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Elasticity and shape equation of a liquid membrane

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Abstract. The density of the elastic energy of a deformed membrane in a liquid state is calculated. The thermodynamic equilibrium of its different parts is taken into account. The shape equation of a closed membrane is deduced. The quantity which keeps its value, when the variations of the energy of the system are calculated, is not the area of the deformed membrane, but its area in the flat tension free state. Because of this, additional terms appear in the second variation around the stable state. The case of a lipid bilayer and its fluctuations is examined for both free and blocked exchange of molecules between the monolayers, comprising the bilayer.

PACS. 87.16.Dg Membranes, bilayers, and vesicles

1 Introduction

The problem, treating the shape, which a membrane can take, is a subject of intensive investigations because of its relation to the shape of biological cells [1]. The main factors determining this shape, are the elastic properties and the spontaneous curvature of the membrane.

Following Helfrich [2], the density of the stretching elastic energy g_s of a piece of a flat membrane with area s is:

$$g_s = \frac{1}{2}k_s\frac{(s-s_0)^2}{ss_0}, \tag{1}$$

where s_0 is the area in its tension free state, and k_s is the stretching elasticity modulus. Again according to Helfrich [2], the density of the bending elastic energy g_c per unit area of a bent membrane in a point with main curvatures c_1 and c_2 is:

$$g_c = \frac{1}{2}k_c(c_1+c_2-c_0)^2 + \overline{k}_c c_1 c_2, \tag{2}$$

where k_c and $\overline{k}_c$ are the bending elasticity and the saddle splay bending elasticity of the membrane, and c_0 is its spontaneous curvature. The order of magnitude of k_c is $k_c \sim D^2 k_s$, where D is the thickness of the membrane. The same estimation is also valid for $\overline{k_c}$. Expressions (1) and (2) are valid for a membrane in its liquid crystal state, when it can be treated as a two-dimensional liquid. This means that its static shear elasticity moduli are equal to zero. Further on in the present work, only membranes of such type will be considered.

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Based on the bending elasticity of the kind of equation (2) and implicitly making the assumption that the stretching elasticity modulus k_s is infinitely large, Ou-Yang Zhong-can and Helfrich [3–5] derived the following equation of shape of a closed membrane:

$$\Delta p + \lambda(c_1+c_2) - k_c(c_1+c_2-c_0)\left[\frac{1}{2}(c_1+c_2)^2 - 2c_1c_2 + \frac{1}{2}c_0(c_1+c_2)\right] - k_c\Delta_s(c_1+c_2) = 0, \tag{3}$$

where, in addition to the quantities defined below equations (1, 2), Δp and λ are Lagrange multipliers, providing conservation of the area of the membrane and of the volume enclosed by the membrane, and Δ_s is the two-dimensional Laplace-Beltrami operator on the surface under consideration.

In the present work, we rederive the shape equation of a closed liquid membrane taking into account the finite value of the stretching elasticity k_s and the related to this fact consequences.

2 The model

We consider an infinitely thin membrane with a given area S_0 in its flat tension free state, elastic moduli of stretching, bending and saddle splay bending k_s, k_c, and $\overline{k_c}$ respectively, and spontaneous curvature c_0. Let s_0 be the area of a "physically infinitely small" piece of the membrane in its tension free state, and let s and c_1 and c_2 be the area and the main curvatures of this piece in its deformed state. We denote the elastic energy of the deformed piece

with $F(s, s_0, c_1, c_2)$, requiring this energy to be zero in its flat tension free state, *i.e.* considering the flat tension free state as a ground state. Let D be the thickness of the real membrane, which we model with the infinitely thin one. The most general expression for $F(s, s_0, c_1, c_2)$, in which all terms, up to the second power of Dc_1, Dc_2 and $(s - s_0)/s_0$ are taken into account, is (Helfrich and Kozlov [6], Safran [7]):

$$F(s, s_0, c_1, c_2) = s_0 \left[\frac{1}{2}k_s\frac{(s-s_0)^2}{(s_0)^2} + \frac{1}{2}k_c(c_1+c_2)^2 - k_c c_0(c_1+c_2) + \overline{k}_c c_1 c_2 + k_s l\frac{s-s_0}{s_0}(c_1+c_2)\right]. \quad (4)$$

