Bending elasticity and bending fluctuations of lipid bilayer containing an additive

Isak Bivas* and Philippe Méléard
UMR-CNRS 6052, École Nationale Supérieure de Chimie de Rennes, 35700 Rennes, France
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The static and dynamic behavior of a bilayer containing an additive is examined theoretically. We have proved that the amplitudes of the thermal shape fluctuations of a quasi spherical lipid vesicle depend on the value of the bending elasticity of the vesicle's membrane at free exchange of molecules between its constituent monolayers. The dependence is calculated of this bending elasticity as a function of the concentration of the additive in the low concentration domain. In the same domain, the autocorrelation function of each vesicle fluctuation mode is found to be dependent on the two-dimensional diffusion coefficient of the bilayer additive.

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I. INTRODUCTION

Biomembranes belong to the main building blocks of living matter. Their mechanical properties, and especially their bending elasticity, determine some of their basic functions: cellular interactions, cell fusion, adhesion, etc. However, due to the complicated structure of the biomembranes, their investigation is usually performed by using simplified models like lipid bilayers. A preponderant method applied for the determination of the bilayer bending elasticity is the analysis of the relevant thermal bending fluctuations. Thus, a theory of the mechanical properties of pure lipid bilayer and of the thermal shape fluctuations of giant lipid vesicle with a membrane of this kind was developed [1–6] (for a recent review see [7]). It is our aim in what follows, to present a further generalization of some of the above theoretical results for the case of a bilayer containing an additive. More specifically, what we are here interested in is a particular case of a model of real biomembranes, comprising different lipids and proteins.

A lipid vesicle is considered with bilayer in a monophase state. It contains $2N$ lipid molecules and $2M$ molecules of an additive, N^{out} and M^{out} of them being in its outer monolayer and N^{in} and M^{in} in its inner monolayer. Let us consider an additive molecule, which is in contact with the molecules of the two monolayers, e.g., the case of transversal proteins. What we can do in this case is to assign a direction to each of the additive molecules. If it is directed ''up'' (''down'') it will be taken as a part of the outer (inner) monolayer. M^{out}, N^{out}, M^{in}, and N^{in} are assumed to be time independent numbers.

Let S^{tf} be the area of the bilayer in its flat tension free state with M molecules of the additive and N lipid molecules in each of its monolayers. Let C be the molar concentration of the additive in the bilayer, $C=M/(M+N)$. The moduli $k_s(C)$ and $\overline{k_c}(C)$ are the stretching and the saddle splay bending elasticity, defined as in the case of a pure lipid bilayer [1]. Following Helfrich [1], a membrane built up of two chemical species is characterized by two bending elasticities: $k_c^{fr}(C)$ when there is a free exchange of the molecules of the lipid and of the additive between the two monolayers comprising the bilayer, and $k_c^{bl}(C)$ when this exchange is not permitted.

First, the concentration of the additive in the bilayer is assumed to be not fluctuating (fluctuations in the monolayers are allowed). In the last section, it is demonstrated that this restriction is not essential.

When the vesicle thermal fluctuations are treated, the membrane is considered as a closed surface $\Sigma(t)$, defined at a time t in the laboratory frame of reference XYZ, whose center O is appropriately placed inside the vesicle. Let (θ,φ) be the polar angles of a direction, piercing the membrane at the moment t in a point with radius vector $\mathbf{R}(\theta,\varphi,t)$. Following the approach described in detail elsewhere [2,3,6,8], $|\mathbf{R}(\theta,\varphi,t)|=R(\theta,\varphi,t)$ is presented as follows:

$$R(\theta,\varphi,t)=R_0[1+u(\theta,\varphi,t)], \tag{1}$$

where R_0 is the radius of a sphere of volume V (not fluctuating), equal to that of the vesicle.

The function $u(\theta,\varphi,t)$ can be decomposed in a series with respect to the spherical harmonics $Y_i^j(\theta,\varphi)$:

$$u(\theta,\varphi,t)=\sum_{i=0}^{i_{max}}\sum_{j=-i}^{i} u_i^j(t)Y_i^j(\theta,\varphi). \tag{2}$$

A cutoff $i_{max}\sim R_0/\lambda$ is introduced in the summation, where λ is some typical intermolecular distance.

