

Steric Interaction of Fluid Membranes in Multilayer Systems

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The out-of-plane fluctuations of fluid membranes are sterically hindered in multilayer systems. The repulsive interaction associated with the steric or excluded-volume effect is studied theoretically by two methods. The interaction energy per unit area of membrane is derived as a function of temperature, membrane curvature elasticity and mean membrane spacing; it is inversely proportional to the square of the latter. Steric repulsion is estimated for lecithin bilayers in water. There and in other cases, it may compete with van der Waals attraction.

1. Introduction

A membrane is fluid when it offers elastic resistance only to curvature but not to shear. Typical examples are lecithin bilayers in water which serve as model systems for biological membranes. Fluid membranes undergo out-of-plane fluctuations analyzable in terms of undulation modes. The out-of-plane fluctuations of lecithin membranes reveal themselves in the slight and constantly changing bumpiness of large thin-walled vesicles observed under a phase contrast microscope [1]. Undulation modes have also found some theoretical attention [2, 3], one reason being that the ripples may use up a small fraction of the effective membrane area.

Under suitable conditions the bilayers composed of amphiphilic molecules and even biological membranes, such as those of the myelin sheath around nerve fibers, tend to form multilayer systems. They are characterized by a parallel arrangement of membranes alternating with thin layers of water. In some respects they are more suitable for experimental and theoretical studies than single membranes. The undulation modes in multilayer systems can be treated in terms of de Gennes' theory [4] of fluctuations in smectic liquid crystals which invokes curvature elasticity and smectic compressibility.

In order to understand the properties of lamellar lipid-water systems and with respect to biological applications it is desirable to know the interaction of parallel membranes embedded in water. Theories of attractive and repulsive forces have been put forward, dealing with long-range van der Waals

interaction [5] and attributing the well-known short-range repulsion to charges at the lipid-water interface [6] and, more recently, to correlation effects in water [7]. They are all based on the concept of a flat membrane and apply to its solid and to its fluid state as long as the intermediate water can be regarded as liquid.

In this article we wish to propose a novel mechanism giving rise to repulsive interaction between fluid membranes. It is the result of the mutual steric hindrance of undulating membranes when they are side by side as in multilayer systems. The effect seems interesting because it should compete in range and possibly in strength with van der Waals attraction which is currently thought to be the farthest-reaching force. It is probably unimportant in the case of solid membranes where we expect out-of-plane fluctuations to be very weak (partly because they are self-impeding, as illustrated by the example of corrugated iron).

Two approaches will be used to derive formulas for the free energy of steric interaction. In both cases the correlation of the undulation modes is omitted in the statistical mechanics of the problem. The first method employs the phenomenological theory [4] of smectic elasticity to calculate the curvature-elastic energy and entropy of each mode. The elastic modulus of smectic compressibility, although a macroscopic quantity, is taken to apply to all modes regardless of wave vector. It is eliminated by means of a self-consistency relation from the total free energy which thus becomes a function of curvature elasticity, membrane spacing, and temperature only. A second theory starts from the steric interaction of a single fluid membrane with two parallel and rigid bounding plates and is then extended to the case of multilayer systems. It is more transparent than the first, but less elegant,

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posing some difficult mathematical problems which are handled only by way of crude approximations. Most of those are discussed in a subsequent section, together with further considerations and possible refinements of the theories. The final results of the two theories are found to be rather similar. In a general discussion numerical estimates are given for lecithin bilayers, steric repulsion is compared to the theoretical strength of van der Waals attraction, and a few remarks are made concerning experimental observations.

2. Theory Employing Smectic Compressibility

We consider a multilayer system made up of fluid membranes of equal spacing and parallel to the xy plane of a cartesian coordinate system. Using the continuum theory of smectic liquid crystals, we may describe small deviations from the ideal order at a given time by the locally varying displacement $u = u(x, y, z)$ of the layers in z direction. For small enough deformations the elastic energy per unit volume is given by [4]:

$$g = \frac{1}{2} B \left(\frac{\partial u}{\partial z} \right)^2 + \frac{1}{2} K \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right)^2. \quad (1)$$

The first term represents the elastic energy of layer compression and the second that of layer curvature, B (dyn cm⁻²) and K (dyn) being the respective moduli. With periodic boundary conditions and the usual Fourier expansion

$$u(\mathbf{r}) = \sum_{\mathbf{q}} u_{\mathbf{q}} \exp(i \mathbf{q} \cdot \mathbf{r}) \quad (2)$$

the equipartition theorem for a sample of volume V has the standard form

$$\frac{1}{2} (B q_{\parallel}^2 + K q_{\perp}^4) \langle |u_{\mathbf{q}}|^2 \rangle V = \frac{1}{2} k_B T, \quad (3)$$

k_B and T being Boltzmann constant and temperature. The wave vector \mathbf{q} has been decomposed into its components \mathbf{q}_{\parallel} parallel to the z axis and \mathbf{q}_{\perp} lying in the xy plane.

This formalism will now be used to deal with the problem of purely steric membrane interaction, although a multilayer system resembles a continuum even less than does an ordinary smectic liquid crystal. We assume that there is no interaction other than the excluded-volume effect. The bulk modulus K can then be expressed by the curvature-elastic modulus k_c (erg) for the single membrane,

also used in the second theory, multiplied by the (average) number n of layers per unit height of stack:

$$K = n k_c. \quad (4)$$

A nonvanishing modulus B of layer compression originates from the excluded-volume effect; it must be furnished by any theory of steric interaction.