In addition, we define the tension $\sigma(s, s_0, c_1, c_2)$ of the curved piece of the membrane *via* the relation:

$$\sigma(s, s_0, c_1, c_2) = \left(\frac{\partial F(s, s_0, c_1, c_2)}{\partial s}\right)_{s_0, c_1, c_2} = k_s\frac{s - s_0[1 - l(c_1+c_2)]}{s_0}. \quad (5)$$

It can be shown, that l is the distance between the surface, presenting the membrane, and the neutral surface of the membrane.

Let an elastic surface Σ be given and in each of its points the main curvatures be defined. Let us consider two "physically infinitely small" pieces of Σ, with areas s^a and s^b, areas in their tension free states s_0^a and s_0^b, and main curvatures c_1^a, c_2^a and c_1^b, c_2^b. The upper indices a and b stand for the first and second piece respectively. Let, in addition, the relation $s_0^a + s_0^b = s_0^{ab}$ be fulfilled. One necessary condition for the surface Σ to be in equilibrium is:

$$\left\{\frac{\partial}{s_0^a}\left[F(s^a, s_0^a, c_1^a, c_2^a) + F(s^b, s_0^{ab} - s_0^a, c_1^b, c_2^b]\right\}_{s^a, s^b, s_0^{ab}, c_1^a, c_2^a, c_1^b, c_2^b} = 0. \quad (6)$$

Equation (6) provides that there will be no lateral redistribution of material (molecules) between the two parts of the membrane. It is a direct consequence of the requirement for equality of the chemical potentials of the molecules comprising the bilayer (see Boruvka and Neumann [8]), or the requirement for mechanical equilibrium of each part of the membrane (see Evans and Skalak [9]). From equations (6, 4), keeping only the terms contributing to the energy $F(s, s_0, c_1, c_2)$, defined *via* equation (4), and neglecting all terms higher than second order with respect to D/R and $(s - s_0)/s_0$, we obtain:

$$\sigma(s, s_0, c_1, c_2) + k_c c_0(c_1 + c_2) = \sigma_0, \quad (7)$$

where σ_0 is the tension of a flat membrane in equilibrium with each part of the surface Σ. The condition for lateral equilibrium for the case of a cylindrical deformation of a symmetric membrane was treated by Kozlov and Markin [10].

From equations (7, 5), disregarding again the higher order terms, for a small enough piece of the membrane we obtain:

$$s_0 = s\left[1 + \left(l + \frac{k_c c_0}{k_s}\right)(c_1 + c_2) - \frac{\sigma_0}{k_s}\right]. \quad (8)$$

This equation is an important result, permitting to obtain the shape equation of the membrane. Let Σ be a closed surface. The integration of equation (8) on it gives:

$$S_0 = S\left(1 - \frac{\sigma_0}{k_s}\right) + \left(l + \frac{k_c c_0}{k_s}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s, \quad (9)$$

where S is the area of Σ and S_0 – its area in the flat tension free state. In the frames of the same approximation, equation (9) can be rewritten in the form:

$$\sigma_0 = k_s\frac{S - S_0}{S_0} + \frac{k_s}{S_0}\left(l + \frac{k_c c_0}{k_s}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s. \quad (10)$$

We define the density of the elastic energy $f(\sigma_0, c_1, c_2)$ of the deformed membrane as follows:

$$f(\sigma_0, c_1, c_2) = \frac{F(s, s_0, c_1, c_2)}{s}. \quad (11)$$

The expression for $f(\sigma_0, c_1, c_2)$ is:

$$f(\sigma_0, c_1, c_2) = \frac{1}{2}\frac{(\sigma_0)^2}{k_s} - k_c c_0(c_1 + c_2) + \frac{1}{2}\left[k_c - l^2 k_s - 2k_c c_0 l - \frac{(k_c c_0)^2}{k_s}\right](c_1 + c_2)^2 + \overline{k}_c c_1 c_2. \quad (12)$$

The modified bending elasticity K_c and the modified spontaneous curvature C_0, participating in equation (12), are given by the relations:

$$K_c = \left[k_c - k_s\left(l + \frac{k_c c_0}{k_s}\right)^2\right], \qquad C_0 = \frac{k_c}{K_c}c_0. \quad (13)$$

The first of these dependencies was deduced by Helfrich and Kozlov [6] for the neutral surface for the case of cylindrical deformation.