II. FORM FLUCTUATIONS OF A VESICLE WITH A LIPID MEMBRANE CONTAINING AN ADDITIVE

The fluctuating membrane is presented by its neutral surface $\Sigma(t)$ [9]. The surface $\Sigma(t)$ is completely determined by the ensemble of amplitudes $u_i^j(t)$ [see Eq. (1)]. A patch of the membrane, containing a sufficiently high number of lipid and additive molecules, is considered having area in its flat tension free state ΔS^{tf}, and principal curvatures c_1 and c_2. The membrane, presented by its neutral surface, is said to be tension free when its tension is equal to zero [9,10]. Let ΔM^{out} and ΔN^{out} be the numbers of additive and lipid molecules in the outer monolayer of this part of the membrane.

*Permanent address: Institute of Solid State Physics, Bulgarian Academy of Sciences, 72 Tzarigradsko Chaussee Blvd., Sofia 1784, Bulgaria. Email address: bivas@issp.bas.bg

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ΔM^{in} and ΔN^{in} are the corresponding numbers in the inner monolayer. The number densities Δm_0 and Δn_0 are then defined as

$$\Delta m_0 = \frac{\Delta M^{out} - \Delta M^{in}}{\Delta S^{tf}}, \quad \Delta n_0 = \frac{\Delta N^{out} - \Delta N^{in}}{\Delta S^{tf}}.$$

Generalizing the flip-flop coefficient for a pure lipid bilayer (introduced by Helfrich [1]), in our case the flip-flop coefficients $\xi_m(C)$ and $\xi_n(C)$ are defined for each of the two constituents of the membrane. Now, at free flip-flop for both constituents, the quantities Δm_0 and Δn_0 can be expressed by the curvatures and the relevant flip-flop coefficients in the following way:

$$\Delta m_0 = \xi_m(C)(c_1 + c_2), \quad \Delta n_0 = \xi_n(C)(c_1 + c_2).$$

Further on, the approach developed initially for the description of one-component bilayer [6] is generalized. Only symmetric membranes are considered. The two-component membrane is symmetric, if its flat tension free state with equal number of molecules of both kinds in the two monolayers is the state with minimum elastic energy. At free flip-flop for the two constituents, the bending elasticity of the membrane is that at free flip-flop k_c^{fr}. For a tension free membrane the bending energy density $g_c^{fr}(c_1, c_2, C)$ is in this case

$$g_c^{fr}(c_1, c_2, C) = \tfrac{1}{2} k_c^{fr}(C)(c_1 + c_2)^2 + \overline{k_c}(C) c_1 c_2. \quad (3)$$

As in the case of a pure lipid bilayer, the saddle splay bending elasticity $\overline{k_c}(C)$ does not depend on the exchange of molecules between the monolayers [11] and does not influence the fluctuations [3]. At fixed curvatures c_1 and c_2 the bending energy at free flip-flop is the minimal one. The bending energy density at arbitrary Δm_0 and Δn_0 close to the minimum is presented in quadratic approximation with respect to Δm_0 and Δn_0 using the moduli $\Phi_{mm}(C)$, $\Phi_{mn}(C)$, and $\Phi_{nn}(C)$,

$$\begin{aligned} g_c(c_1, c_2, C, \Delta m_0, \Delta n_0) &= g_c^{fr}(c_1, c_2, C) + \tfrac{1}{2}\Phi_{mm}(C)[\Delta m_0 - \xi_m(C)(c_1 + c_2)]^2 \\ &+ \Phi_{mn}(C)[\Delta m_0 - \xi_m(C)(c_1 + c_2)][\Delta n_0 - \xi_n(C) \\ &\times (c_1 + c_2)] + \tfrac{1}{2}\Phi_{nn}(C)[\Delta n_0 - \xi_n(C)(c_1 + c_2)]^2. \end{aligned} \quad (4)$$

For convenience, the explicit dependence of the moduli and of the flip-flop coefficients on the concentration C will be omitted till the end of this Section.

