)^2 / A\kappa$ . We have solved numerically eqs 15 for the potential  $U(z)$  of eq 28, with typical values for distearoyl phosphocholine (DSPC):  $\delta = 4\text{--}5.5$  nm<sup>1</sup>;  $A_h/A = 25/12\pi$  nm<sup>-2</sup>, chosen to adjust  $D$  and  $\sigma$  with experiments in the gel phase; and  $z_0 = 0.6$  nm. Reference 2 and Rand, R. P. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 277. For comparison, the analytical values of  $D$  and  $\sigma$  in the low- $\beta$  limit are plotted: dashed line,  $D = z_{\min}$ ; solid line:  $\sigma^2 = \sqrt{\beta/46.14}$ .

with  $\beta_u = (1/12\pi c_H)$ , the subscript u standing for “unbinding”. Equations 17 and 11 yield

$$\begin{aligned} \sigma_{\text{eff}} &= \frac{1}{(384c_H)^{1/4}} \left(\frac{D}{z_0}\right)^{1/4} (D - z_0) \\ &= \frac{z_0}{(384c_H)^{1/4}} \frac{\beta_u^{1/12} \beta^{1/3}}{(\beta_u^{1/3} - \beta_u^{1/3})^{5/4}} \end{aligned} \quad (24)$$

These expressions are plotted as lines in Figure 2.

In the regime of moderate fluctuations,  $\beta \ll \beta_u$ , the relation between  $\sigma_{\text{eff}}$  and  $D_{\text{eff}}$  is approximately linear

$$D_{\text{eff}} = z_0 + (384c_H)^{1/4} \sigma_{\text{eff}} \quad (25)$$

This effective potential approach ceases to be valid at large fluctuations, near the unbinding, where a renormalization approach is necessary, and at low fluctuations, close to the substrate, due to the crude treatment of the hydration repulsion.

**B. More Realistic Interaction Potential.** Since eqs 15 apply to any arbitrary potential, we can take an expression for  $U(z)$  as realistic as possible. To catch the essence of the physics, we suggest the following analytical expression.

The immobile bilayer acts as a hard wall at  $z = 0$ . In fact, it is close to a smooth (rms  $\sim 0.1$  nm) silicon substrate, on which it is immobilized due to a strong van der Waals interaction and possible pinning on defects. Thermal fluctuations (rms  $\sim 0.3$  nm) are neglected here. The floating bilayer thus sees a hard wall and interacts with it through a classical inter-bilayer potential. At long

distance, the dominant term is due to the van der Waals attraction

$$U_{\text{vdW}}(z) = -\frac{A}{12\pi} \left[ \frac{1}{z^2} - \frac{2}{(z + \delta)^2} + \frac{1}{(z + 2\delta)^2} \right] \quad (26)$$

where  $\delta$  is the bilayer thickness,  $z$  the distance between both bilayers, and  $A$  the Hamaker constant. At short distance, for  $z$  typically of order of the hydration length  $z_0 \sim 0.6$  nm or smaller, the potential is dominated by the hydration repulsion due to water molecules inserted between hydrophilic lipid heads:<sup>2</sup>

$$U_h(z) = A_h \exp\left(-2\frac{z}{z_0}\right) \quad (27)$$

We neglect less relevant terms, such as retardation effects<sup>2</sup> or electrostatic interactions: when screened, they become short range and we incorporate them into  $U_h$ . The substrate potential is thus

$$U(z) = U_{\text{vdW}}(z) + U_h(z) \quad (28)$$

The position  $z_{\min}$  of its minimum depends on the values of  $z_0$  and  $A_h/A$ . Analytical solutions are not available for this potential  $U(z)$ . Compared to computer simulations, the present self-consistent method is easier and provides additional insight in the physical mechanism, as we now show in the following section III.C.

**C. Solution of Self-Consistent Equations.** The dimensionless parameter  $\beta$  (eq 22)

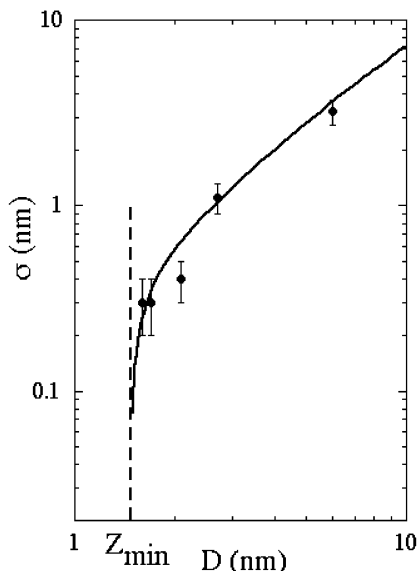
$$\beta = (k_B T)^2 / A\kappa \quad (29)$$

controls the physics of the problem. At low values of  $\beta$ , the bilayer is stiff, barely fluctuating in a strong minimum of potential energy. At high values of  $\beta$ , the bilayer is soft and has large fluctuations, which contribute a significant repulsion term to the free energy, increasing the mean distance  $D$  between bilayers.