Let us assemble the multilayer system from single membranes. We are interested in the free energy ΔF of membrane joining, i.e. the excess energy of the stacked as compared to the separate membranes. It may be divided into an elastic part ΔU and an entropy part $-T\Delta S$

$$\Delta F = \Delta U - T\Delta S, \quad (5)$$

where ΔU is to represent the curvature-elastic energy in the case of purely steric interaction. Formally, the transition from the noninteracting to the interacting state can be carried out at fixed n by going from $B=0$ to some $B \neq 0$. The result is a change of the effective force constants $f_{\mathbf{q}}$ of the undulation modes

$$f_{\mathbf{q}} = V n k_c q_{\perp}^4 \rightarrow V (B q_{\parallel}^2 + n k_c q_{\perp}^4). \quad (6)$$

Its implications have to be examined in some detail. Clearly, the distribution of the values of each amplitude must be Gaussian (a two-dimensional Gaussian in the complex plane in the case of a complex amplitude). If we neglect mode-mode correlation, we have to regard the distribution of the different amplitudes as independent of each other.

From the equipartition theorem (3) the internal energy per mode, $\Delta U_{\mathbf{q}}$, of membrane joining is easily seen to be

$$\Delta U_{\mathbf{q}} = -\frac{1}{2} k_B T \frac{B q_{\parallel}^2}{B q_{\parallel}^2 + n k_c q_{\perp}^4}. \quad (7)$$

The $\Delta U_{\mathbf{q}}$ are negative, while the total free energy of membrane joining will be positive.

The derivation of the entropy $\Delta S_{\mathbf{q}}$ per mode is not so easy and, perhaps, preferably done in terms of real sinusoidal rather than complex modes. We may express the new modes by the old

$$\begin{aligned} a_{\mathbf{q}} \cos \mathbf{q} \cdot \mathbf{r} &= u_{\mathbf{q}} e^{i \mathbf{q} \cdot \mathbf{r}} + u_{-\mathbf{q}} e^{-i \mathbf{q} \cdot \mathbf{r}}, \\ b_{\mathbf{q}} \sin \mathbf{q} \cdot \mathbf{r} &= i(u_{\mathbf{q}} e^{i \mathbf{q} \cdot \mathbf{r}} - u_{-\mathbf{q}} e^{-i \mathbf{q} \cdot \mathbf{r}}) \end{aligned} \quad (8)$$

recalling that $u_{\mathbf{q}}^* = u_{-\mathbf{q}}$. Denoting the amplitude of some sinusoidal mode simply by a , we may

write for the Gaussian

$$w(a) = (1/\sqrt{2\pi}\sigma) \exp\{-a^2/2\sigma^2\}. \quad (9)$$

In this form which is normalized to unity, $w(a)$ da signifies the probability of finding the amplitude in the interval da at a and σ stands for the root mean square of a . The Gaussians (9) must be the product of two other Gaussian, one arising from curvature elasticity (σ_{el}) and the other from the excluded-volume effect (σ_{st}); the mean squares obey the relation

$$1/\sigma^2 = 1/\sigma_{el}^2 + 1/\sigma_{st}^2. \quad (10)$$

It is necessary for a calculation of the entropy to divide the amplitude axis into discrete "cells". Their width a_0 will be irrelevant as only entropy differences are of interest, but it must be small enough to allow classical statistics (e.g. $a_0 \ll \sigma$). The entropy may then be expressed by the sum

$$S = -k_B \sum_n (w a_0)_n \ln(w a_0)_n \quad (11)$$

over all cells.

In the presence of the steric effect a distinction has to be made between accessible and inaccessible cells. Only the former are to be counted in the sum (11). A Gaussian distribution of the density ϱ_t of accessible cells may be written as

$$\varrho_t = (C/a_0) \exp\{-a^2/2\sigma_{st}^2\}, \text{ where } C < 1. \quad (12)$$

Unlike (9) it is not normalized to unity. The constant factor C is limited since ϱ_t must not exceed $1/a_0$. We will use the postulate

$$C = 1. \quad (13)$$

At its origin are the following considerations. We represent the totality of modes in a phase space of sufficiently high dimensionality, the space being divided into small enough cells of the same dimensionality. Looking at the origin, i.e. at the point where all amplitudes are zero, we note that on physical grounds there must be a certain vicinity in which all cells are completely accessible. The vicinity is not infinitesimal, as it should be ideally. However, using the Gaussian character of the amplitude distributions and deliberately disregarding mode-mode correlation, we assume that complete accessibility occurs at the origin and only there. It is then possible to separate formally into one-dimensional cell distributions with complete accessibility at $a = 0$. Our arguments do, of course,

not prove the above postulate, but they show it to be the most plausible assumption if mode-mode correlation is neglected. These problems will be further discussed in section 4.

If the sum (11) is replaced by an integral, counting accessible cells only is tantamount to cancelling the factor $\exp\{-a^2/2\sigma_{st}^2\}$ of w in $\ln(w a_0)_n$. We then have

$$S = \frac{k_B}{\sigma\sqrt{2\pi}} \int_{-\infty}^{+\infty} \exp\left(-\frac{a^2}{2\sigma^2}\right) \left[\frac{a^2}{2\sigma_{el}^2} + \ln \frac{\sigma\sqrt{2\pi}}{a_0} \right] da \quad (14)$$

which, together with (10), yields

$$S = k_B \left[\frac{1}{2} \frac{\sigma_{st}^2}{\sigma_{st}^2 + \sigma_{el}^2} + \ln \frac{\sigma}{a_0} + \frac{1}{2} \ln(2\pi) \right]. \quad (15)$$

The results shows that for the same effective force constant $f \propto 1/\sigma^2$ the entropy per mode depends not only on σ , the spread of the amplitude, but also on the ratio of σ_{st} and σ_{el} . It is larger by $(1/2)k_B$ with pure elasticity ($\sigma_{st} = \infty$) than with the pure steric effect ($\sigma_{el} = \infty$). Returning to ΔS instead of S and to standard notation, we may finally write

$$-T\Delta S_q = \frac{1}{2} k_B T \left[\frac{B q_{\parallel}^2}{B q_{\parallel}^2 + n k_c q_{\perp}^4} - \ln \frac{n k_c q_{\perp}^4}{B q_{\parallel}^2 + n k_c q_{\perp}^4} \right] \quad (16)$$

for the contribution of one mode to the entropy part of the free energy of steric interaction.