We denote with Δp the difference of the pressures outside (p^{out}) and inside (p^{in}) the closed membrane, $\Delta p = p^{out} - p^{in}$.

The total shape energy $G[\sigma_0(\Sigma, S_0), \Sigma, \Delta p]$ is a functional, which depends on the shape of the surface Σ and on the functional $\sigma_0(\Sigma, S_0)$ (see Eq. (10)). The functional $G[\sigma_0(\Sigma, S_0), \Sigma, \Delta p]$ is a sum of the integral of $f(\sigma_0, c_1, c_2)$ on Σ and the term $\Delta p\, V$ where V is the volume, enclosed by Σ. The final expression for G, in the frames of the

considered approximation, is:

$$G[\sigma_0(\Sigma,S_0),\Sigma,\Delta p]=\frac{1}{2}\frac{(\sigma_0)^2}{k_s}S_0 + \oint_\Sigma\left[\frac{1}{2}K_c(c_1+c_2)^2-K_cC_0(c_1+c_2)\right]\mathrm{d}s + \Delta p\int \mathrm{d}V+4\pi\overline{k}_c. \quad (14)$$

In equation (14) it is assumed that Σ is topologically equivalent to a sphere.

One necessary condition for the stability of the surface Σ is the first variation of G to be zero. In the following section, the variations of G will be determined.

3 Variations of the shape energy. Shape equation

To take the variation of the surface Σ we will follow the procedure used by Ou-Yang Zhong-can and Helfrich [5]. Let Σ be parametrized by two generalized coordinates (u,v) and let $\boldsymbol{n}(u,v)$ be the normal unit vector to Σ in a point with coordinates (u,v). Let $\boldsymbol{Y}(u,v)$ be the radius-vector of the point with coordinates (u,v) on Σ. The slightly varied surface Σ' is defined by the ensemble of radius-vectors $\boldsymbol{Y}'(u,v)$:

$$\boldsymbol{Y}'(u,v)=\boldsymbol{Y}(u,v)+\Psi(u,v)\boldsymbol{n}, \quad (15)$$

where $\Psi(u,v)$ is a sufficiently small and smooth function. The quantity σ_0 is given *via* equation (10) and its first variation $\delta\sigma_0$ is:

$$\delta\sigma_0=\oint_\Sigma\left[\frac{k_s}{S_0}(c_1+c_2)+\frac{2k_s}{S_0}\left(l+\frac{k_cc_0}{k_s}\right)c_1c_2\right]\Psi\mathrm{d}s. \quad (16)$$

The first variation δG of the functional $G[\sigma_0(\Sigma,S_0),\Sigma,\Delta p]$ can be calculated using equations (14, 16, 10):

$$\delta G[\sigma_0(\Sigma,S_0),\Sigma,\Delta p]= \sigma_0\oint_\Sigma\left[(c_1+c_2)+2\left(l+\frac{K_cC_0}{k_s}\right)c_1c_2\right]\Psi\mathrm{d}s + \oint_\Sigma\left\{\Delta p+K_c\left[(c_1+c_2)\left(2c_1c_2-\frac{1}{2}(c_1+c_2)^2\right)-2C_0c_1c_2-\Delta_s(c_1+c_2)\right]\right\}\Psi\mathrm{d}s\cdot \quad (17)$$

