Analytical calculation of the lipid bilayer bending modulus

Boris Kheyfets,* Timur Galimzyanov,† Anna Drozdova, and Sergei Mukhin
National University of Science and Technology MISIS, Leninskiy prospekt 4, Moscow 119049, Russia
(Received 29 April 2016; revised manuscript received 2 August 2016; published 18 October 2016)

Bending and Gaussian moduli of a homogenious single-component lipid bilayer are calculated analytically using microscopic model of the lipid hydrocarbon chains. The approach allows for thermodynamic averaging over different chains conformations. Each chain is modeled as a flexible string with finite bending rigidity and an incompressible cross-section area. The interchain steric repulsion is accounted for self-consistently determined single-chain confining parabolic potential. The model provides a simple analytical expression for the membrane bending modulus, which falls within a range of experimental values. An observed dependence of the modulus on hydrocarbon chain length is also reproduced. Correspondence between our microscopic model and the membrane theory of elasticity is established.

DOI: 10.1103/PhysRevE.94.042415

I. INTRODUCTION

Biological membranes play the crucial role in functioning of living cells [1–3]. Apart from the barrier function they are involved as platforms for a large amount of cell proteins and determine the energetic barrier for cell fusion and fission processes. Membrane's basic structural element is lipid bilayer, which motivates vast studies of its mechanical and thermodynamical properties. The presented theoretical study fills a gap between phenomenological liquid crystal and polymer brush models of lipid membrane elasticity.

On the length scales much larger than the membrane thickness, membrane deformations can be described using the elasticity theory of continuum elastic media, which was used, for instance, to explain the red blood cell's shape [4,5]. This theory is based on the Helfrich free-energy density [6]:

$$F_H = \frac{\kappa H^2}{2} - \kappa H_s H + \overline{\kappa} K, \tag{1}$$

where H is a mean curvature, $H_s = c_1 + c_2$, H_s is a spontaneous curvature, and K is a Gaussian curvature $K = c_1 c_2$, both expressed in terms of local principal curvatures c_1 and c_2. The two constants that control the membrane shape are bending modulus κ and saddle-splay modulus $\overline{\kappa}$. However, if neither boundary conditions nor topology of the membrane change, the integral contribution of Gaussian term to the elastic energy is constant due to Gauss-Bonnet theorem [7]. In this case the exact value of $\overline{\kappa}$ is insignificant and the whole term may be omitted.

On smaller length scales elasticity theory can be expanded to take into account the average direction of the lipid molecule, director, by introducing its tilt value [8]. The tilt describes deviation of director from the normal to the membrane surface. This allows us to describe membranes fusion [9,10], fission [3], short wave-length undulations [11], and even lipid domain structure [12–15], to name just a few. Corresponding functional is an extension of the Helfrich model [6] and has been derived by Hamm and Kozlov [8]:

$$F_{HK} = \frac{\kappa \tilde{H}^2}{2} - \kappa H_s \tilde{H} + \overline{\kappa} \tilde{K} + \frac{\kappa_\theta \vec{t}^{\,2}}{2}, \tag{2}$$

where elastic constants κ and $\overline{\kappa}$ are the same as in Helfrich functional Eq. (1), $\vec{t}$ and κ_θ are tilt vector and tilt modulus, respectively, and $\tilde{H}$ and $\tilde{K}$ are renormalized with the local tilt variation. Value of the tilt modulus is assumed to be independent of the lipid type, and approximately equals the surface tension at the oil-water interface [8,16]. Simultaneously, bending modulus strongly depends on the lipid type and lacks analytical description.

As it was shown in the work by Hamm and Kozlov [8] uniform tilt and bending deformations enter additively the deformation energy, hence, being independent. This means that tilt degree of freedom does not contribute to the bending modulus and we might consider pure bending case for our purposes without losing generality of the model.

For a pure bending ($\tilde{K} \equiv 0$, $\vec{t} \equiv 0$) we are left with a single elastic constant and a Helfrich curvature $\tilde{H} \equiv H$ (see Ref. [8]):

$$F_{\text{bend}} = \frac{\kappa H^2}{2} - \kappa H_s H. \tag{3}$$

In this work we have calculated bending modulus analytically from a simple microscopic model.

Various experimental methods are used for bending modulus measurement. In Ref. [17] bending modulus is found by fitting nanotube radii at different values of voltage applied along the lipid nanotube in electrolyte solution. Bending modulus has been also measured using optical [18], neutron spin echo [19], and sensitive micropipet [20] methods, as well as with the help of molecular dynamics simulations by analysis of the undulation [11,21] and tilt and bending fluctuations [22,23].

Several theoretical approaches for bending modulus calculation have been also proposed. In the early work by Szleifer *et al.* [24] a mean-field theory approach was used to find probability distribution function of chain conformations, which minimizes free energy of the membrane. This was the first work when it has been argued that the main contribution to bending energy comes from the hydrophobic chains. Two drawbacks of the work were absence of direct self-assembly

*kheyfboris@misis.ru
†Also at A.N. Frumkin Institute of Physical Chemistry and Electrochemistry RAS.

of lipids in membrane and absence of microscopic expression for bending modulus.

Another theoretical approach [25] starts from an Ising-type model, which is then turned into Landau-Ginzburg free-energy functional. Within this consideration membrane self-assembles due to entropy reduction, and the analytical expression for bending modulus is found. However, lipid conformations are not taken into account, which might be desirable for a microscopic model. Indeed, as we found in this work, lipid conformations might account for 50% of bending modulus.

In another analytical effort [26] bending modulus is split in two contributions: one of an entropic nature, related to the area per head, and the other one is attributed to the lipid-lipid interaction. Both contributions were found numerically using a model of lipid in which rigid beads are connected to each other by a Lenard-Jones potential.

Despite energy functional Eq. (2) having so many applications, there is still no generally accepted microscopic theory of bending modulus and clear analytical estimate for its value. Hence, it is still unclear how the molecular properties of a membrane determine its bending rigidity. In the present work we analytically calculate bending modulus of uniform lipid bilayer membrane using a microscopic model. The lipid molecules are represented with flexible strings. This model previously has been used for calculation of lipid membrane lateral pressure profile [27,28], pore formation phase diagram, and lateral stretching modulus [29].

The flexible strings model has been successful in analytical description of lateral pressure profile in hydrophobic region of lipid [27] and bolalipid [28] membranes, as well as membranes with finite curvature [30]. The calculated profiles were among the first known to us in the literature, giving theoretical explanation to the corresponding MD simulations results of Refs. [31–33]. The profiles are in good agreement with experimental data. Our analytically solvable microscopic model provides correct theoretical estimates for parameters characterizing collective properties of lipid membrane. Those parameters include lipid bilayer area expansion coefficient [27] and bending modulus of the membrane (see current manuscript). They are derived using as an input a single hydrocarbon tail's bending rigidity, incompressible area, and hydrophobic tension.

In the following section we briefly describe flexible strings model, then we describe bending modulus calculation, which is followed by a discussion.

