Membrane Stretching Elasticity of Lipid Vesicle and Thermal Shape Fluctuations

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One of the most widely used methods for determination of the bending elasticity modulus of model lipid membranes is the analysis of the shape fluctuations of nearly spherical lipid vesicles. The theoretical basis of this analysis is given by Milner and Safran. In their theory the stretching effects are not considered. In the present study we generalized their approach including the stretching effects deduced after an application of statistical mechanics of vesicles.

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INTRODUCTION

The mechanical properties of biomembranes determine to a great extent their structure and functioning. According to the model of Singer and Nicolson [1], the biomembrane consists of a lipid bilayer, in which integral proteins float. In the frames of this model, the mechanical properties of the biomembrane are tightly connected with those of the lipid bilayer. Because of its very small thickness compared to the square root of its area, the lipid bilayer may be appropriately modeled as two-dimensional flexible sheet and effectively studied theoretically, experimentally, and by computer simulations.

The notion of membrane tension defined plays a key role in the consideration of the elastic deformation of the lipid bilayers, bending and stretching. The importance of curvature energy to the elasticity of membrane has been emphasized by Helfrich in his pioneering paper from 1973 [2], while the stretching effects were mentioned only in passing. A few years later Brochard, De Gennes and Pfeuty [3] considered the vesicle membrane as compressible and revealed the role of the changes of the local density on the elastic energy. Due to some ambiguity around the definition of the surface tension, the study of this issue is continuously growing [4–10]. In these papers, a number of questions were raised. They concern: the physical meaning of the various definitions of tensions; the consistency between reasonable approximations and theoretical predictions; equivalence of ensembles, which requires implicitly the existence of thermodynamic limits, regimes where equilibrium thermodynamics is far from being justified, etc. These phenomena are still a source of both theoretical and experimental challenges, see [14–17] and refs. therein.

The state of a closed bilayer (e.g. vesicle), that does not contain membrane reservoirs, and of an incompressible membrane conformation is determined by the bending elasticity and constraints on the total surface area and on the enclosed volume. The vesicle has constrained area and constrained volume, since the number of the lipid molecule in the membrane is fixed. While it is technically easy to implement the second constraint, it is difficult to handle the first one. Instead of working with fixed area, an effective tension as Lagrange multiplier conjugated to the fixed membrane area has been used [4, 6], expecting that the two ensembles are equivalent.

The aim of our study is to elucidate the theoretical basis of the analysis, initiated by Milner and Safran [4] in order to take into consideration the role of the membrane compression and tension in terms of stretching elasticity, which are introduced into the theory by nonlocal (anharmonic) terms.

We restrict our considerations to mechanical systems and present some general characteristics of the mean-field approximation in the context of the statistical mechanics of vesicles.

THE TOTAL DEFORMATION ENERGY OF THE VESICLE MEMBRANE

We considered lipid bilayers in liquid crystalline state, in which they can be modeled by two-dimensional liquids. Following Helfrich [2], we consider a small patch of the lipid bilayer with area $\Delta s$, tension $\sigma$, and area in its flat tension-free state $s_0$. Let $c_1$ and $c_2$ be the main curvatures of the patch under consideration. If the patch is tension-free, then the bending energy density $g_c(c_1, c_2)$ can be written in the following form:

$$g_c(c_1, c_2) = \frac{1}{2} K_c (c_1 + c_2 - c_0)^2 + K_c c_1 c_2,$$

(1)

where $K_c$ is the bending elasticity of the bilayer, $c_0$ is the spontaneous curvature of the membrane, and $K_c$ is the saddle splay bending elasticity. Only symmetrical membranes (with $c_0 = 0$) will be considered later on. The bending energy $\Delta G_c$ of the patch is:

$$\Delta G_c = g_c(c_1, c_2) s_0.$$

(2)

If the patch is not tension-free, then its stretching energy density $g_s$ is expressed via its tension $\sigma$ as:

$$g_s(\sigma) = \frac{1}{2} \frac{\sigma^2}{K_s}.$$

(3)
We assume that $\sigma$ is a constant all over the membrane (see [18]). Then $\sigma$ can be expressed as:

$$\sigma = K_s \frac{\Delta s - s_0}{s_0},$$  \hspace{1cm} (4)$$

where $K_s$ is the stretching elasticity of the bilayer. The stretching energy $\Delta G_s$ and the total mechanical energy $\Delta G$ of the patch are:

$$\Delta G_s = g_s(\sigma)s_0,$$  \hspace{1cm} (5)$$

and

$$\Delta G = \Delta G_c + \Delta G_s,$$  \hspace{1cm} (6)$$

respectively.