When $\Delta m_0 = 0$ and $\Delta n_0 = 0$, then the relevant bending elasticity is equal to that at blocked flip-flop k_c^{bl},

$$g_c(c_1, c_2, C, 0, 0) = \tfrac{1}{2} k_c^{bl}(c_1 + c_2)^2 + \overline{k_c}\, c_1 c_2. \quad (5)$$

From Eqs. (3), (4), and (5) the relation between k_c^{bl} and k_c^{fr} is obtained

$$k_c^{bl} = k_c^{fr} + \Phi_{mm}(\xi_m)^2 + 2\Phi_{mn}\xi_n\xi_n + \Phi_{nn}(\xi_n)^2. \quad (6)$$

When the excesses $M^{out} - M^{in}$ and $N^{out} - N^{in}$ are uniformly distributed, the spontaneous curvature c_0^0 of the membrane is

$$c_0^0 = \frac{1}{k_c^{bl}} \left\{ \Phi_{mm} \frac{M^{out} - M^{in}}{S^{tf}} \xi_m + \Phi_{mn} \left[\frac{M^{out} - M^{in}}{S^{tf}} \xi_n + \frac{N^{out} - N^{in}}{S^{tf}} \xi_m \right] + \Phi_{nn} \frac{N^{out} - N^{in}}{S^{tf}} \xi_n \right\}. \quad (7)$$

Let s_0 be the mean area per molecule in a pure lipid tension free bilayer. The mean square amplitudes $\langle [u_i^j(t)]^2 \rangle$, $2 \leqslant i < \sqrt{[(R_0)^2 C / s_0]}$ [$\langle A(t) \rangle$ denoting the time average of any time dependent function $A(t)$], calculated on the basis of the energy in Eq. (4) are

$$\langle [u_i^j(t)]^2 \rangle = \frac{kT}{k_c^{fr}} \frac{1}{(i-1)(i+2)} \times \frac{1}{i(i+1) + \dfrac{\sigma (R_0)^2}{k_c^{fr}} - 2\dfrac{k_c^{bl}}{k_c^{fr}} c_0^0 R_0 + 2\dfrac{k_c^{bl} - k_c^{fr}}{k_c^{fr}}}, \quad (8)$$

where σ is the tension of the membrane, k is the Boltzmann constant, and T is the absolute temperature.

Considering the vesicle membrane as a liquid shell with only one bending elasticity k_c, Milner and Safran [3] established the well known relation between k_c and the mean square amplitudes of the vesicle fluctuation modes. The comparison of their result with Eq. (8) shows that k_c^{fr} plays the role of k_c in their model. The mean square amplitudes $\langle [u_i^j(t)]^2 \rangle$, $i \geqslant 2$, can be measured experimentally [8]. The bending elasticity, deduced from the analysis of these amplitudes, is numerically equal to k_c^{fr}. This result is a generalization of the similar one, obtained earlier for a pure lipid bilayer [5,6,12,13].

III. BENDING ELASTICITY OF A LIPID BILAYER CONTAINING AN ADDITIVE AT LOW CONCENTRATION

Let us consider a flat tension free state of the described above bilayer with concentration $C \ll 1$. The energy density from Eq. (4) becomes

$$\begin{aligned} g_c(0, 0, C, \Delta m_0, \Delta n_0) = [&\tfrac{1}{2}\Phi_{mm}(C)(\Delta m_0)^2 \\ &+ \Phi_{mm}(C)\Delta m_0 \Delta n_0 \\ &+ \tfrac{1}{2}\Phi_{nn}(C)(\Delta n_0)^2]. \end{aligned} \quad (9)$$

Let $\Delta n(\Delta m_0)$ be a function with the property that the energy density $g_c(0, 0, C, \Delta m_0, \Delta n_0)$ attains its minimum

with respect to Δn_0, at fixed Δm_0, when $\Delta n_0 = \Delta n(\Delta m_0)$. For low enough Δm_0 their relationship can be written in the following form:

$$\Delta n(\Delta m_0) = -\{[s^{nm}(C)/s_0]\,\Delta m_0\}. \tag{10}$$

$s^{nm}(C)$ is of the order of the area occupied by one molecule of the additive, and depends on its geometry and its positioning in the bilayer; s_0 is defined in the preceding section.