II. CALCULATION

A. Flexible strings model

The flexible strings model considers lipid as an effective flexible string with a given incompressible area, A_n, and finite bending rigidity, K_f (see Fig. 1), subjected to the confining parabolic self-consistently determined potential. The latter allows for a repulsive entropic force induced between the neighboring lipid molecules, forming a bilayer, due to excluded volume effect (see Fig. 2). Interaction between heads is effectively included into surface tension in the hydrophobic region. Energy functional of the string consists of kinetic energy and bending energy of a given string conformation, as well as potential energy in the confining potential induced by collisions with the neighboring chains:

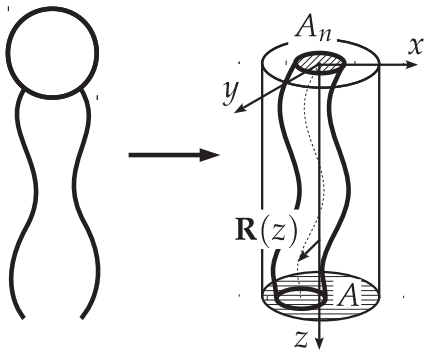

FIG. 1. Hydrocarbon tails of lipid are modeled as a flexible string. Schematic representations.

$$E_t = \int_0^L \left[\frac{\rho \dot{\mathbf{R}}^2}{2} + \frac{K_f}{2} \left(\frac{\partial^2 \mathbf{R}}{\partial z^2} \right)^2 + \frac{B\mathbf{R}^2}{2} \right] dz, \qquad (4)$$

where ρ is a linear density, $\mathbf{R}(z) = \{R_x(z), R_y(z)\}$ is a vector in the plane of the membrane giving deviation of chain's center from the straight line as a function of coordinate z along the "vertical" axis (see Fig. 1), and B is a parameter of the confining potential determined self-consistently (see Fig. 2). Monolayer thickness L depends on membrane curvature J. Self-consistent parabolic potential has been used previously to model a polymer chain in confined geometry [34]. In our case a single chain confinement is due to excluded volume effect of surrounding lipids in a monolayer. Inclusion of kinetic energy term into expression Eq. (4) has pure normalization goal and gives no contribution to observable characteristics [35], since kinetic and potential energies are separable in classical statistical mechanics. Nevertheless, this term leads to correct expression for the eigenfrequencies of transverse vibrations of the Euler beams modeled by the first two terms in Eq. (4).

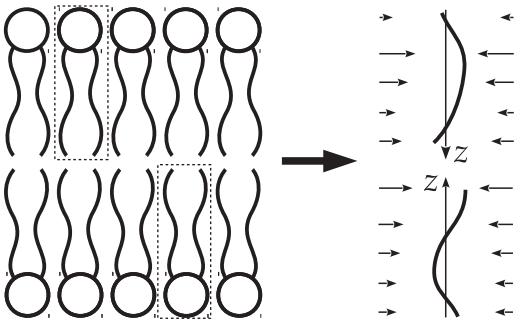

FIG. 2. Collisions with neighboring lipids are modeled by self-consistent confining potential. Potential is quadratic in the string deviation from axis z (arrows size mimics local force strength).

Boundary conditions for the model flexible string take into account the following physical assumptions [36]:

$$\begin{aligned} R_x'(0) &= 0, && \text{a chain terminates perpendicularly to the membrane surface;} \\ R_x'''(0) &= 0, && \text{net force acting on a head is zero;} \\ R_x''(L) &= 0, && \text{net torque acting at a chain's free end is zero;} \\ R_x'''(L) &= 0, && \text{net force acting at a chain free end is zero.} \end{aligned} \tag{5}$$

The same boundary conditions hold for the $R_y(z)$ component. As it is seen from the data on order parameter [21,37], the chains in the vicinity of head groups are not permanently perpendicular to the membrane surface. Yet, the chains in that region are not in a complete disorder: they are constrained by the hydrophobic tension. Lipid monolayer has strongly asymmetric structure. The first boundary condition (orthogonality of the string to the membrane surface) reflects the above-mentioned asymmetry of the monolayer by differing from the boundary condition at the chain's end. Our approximation seems more suitable for corresponding boundary conditions rather than setting lipid chains orientation free in the region of head groups.

Assuming membrane to be locally isotropic in lateral direction, partition function can be split in the product of two equal components, $Z = Z_x Z_y = Z_x^2$, and thus free energy of the lateral oscillations of the chain equals to

$$F_t = -2k_B T \log Z_x. \tag{6}$$

Partition function Z_x could be written as a path integral over all chain conformations:

$$Z_x = \iint e^{-\frac{E\{R_x(z),\dot{R}_x(z)\}}{k_B T}} D R_x D\dot{R}_x. \tag{7}$$

Under the boundary conditions Eq. (5) potential part of the energy functional Eq. (4) can be equivalently rewritten in terms of linear Hermitian operator $\hat{H} = B + K_f \frac{\partial}{\partial z^4}$ in the form

$$E_{t(\text{pot})} = \sum_{\alpha=x,y} E_\alpha, \quad E_\alpha \equiv \int_0^L [R_\alpha(z)\hat{H} R_\alpha(z)] dz. \tag{8}$$

Then, an arbitrary conformation of the chain is expressed as the deviation from the straight line of the centers of the string, $R_{x,y}(z)$, and parameterized by an infinite set of coefficients C_n of the linear expansion of the function $R_{x,y}(z)$ over the eigen functions $R_n(z)$ of the operator $\hat{H}$:

$$\begin{aligned} R_{\alpha=x,y}(z) &= \sum_n C_{n,\alpha} R_n(z), \\ \hat{H} R_n(z) &= E_n R_n(z). \end{aligned} \tag{9}$$

Substituting Eq. (9) into Eq. (4) and using the standard orthogonality property of the eigenfunctions of Hermitian operator $\hat{H}$ allows for simple decomposition of the energy functional into the series:

$$E_t = \sum_n \frac{1}{2}\{\rho \dot{C}_n^2 + E_n C_n^2\}. \tag{10}$$

We thus see that energy of a fluctuating string in a parabolic potential maps on the sum of energies of fictitious "harmonic oscillators" with "rigidities" E_n. Hence, the Boltzmann's probability of the state of a string in an arbitrary conformation $R_{x,y}(z)$, $P(\{R_{x,y}(z)\})$, is proportional to the infinite product of the Boltzmann's probabilities of the states of these oscillators due to obvious relation:

$$\begin{aligned} P(\{R_{x,y}(z)\}) &\propto \exp\left\{-\frac{E_t}{k_B T}\right\} \sim \prod_n \exp\left\{-\frac{\varepsilon_n}{k_B T}\right\}, \\ \varepsilon_n &\equiv \frac{1}{2}\{\rho \dot{C}_n^2 + E_n C_n^2\}. \end{aligned} \tag{11}$$

Therefore, distribution of the coefficients C_n prove to be just Gaussian Boltzmann's distribution functions. This makes the whole thermodynamic theory of the lipid membrane analytically tractable. The corresponding eigen values E_n and eigenfunctions $R_n(z)$ of the operator $\hat{H} = B + K_f \frac{\partial}{\partial z^4}$ are [27]

$$\begin{aligned} n = 0 \Rightarrow & \begin{cases} E_0 = B \\ R_0(z) = \sqrt{1/L}, \end{cases} \\ n \in \mathbb{N} \Rightarrow & \begin{cases} c_n = \pi n - \frac{\pi}{4} \\ E_n = B + c_n^4 \frac{K_f}{L^4} \\ R_n(z) = \sqrt{\frac{2}{L}}\left[\cos\left(c_n \frac{z}{L}\right) + \frac{\cos(c_n)}{\cosh(c_n)} \cosh\left(c_n \frac{z}{L}\right)\right] \end{cases}. \end{aligned} \tag{12}$$

Hence, summation over the infinite number of conformations $R_{x,y}(z)$ in the path integral Eq. (7) is reduced to integration over the infinite-dimensional space of real numbers C_n. This gives the following product of the Gaussian integrals for the partition function:

$$Z_x = \int_{-\infty}^{+\infty} \prod_n e^{-\frac{(\rho \dot{C}_n)^2}{2\rho k_b T} - \frac{C_n^2 E_n}{2k_B T}} \frac{d(\rho \dot{C}_n) dC_n}{2\pi\hbar} = \prod_n \frac{k_B T}{\hbar} \sqrt{\frac{\rho}{E_n}}. \tag{13}$$