Let us consider a vesicle whose membrane is a lipid bilayer. The shape fluctuations of the vesicle deform this bilayer and alter its mechanical energy. The total deformation energy $G(t)$ of the vesicle membrane is obtained by integration of $\Delta G(t)$ on the vesicle surface $S(t)$:

$$G(t) = \int_{S(t)} \Delta G(t).$$  \hspace{1cm} (7)$$

**QUASI-SPHERICAL VESICLES: THE MILNER AND SAFRAN APPROACH**

We consider a nearly spherical lipid vesicle with fixed (i.e. not fluctuating) volume $V$. Let $R = (\frac{4V}{4\pi})^{1/3}$ be the radius of a sphere with the same volume $V$. Let $R(\theta, \varphi)$ is the modulus of the radius-vector of a point on the surface of the vesicle with polar coordinates $(\theta, \varphi)$ in laboratory reference frame with origin 0 placed inside the vesicle. The dimensionless quantity $u(\theta, \varphi, t)$ is defined by the equation:

$$u(\theta, \varphi, t) = \frac{R(\theta, \varphi, t) - R}{R},$$  \hspace{1cm} (8)$$

where $t$ is the time variable. The function $u(\theta, \varphi, t)$ is decomposed in a series of spherical harmonics as follows:

$$u(\theta, \varphi, t) = \sum_{n=0}^{n_{max}} \sum_{m=-n}^{n} u_n^m(t)Y_n^m(\theta, \varphi),$$  \hspace{1cm} (9)$$

where $Y_n^m(\theta, \varphi)$ is the orthonormal basis (for simplicity chosen real) of the spherical harmonics functions. A cutoff $n_{max} \sim R/\lambda$ is introduced in the sum, where $\lambda$ is of the order of the intermolecular distance. As the harmonics with indexes $n = 1$ and $m = -1, 0, 1$ correspond to pure translation of the vesicle, the origin $O$ can be chosen in a way that $u_0^0 = 0$.

As it has been shown by Milner and Safran [4], because of the requirement for volume conservation the amplitude $u_0^0(t)$ can be expressed as:

$$u_0^0(t) = \frac{1}{2V} \sum_{n=2}^{n_{max}} \sum_{m=-n}^{n} [u_n^m(t)]^2.$$

Then the mechanical energy $G(t)$ of the vesicle, obtained after the integration in the rhs of Eq. (7), is:

$$G(t) = \frac{1}{2} K_c \sum_{n=2}^{n_{max}} \sum_{m=-n}^{n} \{(n-1)(n+2)$$

$$\times [n(n+1) + \sigma][u_n^m(t)]^2\},$$  \hspace{1cm} (11)$$

where the quantity $\sigma$ is considered as a Lagrange multiplier, not fluctuating with time, which ensures the mean area of the vesicle membrane to be equal to some prescribed value. In Eq. (11) the following dimensionless expression for $\sigma$:

$$\sigma = \frac{R^2}{K_c}\sigma,$$  \hspace{1cm} (12)$$

where $\sigma$ is the tension of the membrane, has been introduced. The contribution of the saddle-splay elasticity has been disregarded due to the Gauss-Bonnet theorem which assures that if the topology of the vesicle does not change, then the contribution of this elasticity does not depend on the shape fluctuations of the vesicle. The energy $G(t)$ from Eq. (11) can be considered as sum of the energies of not interacting "oscillators". It is worth noting that this energy does not depend on the stretching elasticity, having no stretching elasticity and possessing different from zero tension, which does not depend on the shape fluctuations of the vesicle.

**MODEL-DEPENDENT Derivations**

Later on we consider a model Hamiltonian of the fluctuating vesicle, whose membrane has stretching and bending elasticity as well. Manipulating this Hamiltonian in an appropriate way, i.e. making a mean field approximation (see below), we shall effectively approximate the fluctuating vesicle by a system in form of not interacting harmonic oscillators.
The surface tension

Let us denote by $H(U) = H(u_2^{-2}, u_2^{-1}, \ldots, u_{n_{\text{max}}}^{-1})$ the model Hamiltonian describing a fluctuating vesicle. The symbol $U$ is used as shorthand for the real value functions $(u_2^{-2}, u_2^{-1}, \ldots, u_{n_{\text{max}}}^{-1})$ which are the spherical harmonics amplitudes, appearing in the the expansion of the vesicle shape fluctuations from the equivalent volume sphere with radius $R$ (see Eq. [3]). Further on, the notation $u_n^m$ is used as an abbreviation of $u_n^m(t)$.

One can present the area functional of the membrane $S(U)$ in the form [4, 5, 9]:

$$S(U) = 4\pi R^2 + \Delta S(U),$$

where the quantity $\Delta S(U)$ is the excess area of the vesicle (the difference between the area of the vesicle’s membrane and the area $4\pi R^2$ of a sphere with a volume equal to that of the vesicle).

In terms of $u_n^m$ the excess area of the vesicle $\Delta S(U)$ is presented in the form [4, 5]:

$$\Delta S(U) = \frac{R^2}{2} \left[ \sum_{n=2}^{n_{\text{max}}} \sum_{m=-n}^{n} (n-1)(n+2)(u_n^m)^2 \right].$$

Note that in Eq. [15] the requirement for volume conservation has been used in order to exclude the amplitude $u_0^0$. Evidently, $\Delta S(U) \geq 0$. This property of $\Delta S(U)$ does not depend on the form of the Hamiltonian.