From equation (17) we obtain the generalization of the shape equation derived by Ou-Yang Zhong-can and Helfrich [5], namely:

$$\Delta p+\sigma_0(c_1+c_2)+K_c\left\{(c_1+c_2)\left[2c_1c_2-\frac{1}{2}(c_1+c_2)^2\right]-2\left[C_0-\frac{\sigma_0}{K_c}\left(l+\frac{K_cC_0}{k_s}\right)\right]c_1c_2-\Delta_s(c_1+c_2)\right\}=0, \quad (18)$$

where K_c and C_0 are given by equations (13) and σ_0 – by equation (10). Equation (18) will have the same form as the one derived by Ou-Yang Zhong-can and Helfrich [5], if an effective bending elasticity modulus k_c^{eff} and a spontaneous curvature c_0^{eff}, different from the initial ones, are introduced:

$$k_c^{eff}=K_c \qquad c_0^{eff}=C_0-\frac{\sigma_0}{K_c}\left(l+\frac{K_cC_0}{k_s}\right). \quad (19)$$

In the shape equation (18) derived by us, σ_0 is not a Lagrange multiplier as λ in equation (3), but a functional of the surface Σ (see Eq. (10)). As a consequence, the shape equation is not differential, but integro-differential.

Equation (18) is a shape equation in the case when the pressure difference Δp (but not the volume V) and the area S_0 of the flat tension free membrane are fixed. When the second variation δ^2G of the functional $G[\sigma_0(\Sigma,S_0),\Sigma,\Delta p]$ is calculated, the variation of σ_0 must also be considered, because it is not constant. For the varied surface there is no constraint for conservation of the volume enclosed by it. The calculation of the second variation gives:

$$\delta^2G[\sigma_0(\Sigma,S_0),\Sigma,\Delta p]=\delta^2_{(1)}G+\delta^2_{(2)}G. \quad (20)$$

The variation $\delta^2_{(1)}G$ corresponds to the one obtained by Ou-Yang Zhong-can and Helfrich [5], with effective values of the bending elasticity k_c^{eff} and the spontaneous curvature c_0^{eff} given by equation (19).

The variation $\delta^2_{(2)}G$ is due to the fact that σ_0 is not constant and its variation is different from zero. The result for $\delta^2_{(2)}G$ is:

$$\delta^2_{(2)}G=\frac{k_s}{S_0}\left\{\oint_\Sigma\left[(c_1+c_2)+2\left(l+\frac{K_cC_0}{k_s}\right)c_1c_2\right]\Psi\mathrm{d}s\right\}^2. \quad (21)$$

The calculated by us second variation of the elastic energy differs from the one, calculated by Ou-Yang Zhong-can and Helfrich [5] in the term $\delta^2_{(2)}G$. It is present even in the case when $l=0$ and $C_0=0$.

4 Lipid bilayer

In this section, the results obtained for monolayers will be used to deduce the shape equation of a bilayer.

We will consider a lipid bilayer, consisting of two microscopically identical monolayers, with elastic constants k_c^m, $\overline{k}_c^m$, k_s^m. We choose the surface, representing the bilayer, to coincide with the dividing surface between the two monolayers. In such case, if for the outer monolayer the spontaneous curvature is c_0^m and the coefficient, accounting for the coupling between the stretching and the bending is l^m, then for the inner monolayer they will be $(-c_0^m)$ and $(-l^m)$, respectively. Let the areas of the outer and inner monolayer in their flat tension free states be

S_0^{out} and S_0^{in} and let an area S_0^b be introduced with the property $S_0^{out} + S_0^{in} = 2S_0^b$. We assume that the elastic energy of the bilayer is equal to the sum of the elastic energies of the two monolayers. Evidently the area S is the same for the two monolayers. The quantities σ_0^{out} and σ_0^{in} (see Eq. 10) for the outer and inner monolayer can be defined as follows:

$$\sigma_0^{out} = k_s^m \frac{S - S_0^{out}}{S_0^b} + \frac{k_s^m}{S_0^b}\left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s \tag{22}$$

and

$$\sigma_0^{in} = k_s^m \frac{S - S_0^{in}}{S_0^b} - \frac{k_s^m}{S_0^b}\left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s. \tag{23}$$