Let now $\Delta m_0 = 0$. If $\Delta n_0 \neq 0$, the energy density $g_c(0,0,C,0,\Delta n_0)$ is proportional to $(\Delta n_0)^2$. Let $k_s^{\Delta n}(C)$ have the property

$$g_c(0,0,C,0,\Delta n_0) = \tfrac{1}{8}\, k_s^{\Delta n}(C)(s_0)^2(\Delta n_0)^2. \tag{11}$$

For a pure lipid bilayer, whose stretching elasticity is a sum of the stretching elasticities of its two monolayers, $k_s^{\Delta n}(0) = k_s$, where k_s is the stretching elasticity of the lipid bilayer.

If the bilayer has fixed Δm_0 and Δn_0, its minimal elastic energy state will not be the flat one. Let $\tilde{c}(C,\Delta m_0,\Delta n_0)$ be the curvature of the cylindrically curved state of such a bilayer with the minimal bending energy. This quantity is a linear function of Δm_0 and Δn_0. Let $c_0^m(C)$ and $c_0^n(C)$ be the corresponding proportionality coefficients

$$\tilde{c}(C,\Delta m_0,\Delta n_0) = c_0^m(C)\Delta m_0 + c_0^n(C)\Delta n_0. \tag{12}$$

c_0^m and c_0^n depend on the shape of the molecules comprising the bilayer.

Using the above quantities, the following dependence of k_c^{fr} on C was calculated ($C \ll 1$)

$$k_c^{fr}(C) = k_c^{fr}(0)(1+\nu C) - \Delta k_c \left(\frac{c_0^m(0)}{c_0^n(0)} - \frac{s^{nm}(0)}{s_0} \right)^2 \times [k_s s_0/(2kT)]\, C + O(C^2), \tag{13}$$

where $\Delta k_c = k_c^{bl}(0) - k_c^{fr}(0)$ and ν is a dimensionless quantity of order of 1. Defining $k_c^{aux}(C)$ as

$$k_c^{aux}(C) = k_c^{bl}(C) - \frac{4[k_c^{bl}(C)]^2[c_0^n(C)]^2}{k_s^{\Delta n}(C)(s_0)^2}, \tag{14}$$

and using the relation $k_c^{aux}(0) = k_c^{fr}(0)$, ν was deduced to be

$$\nu = \frac{1}{k_c^{aux}(0)} \left. \frac{\partial [k_c^{aux}(C)]}{\partial C} \right|_{C=0}. \tag{15}$$

To obtain these results, a flat membrane with fixed M^{out} and M^{in} and vanishing concentration of the additive (i.e., with very high number of lipid molecules), in equilibrium with a pure lipid bilayer, was considered. The additive was condensed up to concentration C and the energy of this process was calculated using the fact that at low concentrations the molecules of the additives form a two-dimensional gas in the lipid matrix.

In the general case, the expression $(c_0^m/c_0^n - s^{nm}/s_0)^2$ is nonzero and of the order of 1. Taking the values $s_0 \sim 61.4$ Å^2 [14] and $k_s \approx 200$ mN/m [15], measured for stearoyl-oleoyl-phosphatidylcholine (SOPC) bilayer, the ratio $k_s s_0/2kT \approx 15$ is estimated. Another plausible estimation is $\Delta k_c \sim k_c^{fr} \sim k_c^{bl}$. As can be seen from Eq. (13), the normalized derivative $[\partial k_c^{fr}(C)/\partial C]/k_c^{fr}$ is expected to have high negative values for most of the additives. This prediction is in excellent agreement with the experimental data of Häckl *et al.* [16]. Two particular exceptions have to be noted. The first one is trivial, namely, when the additive is very similar to the lipid. Then $c_0^m = c_0^n$, $s^{nm} = s_0$, $\nu = 0$, and the bending elasticity does not depend on the concentration of the additive. The second one is the case when $c_0^m = 0$ and $s^{nm} = 0$. This case corresponds to symmetric molecules, symmetrically inserted in the bilayer (their plane or center of symmetry coincides with or lies on the dividing surface between the two monolayers of the bilayer). In this case, the derivative $(\partial k_c^{fr}/\partial C)/k_c^{fr} = \nu$ has a modulus of the order of 1.