By definition the vesicle tension $\sigma(U)$ is given by the expression:

$$\sigma(U) = K_s \frac{S(U) - S_0}{S_0},$$

where $S_0$ is the mean area of the vesicle membrane in its flat tension free state.

It will be shown that the experimentally measurable quantity is proportional to:

$$\langle \sigma(U) \rangle_{H(U)} = K_s \frac{\langle S(U) \rangle_{H(U)} - S_0}{S_0}.$$  \hspace{1cm} (17)

With the symbol $\langle A \rangle_{H(U)}$ we denote the thermodynamic average of some quantity $A$ calculated with the Hamiltonian $H(U)$:

$$\langle A \rangle_{H(U)} = \langle Z[H(U)] \rangle^{-1} \int dU A \exp \left[ -\frac{H(U)}{kT} \right],$$ \hspace{1cm} (18)

where

$$Z[H(U)] = \int dU \exp \left[ -\frac{H(U)}{kT} \right]$$ \hspace{1cm} (19)

is the statistical sum of the model. Indeed, one can consider $\langle \sigma(U) \rangle_{H(U)}$ as the mechanical tension and Eq. [17] as the definition of the area compressibility modulus $K_s$, when the mean area of the vesicle membrane $\langle S(U) \rangle_{H(U)}$ deviates from the mean area of its flat tension-free state $S_0$.

Model Hamiltonian and Bogoliubov variational inequalities

Let us consider a model system, having model Hamiltonian, which in the general case is complicated enough and does not allow to find analytical expressions for the investigated thermodynamic quantities. We will replace the model system with a trial one (named approximating), consisting of independent (not interacting) oscillators. We call mean-field approximation the replacement of the model system with the approximating one. Clearly, such a definition does not determine the approximating Hamiltonian in an unequivocal way. It can be expected that the mean-field approximation will be a good approximation when the approximating Hamiltonian is close in some sense to the model one. A natural question arises how to ensure the closeness between these Hamiltonians.

A common and well-known approach in statistical mechanics is to use the Bogoliubov variational inequalities and the approximating Hamiltonian method (presented below) for the construction of an approximating system, whose free energy is close to the free energy of the model system. Namely such an approach will be used for the implementation of the approximating system.

The Hamiltonian under consideration is presented in the following form:

$$H(U) = H_c(U) + H_s(U),$$ \hspace{1cm} (20)

where

$$H_c(U) = \frac{1}{2} K_s \sum_{n=2}^{n_{\text{max}}} \sum_{m=-n}^{n} (n-1)(n+1)(u_n^m)^2$$ \hspace{1cm} (21)

is the bending energy of the vesicle with quasi-spherical geometry, and

$$H_s(U) = \frac{1}{2} \frac{\langle \sigma(U) \rangle^2}{K_s S_0}$$ \hspace{1cm} (22)

is its corresponding stretching energy, expressed via the membrane vesicle tension $\sigma(U)$. The membrane tension $\sigma(U)$ is again assumed to be the same all over the membrane.

The term $H_s(U)$ is nonlinear with respect to the squares of the amplitudes $u_n^m$. To overcome this obstacle we linearize the Hamiltonian $H(U)$ using the Bogoliubov variational inequalities (for historical remarks and a list of different applications see, e.g., [19]). The Bogoliubov variational inequalities are rigorous relations between the free energy $f[H]$ of a valid Hamiltonian $H$ and free energy density $f[H_{\text{app}}(X)]$ of a presumably more simple Hamiltonian $H_{\text{app}}(X)$, depending on a variational parameter $X$. In their most convenient form the inequalities are given by

$$\langle H - H_{\text{app}}(X) \rangle_H \leq f[H] - f[H_{\text{app}}(X)] \leq \langle H - H_{\text{app}}(X) \rangle_{H_{\text{app}}(X)}.$$ \hspace{1cm} (23)
where \( \langle H - H_{\text{app}}(X) \rangle_H \) is the thermodynamic average of the quantity \( H - H_{\text{app}}(X) \), calculated with the Hamiltonian \( H \), \( \langle H - H_{\text{app}}(X) \rangle_{H_{\text{app}}(X)} \) is the thermodynamic average of the same quantity calculated with the Hamiltonian \( H_{\text{app}}(X) \), \( Z[H] \) and \( f[H] = -kT \ln\{Z[H]\} \) are the partition function and the free energy of a system with Hamiltonian \( H \), \( Z[H_{\text{app}}(X)] \) and \( f[H_{\text{app}}(X)] = -kT \ln\{Z[H_{\text{app}}(X)]\} \) are the partition function and the free energy of a system with Hamiltonian \( H_{\text{app}}(X) \). The variational parameter \( X \) must be determined from the condition of the best approximation.