When writing equations (22, 23), the quantity $(S_0^{out} - S_0^{in})/S_0^b$ is assumed to be of the order of $(S - S_0^{out})/S_0^{out}$ and $(S - S_0^{in})/S_0^{in}$ and all the approximations, following from this assumption, have been made (see the discussion in the beginning of Sect. 2). Under these assumptions the elastic energy $E^b(\Sigma, S_0^{out}, S_0^{in})$ of a lipid bilayer represented by an arbitrary surface Σ is:

$$E^b(\Sigma, S_0^{out}, S_0^{in}) = \frac{S_0^b}{2k_s^m}[(\sigma_0^{out})^2 + (\sigma_0^{in})^2] + \oint_\Sigma \left[\frac{1}{2}K_c^b(c_1 + c_2)^2\right]\mathrm{d}s, \tag{24}$$

where

$$K_c^b = 2\left[k_c^m - k_s^m\left(l^m + \frac{(k_c^m c_0^m)^2}{k_s^m}\right)^2\right]. \tag{25}$$

In the case of free flip-flop (the exchange of molecules between the monolayers, comprising the bilayer is permitted) $\sigma_0^{out} = \sigma_0^{in} = \sigma_0^{b,fr}$, where the upper index "b, fr" refers to a bilayer with free flip-flop. In this case, the results for $\sigma_0^{b,fr}(\Sigma)$, $S_0^{out}(\Sigma)$ and $S_0^{in}(\Sigma)$ for an arbitrary surface Σ are:

$$\begin{aligned}
\sigma_0^{b,fr}(\Sigma) &= k_s^m \frac{S(\Sigma) - S_0^b}{S_0^b} \\
S_0^{out,fr}(\Sigma) &= S_0^b + \left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s \\
S_0^{in,fr}(\Sigma) &= S_0^b - \left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s,
\end{aligned} \tag{26}$$

and the elastic energy $E^{b,fr}[\Sigma, S_0^{out,fr}(\Sigma), S_0^{in,fr}(\Sigma)]$ is:

$$E^{b,fr}\left[\Sigma, S_0^{out,fr}(\Sigma), S_0^{in,fr}(\Sigma)\right] = \frac{S_0^b}{k_s^m}\left[\sigma_0^{b,fr}(\Sigma)\right]^2 + \oint_\Sigma \frac{1}{2}K_c^b(c_1 + c_2)^2\mathrm{d}s. \tag{27}$$

The shape equation in the case of free flip-flop, obtained through a variation of the total shape energy $G^{b,fr}(\Sigma) = E^{b,fr}[\Sigma, S_0^{out,fr}(\Sigma), S_0^{in,fr}(\Sigma)] + \Delta pV$ of the bilayer is:

$$\Delta p + 2\sigma_0^{b,fr}(c_1 + c_2) + K_c^b\{(c_1 + c_2)\left[2c_1c_2 - \frac{1}{2}(c_1 + c_2)^2\right] - \Delta_s(c_1 + c_2)\} = 0. \tag{28}$$

Consequently, a lipid bilayer at free flip-flop behaves as a symmetrical membrane with bending and stretching elasticities twice the ones of its constituent monolayers, and an area in the flat tension free state equal to the half sum of the respective areas of the monolayers. This is also true for the second variation of the total shape energy of a surface, satisfying the shape equation (28).

When the flip-flop between the monolayers is forbidden, the quantities S_0^{out} and S_0^{in} are fixed. Let, for a given surface Σ, $C_0^{b,bl}(\Sigma)$ and $\sigma_0^{b,bl}(\Sigma)$, be defined as follows:

$$\begin{aligned}
C_0^{b,bl}(\Sigma) &= \frac{2k_s^m}{K_c^b S_0^b}\left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right) \\
&\quad \times \left[\frac{S_0^{out} - S_0^{in}}{2} - \left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s\right] \\
\sigma_0^{b,bl}(\Sigma) &= k_s^m \frac{S(\Sigma) - S_0^b}{S_0^b},
\end{aligned} \tag{29}$$