Next, using Eq. (4) and definitions of the free energy and partition function in Eqs. (6) and (7), respectively, we differentiate both parts of Eq. (6) with respect to, so far unknown, parameter B and readily obtain the following self-consistency condition for parameter B:

$$\frac{\partial F_t}{\partial B} = L\langle R_x^2 \rangle, \tag{14}$$

where brackets denote thermodynamic (Boltzmann) average over chain conformations. The right-hand side of Eq. (14) is directly expressed via the thermodynamic average area per lipid A in the membrane plane and effective incompressible area of lipid chain A_n:

$$\pi\langle R_x^2 + R_y^2 \rangle = 2\pi\langle R_x^2 \rangle = (\sqrt{A} - \sqrt{A_n})^2, \tag{15}$$

and an isotropic in x-y plane membrane is considered for simplicity. Using this relation one can rewrite Eq. (14) in the explicit form using Eqs. (9) and (13):

$$\sum_{n=0} \frac{1}{b + c_n^4} = \nu(\sqrt{a} - 1)^2, \tag{16}$$

where we introduced dimensionless parameters,

$$a = \frac{A}{A_n}, \quad b = B\frac{L^4}{K_f}, \quad \nu = \frac{K_f A_n}{\pi k_B T L^3}. \tag{17}$$

For the reference we consider DPPC lipid at $T = 323$ K and estimate thickness of its hydrophobic region to be $L = 13$ Å [38], effective incompressible area of lipid chain $A_n = 10\,\text{Å}^2$, and effective bending rigidity of the string as $K_f = 4.5 k_B T L$ at room temperature T [39]. With these values, the dimensionless parameter of our analytical theory, ν, can be estimated as $\nu \sim 0.08$, which allows us to solve Eq. (16) iteratively, approximating the answer by iterations over two lowest inverse powers of the large parameter b:

$$\frac{3}{4b} + \frac{1}{2\sqrt{2}b^{3/4}} = \nu(\sqrt{a} - 1)^2. \tag{18}$$

The approximate solution of Eq. (18) is given by the following expression:

$$B \equiv \frac{K_f}{L^4} b = \frac{K_f}{L^4}\left[\frac{1}{4\nu^{4/3}(\sqrt{a}-1)^{8/3}} + \frac{1}{\nu(\sqrt{a}-1)^2}\right]. \tag{19}$$

Free energy of the string is equal to the sum of lipid tail free energy Eq. (6) and hydrophobic tension energy γA:

$$F_T = F_t + \gamma A. \tag{20}$$

Equilibrium area per lipid can be found by minimizing Eq. (20) over the area per lipid A in the membrane plane, which leads to the following balance equation for a membrane with zero external lateral pressure applied at the membrane perimeter (self-assembly condition):

$$\frac{\partial F_T}{\partial A} = \frac{\partial F_t}{\partial A} + \gamma = 0 \Rightarrow \gamma = -\frac{\partial F_t}{\partial A} \equiv P_t. \tag{21}$$

Condition Eq. (21) simply means that in equilibrium repulsion of the chains, P_t, should be balanced by hydrophobic tension, i.e., the attraction of the heads.

Solving self-assembly condition Eq. (21) with respect to the substitution of the relation Eq. (19) into expression Eq. (13) and Eq. (12) using Eq. (6), one computes area per lipid in a membrane. This model gives results close to experimental ones: for the values of parameters mentioned after Eq. (17), and using $\gamma = 17$ erg/cm^2 [11,40,41], we reproduce just the experimental value [42] for DPPC area per chain (at $T = 323$ K), $A = 64\,\text{Å}^2$.

B. Bending modulus

At first we consider pure bending of flat single-component bilayer. In that case one can expand free energy of the lipid in series by infinitesimally small value of curvature H:

$$F_{\text{bend}} = F_0 + \left.\frac{\partial F_t}{\partial H}\right|_{H=0} H + \frac{1}{2}\left.\frac{\partial^2 F_t}{\partial H^2}\right|_{H=0} H^2. \tag{22}$$

Comparing Eq. (22) with the first term of the pure bending energy functional of phenomenological elasticity theory, Eq. (3), one finds that

$$\kappa = \frac{1}{A_0}\left.\frac{\partial^2 F_t}{\partial H^2}\right|_{H=0}, \tag{23}$$

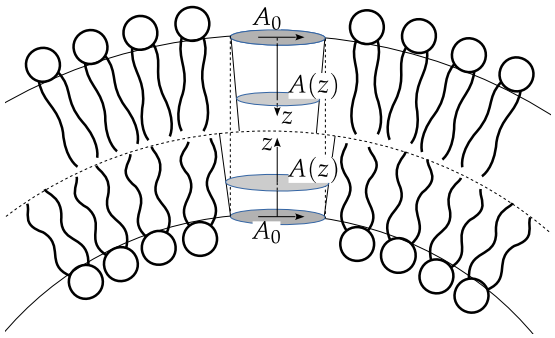

FIG. 3. Schematic representation of the lipid bilayer connected to a lipid reservoir. Area per lipid at the head group neutral surfaces of each monolayer is unchanged under bending provided there is a lipid reservoir. Condition Eqs. (26) and (27) for the monolayers are sketched in the figure.

where A_0 is the area per chain in the flat bilayer calculated above. We do not consider twisting or tilting deformations since we consider isotropic membrane. Twisting deformation of the string and tilting deformation of membrane does not couple to membrane curvature deformation and membrane monolayer transversal heterogeneity, so that kind of degrees of freedom are simply integrated out and give only constant contribution into the system's free energy.

Next, equating the second term in Eq. (22) with the second term in Eq. (3) and using already derived relation for κ in Eq. (23), we find

$$H_s = -\frac{\left.\frac{\partial F_t}{\partial H}\right|_{H=0}}{\left.\frac{\partial^2 F_t}{\partial H^2}\right|_{H=0}}. \tag{24}$$

Bilayer elastic modulus is shown to be twice that of one of the monolayers if the two monolayers are completely decoupled [43–48]. Hence,

$$\kappa^{\text{bilayer}} = 2\kappa^{\text{monolayer}} = \frac{2}{A_0}\left.\frac{\partial^2 F_t}{\partial H^2}\right|_{H=0}. \tag{25}$$

Henceforth, our aim is to find bending modulus of the membrane by calculating first and second derivatives of the membrane's free energy in our microscopic flexible strings model, and substituting the result into Eqs. (23)–(25).

As a first step, let us introduce bending in the flexible strings model. This is done by considering area per lipid being dependent, i.e., on the distance (depth) from head-group region inside lipid monolayer, together with the assumptions of lipid volume conservation and fixed area per head group on both sides of bilayer. Following standard formula of differential geometry for parallel bent surfaces, see, e.g., Ref. [8], we write

$$A(z) = A_0[1 - zH + z^2 K] \Rightarrow \frac{\delta A(z)}{A_0} = -zH + z^2 K, \tag{26}$$

where z axis is directed from neutral surface toward the opposite side of monolayer (see Fig. 3), and $\delta A(z)$ is an area change of the flat surface due to small mean and Gaussian curvatures H and K, respectively. The neutral

surface is defined as surface of zero lateral expansion under bending deformation of the membrane, which is taken at the hydrophilic-hydrophobic interface [8]. Further, using equation of volume per lipid conservation under the bending [8], and solving it for the thickness L of the curved monolayer up to a second order in curvature, one can obtain

$$\delta L \equiv L - L_0 = \frac{L_0^2 H}{2} + \frac{L_0^3 H^2}{2} - \frac{L_0^3 K}{3}$$
$$\Rightarrow \frac{\delta L}{L_0} = \frac{L_0 H}{2} + \frac{L_0^2 H^2}{2} - \frac{L_0^2 K}{3}. \tag{27}$$

Relation Eqs. (26) and (27) hold for both monolayers provided that opposite signs are assigned to the mean curvatures of the opposite monolayers of the curved bilayer. Figure 3 illustrates bending of a bilayer described by equations Eqs. (26) and (27).