Adding (7) and (16), we find that ΔU_q is cancelled by the first term of $-T\Delta S_q$. Consequently, the free energy per mode takes the simple form

$$\Delta F_q = -\frac{1}{2} k_B T \ln \frac{n k_c q_{\perp}^4}{B q_{\parallel}^2 + n k_c q_{\perp}^4}. \quad (17)$$

It is interesting to note that the same formula for ΔF_q can be deduced in another way which is much easier but physically obscure. Upon redefining ΔU and ΔS so that ΔU contains both the "true" elastic energy of curvature and the "apparent" one of layer compression, we obtain $\Delta U_q \equiv 0$ from the equipartition theorem (3). If we now interpret the narrowing of the amplitude spread as an ordinary elastic effect, we arrive immediately at a formula for $-T\Delta S_q$ equal to (17).

It remains to add the contributions of all modes to obtain the total values of ΔF and, for completeness and comparison, of ΔU . We will denote these by the subscript 1 to indicate that they are obtained by the first theory. Replacing the sum

over the modes by an integral

$$\sum_q \rightarrow \frac{V}{(2\pi)^3} \int_{-q_{\parallel c}}^{q_{\parallel c}} \int_0^{q_{\perp c}} \pi dq_{\perp}^2 dq_{\parallel} \quad (18)$$

where $q_{\parallel c}$ and $q_{\perp c}$ are cutoff values, we have

$$(\Delta F)_1 = - \frac{V k_B T}{8\pi^2} \int_0^{q_{\parallel c}} \int_0^{q_{\perp c}} \ln \frac{q_{\perp}^4}{(q_{\parallel}/\lambda)^2 + q_{\perp}^4} dq_{\perp}^2 dq_{\parallel} \quad (19)$$

when use is made of de Gennes' penetration length

$$\lambda = (K/B)^{1/2} = (n k_c/B)^{1/2}. \quad (20)$$

The double integration yields

$$\begin{aligned} (\Delta F)_1 = & - \frac{V k_B T}{8\pi^2} q_{\perp c}^2 q_{\parallel c} \left[\ln \frac{q_{\perp c}^4}{(q_{\parallel c}/\lambda)^2 + q_{\perp c}^4} + 1 \right] \\ & + \frac{V k_B T}{8\pi^2} \lambda \left[\frac{q_{\parallel c}^2}{\lambda^2} \arctan \frac{q_{\perp c}^2}{(q_{\parallel c}/\lambda)} + q_{\perp c}^4 \operatorname{arccot} \frac{q_{\perp c}^2}{(q_{\parallel c}/\lambda)} \right] \end{aligned} \quad (21)$$

On the assumption

$$q_{\parallel c}/\lambda q_{\perp c}^2 \ll 1, \quad (22)$$

to be justified below, the formula for $(\Delta F)_1$ can be greatly simplified by expanding it in powers of $(q_{\parallel c}/\lambda q_{\perp c}^2)^2$. The zero-order term gives

$$(\Delta F)_1 = \frac{V k_B T}{16\pi} \frac{q_{\parallel c}^2}{\lambda}. \quad (23)$$

The first-order contributions of the expansion cancel each other and higher orders can be safely neglected if (22) is valid.

The total internal energy of membrane joining may be expressed by the integral of (7),

$$(\Delta U)_1 = - \frac{V k_B T}{8\pi^2} \int_0^{q_{\parallel c}} \int_0^{q_{\perp c}} \frac{(q_{\parallel}/\lambda)^2}{(q_{\parallel}/\lambda)^2 + q_{\perp}^4} dq_{\perp}^2 dq_{\parallel}. \quad (24)$$

Integration leads to

$$\begin{aligned} (\Delta U)_1 = & - \frac{V k_B T}{8\pi} \frac{\lambda}{2} \left[\frac{q_{\parallel c}}{\lambda} q_{\perp c}^2 \right. \\ & \left. + \left(\frac{q_{\parallel c}}{\lambda} \right)^2 \frac{\pi}{2} - \left(q_{\perp c}^4 + \frac{q_{\parallel c}^2}{\lambda^2} \right) \operatorname{arccot} \frac{q_{\perp c}^2}{q_{\parallel c}/\lambda} \right]. \end{aligned} \quad (25)$$

Expansion in powers of $q_{\parallel c}/\lambda q_{\perp c}^2$, together with assumption (22), gives the simplified version

$$(\Delta U)_1 = - \frac{V k_B T}{32\pi} \frac{q_{\parallel c}^2}{\lambda}. \quad (26)$$

The next nonvanishing term is a second power of $q_{\parallel c}/\lambda q_{\perp c}^2$. The internal energy of membrane joining is, of course, negative and its magnitude is just

half the positive free energy, as follows from comparing (26) and (23).