IV. DYNAMICS OF THE VESICLE SHAPE FLUCTUATIONS

The theoretical description of the dynamics of a fluctuating vesicle was first developed by Schneider *et al.* [2] and Milner and Safran [3] under the already mentioned assumption that the vesicle membrane is a shell characterized by a single bending modulus k_c.

In a previous work [6], we took into account the intermonolayer friction on the correlation function $\langle u_i^j(t)\, u_i^j(t+\tau)\rangle$ and obtained the following result for the pure lipid bilayer:

$$\langle u_i^j(t)\, u_i^j(t+\tau)\rangle = \langle [u_i^j(t)]^2\rangle [A_{ij}^{\omega} \exp(-\omega_i^j \tau) + A_{ij}^{\Omega} \exp(-\Omega_i^j \tau)], \tag{16}$$

where Ω_i^j, ω_i^j, A_{ij}^{ω}, and A_{ij}^{Ω} were expressed by the friction coefficient between the monolayers, the flip-flop coefficient, and the bending elasticities of the pure lipid bilayer. Considering the case of a bilayer containing an additive, we proved that the generalization of this result is as follows: for an arbitrary concentration $C \neq 0$ the correlation function $\langle u_i^j(t)\, u_i^j(t+\tau)\rangle$ consists of three exponents instead of two.

Let $q_{\uparrow\uparrow}$, $q_{\uparrow\downarrow}$, q_m, and q_n be the friction coefficients between the additive and the lipid in the same monolayer, the additive and the lipid of the opposite monolayer, the additive in the inner and the additive in the outer monolayer, and the lipid in the inner and the lipid in the outer monolayer, respectively. In the low concentration limit of the additive, the friction coefficients depend on C as

$$q_{\uparrow\uparrow}(C) = q_{\uparrow\uparrow}^{(1)} C + O(C^2), \quad q_{\uparrow\downarrow}(C) = q_{\uparrow\downarrow}^{(1)} C + O(C^2),$$

$$q_m(C) = \tfrac{1}{2}\, q_m^{(2)} C^2 + O(C^3), \quad q_n(C) = q_n^{(0)} + q_n^{(1)} C + O(C^2),$$

where $q_{\uparrow\uparrow}^{(1)}$, $q_{\uparrow\downarrow}^{(1)}$, $q_m^{(2)}$, $q_n^{(0)}$, and $q_n^{(1)}$ are constants, characterizing the lipid and the additive molecules. Taking into account that the ratio $kT/[s_0(q_{\uparrow\uparrow}^{(1)} + q_{\uparrow\downarrow}^{(1)})]$ is equal to the two-dimensional diffusion coefficient of the additive D, the

following result is obtained for the correlation $\langle u_i^j(t)u_i^j(t+\tau)\rangle$ at low concentrations C:

$$\langle u_i^j(t)u_i^j(t+\tau)\rangle=\langle[u_i^j(t)]^2\rangle\left\{A_{ij}^{\omega}[1+O(C)]\exp(-\omega_i^j\tau)+A_{ij}^{\Omega}[1+O(C)]\exp(-\Omega_i^j\tau)+A_{ij}^{add}(C)\exp\left[-D\frac{i(i+1)}{(R_0)^2}\tau\right]\right\}, \quad (17)$$

