Hydration *vs.* Protrusion Forces Between Lipid Bilayers.

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Abstract. – For separation of the order of 1 nm, lipid bilayers exhibit a strong repulsive interaction which could arise from direct hydration forces or from thermally excited protrusions. A simple model for the competition between these two forces is introduced. In this model, collective protrusion modes consisting of the correlated displacements of several molecules are included. The model is studied by Monte Carlo simulations and functional renormalization. Two different regimes are found in which the repulsion is dominated by protrusion and by hydration forces, respectively. Both regimes should be accessible to experiments by changing the temperature or the lipid-solvent composition.

It has been well established by many experiments that lipid bilayers and other model membranes in aqueous solution experience a strong repulsion at smaller separations [1]. The corresponding force per unit area or disjoining pressure, P, has been observed to decay exponentially as $P \approx P_t \exp[-l/l_t]$ with the mean separation l of the two membranes (here and below, the subscript t stands for «total»). It was believed that this repulsive interaction between the membranes arises because the polar head groups of the lipid molecules perturb the structure of the adjacent water [2]. Therefore, this repulsion was termed the hydration interaction.

Recently, an alternative explanation for this repulsive force has been proposed: it has been argued that it arises from the molecular roughness of the bilayers and is thus produced by local protrusions of the lipid molecules [3]. The molecular roughness of lipid bilayers had been previously observed in molecular-dynamics simulations [4]; in addition, recent X-ray and neutron scattering experiments have also been interpreted in terms of such a roughness [5, 6].

The protrusion picture appears to be quite different from the hydration picture. Therefore, the true nature of the short-ranged repulsive forces between lipid bilayers has now become rather controversial [7, 8]. In fact, other theories have also been proposed which focus on the intrinsic structure of the lipid water interface [9].

In this letter, we will argue that, in general, *both* hydration *and* protrusion will contribute towards the repulsive interaction of the membranes. In order to show this, we introduce and study a new model for these interactions. In our model, each lipid molecule acts to perturb the structure of the adjacent water. As will be explained more precisely below, the decay length of this perturbation defines the hydration length l_{hy}. In addition, we include thermally excited protrusions of the lipid molecules. These protrusions introduce another length scale, the protrusion length l_{pr}. So far, this scale has only been estimated within a single-mode picture [3]. In contrast, we include *collective* protrusion modes and find that these collective

excitations *enhance* l_{pr} by about 50 percent. The competition between the two length scales l_{hy} and l_{pr} leads to two different regimes for the physical decay length l_t, see (8) and (9) below.

In order to give a precise definition of the hydration length, consider a membrane which has been immobilized on a planar surface. The lipid/water interface of such a bilayer is characterized by a density profile which represents the lateral average of some local density and thus depends only on the coordinate, say y, perpendicular to this interface.

If the lipid/water interface were smooth and planar on the scale of the water molecules, the density profile would exhibit oscillations which represent the successive packing of planar water layers on top of the lipid bilayer [10]. However, the surface of the immobilized bilayer is rough on the scale of the water molecules since it exhibits «hills» and «valleys», the size of which is set by the lipid head groups. Therefore, the water layers are corrugated and the lateral average leads to a density profile for which the oscillatory part is strongly suppressed ([1]). The decay of this profile should then be characterized by an exponential tail $\sim \exp[-y/l_{hy}]$ which defines the hydration length l_{hy}.

If two such immobilized bilayers are pushed against each other by the external pressure P, the density profiles of their surfaces overlap. This leads to a repulsive interaction of the form $\sim \exp[-l/l_{hy}]$ between these two lipid/water interfaces where l denotes their separation. This interaction is consistent with surface force measurements for bilayers immobilized on mica surfaces (for which an oscillatory behaviour has never been observed) [8].

Thus, the interaction potential $V(l)$ of two immobilized membranes is taken to be

$$V(l) = Pl + V_{hy} \exp[-l/l_{hy}] \quad \text{for } l > 0 \tag{1}$$

with the hard-wall condition $V(l) = \infty$, for $l < 0$, where the parameter V_{hy} describes the amplitude of the repulsive hydration interaction. This form is valid for separations of the order of 1 nm, for which one can ignore the long-ranged part of the van der Waals forces. Now, we want to study how this interaction is changed by thermally excited fluctuations.