The use of these inequalities in the statistical mechanics of a lipid vesicle has been announced in \([20]\). 

### The approximating Hamiltonian

The model Hamiltonian \( H(U) \) has been rewritten in an alternative form that makes the application of the Bogoliubov variational inequalities (see \([20]\)) more convenient:

\[
H(U) = T(U) + \langle A(U) \rangle^2,
\]

where

\[
T(U) = \frac{1}{2} K_c \sum_{n=2}^{n_{\text{max}}} \sum_{m=-n}^{n} (n-1)(n+2)[n(n+1)+\sigma_0](u_n^m)^2.
\]

Here and further on the bare over the quantity means dimensionless due to the multiplier \( R^2/K_c \):

\[
\sigma_0 = \frac{R^2}{K_c} \sigma_0
\]

\[
\sigma_0 = K_s \frac{4\pi R^2 - S_0}{S_0},
\]

and

\[
A(U) = \sqrt{\frac{K_s}{2S_0}} \Delta S(U).
\]

Evidently the following identity holds:

\[
\langle A(U) \rangle^2 = 2X A(U) - X^2 + \langle A(U) - X \rangle^2,
\]

where \( X \) is an arbitrary real number. We define the linearized Hamiltonian \( H_{\text{app}}(U, X) \) as:

\[
H_{\text{app}}(U, X) = T(U) + 2X A(U) - X^2
\]

The last equation is obtained from Eq. \([24]\) by removing the term \( \langle A(U) - X \rangle^2 \) from the right-hand-side. The defined in this way Hamiltonian \( H_{\text{app}}(U, X) \) is linear with respect to \( (u_n^m)^2 \).

From Eqs. \([25]\), \([28]\), and \([29]\) we obtain:

\[
H_{\text{app}}(U, X) = \sum_{n=2}^{n_{\text{max}}} \sum_{m=-n}^{n} a_n(X) (u_n^m)^2 - X^2,
\]

where

\[
a_n(X) = \frac{1}{2} K_c (n-1)(n+2)[n(n+1)+\Sigma_{\text{app}}(X)],
\]

and

\[
\Sigma_{\text{app}}(X) = \sigma_0 + \sigma_1 X
\]

with \( \sigma_0 \) from Eq. \([26]\), and

\[
\sigma_1 = \frac{R^2}{K_c} \sqrt{\frac{2K_s}{S_0}}.
\]

Since the thermal average of a nonnegative quantity is nonnegative it follows that:

\[
\langle H(U) - H_{\text{app}}(U, X) \rangle_H = \langle [A(U) - X]^2 \rangle_H \geq 0.
\]

Then Eqs. \([23]\) and \([35]\) imply that for each \( X \):

\[
0 \leq f[H(U)] - f[H_{\text{app}}(U, X)] \leq \langle [A(U) - X]^2 \rangle_{H_{\text{app}}(U, X)}.
\]

We define \( \tilde{X} \) as the solution of the equation:

\[
\frac{\partial f[H_{\text{app}}(U, X)]}{\partial X} = 0
\]

It can be shown that the equation above has only one solution, namely \( \tilde{X} \) and \( \tilde{X} \) satisfies the condition:

\[
f[H_{\text{app}}(U, \tilde{X})] = \max_X f[H_{\text{app}}(U, X)].
\]

From Eq. \([36]\) it follows that:

\[
f[H_{\text{app}}(U, \tilde{X})] \leq f[H(U)].
\]

Consequentially, the free energy \( f[H_{\text{app}}(U, \tilde{X})] \) of the ensemble of not interacting oscillators is the best approximation from below of the free energy, corresponding to the model Hamiltonian \( H(U) \).

### The self-consistent equation

To present Eq. \([37]\) in explicit form we start from the Helmholtz free energy \( f[H_{\text{app}}(U, X)] \):

\[
f[H_{\text{app}}(U, X)] = -kT \ln\{Z[H_{\text{app}}(U, X)]\},
\]

where \( Z[H_{\text{app}}(U, X)] \) is the partition function of the approximating system:

\[
Z[H_{\text{app}}(U, X)] = \int dU \left\{ \exp \left[ -\frac{H_{\text{app}}(U, X)}{kT} \right] \right\},
\]

As a result we obtain:

\[
f[H_{\text{app}}(U, X)] = -kT \times \ln \left\{ \int dU \exp \left[ -\frac{T(U) + 2X A(U)}{kT} \right] \right\} - X^2,
\]

\[
(\text{42})
\]
and:
\[
\frac{\partial f[H_{\text{app}}(U,X)]}{\partial X} = 2((A(U))_{H_{\text{app}}(U,X)} - X) = 0.
\] (43)
Consequently, Eq. (37) can be written in the following equivalent form:
\[
\langle A(U) \rangle_{H_{\text{app}}(U,X)} - X = 0.
\] (44)
This is a typical self-consistent equation for the parameter \(X\).