where the upper index "b, bl" refers to a bilayer with blocked flip-flop. The estimation of $C_0^{b,bl}$ gives $C_0^{b,bl} \sim (S_0^b)^{-\frac{1}{2}}$. At blocked flip-flop, the elastic energy $E^{b,bl}(\Sigma, S_0^{out}, S_0^{in})$ is:

$$\begin{aligned}
E^{b,bl}(\Sigma, S_0^{out}, S_0^{in}) &= \frac{S_0^b}{k_s^m}[\sigma_0^{b,bl}(\Sigma)]^2 + \oint_\Sigma \frac{1}{2}K_c^b(c_1 + c_2)^2\mathrm{d}s \\
&+ \frac{k_s^m}{S_0^b}\left[\frac{S_0^{out} - S_0^{in}}{2} - \left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\oint_\Sigma (c_1 + c_2)\mathrm{d}s\right]^2.
\end{aligned} \tag{30}$$

After variation of the appropriate total shape energy $G^{b,bl}(\Sigma, S_0^{out}, S_0^{in}) = E^{b,bl}(\Sigma, S_0^{out}, S_0^{in}) + \Delta pV$, the shape equation of a lipid bilayer with blocked flip-flop can be written:

$$\Delta p + 2\sigma_0^{b,bl}(c_1 + c_2) + K_c^b\left\{(c_1 + c_2)\left[2c_1c_2 - \frac{1}{2}(c_1 + c_2)^2\right] - 2C_0^{b,bl}c_1c_2 - \Delta_s(c_1 + c_2)\right\} = 0. \tag{31}$$

The comparison of equations (26, 28, 29, 31) shows that, concerning the shape equation, the difference between the free and blocked flip-flop is only in the appearance of an effective spontaneous curvature $C_0^{b,bl}$, given by equation (29). From equation (31) it follows that a membrane exists with modified bending elasticity modulus K_c, σ_0, and spontaneous curvature c_0^{eff} æææ (see Eq. (19)), equal to K_c^b, $\sigma_0^{b,bl}$, and $C_0^{b,bl}$ of a lipid bilayer. This membrane

has to satisfy the shape equation for the same shape, as the lipid bilayer. For a membrane with $c_0 \sim (S_0)^{-\frac{1}{2}}$ the opposite is also true, *i.e.* a lipid bilayer can be chosen in such a way that both to satisfy the shape equation for the same shape. As for the second variation of the total shape energy of the bilayer, $\delta^2 G^b$, it can be written as $\delta^2 G^b = \delta^2_{(1)} G^b + \delta^2_{(2)} G^b$, where $\delta^2_{(1)} G^b$ corresponds to the one obtained by Ou-Yang Zhong-can and Helfrich [5], with effective values of the bending elasticity K_c^b and the spontaneous curvature $C_0^{b,bl}$ given by equation (29), and $\delta_{(2)} G^b$ is:

$$\delta^2 G^b = 2\frac{k_s^m}{S_0^b}\left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)^2 \left[\oint_\Sigma 2c_1 c_2 \Psi \mathrm{d}s\right]^2 \geq 0. \quad (32)$$

Consequently, the ensemble of the solutions of the shape equation (31) of bilayers with blocked flip-flop coincides with the catalog of the not trivial solutions of the shape equation (18) of membranes with given parameters (elasticity moduli and spontaneous curvature). In both cases the problem of the stability of the found shapes needs additional examination.

5 Thermal form fluctuations of a quasi-spherical lipid vesicle

The problem of the thermal form fluctuations of a quasi-spherical vesicle, whose membrane has given elastic moduli, spontaneous curvature and mean area S was solved theoretically by Milner and Safran [11]. We will study the case when the membrane is a lipid bilayer. The experiments in which these fluctuations are measured usually take about of 10 minutes [12], while the typical times for the flip-flop of the bilayer are of the order of many hours. This is the reason why the most plausible assumption is that S_0^{out} and S_0^{in} remain constant. Another assumption, which we will make, is the conservation of the volume V of the vesicle. It was used by Milner and Safran [11] as well.