If the wavelength $1/q$ of the fluctuation is large compared to the bilayer thickness, $a_{mem} \simeq 5$ nm, the membrane roughness is primarily determined by bending modes for which the surface area of the membrane remains unchanged [11]. However, the concept of a bending mode is not longer well-defined as soon as q becomes of the order of $1/a_{mem}$. On these latter scales, the molecular structure of the lipid/water interface and, thus, the relative displacements or protrusions of the lipid molecules should be taken into account. In contrast to bending modes, protrusions change the surface area of the membrane and are thus governed by an effective interfacial tension.

The thermally excited fluctuations which are studied here are pure protrusion modes in which the molecules are displaced but not tilted. Tilts determine the membrane curvature. Protrusions of a curved bilayer will act to reduce the bending rigidity ([2]). This interplay of

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([1]) As a simple example, consider a density variation of the form $\sim f(y + y_0(x_1, x_2))$ with $f(y) = \exp[-y/l](1 + \cos[2\pi y/a_w])$, where x_1 and x_2 are the lateral coordinates and a_w denotes the size of the water molecule, and take the Gaussian distribution $\sim \exp[-2(y_0/a_h)^2]$ for the variable y_0 which is governed by half of the size a_h of the lipid headgroup. The lateral average then leads to $\langle f(y + y_0) \rangle \sim \exp[-y/l](1 + \exp[-(\pi a_h/\sqrt{2}a_w)^2]\cos[2\pi y/a_w + \text{const}])$. Because of the Gaussian prefactor, the oscillatory part is completely suppressed for $a_h > a_w$.

([2]) The reduction of the bending rigidity should increase with the roughness of the lipid/water interfaces. This can be explicitly shown within a continuum model for small displacements from the planar state.

protrusion and bending modes goes beyond the scope of this paper but will be addressed in future work.

The protrusion-induced interaction between two membranes can be estimated within a simple picture which is equivalent to the self-consistent approach of ref. [3]. Consider the protrusion of a single lipid molecule. The hydrophobic part of this molecule is taken to have the shape of a small column with constant cross-section; the area of this cross-section is denoted by A_0 and its circumference by a_0. It will also be assumed that the headgroup of the lipid is rather rigid and thus does not deform significantly as the lipid molecule protrudes. The protrusion energy is then independent of the molecular shape of the strong headgroup [3].

If such a protrusion bridges the gap of size l between the two membranes, its energy is $\Delta E = \sigma a_0 l$, where σ represents the free energy of the interface between the non-polar part of the molecule and the polar solvent. The probability for such a fluctuations is $\sim \exp[-\Delta E/T] = \exp[-l/l_{sc}]$ with the length scale $l_{sc} = T/a_0 \sigma$. For a molecule with circumference $a_0 \simeq 3$ nm and interfacial free energy $\sigma \simeq 0.02$ J/m^2, this length scale is $l_{sc} \simeq 0.07$ nm at room temperature $T = 4.12 \times 10^{-21}$ J.

Within the single-mode picture, the length scale l_{sc} is in fact equal to the protrusion length l_{pr}. One should note, however, that this picture does not include any collective protrusions involving several molecules nor does it include any direct interaction arising from hydration.

We will now go beyond the single-mode picture and consider a rough membrane in which all molecules can be displaced with respect to the flat state. In addition, we will include the direct interaction between the membranes as given by (1). The separation of the protruding molecule i from the other membrane is now described by the local displacement field l_i which varies along the membrane surface. Each molecule is supposed to interact with n nearest neighbours. The energy of such a membrane configuration is given by

$$\mathcal{H}\{l\}/T = \sum_{\langle ij \rangle} (a_0 \sigma/Tn)|l_i - l_j| + \sum_i A_0 V(l_i)/T \tag{2}$$

and its statistical weight is given by the Boltzmann factor $\sim \exp[-\mathcal{H}\{l\}/T]$.

A model similar to (2) has been previously studied in the context of *wetting* in a lattice gas (or Ising) model [13]. This model belongs to the same universality class as the so-called Gaussian model for which the discrete sites i are replaced by a continuous coordinate and the term $(a_0 \sigma/Tn)|l_i - l_j|$ is replaced by $(\Sigma/2T)(\nabla l)^2$. The parameter Σ represents the effective interfacial tension on large scales; in the present context, one has $\Sigma = c_\Sigma T/l_{sc}^2$ with $c_\Sigma \simeq 0.067$, see below. Using the above estimate for l_{sc}, one then has $\Sigma \simeq 0.056$ J/m^2.