In a last step we eliminate the layer compressibility modulus B which through λ still enters formula (23) for the free energy of steric interaction. We first relate the cutoff wave number $q_{\parallel c}$ to the number n of membranes per unit height of stack by the obvious equation

$$q_{\parallel c} = n\pi. \quad (27)$$

With this and (20), Eq. (23) transforms into

$$(\Delta F)_1 = \frac{V k_B T n^2 \pi}{16} \sqrt{\frac{B}{n k_c}}. \quad (28)$$

Let us now permit the mean membrane spacing to be a function of position, while keeping constant the overall average of membrane spacing in the sample. The undulation modes are deformations of this type. The corresponding elastic modulus is proportional to the second derivative of $\Delta F/V$ with respect to mean membrane separation d . It must be identical with B , the exact relation being

$$B = \frac{1}{n^2} \frac{\partial^2}{\partial d^2} \frac{(\Delta F)_1}{V}. \quad (29)$$

Inserting (29) in (28) and substituting membrane area A for sample volume V by means of

$$A = nV \quad (30)$$

we arrive after some manipulations at

$$\frac{(\Delta F)_1}{A} = \frac{3\pi^2}{128} \frac{(k_B T)^2}{k_c d^2}. \quad (31)$$

This is the free energy of steric interaction per unit area of membrane in a multilayer system. The energy $(\Delta F)_1/A$ is independent of membrane thickness, while B and $(\Delta F)_1/V$ are not.

3. Theory Starting from Single Membrane between Rigid Plates

In this section the derivation of steric interaction via the formalism of smectic compressibility is complemented by another method starting from a single fluid membrane between parallel rigid plates.

The second approach is less direct, but apart from the usefulness of a control it gives more insight into the physical mechanism. It also suggests a refinement of both theories, to be discussed in the next section.

The single membrane is thought to lie in the xy plane and its local displacements in z direction are expressed by $u(x, y)$. If the deformations are not too strong, the associated curvature-elastic energy per unit area of plane and, equally, of membrane can be expressed by [8]

$$g = \frac{1}{2} k_c \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right)^2 \quad (32)$$

k_c (erg) being again the elastic modulus of membrane curvature. Adopting periodic boundary conditions, we use the standard Fourier expansion

$$u(\mathbf{r}) = \sum_{\mathbf{q}} u_{\mathbf{q}} \exp(i \mathbf{q} \cdot \mathbf{r}). \quad (33)$$

The modes thus defined satisfy the equipartition theorem in the form

$$\frac{1}{2} A k_c q^4 \langle |u_{\mathbf{q}}|^2 \rangle = \frac{1}{2} k_B T. \quad (34)$$

All this is similar to but simpler than Eqs. (1) to (3) for the smectic liquid crystal. The new vectors have, of course, only two components: $\mathbf{r} = (x, y)$ and $\mathbf{q} = \mathbf{q}_{\perp}$.

The thermal undulations of the membrane are now restricted by two parallel rigid plates of separation $2d$ positioned at $z = \pm d$. The membrane thickness is regarded as negligible. To describe how the plates affect out-of-plane fluctuations we employ the ratio

$$R(q) = \frac{\langle |u_{\mathbf{q}}|^2 \rangle_{\text{restricted}}}{\langle |u_{\mathbf{q}}|^2 \rangle_{\text{free}}} \quad (35)$$

of the mean square of the mode amplitudes with and without steric restriction. The presence of the rigid plates enforces

$$-d \leq u(\mathbf{r}) \leq +d. \quad (36)$$

As this condition is difficult to handle mathematically, it is replaced here by the approximation

$$\langle u^2(\mathbf{r}) \rangle = \mu d^2. \quad (37)$$

The purpose of (37) is to restrict the local displacements $u(\mathbf{r})$ largely but not totally to an interval of width $2d$, i.e. to the space available between the plates. The factor μ in front of d^2 must be smaller than unity, but not by too much. We postpone its evaluation and a discussion of the adequacy of (37)

to section 4. Equivalent to Eq. (37) is

$$\frac{1}{4\pi} \int_0^{q_{\perp c}} R(q) \frac{k_B T}{k_c q^4} dq^2 = \mu d^2, \quad (38)$$

where an integral over all modes is taken and use is made of (34) and (35). The cutoff $q_{\perp c}$ is the same as in the last section.

It would be attractive to derive the general form of the function $R(q)$ by minimizing a free energy. However, there is no evident way to treat the steric effect in such a calculus. Under these circumstances another phenomenological approach is taken. It consists in adding to the variable curvature-elastic force constant of the modes an invariable apparent force constant attributed to the steric effect. The force constants being inversely proportional to $\langle |u_{\mathbf{q}}|^2 \rangle$, we may thus write

$$\frac{1}{\langle |u_{\mathbf{q}}|^2 \rangle_{\text{restricted}}} = \frac{1}{\langle |u_{\mathbf{q}}|^2 \rangle_{\text{free}}} + \alpha. \quad (39)$$

Insertion in (35) leads to the function

$$R(q) = 1/(1 + \alpha \langle |u_{\mathbf{q}}|^2 \rangle_{\text{free}}). \quad (40)$$

With (34) this becomes

$$R(q) = q^4/(q^4 + \tilde{q}^4) \quad (41)$$

where \tilde{q}^4 symbolizes

$$\tilde{q}^4 = \alpha k_B T / A k_c. \quad (42)$$

The characteristic wave vector \tilde{q} of the spectrum is determined by condition (38). Insertion into this equation and integration give

$$\arctan(q_{\perp c}^2/\tilde{q}^2) = 4\pi \mu \tilde{q}^2 k_c d^2 / k_B T \quad (43)$$

which for

$$\tilde{q}/q_{\perp c} \ll 1 \quad (44)$$

leads to the simplified relation

$$\tilde{q}^2 = k_B T / 8\mu k_c d^2. \quad (45)$$

The inequality (44) will be discussed below, together with the related inequality (22).