As the next step, we find self-consistent coefficient $B(z)$ as z-dependent function of membrane depth. For this, we add to the area A the z-dependent area change $\delta A(z)$ caused by the curvature, Eq. (26), and find corresponding change of the self-consistent parameter B_0 by expanding around its flat bilayer area dependence, $B(z) = B_0 + \delta B(z)$:

$$B(z) = B_0 + \left.\frac{\partial B_0}{\partial A}\right|_{A=A_0} \delta A + \left.\frac{\partial B_0}{\partial L}\right|_{L=L_0} \delta L + \frac{1}{2}\left.\frac{\partial^2 B_0}{\partial A^2}\right|_{A=A_0} (\delta A)^2 + \frac{1}{2}\left.\frac{\partial^2 B_0}{\partial L^2}\right|_{L=L_0} (\delta L)^2 + \left.\frac{\partial^2 B_0}{\partial A \partial L}\right|_{\substack{A=A_0 \\ L=L_0}} \delta A \delta L, \tag{28}$$

where δA and δL are defined by Eqs. (26) and (27), respectively. Hence, coefficient $B(z)$ becomes function of the mean and Gaussian curvatures, H and K, due to Eqs. (26) and (27). Thus, the curvatures H and K enter the free energy of lipid tails F_t via dependence of B on δA and δL. In turn, dependencies of B_0 on A_0 and L_0, implied in Eq. (28) above, follow directly from Eqs. (17) and (19).

Let us now proceed with the calculation of bending modulus [see Eq. (25)]. The general expression for $\frac{\partial^2 F_t}{\partial H^2}$ can be obtained from Eq. (6):

$$\frac{\partial^2 F_t}{\partial H^2} = \frac{2k_B T}{Z_x^2}\left(\frac{\partial Z_x}{\partial H}\right)^2 - \frac{2k_B T}{Z_x}\frac{\partial^2 Z_x}{\partial H^2}. \tag{29}$$

Substituting here expression for Z_x in Eq. (7) and evaluating its derivatives, we find from Eq. (29)

$$\left.\frac{\partial^2 F_t}{\partial H^2}\right|_{H=0} = \frac{2}{k_B T}\left[\left\langle \left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0}\right\rangle^2 - \left\langle \left(\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0}\right)^2\right\rangle + k_B T\left\langle \left.\frac{\partial^2 E_{tx}}{\partial H^2}\right|_{H=0}\right\rangle\right], \tag{30}$$

where $\langle\rangle$ denotes thermodynamic average over chain conformations with distribution Eq. (11) properly normalized by division with partition sum Eq. (13). Taking into account the boundary conditions for a string and assuming that kinetic energy of the string does not change under bending, we obtain explicit expression for the first derivative of the functional E_{tx} Eq. (4) with respect to curvature:

$$\frac{\partial E_{tx}}{\partial H} = \int_0^L \frac{\partial B}{\partial H}\frac{R_x^2}{2}\,dz + \frac{\partial L}{\partial H}\frac{B(L)R_x^2(L)}{2}. \tag{31}$$

Note that one should not differentiate R_x with respect to H, since the whole expression $\frac{\partial E_{tx}}{\partial H}$ enters the functional integral over R_x in Eq. (30). Derivative of B with respect to curvature H is found from the corresponding derivatives:

$$\frac{\partial B}{\partial H} = \left.\frac{\partial B_0}{\partial A}\right|_{H=0}\frac{\partial A}{\partial H} + \frac{\partial B_0}{\partial L}\frac{\partial L}{\partial H}, \tag{32}$$

where B_0 is self-consistent coefficient of lipid mean-field potential of the flat membrane [see Eq. (19)]. We omit here an explicit expression for $\frac{\partial^2 E_{tx}}{\partial H^2}$, which we had derived via a cumbersome but straightforward procedure. See Appendices B and C for details on how to compute Eq. (30). The resulting expression for bending modulus reads as

$$\kappa^{\text{bilayer}} = 2\frac{k_B T}{A_0}\left\{\sum_n \frac{1}{E_n}\int_0^{L_0}\left.\frac{\partial^2 B}{\partial H^2}\right|_{H=0} R_n^2\,dz + \left.\frac{\partial^2 L}{\partial H^2}\right|_{H=0} B_0 \sum_n \frac{R_n(L_0)^2}{E_n} + 2\left.\frac{\partial L}{\partial H}\right|_{H=0}\left.\frac{\partial B}{\partial H}\right|_{L=L_0}\sum_n \frac{R_n(L_0)^2}{E_n} + 2\left(\left.\frac{\partial L}{\partial H}\right|_{H=0}\right)^2 B_0 \sum_n R_n(L_0)\left.\frac{\partial R_n}{\partial L}\right|_{L=L_0}\frac{1}{E_n} - \sum_{nm}\frac{\left(\int_0^{L_0}\left.\frac{\partial B}{\partial H}\right|_{H=0} R_n R_m\,dz + \left.\frac{\partial L}{\partial H}\right|_{H=0} B_0 R_n(L_0) R_m(L_0)\right)^2}{E_n E_m}\right\} \tag{33}$$

(see Eq. (12) with expressions for R_n, and Eq. (28), which provides dependence of B on H via Eq. (26), and Eq. (27) for the H-dependent corrections $\delta A(z,H)$ and $\delta L(H)$ as explained above).

Substituting typical values of the lipid bilayer parameters mentioned above into Eq. (33) we evaluate bending modulus: $\kappa^{\text{bilayer}} \sim 25 k_B T$ for DPPC bilayer (see Appendix C for details), which is consistent with experimental measurements [18,19].

All contributions to the bending modulus Eq. (33) turn out to be of the same order and general expression for the bending modulus is rather bulky. However, it could be simplified in the limit of small parameter ν, which was defined in Eq. (17) and was evaluated to be ~ 0.08. In this case we find the approximate expression

$$\kappa^{\text{bilayer}} = k_B T \frac{L_0^2}{A_n}\left.\frac{2}{(\sqrt{a}-1)^{5/2}\nu^{1/4}}\right|_{H=0}. \tag{34}$$

This approximate expression equals numerically to the exact expression Eq. (33) within a 15% error, assuming that the values of all the parameters of the bilayer are in close vicinity of the values listed in the paragraph after Eq. (17).

TABLE I. Experimental data used to test Eq. (39) at 50°C. Note that generally the dependence of elastic properties on temperature above main phase transition can be considered weak [18,19,54] (within a given study). We used DMPC's value for K_A for DPPC for a lack of a better source.

	h_{pp}	κ	K_A
DPPC	38.3 Å at 50°C [42]	12.5×10^{-20} J at 50°C [18,19]	≈234 mN/m
DMPC	35.3 Å at 30°C [55]	6.9×10^{-20} J at 30°C [55]	234 mN/m at 30°C [51]
DLPC	30.8 Å at 30°C [55]	5.5×10^{-20} J at 30°C [55]	239 mN/m at 30°C [51]

III. DISCUSSION

A. Bending modulus dependence on the chain length

Although many different scaling exponents have been reported in literature [49,50], recent experimental studies assert that bending modulus is proportional to the square of thickness of the membrane [51,52],

$$\kappa \sim L_0^2. \tag{35}$$

Our simplified result Eq. (34), although it contains relation Eq. (35) explicitly, does not allow us to check it, since the dependence of area per lipid, a, on chain length, L_0, cannot be found analytically in the general case. However, the exact relation can be obtained in a rigid rods approximation [29].