After simple calculations, from Eqs. (31) and (41) we obtain for the free energy \(f[H_{\text{app}}(U,X)]\) of the approximating system:
\[
f[H_{\text{app}}(U,X)] = kT \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{2} \ln \left\{ (n-1)(n+2)[n(n+1) + \Sigma_{\text{app}}(X)] \right\}
- \left( \bar{X} \right)^2 + kT \frac{N}{2} \ln \left( \frac{K_c}{2\pi kT} \right).
\] (45)
In the equation above \(N \approx (n_{\text{max}})^2\) is the number of lipid molecules in the vesicle membrane.

Further on, in order to be unambiguous we will use notations linearized and approximating Hamiltonian for \(H_{\text{app}}(U,X)\), respectively.

The mean squares \((\langle u_n^m \rangle^2)_{H_{\text{app}}(U,X)}\), calculated by the linearized Hamiltonian \(H_{\text{app}}(U,X)\), are:
\[
\langle (u_n^m)^2 \rangle_{H_{\text{app}}(U,X)} = \frac{kT}{K_c} \frac{1}{(n-1)(n+2)[n(n+1) + \Sigma_{\text{app}}(X)]}.
\] (46)
Except for difference in the interpretation of the last term in the denominator of (46) the result for \((\langle u_n^m \rangle^2)_{H_{\text{app}}(U,X)}\) has been obtained by many authors [4, 6, 10].

As it can be seen after comparison with Eq. (12), the result for \((\langle u_n^m \rangle^2)_{H_{\text{app}}(U,X)}\) formally reproduces the result of Milner and Safran. The difference between our and their result consists in the interpretation. In their theory \(\Sigma\) is considered as a Lagrange multiplier, while in our theory \(\Sigma_{\text{app}}(X)\) with \(X = \bar{X}\) is obtained by application of the principles of the statistical mechanics to concrete model Hamiltonian \(H(U)\).

Let us for convenience introduce the shorthand
\[
\Sigma_{\text{app}} = \Sigma_{\text{app}}(\bar{X}).
\] (47)
Then \(\Sigma_{\text{app}}\) can be obtained from the self-consistent equation (44) as follows. With the help of Eqs. (28), (31), and (40), Eq. (44) can be rewritten as:
\[
X = \frac{kT \sigma_1}{4} \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{n(n+1) + \Sigma_{\text{app}}(X)}.
\] (48)
By multiplying the two sides of Eq. (48) with \(\sigma_1\) (see Eq. (24)) and adding to them \(\sigma_0\) (see Eq. (20)), we get the following form the self-consistent equation (44), written in a different way:
\[
\Sigma_{\text{app}} = \sigma_0 + \frac{kT K_s}{2 S_0 (K_c)^2} \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{n(n+1) + \Sigma_{\text{app}}}.
\] (49)
The dependence of \((\langle u_n^m \rangle^2)_{H_{\text{app}}(U,X)}\) on \(K_s\) is hidden in \(\Sigma_{\text{app}}\) (see Eqs. (49) and (50)).

Inserting Eq. (20) into Eq. (49), we obtain:
\[
\Sigma_{\text{app}} = \frac{R^2 K_s}{K_c S_0} \left\{ 4\pi R^2 + \frac{kT R^2}{2} \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{n(n+1) + \Sigma_{\text{app}}} \right\}.
\] (50)
We introduce the new quantity \(\Sigma^0_{\text{app}}\) through the relation:
\[
S_0 = 4\pi R^2 + \frac{kT R^2}{2} \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{n(n+1) + \Sigma^0_{\text{app}}}.
\] (51)
Its physical meaning will be revealed later (see the comment concerning Eq. (54)).

Using Eq. (31), Eq. (40) can be presented in the following form:
\[
\Sigma_{\text{app}} = \frac{R^4}{2 K_c S_0} \frac{K_s kT}{K_c}
\times \sum_{n=2}^{n_{\text{max}}} \frac{(2n+1)(\Sigma^0_{\text{app}} - \Sigma_{\text{app}})}{n(n+1) + \Sigma^0_{\text{app}}}.
\] (52)
Eq. (52) shows the dependence \(\Sigma_{\text{app}}(K_c)\) for different values of \(\Sigma_{\text{app}}\) (respectively \(S_0\)) at fixed \(K_c\), \(R\), and \(T\). For three different values of \(S_0\), \(\Sigma_{\text{app}}\) is schematically presented on Fig. (1). The obtained results allow to determine the values of \(\Sigma_{\text{app}}(K_s)\) when \(K_s \to 0\) and \(K_s \to \infty\). For the first limit the result is:
\[
\lim_{K_s \to 0} \Sigma_{\text{app}}(K_s) = 0.
\] (53)
The analogous case in the Milner and Safran approach is the case when \(\Sigma = 0\) (see Eqs. (11) and (12)).

The second limit is exactly the auxiliary tension \(\Sigma^0_{\text{app}}\):
\[
\lim_{K_s \to \infty} \Sigma_{\text{app}}(K_s) = \Sigma^0_{\text{app}}.
\] (54)
This case has no analogue in the theory of Milner and Safran.