Eliminating  $z$  from the three self-consistent equations (15), we can express two of the three physical quantities  $D$ ,  $\sigma$ , and  $\beta$  as a function of the third one. At first glance, it would appear more natural to determine both functions  $D(\beta)$  and  $\sigma(\beta)$ . Nevertheless, all arguments, both experimental and numerical, suggest to perform the opposite calculation. Experimentally,  $D$  and  $\sigma$  are measurable quantities. On the other hand,  $\beta$  is much more difficult to measure, especially for bilayers in the gel phase, as will be discussed below. If the present self-consistent approach determines  $\beta$  vs  $D$ , we can hope to use measurements of  $D$  to estimate  $\beta$ .

Numerically, we can determine  $D$  and  $\sigma$  for various values of  $\beta$  or determine  $\beta$  and  $c_{\perp} = (D/\sigma)$  for various values of  $D$ . Both approaches are of course correct; integrals calculations and resolution of Eqs. (15) are performed using Newton’s classical algorithm. However, we chose the latter approach, which has two advantages over the former. First, since  $\beta$  appears only in the right-hand side, the resolution is easier. Second, in cases where  $D$  diverges, the resolution is numerically more stable.

Figure 2 plots  $D$  and  $\sigma$  versus  $\beta$  for typical values of the parameters entering in the potential  $U(z)$  given by eqs 26–28. At low  $\beta$  we recover the limit of a membrane fluctuating around the microscopic potential energy minimum  $D \approx z_{\min}$  (dashed line), with the amplitude



**Figure 3.** Relation between interbilayer distance  $D$  and fluctuation amplitude  $\sigma$ : solid line, numerical self-consistent resolution, same numerical data as in Figure 2; symbols, experimental data from a single sample,<sup>1</sup> one symbol per temperature, from 25.4 to 64.1 °C.

described by eq 17 (solid line). As expected, when  $\beta$  increases, the entropic repulsion increases; hence both  $D$  and  $\sigma$  increase. Eliminating  $\beta$  yields a relation between  $D$  and  $\sigma$ , plotted as solid line in Figure 3. In the unbinding limit  $D \rightarrow \infty$ , the present approach ceases to be valid for the same reason as the effective potential; it requires a more subtle treatment of scale separation.<sup>21</sup>

#### IV. Discussion

We first discuss the advantages and self-consistency of the present theory, as well as the agreement with the analytical and numerical results of the literature. We then compare its predictions with the experiments of ref 1 to try and estimate how the bending modulus  $\kappa$  varies with temperature. We finally compare this estimate with the literature.

**A. Discussion of the Self-Consistent Theory.** Podgornik and Parsegian<sup>22</sup> already determined self-consistently the fluctuation spectrum (see also ref 23). However, their approach assumes a potential which is either parabolic (or so close to parabolic that deviations can be treated as perturbations), with a Gaussian (normal) probability distribution of distances; this approximation has been termed “uncontrolled” and hence “too inaccurate to extract fundamental interbilayer interactions from data”.<sup>11,12</sup> Their expression for  $\sigma^2\mu^{-2}$  is linear in the interaction potential, corresponding in our approach to an expansion in  $1/\beta = \kappa A/(k_B T)^2$ .

Our approach is based on the same principle, but with fewer assumptions. It is valid for arbitrary potentials; instead of a first-order perturbation, we use explicitly the Boltzmann weight  $\exp[-\alpha U(z)]$ . This was already tested and compared with simulations and experiments for surface-tension dominated capillary waves on thin liquid films and adsorption isotherms.<sup>15,16</sup>

For hard confinement (single membrane between two hard walls), we obtain almost identical results,<sup>22</sup> but in addition, we capture important results from simulations; for instance, we obtain Helfrich’s parameter  $c_H = 0.08$  and  $\mu = 0.19$  very accurately without any fit parameter. In addition, we can describe the crossover from hard to soft confinement, clearly visible, e.g., in Figures 2 and 3. Our approach even applies to small  $\beta$ , i.e., dominant interaction potential where  $\sigma/D$  is essentially influenced by the potential, see Figures 2, 3, and 4. Since we also study asymmetric situations, where not only  $\sigma$  but also the mean distance  $D$  is determined self-consistently, we capture the large asymmetry of the probability distribution evidenced in simulations.<sup>11</sup> Detailed results on the dependence of  $\sigma$  and  $D$  on the interaction potential will be presented elsewhere.