Let R_0 be the radius of a sphere with the same volume as the vesicle $V = 4\pi(R_0)^2/3)$. Let XYZ be a laboratory reference frame, the origin O being inside the vesicle. Observing the fluctuating vesicle with a 3-dimensional technique, we define $R(\theta, \varphi, t)$ to be the modulus of the radius-vector of a point on the surface of the vesicle in the direction (θ, φ) (spherical coordinates) at a moment t:

$$R(\theta, \varphi, t) = R_0[1 + u(\theta, \varphi, t)]. \quad (33)$$

Let $Y_n^m(\theta)$, $n \geq |m| \geq 0$, be the orthonormal spherical harmonics (for details see Bivas *et al.* [13]). The amplitudes $u(\theta, \varphi, t)$ can be expanded in a series of Y_n^m:

$$u(\theta, \varphi, t) = \sum_{n=0}^{n_{max}} \sum_{|m| \leq n} u_n^m(t) \cdot Y_n^m(\theta, \varphi). \quad (34)$$

Using the assumptions, mentioned above the elastic energy $E^b(\Sigma(t), S_0^{out}, S_0^{in})$ can be expressed by the ensemble of amplitudes $u_n^m(t)$:

$$E^b(\Sigma(t), S_0^{out}, S_0^{in}) = \frac{k_s^m}{S_0^b}\left[(S - S_0^b)^2 - \frac{2S}{R_0}\left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)(S_0^{out} - S_0^{in})\right] + \frac{1}{2}K_c^b \sum_{n=2}^{n_{max}} \sum_{m=-n}^{n} (n-1)n(n+1)(n+2)|u_n^m(t)|^2. \quad (35)$$

We denote:

$$\sigma(S) = 2k_s^m \frac{(S - S_0^b)}{S_0^b}$$
$$c_0^{fluct} = \frac{k_s^m}{K_c^b}\left(l^m + \frac{k_c^m c_0^m}{k_s^m}\right)\frac{S_0^{out} - S_0^{in}}{S_0^b}. \quad (36)$$

Milner and Safran [11] assumed that the mean square fluctuations $\overline{|u_n^m(t)|^2}$ at fixed area are the same as those at fixed tension σ. This assumption is valid when the normalized tension $\overline{\sigma} = \sigma(R_0)^2/K_c$ is sufficiently higher than -6 [14,15]. In the frames of this assumption we obtain the well-known expression for the mean square amplitudes $\overline{|u_n^m(t)|^2}$ [11]:

$$\overline{|u_n^m(t)|^2} = \frac{kT}{K_c^b}\frac{1}{(n-1)(n+2)[n(n+1) + \overline{\sigma} - 2c_0^{fluct}R_0]}. \quad (37)$$

The effective value of σ, participating in this equation, is:

$$\sigma = 2k_s^m \frac{\overline{S} - S_0^b}{S_0^b}, \quad (38)$$

where $\overline{S}$ is expressed by the mean square amplitudes $\overline{[u_n^m(t)]^2}$ as [11]:

$$\overline{S} = 4\pi(R_0)^2 + \frac{(R_0)^2}{2}\sum_{n=-2}^{n_{max}} \sum_{m=-n}^{n} \overline{[u_n^m(t)]^2}. \quad (39)$$

Seifert [16], making a critical analysis of the theory of Milner and Safran, has shown that if the orders higher than the second one in the development of the energy with respect to the amplitudes u_n^m are neglected and the excess area of the vesicle is much less than the area of the vesicle the results of Milner and Safran are true (excess area is the difference between the area of the vesicle and the area of the sphere with the vesicle's volume). Equation (37) shows, that when the results of the theory of Milner and Safran are applicable the relevant bending elasticity K_c^b for lipid bilayers is the same in both the shape equation, and the equation for the mean square amplitudes of the fluctuating modes. It is equal to the doubled value of the modified, due to the asymmetry, bending elasticity of the monolayer. This is exactly the bending elasticity at free flip-flop introduced by Helfrich [2]. The value of the spontaneous curvature c_0^{eff} in the shape equation does not coincide with the value c_0^{fluct}.

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