We note, that by taking these limits the relation \(K_c \sim K_s \times d^2\) where \(d\) is the thickness of the membrane [2] no longer holds. Our aim is to check the behavior of the vesicle when the often used approximations of not stretchable or infinitely stretchable membrane. Evidently the above
relation cannot be kept at such approximations. Our results show that in both cases the tension of the membrane (see Eqs. (16) and (17)).

From Eqs. (15) and (46) it follows that:

\[
\langle \Delta S(U) \rangle_{H_{app}(U, \bar{X})} = \frac{kT}{K_c} \frac{R^2}{2} \sum_{n=2}^{n_{max}} \frac{2n + 1}{n(n + 1) + \Sigma_{app}}.
\]  (55)

With the help of Eqs. (14), (15), (46), and (55), Eq. (50) becomes:

\[
\Sigma_{app} = \frac{R^2}{K_c} \frac{\langle S(U) \rangle_{H_{app}(U, \bar{X})} - S_0}{S_0} = \frac{R^2}{K_c} \frac{\langle \sigma(U) \rangle_{H_{app}(U, \bar{X})}}{S_0},
\]  (56)

where \(\sigma(U)\) is the true (not normalized) tension of the membrane (see Eqs. (10) and (17)).

The self-consistent equation can be written in the following form:

\[
K_s = \frac{\Sigma_{app} S_0}{K_c} \frac{R^2}{2} \sum_{n=2}^{n_{max}} \frac{(2n+1)(\Sigma_{app} - \Sigma_{app})}{n(n+1)+\Sigma_{app}}.
\]  (57)

It is clear that when the denominator of the fraction on the rhs of the above equation tends to zero, then \(K_s \to \infty\). The denominator is zero only if \(\Sigma_{app} = \Sigma_{app}\) (see the asymptotes on Fig. 1).

In order to obtain experimentally \(K_s\) it is sufficient to calculate the dependence of \(\Sigma_{app}\) on \(K_c\) and \(K_s\) (i.e. to obtain from Eq. (10) \(\Sigma_{app}(K_c, K_s)\)) and after that to calculate the dependence of \(\langle (u_n^m)^2 \rangle_{H_{app}(U, \bar{X})}\) on \(K_c\), \(K_s\), and \(\Sigma_{app}(K_c, K_s)\). (i.e. to obtain from Eq. (10), with \(X = \bar{X}, \langle (u_n^m)^2 \rangle_{H_{app}(U, \bar{X})}[K_c, K_s, \Sigma_{app}(K_c, K_s)]\).

In the general case \(\Sigma_{app}\) depends on \(K_c\) (bending elasticity), \(K_s\) (stretching elasticity), \(R\) (the radius of the vesicle), and \(S_0\) (the area of the vesicle membrane in its flat tension-free state). We consider the case when the last two parameters can be determined or measured by independent methods and are not correlated with \(K_c\) and \(K_s\). Fitting \(\langle (u_n^m)^2 \rangle_{H_{app}(U, \bar{X})}\) with \(K_c\) and \(K_s\), we can determine the stretching elasticity of the membrane, deduced from the analysis of the thermal fluctuations of the vesicle shape.

The stretching elasticity \(K_s\)

One of the key points of this work is the elucidation of the role of the self-consistent equation. It is shown that this equation permits to express the stretching elasticity \(K_s\) via experimentally accessible quantities.

FIG. 1. Schematic representation of the dependence of the vesicle tension \(\Sigma_{app}\) (see Eq. (50)) on its stretching elasticity \(K_s\). The three curves 1, 2, and 3 refer to three vesicles with identical radiuses \(R\), bending elasticities \(K_c\), and temperature \(T\), and with different tension-free areas \(S_0\) of their membranes. \(S_0\) of curve 1 is less than that of curve 2, which is less than this of curve 3. When \(K_s\) tends to \(\infty\), the dependences of this kind tends to horizontal asymptotes: \(\Sigma_{app} = \Sigma_{app}\) (see below). In the present case the asymptotes, corresponding to the curves 1, 2, and 3, are denoted as 1-asymptote, 2-asymptote, and 3-asymptote, respectively.