We have studied the model as defined by (2) via Monte Carlo (MC) simulations and the equivalent Gaussian model by functional renormalization group methods. As described below, both methods give very consistent results.

It will be convenient to use dimensionless variables. Thus, let us introduce the rescaled coordinate $z \equiv l/l_{sc}$ with the scale $l_{sc} = T/a_0 \sigma$. One then arrives at the effective Hamiltonian

$$\mathcal{H}\{z\}/T = \sum_{\langle ij \rangle} |z_i - z_j|/n + \sum_i U(z_i) \tag{3}$$

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[3] The opposite limit of very flexible headgroups attached to a planar hydrocarbon/solvent interface has been studied in ref. [12].
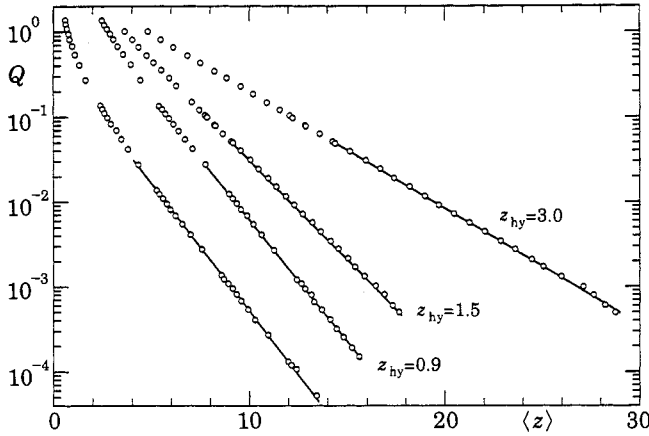
Fig. 1. – Monte Carlo data for the pressure Q as a function of the mean separation $\langle z \rangle$. Apart from the hard-wall case, three sets of data are displayed corresponding to the hydration interaction as given by (4) with amplitude $U_{\mathrm{hy}} = 13.5$ and three different values of the hydration length z_{hy}. The lines represent fits according to the functional form in (5) and (6).

with the rescaled interaction

$$U(z) = Qz + U_{\mathrm{hy}} \exp\left[-z/z_{\mathrm{hy}}\right]. \tag{4}$$

This interaction depends on the rescaled pressure $Q \equiv A_0 P / a_0 \sigma$ and on the rescaled hydration parameters $U_{\mathrm{hy}} \equiv A_0 V_{\mathrm{hy}}/T$ and $z_{\mathrm{hy}} \equiv l_{\mathrm{hy}}/l_{\mathrm{sc}}$. Using the values $a_0 \simeq 3\,\mathrm{nm}$, $A_0 \simeq$ $\simeq 0.7\,\mathrm{nm}^2$ and $\sigma \simeq 0.02\,\mathrm{J/m}^2$, one obtains the pressure scale $P_{\mathrm{sc}} \equiv a_0 \sigma / A_0 \simeq 8.6 \cdot 10^7\,\mathrm{J/m}^3$ which lies within the range of the experimentally observed values for P_{t}.

First, let us consider the case $V_{\mathrm{hy}} = U_{\mathrm{hy}} = 0$ for which the repulsive interaction is determined by the protrusions alone which transform the hard wall into a smoothly decaying repulsive interaction. The corresponding MC data are shown in fig. 1 for $n = 6$, *i.e.* for the case of six nearest neighbours per lipid molecule. The MC code used here is fully vectorized and has been previously described in ref. [13].