In this case probability of the bended string is zero and we are left with the single eigenfunction, R_0, being a constant [see Eq. (12)]. This means that the rigid rod can oscillate in a lateral direction only as a whole without changing its straight shape, which leads to a simpler formula for a self-consistent lipid-lipid interaction parameter,

$$B = \frac{k_B T}{A_n L_0} \frac{\pi}{(\sqrt{a}-1)^2} \tag{36}$$

(limit $K_f \to \infty$ cannot be utilized explicitly in Eq. (19) since it was derived in the limit of small $\nu \ll 0$), and allows one to find analytical expression for area per lipid:

$$a = \frac{1}{4}\left[1 + \sqrt{1 + 4\frac{k_B T}{A_n \gamma}}\right]^2 \gamma L_0. \tag{37}$$

Bending modulus for rigid rods membrane can be calculated using the general result Eq. (33):

$$\kappa^{\text{bilayer}} = \frac{k_B T L_0^2}{A_n} \frac{8a - 2\sqrt{a} - 3}{6a(\sqrt{a}-1)^2} \sim L_0^2, \tag{38}$$

in full agreement with Eq. (35).

Furthermore, in a polymer brush model, the exact relation is found to be [51]

$$\kappa = \frac{K_A L_0^2}{24}, \tag{39}$$

where K_A is a stretch modulus that has been shown to be determined by a tension at the hydrophobic interface, γ, and independent of the bilayer thickness [51,53]. It has also been shown within flexible strings model for rigid rods and liquid-disordered limits [29].

Relation Eq. (39) has been confirmed in experiment [51,52] by relating L_0 with an x-ray peak-to-peak head-group separation, h_{pp}, as $L_0 = h_{pp} - h_o$, with the offset $h_0 = 1$ nm. However, in these studies the lipids with different chain saturation were used. Equation (39) has been derived [51] within polymer brushes model, which does not take into account chain unsaturation directly.

For the sake of transparency, to avoid unknown effect of unsaturated bonds, we consider saturated lipids only. These are DLPC (12:0), DMPC (14:0), DPPC (16:0) lipids. DPPC undergoes lipid gel phase transition at 41° C. For this reason we consider all these lipids at 50° C (see Table I) since elastic properties of lipid membrane does not change significantly above main transition temperature [18,19,54].

Calculation results are presented at Fig. 4. It is clear from that figure, that our results at 50°C match experimental data for saturated lipids better than polymer brush model.

One can easily obtain from the data Fig. 4: $m = \frac{\kappa}{K_A L_0^2} = 1/6$ in place of $m = 1/24$ of polymer brushes [see Eq. (39)]. There is a common agreement that this coefficient in membranes lies in the interval between 1/12 to 1/48 [53]. 1/12 is derived for flexible film model, while 1/48 is obtained with naive approach, in which each monolayer is considered as elastic film [56]. The later approach doesn't take into account that lipid membrane's essential asymmetry (see Fig. 3)—it's neutral surface is located near the head-group region. One can easily show that in this case the coefficient is equal to $m = 1/12$ (see Appendix D).

All these results are obtained using simple geometric considerations (see Appendix D). Polymer brushes theory [51] brings an idea to the table—it takes into account two contributions into the elastic energy. Fist one stands for the hydrophobic surface stretching energy and second one stands

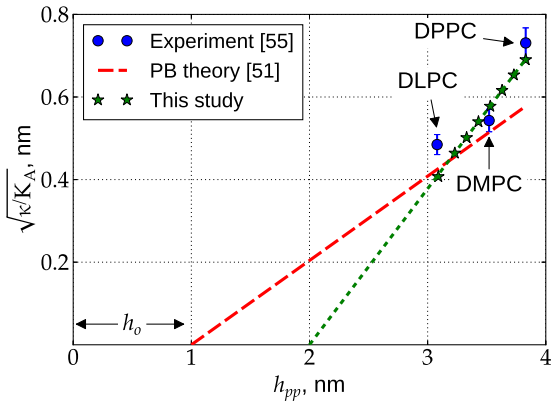

FIG. 4. Dependence of membrane bending—to stretch modulus ratio on the head-to-head thickness of fully saturated lipid bilayers at $T = 323$ K. Stars are our model results; long dashes—polymer brush model results [51].

for the entropic repulsion of lipid tails:

$$F_{\rm pb} = \gamma A + \frac{B}{A^2} \tag{40}$$

(see the equation on p. 337 in Ref. [51]). That model gives coefficient $m = 1/24$.

However, this model does not incorporate the neutral surface conception—membrane patch assumed to conserve its geometry under the bending (see text after Eq. (A1) in Ref. [51]), stretching transversely uniformly. Moreover, all of these models, namely simple geometric and polymer brushes model, omit the fact that lateral pressure profile in membranes has a pressure peak in the midplane region [27,57], which means higher value of entropic repulsion and consequently higher lateral compression modulus in the region of lipid tail ends.

Here we show the result of the expansion of polymer brush model, which takes into account monolayer asymmetry (see Fig. 3). We model the asymmetry by introducing the transversal inhomogeneity of the entropic repulsion coefficient $B(z) = B_0 + B_1 z^n$ with arbitrary n, characterizing the sharpness of the lateral pressure profile peak in the midplane region. Equation (40) takes the following form:

$$F = \gamma A + \int_0^L \frac{B_0 + B_1 z^n}{A^2(z)} dz. \tag{41}$$

Equilibrium area is given by the corresponding equation:

$$\frac{\partial F}{\partial A} = 0 \quad \Rightarrow \quad A_0 = \sqrt[3]{2\frac{B_0 L + B_1 L^{1+n} + B_0 L n}{\gamma(1+n)}}, \tag{42}$$

which leads to the following value of the bilayer compression and stretching modulus:

$$K_A = \frac{1}{A_0}\frac{\partial^2 F}{\partial A^2}\bigg|_{A=A_0} = 6\gamma. \tag{43}$$

Membrane bending leads to the deformation of the membrane patch according to Eq. (26). This leads to the expression of the free energy of bended membrane:

$$F_J = 2\gamma A_0 + \int_0^L \frac{B_0 + B_1 z^n}{A_t^2}\,{\rm d}z + \int_0^L \frac{B_0 + B_1 z^n}{A_b^2} dz, \tag{44}$$

where $A_t = A_0(1 - Hz)$ and $A_b = A_0(1 + zH)$ stand for top and bottom monolayers area dependence. Expansion of Eq. (44) in terms of curvature H, and using it in Eqs. (25) and (43) leads to a general expression for m:

$$m = \frac{(n+1)(B_0(n+3) + 3B_1 L^n)}{12(n+3)(B_0(n+1) + B_1 L^n)}. \tag{45}$$

One can show that this result does not depend on the power of $A(z)$, which models entropic repulsion in Eq. (41). For $B_1 = 0$ one obtains $m = 1/12$ in agreement with a simple homogenious model (see Appendix D). The opposite limit of strong lateral pressure profile peak, $B_1 L_0^n \ll B_0$, leads to

$$m = \frac{n+1}{4(n+3)}, \tag{46}$$

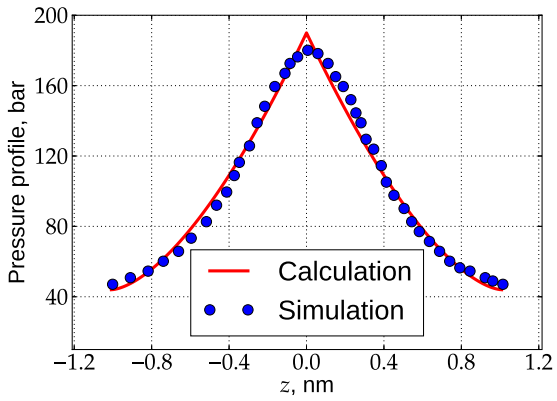

FIG. 5. Dots: simulation data quoted from Ref. [32]—see lateral region for DPPC lipids in Fig. 9 there. Line: fit of toy model, Eq. (41). The fit leads to $n = 1.6$, which results [see Eq. (45)] in $m = 1/8.6$. This value is close to the one obtained with flexible strings model.