FIG. 2. The dependence of the ratio correlator/free energy on the normalized tension \(\Sigma_{app}\) is shown. The numerical results are obtained with the given above numerical values of the quantities \(K_s, K_c, R, S_0\).

\[\Sigma_{app} = \frac{R^2}{K_c} \frac{\langle S(U) \rangle_{H_{app}(U, \bar{X})} - S_0}{S_0} = \frac{R^2}{K_c} \frac{\langle \sigma(U) \rangle_{H_{app}(U, \bar{X})}}{S_0},\]
THE CLOSEDNESS OF THE MODEL HAMILTONIAN TO THE APPROXIMATING HAMILTONIAN

We continue with the calculation of \( \langle [\mathcal{A}(U) - \bar{X}]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} \). Obviously, we have:

\[
\langle [\mathcal{A}(U) - \bar{X}]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X}, \sigma_0)} = \langle [\mathcal{A}(U)]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X}, \sigma_0)} - 2\langle \mathcal{A}(U) \rangle_{\text{H}_{\text{app}}(U, \bar{X}, \sigma_0)} \bar{X} + \langle \bar{X} \rangle^2.
\]

From Eqs. (28), (34), and (46) we obtain:

\[
\langle \mathcal{A}(U) \rangle_{\text{H}_{\text{app}}(U, \bar{X}, \sigma_0)} = \frac{K_s}{2S_0} R^2 \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{[n(n+1)+\Sigma_{\text{app}}]}.
\]

From Eqs. (44) it follows that:

\[
\langle \mathcal{A}(U) \rangle_{\text{H}_{\text{app}}(U, \bar{X})} = \bar{X}.
\]

But from Eqs. (14) and (28) it follows that:

\[
\langle [\mathcal{A}(U)]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} = \frac{K_s}{2S_0} R^2 \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{[n(n+1)+\Sigma_{\text{app}}]}.
\]

Taking into account that the amplitudes \( u_m^n \) are not correlated (the approximating Hamiltonian presents a system of not interacting oscillators) and have a Gaussian distribution, we obtain that:

\[
\langle (u_m^n)^4 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} = 3 \langle (u_m^n)^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})}^2.
\]

After some tedious but simple calculations it follows that one gets

\[
\langle [\mathcal{A}(U)]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} = \frac{K_s}{2S_0} R^2 \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{[n(n+1)+\Sigma_{\text{app}}]}.
\]

Finally, we have:

\[
\langle [\mathcal{A}(U) - \bar{X}]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} = \frac{K_s}{2S_0} R^2 \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{[n(n+1)+\Sigma_{\text{app}}]}.
\]

with \( X = \bar{X} \). Thus, the use of an approximating Hamiltonian is justified when the correlator is small enough. We will try to put these qualitative considerations on a more quantitative basis.

Let us consider the behavior of the correlator Eq. (64) at the extreme values of \( K_s \) (0 and \( \infty \)). From Eq. (61) it follows that when \( K_s \to 0 \) at fixed \( kT \), \( K_c \), \( R \), and \( S_0 \), then the correlator in Eq. (61) also tends to zero. From the same equation it follows that when \( K_s \to \infty \), taking into account the result from Eq. (61) that \( \Sigma_{\text{app}} \) tends to a limited value, the correlator also tends to \( \infty \).

In order to estimate the correlator in Eq. (61), measured in units \( kT \), we shall use the following numerical values of the quantities:

\[
K_s \sim 100 \text{ erg/cm}^2; \\
K_c \sim 10^{-12} \text{erg}; \\
R \sim 10^{-3} \text{cm}; \\
S_0 \sim 4\pi R^2 \sim 1.256 \times 10^{-5} \text{cm}^2; \\
\sigma_1 = 4 \times 10^9 \text{erg}^{-0.5}.
\]

These values are typical for the experiments where analysis of the shape fluctuations of nearly spherical lipid vesicles were carried out [21]. With these values the quantity \( \langle [\mathcal{A}(U) - \bar{X}]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} \) from Eq. (61) is estimated as follows:

\[
\langle [\mathcal{A}(U) - \bar{X}]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})} = 8 \times 10^{-10} \sum_{n=2}^{n_{\text{max}}} \frac{2n+1}{[n(n+1)+\Sigma_{\text{app}}]}^2.
\]

The value of the correlator is a measure of the error in the determination of the free energy \( f[\text{H}_{\text{app}}(U, \bar{X})] \) in Eq. (13) with \( X = \bar{X} \). We assume that it is small enough when the following inequality is fulfilled:

\[
\frac{\langle [\mathcal{A}(U) - \bar{X}]^2 \rangle_{\text{H}_{\text{app}}(U, \bar{X})}}{f[\text{H}_{\text{app}}(U, \bar{X})]} \ll 1
\]

Our numerical calculations showed that for the values of the quantities \( K_s, K_c, R, S_0 \) and \( \sigma_1 \) given above, the inequality in Eq. (66) is valid. Consequently, the mean-field approximation, applied to the studied system, shows very good results.

RESULTS AND CONCLUSIONS

In the Milner and Safran phenomenological theory [4, 5], the “effective tension” \( \mathcal{F} \) is a free parameter which simulates an area constraint on the fluctuation amplitudes in Eq. (13). The mean square amplitudes are used to determine experimentally the bending elasticity \( K_s \). The question is whether it is possible to include the experimental determination of the stretching elasticity \( K_c \) in this scenario? Having this in mind it is necessary to take into consideration the area dilation energy in the Hamiltonian of the fluctuating system.
the simplest Hamiltonian approach the membrane area constraint is is guaranteed by a Lagrange multiplier $\sigma$ conjugated to the real area $S(U)$ [3, 10, 11, 17]. This Lagrange multiplier fixes the mean area $\langle S(U) \rangle_{H(U)}$, of the vesicle and is known as the "intrinsic tension" [11] or "internal tension" [17]. Its value cannot be directly experimentally measured. Its relation with the multiple other definitions of membrane surface tension is a matter of a longstanding debate (see e.g. [10, 11, 17] and refs. therein).