The functional dependence of the pressure $P \sim Q$ on the mean separation $l \sim \langle z \rangle$ can be obtained by functional renormalization [14]. For the hard-wall case, one obtains the expression

$$Q \approx Q_{\mathrm{pr}} \exp\left[-\langle z \rangle / z_{\mathrm{pr}}\right]/\langle z \rangle^{1/4} \tag{5}$$

for large $\langle z \rangle$ with the amplitude Q_{pr} and the decay length z_{pr}. Thus one has a confluent power law $\sim 1/\langle z \rangle^{1/4}$ for the hard-wall case which represents the leading correction to the exponential decay. Fitting the form as given by (5) to the MC data for $n = 6$, one obtains the amplitude $Q_{\mathrm{pr}} = 0.57 \pm 0.03$ and the decay length $z_{\mathrm{pr}} = 1.54 \pm 0.03$, compare fig. 1. The numerical error has been estimated by fitting over different ranges of $\langle z \rangle$-values. We have also studied the hard-wall potential for $n = 4$; for fixed Q, the $\langle z \rangle$-values are increased by a few percent but the value for z_{pr} is recovered within the numerical accuracy. The coefficient c_{Σ} for the effective interfacial tension Σ is related to z_{pr} by $c_{\Sigma} = 1/2\pi z_{\mathrm{pr}}^2 = 0.067 \pm 0.003$.

Now, let us include the hydration interaction as given by (4) with $U_{\mathrm{hy}} > 0$. In this case, functional renormalization shows that the $\langle z \rangle$-dependence of the repulsive force is still described by (5), *provided* one has $z_{\mathrm{pr}} > 2z_{\mathrm{hy}}$. We have checked this explicitly by MC simulations for the case $U_{\mathrm{hy}} = 0.6$ and $z_{\mathrm{hy}} = 0.375$. Thus, one has a protrusion-dominated regime with $z_{\mathrm{pr}} > 2z_{\mathrm{hy}}$ for which the repulsive interaction decays as in (5).
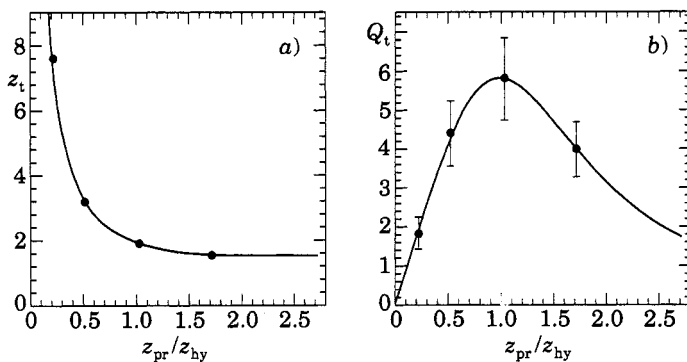
Fig. 2. – *a*) The decay length z_t and *b*) the pressure amplitude Q_t as a function of the ratio $z_{pr}/z_{hy} = l_{pr}/l_{hy}$. The four dots represent the best fits to the Monte Carlo data; the error bars for Q_t are relatively large, while the error bars for z_t are smaller than the size of the symbols. The curve in *a*) represents the functional dependence in (6); the curve in *b*) is given by (7) with $U_{hy} = 13.5$ and $c_t = 0.85$.

However, as soon as the hydration length exceeds half of the protrusion length, *i.e.* for $z_{hy} > z_{pr}/2$, one enters a second regime in which the decay length z_t depends both on z_{pr} and on z_{hy}. For this hydration-dominated regime, functional renormalization gives the pressure

$$Q \approx Q_t \exp\left[-\langle z \rangle / z_t\right] \quad \text{with} \quad z_t = \left[(z_{hy}/z_{pr}) + (z_{pr}/4z_{hy})\right] z_{pr} \tag{6}$$

for large $\langle z \rangle$. The dependence of the decay length z_t on z_{pr}/z_{hy} is shown in fig. 2*a*). This figure contains four points which have been obtained by fitting the MC data for $Q = Q(\langle z \rangle)$ as obtained for $U_{hy} = 13.5$ and $z_{hy} = 0.9, 1.5, 3$, and 7.5, respectively, compare fig 1. Inspection of fig. 2*a*) shows that the values of z_t as obtained from the MC data are very well fitted by the expression in (6). Note that there are *no adjustable parameters* in this fit. It has been explicitly checked for $U_{hy} = 0.6$ and $z_{hy} = 0.9$ that the numerically obtained value for z_t is indeed independent of U_{hy} as predicted by (6).