which leads to m equal to $1/8$, $3/20$, and $1/6$ for $n = 1 \ldots 3$, respectively, with a limit of $m = 1/4$ for $n \to \infty$.

The fitting of the lipid tail region of the lateral pressure profile data for DPPC lipid taken from Ref. [32] (see Fig. 5) gives the following parameters (in arbitrary units): $B_0 = 44$, $B_1 = 146$, $n = 1.6$, leading to $m = 1/8.6$ [see Eq. (45)] versus $m = 1/6$ in our model. We consider it a good qualitative agreement, giving simplicity of the toy model. Note that $m = 1/8.6$ predicted by the toy model already exceeds the previous theoretical limit of $m = 1/12$.

In essence, we show that the relation between κ and K_A for lipid bilayer might be underestimated due a peak in lateral pressure profile in the midplane region. Our calculation (see Fig. 4) suggests $m = 1/6$, so it is in the range of theoretically possible values. Comparing with molecular dynamic data for lateral pressure profile shows qualitative agreement with our model, giving $m = 1/6$. Flexible strings model calculations, however, are more rigor compared to free energy Eq. (41), since it uses proper statistical averaging in calculation of entropic repulsion. It also takes into account the change in thickness of the membrane with the bending, $L(H)$.

B. Gaussian modulus

As already mentioned in the Introduction, the value of Gaussian modulus is insignificant if the topology and boundary conditions of the membrane are fixed. However, topology does change in fusion and fission events, which are omnipresent in cell biology. The associated energy changes are $-4\pi\overline{\kappa}$ for fusion and $4\pi\overline{\kappa}$ for fission [58]. Hence, the value of Gaussian modulus is important in elucidating diverse biological phenomena like intracellular protein trafficking, secretion, fertilization, and viral infection. And indeed it has been shown that Gaussian energy significantly influences early stage of the fusion—stalk formation [59].

There are several experimental approaches to measure Gaussian modulus [48,60] as well as few molecular dynamics approaches [61,62]. To the best of our knowledge, there is no microscopic theory of Gaussian modulus. However, method applied in this paper to calculate bending modulus might also be used to calculate Gaussian bending modulus.

For this let us consider bilayer *local* deformations without bending, $H = 0$. In this case monolayer Gaussian modulus is simply

$$\overline{\kappa} = \frac{1}{A_0}\frac{\partial F_t}{\partial K}\bigg|_{K=0}. \tag{47}$$

The general expression for $\frac{\partial F_t}{\partial K}$ might be obtained from Eq. (6):

$$\frac{\partial F_t}{\partial K} = -2k_BT\frac{1}{Z_x}\frac{\partial Z_x}{\partial K}. \tag{48}$$

Substituting here the expression for Z_x Eq. (7) and upon evaluating the result at zero curvature, Eq. (48) becomes

$$\frac{\partial F_t}{\partial K}\bigg|_{K=0} = 2\left\langle \frac{\partial E_{tx}}{\partial K}\bigg|_{K=0}\right\rangle. \tag{49}$$

Modulo a substitution of H with K, the expression for $\frac{\partial E_{tx}}{\partial K}$ is the same as in Eq. (31). After averaging over lipid conformations (see Appendix A), one obtains

$$\overline{\kappa}^{\text{monolayer}} = \frac{k_BT}{A_0}\left\{\sum_{n=0}\frac{1}{E_n}\int_0^{L_0}\frac{\partial B}{\partial K}\bigg|_{K=0}R_n^2\,\mathrm{d}z + \frac{\partial L}{\partial K}\bigg|_{K=0}B_0\sum_{n=0}\frac{R_n^2(L)}{E_n}\right\}. \tag{50}$$

For the reference parameters one obtains $\overline{\kappa} = -2.9k_BT$ and $\overline{\kappa}/\kappa = -0.23$ for the monolayer. This value of Gaussian modulus falls within the range found in the literature (for a survey of reported values see Ref. [62]). The discrepancy might be due to the omission of van der Waals forces in the free energy of the string Eq. (20).

C. Limitations of the model

The major limitations of our model follow from the effective medium approximation, an approach which is well known in physics of random alloys (see, e.g., Ref. [63]). Our approximation is valid on the length and timescales large enough with respect to the characteristic subatomic scales of the constituent molecular structure of lipid membranes. Moreover, flexible string approach can be viewed as an extreme case of coarse-grained model. Flexible string approach can be viewed as an extreme case of coarse-grained model. Coarse-grained models are common in the study of lipid membranes [33,56]. Simulating lipids with a few beads has been successfully used to explain a number of properties [62,64,65]. The advantage of our model is that it permits calculation of the averages over rather random subatomic configurations of lipid chains in a membrane, nevertheless providing physical description of their collective properties. Such collective properties include, e.g., lateral pressure profile in the membrane, orientational order parameter of the lipid chain, elastic moduli of the membrane, etc.

Another limitation is that we have rather rough estimation of effective elastic string rigidity K_f. However, calculations show that the value of the bending modulus is not too sensitive to the value of string rigidity: it can be easily seen from the rigid rods limit [Eqs. (36)–(38)], at which K_f is taken to be infinite. At this limit the calculated values of bending rigidity does not deviate strongly from finite K_f description.

Finally, there is a limitation due to the boundary conditions at the head region. We assume lipid chains on the average are perpendicular to the membrane surface. This approximation is supported by data on the order parameter [21,37], which shows that chains in that region are partially ordered in comparison to the midplane region.

IV. SUMMARY

Bending modulus of lipid bilayer has been calculated analytically from a microscopic model. A simple approximate expression for the bending modulus as function of molecule's characteristic parameters has been obtained. The model allows for self-assembly of the membrane lipids and provides explicit averaging over lipid tails conformations. An effective lipid-lipid interaction strength due to excluded volume effect is found self-consistently. In our model the free-energy increase is caused by membrane bending and arises due to suppression of the entropy of the chains accessible conformations. Calculated bending modulus of liquid-crystalline bilayer lipid membrane falls within the range of experimental values.

The theory has only two adjustable parameters (yet in strongly limited intervals): bending rigidity, K_f, of the effective string (that models hydrocarbon lipid tail), and incompressible area of the string, A_n. A single adjustment of solely these two parameters allows us to reproduce at once the areas per lipid and bending moduli of DPPC, DLPC, and DMPC lipid bilayers (see Fig. 4).