The reduction of the mode amplitudes being caused by the steric effect, we re-use the arguments and the formalism laid down in Eqs. (8) to (17) to obtain a formula for the free energy of steric interaction. In analogy to (17) we have for one mode

$$\Delta F_{\mathbf{q}} = -\frac{1}{2} k_B T \ln R(q). \quad (46)$$

The sum over all modes is expressed by the integral

$$(\Delta F)_{rp} = -\frac{A k_B T}{8\pi} \int_0^{q_{\perp c}} \ln R(q) dq^2$$

$$= -\frac{A k_B T}{8\pi} \left(q_{\perp c}^2 \ln \frac{q_{\perp c}^4}{q_{\perp c}^4 + \tilde{q}^4} + 2\tilde{q}^2 \arctan \frac{q_{\perp c}^2}{\tilde{q}^2} \right).$$

The subscript *rp* is to indicate that we are dealing with a single membrane between rigid plates. Again utilizing (44) we derive for the free energy of steric interaction per unit area of membrane

$$(\Delta F)_{rp}/A = (k_B T)^2/64\mu k_c d^2 \quad (48)$$

where use has been made of (45). It can also be shown that $(\Delta U)_{rp} = -(1/2)(\Delta F)_{rp}$ as in the first theory.

Clearly, the second theory recovers all the functional dependences of ΔF on d , k_c and T found by the first. It is possible to extend (48) immediately to a hypothetical system of two fluid membranes of average separation d , now with periodic boundary conditions in all three directions. The undulations $u_1(x, y)$ and $u_2(x, y)$ of the membranes may be transformed into equal and opposite joint displacements $u_1 + u_2$ and $u_1 - u_2$. The former does not involve steric interaction. The latter is readily seen to be mathematically equivalent to the motion of a single membrane between rigid plates of distance $2d$, this time with the elastic modulus $k_c/2$ instead of k_c . Since the doubled energy of interaction has to be shared by two membranes, Eq. (48) remains valid for the two-membrane system.

Rather difficult considerations are needed for periodic systems of more than two membranes. Generally, $\Delta F/A$ may be expected to lie in the interval

$$\frac{(\Delta F)_{rp}}{A} < \frac{\Delta F}{A} < \frac{4(\Delta F)_{rp}}{A}. \quad (49)$$

The energy per area should be larger than in the two-membrane system. The upper limit reflects the fact that membranes of mean spacing d cease to interact sterically when they are squeezed into non-overlapping intervals of width d , as opposed to the original width $2d$.

For m orthogonal joint motions that can be defined in the case of m membranes,

$$\sum_{i=1}^m \kappa_i u_i, \quad m = \text{number of membranes} \quad (50)$$

only one, characterized by $\kappa_1 = \kappa_2 = \dots = \kappa_m$, does not produce steric interactions. In a m -layer system we have $(m-1)$ joint motions with steric interaction. This suggests, for $m \rightarrow \infty$,

$$\Delta F/A \approx 2(\Delta F)_{rp}/A \quad (51)$$

if we start from the joint displacements $u_{i+1} - u_i$ of adjacent membranes. However, as the axes of $u_{i+1} - u_i$ and $u_i - u_{i-1}$ form an angle in m -dimensional space of 60° instead of 90° , the factor of 2 in (51) may have to be replaced by $2 \cos(\pi/3) = 1.73$. A better theory may raise the factor again, but this is not pursued here. In view of other simplifications, such as the neglect of mode-mode correlation and those discussed in the next section, we adopt the factor of 2 as an approximation. It represents the geometric mean of the two limits given by (49). Accordingly, we write for the free energy of steric interaction in a multilayer system

$$(\Delta F)_2/A \approx (k_B T)^2/32\mu k_c d^2. \quad (52)$$

The subscript indicates that this is the result obtained by the second theory. A numerical comparison with $(\Delta F)_1$ requires knowledge of μ .

4. Further Considerations and Final Formula

This section is devoted to an evaluation of the factor μ and to a discussion of mode-mode correlation, including an attempt to estimate a correction applying to both theories of steric interaction. It concludes with a comparison of their results (the two turn out to be quite similar) and a final approximative formula for the free energy of steric interaction in multilayer systems, to be used in the following section. We begin with a brief digression, a simple scaling argument confirming the $1/d^2$ dependence of the excluded-volume effect and elucidating the roles of membrane thickness and cutoff $q_{\perp c}$.

Let us start with the observation that the total curvature-elastic energy of any finite system of fluid membranes is independent of its size or scale. Upon blowing up all lengths by, say, a factor of two, the local curvatures will be halved, but a given piece of membrane will become four times as large. Because of (32) the two effects must cancel in the integration over the entire configuration. It is assumed here that the thickness of the fluid membranes is negligible compared to their mean

spacing. The requirement can be dropped in the case of multilayers systems if the fluctuations are weak enough for removing the thickness from the problem by subtracting it. We will take advantage of this possibility, as we have already done implicitly in Section 2.