**B. Comparison with Experiments: Determining Bending Rigidity.** Fragneto et al. have recently studied a single DSPC bilayer floating close to an immobile one, adsorbed on a hard wall.<sup>1</sup> The equilibrium distance  $D$  between two bilayers and the rms fluctuation amplitude  $\sigma$ , measured by neutron reflectivity for different temperatures between 25.4 and 64.1 °C, were both spectacularly larger at a swelling temperature  $T_s$  than on either the lower or the higher temperature phases. Bilayers of DPSC, or similar saturated phosphocholines, undergo two transition temperatures: the “pretransition” temperature  $T_p$  which separates the gel phase from the ripple phase; and the “main” or “melting” transition temperature  $T_m$  which separates the ripple phase from the fluid phase. Hence the question: What is the major contribution to the observed swelling? Is it thermal fluctuations, or rippling, that is static fluctuations?

Answering this crude question requires experiments including the measurement of bilayer position and fluctuation versus temperature, for molecules other than DSPC (experiments in progress). However, we have no clear answer yet, we try to apply our theory to these data and neglect the role of ripple. This leads to an estimation of the bilayer bending rigidity modulus  $\kappa$  that we compare to measurements valid in the bilayer’s fluid phase,<sup>24,25</sup> in gel phase,<sup>26</sup> or in a wide range of temperatures.<sup>27</sup>

The experimental setup of Fragneto et al.,<sup>1</sup> as well as the measured quantities  $D$  and  $\sigma$ , allows easier comparison with theory than other configurations, especially multilamellar.<sup>28,29</sup> We first test the consistence between data and the above predictions. We then determine  $\beta(T)$  from the measurements of  $D$ . Finally, we use  $\beta(T)$  to estimate  $\kappa(T)$  as well as possible.

Are these experiments consistent with the calculations of section III? To test it, we plot experimental measurements of  $D$  and  $\sigma$  as symbols in Figure 3. There is no adjustable parameter; the agreement with the self-consistent results is remarkable.

If we go further, from the measured values of  $D$ , we can deduce the value of  $\beta$  by solving the self-consistent equations (see Figure 4). We determine the error bars by solving the equations for extremal values of  $D$ . The error bars decrease from near 95% at 25.4 °C, due to the incertitude in experimental values of  $D$ , to 4% at 52.5 °C.

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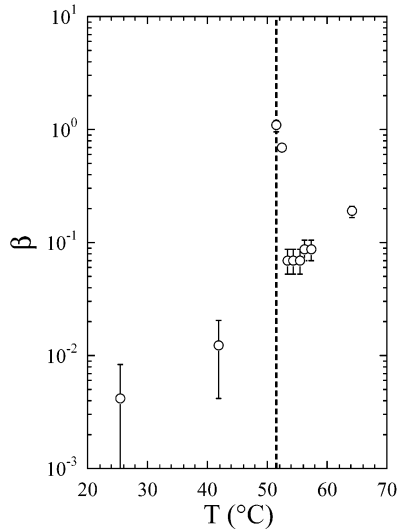
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**Figure 4.** Analysis of the same experimental data as in Figure 3. Using the present self-consistent theory (Figure 2), we deduce  $\beta(T)$  from measurements of  $D(T)$ .<sup>1</sup> Like  $D$  and  $\sigma$ ,  $\beta$  reaches its maximum value at 51.5 °C, close to the melting transition. Error bars reflect the sensitivity of  $\beta$  to incertitude ( $\sim 0.1$  nm) in experimental measurements of  $D$ .

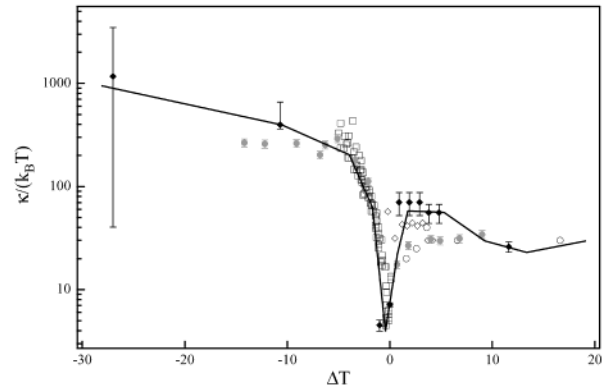
We observe a significant 250-fold increase in  $\beta$  around 51.5 °C; note the logarithmic scale.

How can we understand this maximum in  $\beta$ ? Remember that  $\beta = (k_B T)^2 / A\kappa$ . The  $T^2$  term varies only 25% over the experimental range, and of course it increases monotonically with  $T$ . The Hamaker constant  $A$  can significantly change at the melting transition, due to density changes, but we do not expect a marked minimum, nor a divergence. Most of the variation in  $\beta$  thus probably comes from a marked minimum in  $\kappa$ .