Calculated Gaussian modulus is in qualitative agreement with the data found in literature. To the best of our knowledge this is a first calculation of Gaussian modulus from a microscopic model. Correspondence with the Helfrich free-energy functional Eq. (3) of lipid membrane energy is established by Eqs. (22)–(24) using our microscopic model calculation of the free energy of the bent membrane.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Ministry of Education and Science of the Russian Federation in the framework of Increase Competitiveness Program of MISiS and partial support of this work by the Government of Russian Federation through Goszadanie research project (Grant No. 3.2007.2014/K) and the Russian Foundation for Basic Research (Project No.130440327N (KOMFI)).

APPENDIX A: AVERAGING OVER CONFORMATIONS OF THE STRING

In order to compute averages in the expression Eq. (30) one should expand arbitrary chain conformation R_x in elementary modes Eq. (12):

$$R_x = \sum_{n=0} C_n R_n, \quad \dot{R}_x = \sum_{n=0}\dot{C}_n R_n. \tag{A1}$$

In order to evaluate Eq. (30) one needs to compute $\langle C_n^2\rangle$ and $\langle C_n^4\rangle$ (see Appendix B). We begin with a simple result of

average "coordinate" C_n:

$$\langle C_n\rangle = \frac{\int_{-\infty}^{+\infty} C_n e^{-\frac{C_n^2 E_n}{2k_BT}} dC_n}{\int_{-\infty}^{+\infty} e^{-\frac{C_n^2 E_n}{2k_BT}} dC_n} = 0, \tag{A2}$$

which is due to the numerator containing an integral over all axis of odd function $C_n \exp(-\frac{C_n^2 E_n}{2k_BT})$. The situation is changed if we square C_n:

$$\langle C_n^2\rangle = \frac{\int_{-\infty}^{+\infty} C_n^2 e^{-\frac{C_n^2 E_n}{2k_BT}} dC_n}{\int_{-\infty}^{+\infty} e^{-\frac{C_n^2 E_n}{2k_BT}} dC_n} = \frac{k_BT}{E_n}, \tag{A3}$$

where the denominator is a Gaussian integral, and the numerator might be reduced to a Gaussian integral. The same technique applies to C_n^4:

$$\langle C_n^4\rangle = \frac{\int_{-\infty}^{+\infty} C_n^4 e^{-\frac{C_n^2 E_n}{2k_BT}} dC_n}{\int_{-\infty}^{+\infty} e^{-\frac{C_n^2 E_n}{2k_BT}} dC_n} = 3\left(\frac{k_BT}{E_n}\right)^2. \tag{A4}$$

The average of the product is zero unless coordinates are the same:

$$\begin{aligned}\langle C_nC_m\rangle &= \frac{\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} C_nC_m e^{-\frac{C_n^2E_n+C_m^2E_n}{2k_BT}} dC_ndC_m}{\int_{-\infty}^{+\infty} e^{-\frac{C_n^2E_n+C_m^2E_m}{2k_BT}} dC_ndC_m}\\ &\equiv \langle C_n^2\rangle\delta_{nm},\end{aligned} \tag{A5}$$

where δ_{nm} is Kronecker's symbol. By analogy,

$$\begin{aligned}&\langle C_iC_jC_nC_m\rangle\\ &\quad= \langle C_iC_jC_nC_m\rangle(\delta_{ij}\delta_{nm}+\delta_{in}\delta_{jm}+\delta_{im}\delta_{jn}+\delta_{ijnm}),\end{aligned}$$

hence,

$$\sum_{i,j,n,m}\langle C_iC_jC_nC_m\rangle = 3\sum_{n,m}\langle C_n^2\rangle\langle C_m^2\rangle + \sum_k\langle C_k^4\rangle. \tag{A6}$$

APPENDIX B: GENERAL EXPRESSION FOR THE BENDING MODULUS

At zero curvature, Eq. (31) becomes

$$\begin{aligned}\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0} &= \underbrace{\int_0^{L_0}\left.\frac{\partial B}{\partial H}\right|_{H=0}\frac{R_x^2}{2}\,\mathrm{d}z}_{f}\\ &\quad+\underbrace{\left.\frac{\partial L}{\partial H}\right|_{H=0}\frac{B(L_0)R_x(L_0)^2}{2}}_{g}.\end{aligned} \tag{B1}$$

Averaging over all conformations turns it into [see Eq. (18)]

$$\begin{aligned}\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0} &= \underbrace{\sum_n\langle C_n^2\rangle\frac{1}{2}\int_0^{L_0}\left.\frac{\partial B}{\partial H}\right|_{H=0}R_n^2\,\mathrm{d}z}_{\langle f\rangle}\\ &\quad+\underbrace{\left.\frac{\partial L}{\partial H}\right|_{H=0}\frac{B(L_0)}{2}\sum_n\langle C_n^2\rangle R_n(L_0)^2}_{\langle g\rangle}.\end{aligned} \tag{B2}$$

Raising it to the second power gives

$$\left\langle\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0}\right\rangle^2 = \langle f\rangle^2 + 2\langle f\rangle\langle g\rangle + \langle g\rangle^2, \tag{B3}$$

whereas the following relation holds:

$$\left\langle\left(\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0}\right)^2\right\rangle = \langle f^2\rangle + 2\langle fg\rangle + \langle g^2\rangle. \tag{B4}$$

In order to compute difference between Eqs. (B3) and (B4) [see Eq. (30)], let us consider $\langle f\rangle^2 - \langle f^2\rangle$:

$$\begin{aligned}f &= \frac{1}{2}\sum_{n,m}C_nC_m\int_0^{L_0}\left.\frac{\partial B}{\partial H}\right|_{J=0}R_nR_m\,\mathrm{d}z = \sum_{n,m}C_nC_mF_{nm},\\ \langle f\rangle &= \sum_n\langle C_n^2\rangle F_{nn},\\ \langle f\rangle^2 &= \sum_{nm}\langle C_n^2\rangle\langle C_m^2\rangle F_{nn}F_{mm},\\ f^2 &= \sum_{nmkl}C_nC_mC_kC_lF_{nm}F_{kl},\\ \langle f^2\rangle &= \sum_{nmkl}\langle C_nC_mC_kC_l\rangle F_{nm}F_{kl}\\ &= \sum_{n\neq m}\langle C_n^2\rangle\langle C_m^2\rangle F_{nn}F_{mm} + 2\sum_{n\neq m}\langle C_n^2\rangle\langle C_m^2\rangle F_{nm}^2\\ &\quad+\sum_n\langle C_n^4\rangle F_{nn}^2\\ &= \sum_{nm}\langle C_n^2\rangle\langle C_m^2\rangle F_{nn}F_{mm} - \sum_n\langle C_n^2\rangle^2F_{nn}^2\\ &\quad+2\sum_{n\neq m}\langle C_n^2\rangle\langle C_m^2\rangle F_{nm}^2 + \sum_n\langle C_n^4\rangle F_{nn}^2\\ &= \langle f\rangle^2 + \sum_n\left(\langle C_n^4\rangle - \langle C_n^2\rangle^2\right)F_{nn}^2 + 2\sum_{n\neq m}\langle C_n^2\rangle\langle C_m^2\rangle F_{nm}^2\end{aligned}$$

[see Eq. (A6)]. And from the last equation above it follows that

$$\begin{aligned}\langle f\rangle^2 - \langle f^2\rangle &= \sum_n\left(\langle C_n^2\rangle^2 - \langle C_n^4\rangle\right)F_{nn}^2 - 2\sum_{n\neq m}\langle C_n^2\rangle\langle C_m^2\rangle F_{nm}^2\\ &= -2(k_BT)^2\sum_{n,m}\frac{F_{nm}^2}{E_nE_m}.\end{aligned} \tag{B5}$$

Calculating $\langle 2fg\rangle$ and $\langle g^2\rangle$ analogously, one finds

$$\begin{aligned}&\left\langle\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0}\right\rangle^2 - \left\langle\left(\left.\frac{\partial E_{tx}}{\partial H}\right|_{H=0}\right)^2\right\rangle\\ &\quad= -\frac{(k_BT)^2}{2}\sum_{nm}\\ &\qquad\times\frac{\left(\int_0^{L_0}\left.\frac{\partial B}{\partial H}\right|_{H=0}R_nR_m\,\mathrm{d}z + \left.\frac{\partial L}{\partial H}\right|_{H=0}B_0R_n(L_0)R_m(L_0)\right)^2}{E_nE_m}.\end{aligned} \tag{B6}$$

The last term in the right-hand side of Eq. (30) is computed in the same way directly using Eq. (31).