Let us make, for a moment, the false but simplifying assumption that the number of undulation modes of our fluctuating array of parallel membranes does not depend on its scale. Then the relative decrease of the mode amplitudes caused by mutual steric hindrance of the membranes is completely independent of the scale of the system. It follows that the total change of entropy associated with the excluded volume effect is also invariant. Therefore, the expected increase of free energy per unit area, $(\Delta F)/A$, should vary as

$$(\Delta F)/A \propto 1/d^2 \quad (53)$$

where d is again the mean membrane separation, i.e. the thickness of the intermediate water layers. Returning to our gedanken-experiment of blowing up all lengths by a factor of two, we now admit the fourfold increase of the number of undulation modes that takes place in reality. Since the additional modes are on the side of large q and

$$\langle |u_q|^2 \rangle \propto 1/q^4,$$

they should have only a negligible effect on the coarse features of the equilibrium distribution of membrane configurations, provided the mean spacing d is always much larger than the length $\pi/q_{\perp c}$ corresponding to the cutoff $q_{\perp c}$. This length is determined by molecular properties and cannot be smaller than the spacing between the molecules or equivalent units.

It is interesting to study a primitive version of the single-membrane model which will be referred to as the point model. The simpler model will be used to evaluate the factor $\mu = \langle u^2(\mathbf{r}) \rangle / d^2$, still missing in the single-membrane model. It will also shed some light on the general problems of mode-mode correlation and our treatment of the steric effect.

In the point model the fluid membrane is kept between two identical point lattices of distance $2d$. In constructing the model, we first introduce periodic boundary conditions for a square piece of membrane. Only the undulation modes with $|q_x|$ and $|q_y|$ smaller than some limiting value are

admitted. It is then possible to introduce local deformations of the form

$$u_j(\mathbf{r}) = \sum_{\mathbf{q}} c_j \frac{d}{N} \exp \{i \mathbf{q}(\mathbf{r} - \mathbf{r}_j)\} \quad (54)$$

where the summation is over N modes. The relative displacements c_j of the points \mathbf{r}_j are confined to the intervals

$$-1 < c_j < 1, \quad j = 1, \dots, N. \quad (55)$$

Exactly N orthogonal local deformations can be defined. They are arranged on a point lattice, preferably a square lattice, whose displacement by $\pm d$ gives the bounding lattices. The restoring force of curvature elasticity is omitted in the point model. Therefore, all values of c_j in the interval (55) are equally probable if we are dealing with a statistical problem. It is easy to derive the mean squares

$$\langle c_j^2 \rangle = \frac{1}{3} \quad (56)$$

and

$$\langle u^2(\mathbf{r}) \rangle = \langle u^2(\mathbf{r}_j) \rangle = d^2/3. \quad (57)$$

A Fourier analysis gives

$$u_q = \sum_j c_j \frac{d}{N} e^{-i \mathbf{q} \mathbf{r}_j} \quad (58)$$

where u_q is defined as in (33). This leads to

$$\langle |u_q|^2 \rangle = \frac{1}{3} d^2/N \quad (59)$$

and, for very large N , to a Gaussian distribution of the amplitudes u_q (here in the complex plane).

At first sight, one might conclude from (57) that the factor μ , introduced in Eq. (37), should be $1/3$. However, the point model restricts the displacement to the space between the plates only at N selected points. By suitably choosing the c_j of two or more neighbouring points, the displacement $u(\mathbf{r})$ in the region between them can be made to transgress the limits $+d$ or $-d$ quite substantially. To take into account that many of the configurations permitted in the point model are forbidden in the case of rigid plates, we will use $\mu = 1/6$ in the final estimates. There is a formal reason for this particular choice: A simple sinusoidal deformation of the type $\sin q_x x \sin q_y y$ with just one fluctuating amplitude gives $\mu = 1/12$. The chosen value of $1/6$ is the geometric mean between the extreme values of $1/3$ and $1/12$. A calculation of the best value of μ would be difficult and is not attempted here.

As mentioned before, the point model is also useful to examine some general problems of steric interaction (though not very profoundly). This is because the displacements at the N specified points are completely uncorrelated, which allows the entropy to be calculated exactly. The distribution functions of the displacements are rectangular instead of Gaussian. The entropy S_p associated with a single point is readily seen to be

$$S_p = k_B \ln(2d/d_0) \quad (60)$$

where d_0 is the width of the elementary "cell", here expressed by an interval of displacement, not of amplitude. S_p is to be compared with the entropy of a Gaussian distribution of the same root mean square $\sigma = d/\sqrt{3}$ and the same cell width d_0 . From (15) we obtain

$$S_p - S_{st} = k_B \left[\ln 2 - \frac{1}{2} \ln \frac{2\pi}{3} \right] = 0.32 k_B \quad (61)$$

and

$$S_p - S_{el} = (0.32 - 0.5) k_B = -0.18 k_B \quad (62)$$

where S_{st} and S_{el} are the entropies for the purely steric and for the purely elastic effect, respectively. S_{st} and S_{el} are identical to the entropies of each of the N undulation modes, as can be shown with the help of (59) and, for sinusoidal modes, of (8). However, the detour involving the ratio a_0/d_0 is not necessary. Our basic assumptions concerning the neglect of mode-mode correlation, Eqs. (12) and (13), if made for one representation in phase space (N amplitudes), are equally valid for the other (N displacements).