Going from  $\beta$  to  $\kappa$  is difficult because of the incertitude on the bilayer–bilayer interaction potential, especially on the Hamaker constant  $A$ . The best we can do is to assume that the variations in  $A$  are much smaller than the variations in  $\kappa$ . This means that we can inverse eq 22 and extract relative values of  $(k_B T)^2 / A\beta = \kappa$ , up to an unknown but almost constant normalization factor  $A$ . Results are displayed in Figure 5. Far from  $T_s$  the distance  $D$  is fixed by the minimum in the potential and the mean width  $\sigma$  is determined only by the curvature of the potential. Thus, in this case the steric repulsion potential does not play any significant role, so that we have a large error in  $\kappa$ , although we know  $D$  very well. However, close to  $T_s$  the value of  $D$  depends sensitively on  $\kappa$  yielding a variability in  $D$  from sample to sample, although all values of  $D$  indicate the same value of  $\kappa$ .

## V. Conclusion

**A. Summary.** In summary, we have considered a two-dimensional membrane in an external one-dimensional potential. We have studied its thermal fluctuations at a temperature much lower than its unbinding transition. Our main assumption was that we could separate the bending modes at small in-plane scales from the effect of the potential at large in-plane scales.<sup>15</sup> We obtained the following results. (i) We derived the eqs 15 to determine self-consistently the free energy, the average position  $D$ , and the fluctuation amplitude  $\sigma$ . They are easy to solve numerically for arbitrary expressions of the potential.



**Figure 5.** Bending modulus  $\kappa$ , in units of  $k_B T$ , versus temperature: open squares,  $\kappa$  of DMPC vesicles measured in low-temperature phase by micromanipulation, from ref 26; open circles,  $\kappa$  of DMPC vesicles measured in fluid phase by optical analysis of thermal fluctuations, from ref 24; open diamonds,  $\kappa$  of DPPC cylindrical tubes measured in fluid phase by electric-field induced bending, from ref 25; closed gray square,  $\kappa$  of DPPC vesicles measured by optical micromanipulation from ref 27; closed diamonds, relative values of  $\kappa$  for DSPC bilayers, deduced from  $\beta(T)$  (Figure 4) using eq 22; error bars reflect the error bars in  $\beta$ . The ordinate has been normalized, to superimpose data in the high-temperature phase, since the Hamaker constant  $A$  in eq 22 is unknown. To enable comparison between different molecules, we plot the present DSPC data versus  $\Delta T = T - T_s$ , and DPPC and DMPC data versus  $\Delta T = T - T_m$ . The solid line is a guide for the eye.

(ii) We check this equations for well-known cases as membrane in a quadratic potential, membrane in a linear potential, and membrane between two hard walls. (iii) We solve them in the more realistic case of a bilayer–bilayer interaction potential and derived  $D$  and  $\sigma$  as a function of the dimensionless parameter  $\beta = (k_B T)^2 / A\kappa$ , where  $T$  is the temperature,  $A$  the Hamaker constant, and  $\kappa$  the bending rigidity modulus. (iv) We have extracted from the data of ref 1 an estimation of the bilayer’s bending modulus  $\kappa$  for the same sample at different temperatures ranging from the gel to the fluid phase. We found that  $\kappa$  undergoes a sharp minimum.

The coherence of this picture validates the initial assumption of our theory, namely, the possibility to treat separately small and large length scales. The analytical solutions of our equations agree with that of the literature, when they are known: membrane between two hard walls, membrane in a parabolic potential, membrane in a linear potential. The agreement between our estimate of entropic interaction and the literature’s simulations<sup>11</sup> suggests a numerical determination of Helfrich’s prefactor  $\chi_H$ .

**B. Perspectives.** Our equations are valid only for moderate fluctuations. The self-consistent treatment of large fluctuations, and hence the unbinding transition,<sup>4</sup> requires a more detailed theory.<sup>21</sup> Other future developments of the present theory could also include the effect of a possible tension in the membrane.

Future experimental developments<sup>30</sup> include the measurement of bilayer position and fluctuation versus temperature, for molecules other than DSPC, to clarify the interpretation of the swelling temperature. Beside reflectivity measurements, off-specular X-ray scattering experiments could determine, precisely, not only its amplitude  $\sigma$  integrated over all modes but also the whole fluctuation spectrum  $\tilde{\sigma}(q)$ ; this would reduce further the error bars in the determination of  $\beta$ . The determination of the whole spectrum would also provide a direct test of

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the present theory, another independent determination of  $\kappa$ , and a validation of the hypothesis of separation between small and large length scales. In turn, we expect that the present prediction of the fluctuation spectrum, and especially of the in-plane correlation length, would be particularly helpful to analyze these off-specular X-ray scattering experiments.

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