with

$$A_{ij}^{add}(C)=\frac{(i-1)(i+2)}{i(i+1)+\dfrac{\sigma(R_0)^2}{k_c^{fr}}-2\dfrac{k_c^{bl}}{k_c^{fr}}c_0^0R_0+2\dfrac{\Delta k_c}{k_c^{fr}}}\times\frac{\Delta k_c}{k_c^{fr}}\left(\frac{c_0^m(0)}{c_0^n(0)}-\frac{s^{nm}(0)}{s_0}\right)^2\frac{k_ss_0}{2kT}C+O(C^2), \quad (18)$$

where A_{ij}^{ω}, A_{ij}^{Ω}, ω, and Ω are those of Eq. (16) for the pure lipid bilayer. The comparison with Eq. (13) shows that when $(c_0^m(0)/c_0^n(0)-s^{nm}(0)/s_0)^2\geqslant 1$, $A_{ij}^{add}(C)$ can be presented approximately as

$$A_{ij}^{add}(C)\approx-\frac{1}{k_c^{fr}}\frac{\partial[k_c^{fr}(C)]}{\partial C}\bigg|_{C=0}C. \quad (19)$$

These results reveal that the analysis of the thermal shape fluctuations of a lipid vesicle with bilayer containing an additive, is an appropriate tool for the determination of the diffusion coefficient of the inclusion in the membrane.

The experimentally measured quantities are $\langle[u_i^j(t)]^2\rangle$ and $\langle u_i^j(t)u_i^j(t+\tau)\rangle$ [8,17]. The experimental error for the two quantities are of the same order. For small values of i, they are $\sim 0.1\langle[u_i^j(t)]^2\rangle$. Obviously, the value of $A_{ij}^{add}(C)$ has to be significantly greater than this error. Consequently, the concentration C must be chosen so that $A_{ij}^{add}(C)$, calculated from the experimental data by means of Eq. (19), satisfies this condition.

V. INFLUENCE OF THE CONCENTRATION FLUCTUATIONS OF THE ADDITIVE IN THE BILAYER

The results presented so far, were obtained under the assumption, that the concentration of the additive is the same all over the membrane. The area of validity of this assumption is outlined in this section.

Let $\overline{C}$ be the mean concentration of the additive in the membrane and $\langle(u_i^j[C(\theta,\varphi,t)])^2\rangle$ and $\langle[u_i^j(\overline{C})]^2\rangle$ be the mean squares of the amplitude u_i^j when the fluctuations of the density C of the additive in the bilayer are permitted and forbidden (i.e., $C=\overline{C}$ all over the membrane), respectively. If the system is not in the vicinity of a critical point, the following relationship between these quantities is obtained:

$$\langle(u_i^j[C(\theta,\varphi,t)])^2\rangle=\langle[u_i^j(\overline{C})]^2\rangle\left\{1+O\left[\frac{k_c^{bl}(\overline{C})(R_0)^2}{kTs_0}\right]\right\}.$$

Taking $R_0=10\ \mu$m (a typical radius value for a giant vesicle, whose thermal shape fluctuations are studied), $s_0=61.4$ Å^2, and $k_c^{bl}\sim 20\ kT$, it is seen that the corrections due to the fluctuations of the density of the additive are of the order of 10^{-7} and can be neglected.

The density fluctuations of the additive in the bilayer result in one more exponent in the expression for $\langle u_i^j(t)u_i^j(t+\tau)\rangle$ in Eq. (17). Far enough from any critical point, the corresponding preexponential factor is of the order of $[k_c^{bl}/kT][s_0/(R_0)^2]\langle[u_i^j(t)]^2\rangle\approx 10^{-7}\langle[u_i^j(t)]^2\rangle$. Consequently, this exponent can be neglected too.

On account of these estimations, it follows that the results in the present work are valid everywhere in the one-phase region of the lipid/additive phase diagram except in the vicinity of critical points, if they exist.

VI. CONCLUDING REMARKS

The results of the present work allow better interpretation of the information deduced from the analysis of the bilayer bending fluctuations. They demonstrate that most additives decrease the bending elasticity of the bilayer, even at relatively low concentrations in the membrane. On the basis of our results, a method is proposed for the determination of the diffusion coefficient of an inclusion in the bilayer.

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