Equations (61) and (62) seem to indicate that steric interaction diminishes the entropy less than expected on the basis of (12) and (13) though still more than the purely elastic effect. In the point model the statistical cells in N -dimensional phase space are completely accessible inside an N -dimensional cube, while the cells outside are all inaccessible. Returning to the model of a membrane bounded by rigid plates, one finds without much difficulty two important differences: First, the distribution function of the N points is not a rectangle between $z = \pm d/\sqrt{2}$, as a result of $\langle u^2(\mathbf{r}) \rangle = d^2/6$, but a continuous function reaching zero at $z = \pm d$. The broadening at fixed $\langle u^2(\mathbf{r}) \rangle$ is readily seen to be linked with a decrease of the total number of accessible cells. Second, the displacements at the N selected points are no

longer completely uncorrelated. This has the effect of further reducing the entropy per point or undulation mode, pushing it perhaps even below S_{st} . Therefore, we may conclude that the entropies per undulation mode as calculated on the basis of the simplifying assumptions (12) and (13) do not represent serious underestimates.

A rectangular spectrum $\langle |u_q|^2 \rangle$ was employed in estimating $\mu = \langle u^2(\mathbf{r}) \rangle / d^2$ for the single-membrane model. However, the spectrum of this model, although fairly flat for $q < \tilde{q}$, tails off as $1/q^4$ in the limit of large q . The deviation from the rectangular shape is likely to make the ratio $\langle u^2(\mathbf{r}) \rangle / d^2$ even smaller than $1/6$, causing an additional rise of the free energy of steric interaction at given d . The correction is treated separately and not incorporated into μ because it is expected to apply to both theories of steric interaction. It is extremely difficult to deal with and the following attempt is of a very preliminary nature.

In order to visualize the additional effect, we compare a spectrum given by (34) and (41) with a rectangular one. The two spectra are taken to have the same total intensity and the same density at $q = 0$, i.e. equal $\langle u^2(\mathbf{r}) \rangle$ and equal $\langle |u_0|^2 \rangle$. (Such a rectangular spectrum breaks off abruptly at $q^2 = (\pi/2) \tilde{q}^2$.) We first consider the undulations due to the modes of wave numbers above some $q \gg \tilde{q}$. They will give rise to relatively weak ripples superimposed on the other fluctuations of mostly much longer wavelengths. Assuming for the moment that the wavelengths of the ripples are very much shorter than those of all the other undulations, we could say that the extrema of the displacements of the two parts have to be added linearly. This is because at least one short-wave maximum (minimum) exists in the immediate neighborhood of each long-wave maximum (minimum), regardless of the local phase relation of the two contributions at the exact position of the long-wave extremum. In Eq. (37) relating d and $\langle u^2(\mathbf{r}) \rangle$ we could then replace $(\langle u^2(\mathbf{r}) \rangle)^{1/2}$ by

$$\sqrt{\langle u_1^2(\mathbf{r}) \rangle} + \sqrt{\langle u_2^2(\mathbf{r}) \rangle} > \sqrt{\langle u^2(\mathbf{r}) \rangle}, \quad (63)$$

the subscripts denoting the two parts. The result is an increase of d at fixed $\langle u^2(\mathbf{r}) \rangle$.

The actual situation is much more complicated. On the one hand, the spectrum in question is continuous. We cannot simply cut it in two at some point and expect linear additivity of the two parts. This consideration would indicate that the increase

of d should be smaller than suggested by (63). On the other hand, as the short-wave contribution has more extrema than the long-wave part, we may be able to select the most prominent ripple of all those sitting on one long-wavelength bulge. It may also be necessary to cut the spectrum more than once, so that several sections of more or less linear additivity are obtained. The latter two arguments favor an increase of d beyond that predicted by (63).

As any useful calculations seem very difficult, we restrict ourselves to a crude estimate based on the model of one cut and linear additivity. It appears reasonable to place the cut somewhere above \tilde{q}^2 where $\langle |u_q|^2 \rangle$ starts to drop rapidly, so rapidly in fact that further cuts should make little difference. Writing

$$\sqrt{\langle u_1^2(\mathbf{r}) \rangle} + \sqrt{\langle u_2^2(\mathbf{r}) \rangle} = D \sqrt{\langle u^2(\mathbf{r}) \rangle} \quad (64)$$

we have $D^2 = 2, 1.66$ and 1.48 for $\langle u_2^2(\mathbf{r}) \rangle / \langle u^2(\mathbf{r}) \rangle = 1/2, 1/8$, and $1/16$, respectively. The first case corresponds to a cut near \tilde{q} , the others to cuts at larger wave numbers. In this range, the dependence of D^2 on the position of the cut is rather weak. D^2 is readily seen to be the correction factor of the free energy of steric interaction for fixed d . It should apply to both formulas, Eqs. (52) and (31), because of the similarity of the spectra of the two theories. We will use $D^2 = 2$.

Correcting Eq. (31) for the effect of linear additivity just discussed gives

$$\frac{(\Delta F)_1}{A} \approx \frac{3\pi^2}{64} \frac{(k_B T)^2}{k_c d^2}. \quad (65)$$

Applying the same correction to Eq. (52) and inserting $\mu = 1/6$ leads to

$$\frac{(\Delta F)_2}{A} \approx \frac{3}{8} \frac{(k_B T)^2}{k_c d^2}. \quad (66)$$

The numerical factors differ by about 20 percent. The fairly good agreement of the two theories is of course reassuring. If the mean is taken to obtain a final formula, we have for the free energy of steric interaction per unit area of membrane in multilayer systems

$$\frac{(\Delta F)_{ml}}{A} = 0.42 \frac{(k_B T)^2}{k_c d^2}. \quad (67)$$

The numerical factor in the final formula is not too reliable; we think that the correct value could

be between twice and half as large. Most of the uncertainty is, perhaps, due to the correction attempted in the last paragraph which cannot be checked by comparing the results of the two theories.