Another approach for description of the fluctuating system is to present the constraint $S(U) = \text{const}$ exactly by a $\delta$-function [3, 8]. It must be pointed out that it is a quite subtle to study the thermodynamics of the system in the later case. This requires to use the integral representation of the $\delta$–function in the partition function. The corresponding calculations can be evaluated using the method of steepest descend in the corresponding integral and are exactly valid in the thermodynamic limit [3, 8, 22].

The methods, involving Lagrange multipliers allow the easier analytical of calculation the partition function for fluctuating vesicles with a fixed area, (see also refs. [4, 6, 21]). However, this means to change the statistical ensemble.

Although the two approaches model two different physical situations, it is widely assumed that the ensembles give equivalent results in the thermodynamic limit, i.e., in the limit of infinitely large membranes [11, 17]. It is questionable whether both approaches are totally equivalent. The problem resembles the well-known problem for the equivalence of spherical and of mean spherical models of ferromagnetism which belong to different ensembles. It is well known that these two ensembles are, in general, statistically inequivalent (for a discussion and list of references see Chapter 3 of the monograph [19]). In the membrane fluctuation theories the above mentioned equivalence problem should be carefully reconsidered. In addition there is one more profound obstacle, namely infinitely large membrane at finite tension is an object not at thermodynamic equilibrium [14] and the description in different ensembles should not give equivalent results.

Our considerations avoids these problems being in accordance with the prescriptions of the statistical mechanics of finite-size systems. The considered Hamiltonian $H(U)$ is nonlinear with respect to the squares $(u_n^m)^2$ of the amplitudes $u_n^m$, due to the nonlinearity of $H_s(U)$. This causes certain mathematical problems. To avoid this obstacle, one can follow the common approach based on the Hubbard-Stratonovich transformation, with the subsequent use of the saddle-point point approximation [3, 12]. It turns out that the problem is exactly solvable (only) in the thermodynamic limit [3, 8, 11, 12]. Let us recall that this aspect of the theory was already discussed in the context of the spherical model of phase transitions in 1976 [3].

In the present paper we linearize the Hamiltonian in Eq. (22) using different approach. It is based on the Bogoliubov variational inequalities. In our opinion this yields a more clear picture of the proposed approximation. Moreover, the approximation is not related with the notion of the thermodynamic limit.

In our approach the problem is reduced to solving a self-consistent equation for the auxiliary variable $X$. For $X = \tilde{X}$ this equation has a simple physical interpretation if it is presented in the form:

$$\left[\frac{1}{2} \sum_{n=m}^{n=\infty} \sum_{m=-n}^{n} (n-1)(n+2)\langle (u_n^m)^2 \rangle_{H_{app}(U, \tilde{X})} + 4\pi \right] R^2 = A(\tilde{X}),$$

where

$$A(\tilde{X}) = 4\pi R^2 + \sqrt{\frac{2S_0}{K_c}} \tilde{X}.$$  

Comparing with the approach where the microscopic area is fixed in the partition function through delta-function (see, e.g., Eq. (47) in [3] and Eq. (15) in [8]), we see that the equality (67) (valid for membrane parameters: $S_0, K_s, K_c, R$, and temperature $T$) imposes a "soft" constraint on the amplitudes of the shape fluctuations of the vesicle. It assures that the mean area of the membrane (lhs of Eq. (67)) is equal to the area $A(\tilde{X})$ (rhs of Eq. (67)). If $\sum_{\text{app}}(X)$ is considered as a fitting parameter, then the expressions from Eq. (40) for $\langle (u_n^m)^2 \rangle_{H_{app}(U, X)}$ can be used to determine not only the bending elasticity $K_c$, but also the stretching elasticity $K_s$, by using the measurable shape fluctuations of quasi-spherical vesicles. One possible method for carrying out such measurements is the treatment of the images in order to extract the vesicles’ contours by means of phase contrast microscopy combined with fast image processing [3].

In the present paper we showed that theory a la Milner and Safran can be deduced by an approach based on the Bogolyubov inequalities and the approximating Hamiltonian method which is in accordance to the principles of statistical mechanics. Our consideration reveals the principal possibility to extract the value of the stretching elasticity modulus $K_s$ of the vesicle membrane from the analysis of the shape fluctuations of nearly spherical lipids vesicles. It was proved that there is an interval of values of the vesicle membrane tension, for which the application of this method give precise enough results. Quite unexpectedly this approach is applicable when the stretching elasticity $K_s$ of the membrane tends to zero.

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