The amplitude Q_t in (6) is given by

$$Q_t = c_t^\varrho U_{hy}^{1-\varrho} z_{hy}^{2\varrho - 1} \quad \text{with} \quad \varrho \equiv (z_{pr}/z_{hy})^2 / \left[(z_{pr}/z_{hy})^2 + 4\right]. \tag{7}$$

The dependence of the amplitude Q_t on z_{pr}/z_{hy} is shown in fig. 2*b*) for $n = 6$ and $U_{hy} = 13.5$. The coefficient c_t is found to be $c_t = 0.85 \pm 0.15$ from a fit to the numerically obtained amplitudes displayed in fig. 2*b*). The same estimate for c_t is found by fitting the data for $U_{hy} = 0.6$.

Inspection of fig. 2*b*) shows that the amplitude Q_t is *not monotonic* as a function of z_{pr}/z_{hy}. This can be easily understood from the explicit expression given in (7): for small $x \equiv z_{pr}/z_{hy}$, one has $\varrho \approx x^2/4$ and $Q_t \approx U_{hy}/z_{hy} = U_{hy} x/z_{pr}$; for large x, one has $\varrho \approx 1$ and $Q_t \approx C_t z_{hy} = C_t z_{pr}/x$. One should note, however, that (7) is only valid for $z_{pr}/z_{hy} < 2$.

We have also determined the roughness $\xi_\perp$ of the lipid/water interface as a function of the pressure Q which behaves as $\xi_\perp^2 \approx l_{pr}^2 \ln(Q/Q_\perp)^{1/2}$ for small Q. The corresponding MC data will be presented elsewhere [15].

The model as given by (2) describes the interaction of one protruding and one immobilized membrane. For the interaction between two flexible lipid bilayers, there are two changes. On the one hand, the protrusion of one bilayer should involve both lipid/water interfaces bounding this bilayer in order to avoid bilayer cavities which would cost a lot of energy. If the bilayer were incompressible and the two lipid/water interfaces bounding this bilayer has a

constant separation, the interfacial free energy σ for its protrusions would be *increased* by a factor of two and the effective tension $\Sigma \sim \sigma^2$ by a factor of four. On the other hand, if both bilayers exhibit protrusions, the effective interfacial tension for the *relative* displacement field is *decreased* by a factor of two. Thus, these two effects compensate each other to a certain extent and would lead, for incompressible bilayers, to an overall increase of σ by a factor of $\sqrt{2}$. The finite compressibility of these membranes will act to reduce this factor.

The physical decay length or the effective repulsion between the membranes is given by $l_t = z_t l_{sc}$ with $l_{sc} = T/a_0 \sigma$. It then follows from (5) that the protrusion-dominated regime with $l_{pr} > 2l_{hy}$ is characterized by the decay length

$$l_t = l_{pr} = z_{pr} l_{sc} = z_{pr} T/a_0 \sigma \quad \text{with } z_{pr} = 1.54 \pm 0.03 . \tag{8}$$

Thus, compared to the single-mode picture, the decay length is enhanced by about 50 percent as a result of the collective protrusion modes. Within the hydration-dominated regime with $l_{hy} > l_{pr}/2$, on the other hand, one obtains from (6) that the physical decay length is given by

$$l_t = l_{hy} + l_{pr}^2/4l_{hy} = l_{hy} + z_{pr}^2 T^2/4(a_0 \sigma)^2 l_{hy} . \tag{9}$$

Inspection of (8) and (9) shows that the physical decay length l_t is determined by the relative size of the protrusion length l_{pr} and the hydration length l_{hy}. The protrusion length depends on temperature and on the molecular structure of the lipid bilayer, the hydration length on the structure of the solvent.

At fixed temperature T, the repulsive interaction will be dominated by protrusion and by hydration forces i) for small and for large values of the parameter $a_0 \sigma$, respectively (where $a_0 \sigma$ represents an effective edge tension of the lipid molecule), and ii) for small and for large values of the hydration length, respectively. Thus, depending on the lipid and on the solvent, one may probe either of both interaction regimes.

For fixed lipid and solvent, on the other hand, one will have a transition at a characteristic temperature $T = T_*$ which is implicitly given by $l_{pr}(T_*) = 2l_{hy}(T_*)$. It follows from (8) and (9) that $T_* = 2a_0 l_{hy} \sigma/z_{pr}$ if one ignores the T-dependence of the interfacial free energy σ and of the hydration length l_{hy}. For the hydration-dominated regime at low temperatures $T < T_*$, the physical decay length l_t increases quadratically with increasing T, see (9), while it increases linearly with T for the protrusion-dominated regime at $T > T_*$, see (8).

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