5. Discussion

The most interesting and unequivocal property of the steric interaction of fluid membranes is its long range. We have shown by three quite different methods that in multilayer systems the interaction energy per unit area of membrane varies with the inverse square of membrane spacing. In deriving the dependence it was assumed either that the membrane thickness is negligible with respect to membrane separation or that the local tilt of the membranes induced by undulations remains, in effect, well below $\pi/2$. In practice, it is enough to verify that the second requirement is satisfied at the minimum spacing of interest ($\gtrsim \pi/q_{\perp c}$) because the average tilt angle can be shown to increase only logarithmically with membrane spacing.

It has also become quite clear that the statistical thermodynamics of the excluded-volume effect of parallel undulating fluid membranes is far from simple. We have tried to point out the various problems and to find approximate solutions. However, there remain a number of delicate points calling for further study. Accuracy is of special importance as the following estimates for lecithin membranes will show steric repulsion to be in close competition with van der Waals attraction.

A numerical estimate is made for egg lecithin, the only truly fluid membrane for which the elastic modulus of curvature elasticity has been measured at least once. The following numbers will be used

$$\begin{aligned} k_B T &= 4 \cdot 10^{-14} \text{ erg (room temperature),} \\ k_c &= 2 \cdot 10^{-12} \text{ erg (see Ref. [9]),} \\ q_{\perp c} &= \pi/a, \\ a &= 5 \cdot 10^{-8} \text{ cm (hydrocarbon chain} \\ &\quad \text{spacing).} \end{aligned} \quad (68)$$

Insertion of the first two numbers in Eq. (67) results in

$$(\Delta F)_{ml}/A = 3.4 \cdot 10^{-16} \text{ erg}/d^2. \quad (69)$$

It may be deduced from the value of the cutoff wave vectors that the simplifying assumptions (22)

and (44) should apply down to membrane spacings considerably smaller than the membrane thickness of about 35 Å. It can also be shown that the average tilt angle of the membranes is much smaller than $\pi/2$ for spacings of the order of membrane thickness.

Steric repulsion has to be compared with van der Waals attraction. Considering a multilayer system of flat and uniform lipid layers alternating with water Ninham and Parsegian [5] calculated

$$(\Delta F)_{\text{v. d. Waals}}/A = -1.4 \cdot 10^{-15} \text{ erg}/d^2. \quad (70)$$

According to the two theoretical predictions (69) and (70) van der Waals attraction would be 4 times stronger than steric repulsion.

The experimental situation concerning the lecithins is ambiguous. Le Neveu, Rand, and Parsegian [10] have recently determined the period as well as the membrane spacing of egg lecithin multilayer systems as functions of the concentration of dextran in an aqueous environment. The dextran molecules did not seem to penetrate into the multilayer system. By lowering the dextran concentration and, thereby, the osmotic stress the authors measured a maximum spacing of 27.5 Å which could not be exceeded by adding more water. They interpreted this number as the equilibrium spacing representing a balance between van der Waals attraction and short-range repulsion. With the help of an extrapolation method they inferred an attractive force about half as strong as the theoretical value derived from (70).

Those findings are in conflict with the repeated observation that various lecithins, among them egg lecithin, form large vesicles when brought into a big volume of water. The formation occurs either spontaneously [1, 9] or under slight and probably immaterial agitation [11, 12]. Also, the complete mathematical analysis [13, 14] of photographed shapes of axi-symmetric, oblate vesicles of egg lecithin whose membrane was in contact with itself over a certain area did not provide any evidence of mutual attraction. The membranes of these vesicles were judged from experience to consist of only a few bilayers. Accordingly, the total curvature-elastic energy residing in a membrane was probably of the order of a few 10^{-10} erg [13, 14]. For the approximate area of contact of $100 \mu^2$ and a membrane spacing of 27.5 Å, one computes from (70) a cohesion energy of $185 \cdot 10^{-10}$ erg. A cohesion of

this strength should have a dramatic influence on vesicle shape, but none was detected.

Independently of the specific problems of lecithin-water systems, steric interaction of fluid membranes appears to be an effect which has to be taken into account in future work on bilayer and monolayer interaction. Furthermore, human red blood cells were found [15] not to cohere in physiological saline solution free of macromolecules such as fibrinogens. Although this may be due to electrostatic repulsion, i.e. electric double layers, we remark that Brochard and Lennon [16] reported a value of the curvature-elastic modulus of the human red cell membrane about ten times smaller than that of egg lecithin. To the extent that the membrane can be regarded as fluid, and the value is reliable, a very strong steric repulsion may be anticipated.

In conclusion, we note that the energy of van der Waals attraction between widely spaced membranes varies as $1/d^4$ rather than $1/d^2$. There are different predictions [5] for the transition from one regime to the other. Since the energy of the steric effect is proportional to $1/d^2$ at all membrane spacings d , repulsion will always become stronger than van der Waals attraction at large enough d . Also, the effective membrane separation of the steric effect is smaller than that of van der Waals interaction because the region of strong short-range repulsion should be counted to the membrane thickness in considering steric interaction. Very wide regions of predominant electrostatic repulsion extending up to 1000 Å are possible [17] if the polar heads of the amphiphilic molecules forming a bilayer are ions rather than zwitterions like the lecithins. All these facts are in favor of net repulsion even if the van der Waals forces are stronger than the steric ones at small spacings. Concerning the vesicle experiments just mentioned, it cannot be ruled out entirely that the apparent absence of cohesion was due to ionic impurities in the bilayers. However, vesicles swelled from lecithin and no traces of cohesion were seen in NaCl solution of up to 0.5 mole per liter, the highest concentration used [18].

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