APPENDIX C: ANALYTICAL EXPRESSIONS FOR DERIVATIVES OF *B*

The basic approach is first to rewrite expressions in dimensionless units and then to compute the dimensionless derivative using self-consistency Eq. (18). For example,

$$\left.\frac{\partial B}{\partial A}\right|_{H=0} = \frac{K_f}{L_0^4}\frac{1}{A_n}\left.\frac{\partial b}{\partial a}\right|_{H=0},$$
$$\left.\frac{\partial b}{\partial a}\right|_{H=0} = -\frac{16(\sqrt{a_0}-1)b^2\nu}{3\sqrt{a_0}(4+\sqrt{2}b^{1/4})}.$$

Here is an example of computing $\frac{\partial B}{\partial L}$:

$$\left.\frac{\partial B}{\partial L}\right|_{H=0} = \left.\frac{\partial\left(\frac{K_f}{L^4}b\right)}{\partial L}\right|_{H=0} = \frac{K_f}{L_0^5}\left(-4b + L_0\left.\frac{\partial b}{\partial L}\right|_{H=0}\right),$$
$$\left.\frac{\partial b}{\partial L}\right|_{H=0} = \frac{1}{L_0}\frac{16(\sqrt{a_0}-1)^2b^2\nu}{4+\sqrt{2}b^{1/4}}.$$

Other expressions $\frac{\partial^2 B}{\partial A^2}|_{H=0}$, $\frac{\partial^2 B}{\partial L^2}|_{H=0}$, and $\frac{\partial^2 B}{\partial A\partial L}|_{H=0}$ might be calculated in the same way.

APPENDIX D: DERIVATION OF RELATION BETWEEN BENDING MODULUS AND STRETCH MODULUS FOR BILAYER WITH NEUTRAL SURFACE FIXED IN THE HEADS

For simplicity we consider cylindrical bilayer at Fig. 3 and neglect the change in membrane thickness. The bending energy of a monolayer is equal to

$$\Delta F_b = \frac{\kappa^m H^2}{2} = \frac{\kappa^m}{2R^2}, \tag{D1}$$

where κ^m is bending energy of monolayer, and H is a mean curvature of cylindric membrane with radius R (R ends in the membrane midplane). The same energy can be expressed in terms of the mean-square strain on the monolayer:

$$\Delta F_s = \frac{K_A^m\langle u_{xx}^2\rangle}{2}. \tag{D2}$$

Due to the fact that neutral surface of the monolayer is located in the head-group region, the strain u_{xx} varies linearly with z from $u_{xx}=0$ at the neutral surface $z=0$ to $u_{xx}=u_m$ at midplane region: $z=L^m$. Its mean-square value is equal to $u_m^2/3$. Inserting this into Eq. (D4) and equating stretch energy Eq. (D4) to Eq. (D3), we obtain the following relationship of κ^m and K_A moduli:

$$\kappa^m = \frac{K_A^m(L^m)^2}{2}. \tag{D3}$$

Switching to the values for bilayer,

$$\kappa = 2\kappa^m, \quad K_A = 2K_A^m, \quad L = 2L^m, \tag{D4}$$

finally leads to

$$\kappa = \frac{K_A L^2}{12}. \tag{D5}$$

[1] B. Alberts, *Molecular Biology of the Cell* (Garland Science, New York, 2008).
[2] P. V. Bashkirov, S. A. Akimov, A. I. Evseev, S. L. Schmid, J. Zimmerberg, and V. A. Frolov, Cell **135**, 1276 (2008).
[3] V. A. Frolov, A. Escalada, S. A. Akimov, and A. V. Shnyrova, Chem. Phys. Lipids **185**, 129 (2015).
[4] H. J. Deuling and W. Helfrich, Biophys. J. **16**, 861 (1976).
[5] H. Naito, M. Okuda, and Ou-Yang Zhong-can, Phys. Rev. E **48**, 2304 (1993).
[6] W. Helfrich, Z. Naturforsch. C **28**, 693 (1973).
[7] M. P. D. Carmo, *Differential Geometry of Curves and Surfaces*, 1st ed. (Pearson, Englewood Cliffs, NJ, 1976).
[8] M. Hamm and M. M. Kozlov, Eur. Phys. J. E **3**, 323 (2000).
[9] Y. Kozlovsky and M. M. Kozlov, Biophys. J. **82**, 882 (2002).
[10] Y. Kozlovsky, L. V. Chernomordik, and M. M. Kozlov, Biophys. J. **83**, 2634 (2002).
[11] E. R. May, A. Narang, and D. I. Kopelevich, Phys. Rev. E **76**, 021913 (2007).
[12] T. R. Galimzyanov, R. J. Molotkovsky, and S. A. Akimov, Biochem. Moscow Suppl. Ser. A **5**, 385 (2011).
[13] T. R. Galimzyanov, R. J. Molotkovsky, P. I. Kuzmin, and S. A. Akimov, Biochem. Moscow Suppl. Ser. A **5**, 286 (2011).
[14] T. R. Galimzyanov, R. J. Molotkovsky, B. B. Kheyfets, and S. A. Akimov, JETP Lett. **96**, 681 (2013).
[15] T. R. Galimzyanov, R. J. Molotkovsky, M. E. Bozdaganyan, F. S. Cohen, P. Pohl, and S. A. Akimov, Phys. Rev. Lett. **115**, 088101 (2015).
[16] S. May, Y. Kozlovsky, A. Ben-Shaul, and M. M. Kozlov, Eur. Phys. J. E **14**, 299 (2004).
[17] P. V. Bashkirov, Biochem. Moscow Suppl. Ser. A **1**, 176 (2007).
[18] C. H. Lee, W. C. Lin, and J. Wang, Phys. Rev. E **64**, 020901(R) (2001).
[19] H. Seto, N. L. Yamada, M. Nagao, M. Hishida, and T. Takeda, Eur. Phys. J. E **26**, 217 (2008).
[20] E. Evans and W. Rawicz, Phys. Rev. Lett. **64**, 2094 (1990).
[21] E. Lindahl and O. Edholm, Biophys. J. **79**, 426 (2000).
[22] G. Khelashvili, B. Kollmitzer, P. Heftberger, G. Pabst, and D. Harries, J. Chem. Theory Comput. **9**, 3866 (2013).
[23] M. C. Watson, E. G. Brandt, P. M. Welch, and F. L. H. Brown, Phys. Rev. Lett. **109**, 028102 (2012).
[24] I. Szleifer, D. Kramer, A. Ben-Shaul, W. M. Gelbart, and S. A. Safran, J. Chem. Phys. **92**, 6800 (1990).

[25] H.-J. Woo, C. Carraro, and D. Chandler, Phys. Rev. E **53**, R41 (1996).
[26] A. Würger, Phys. Rev. Lett. **85**, 337 (2000).
[27] S. I. Mukhin and S. Baoukina, Phys. Rev. E **71**, 061918 (2005).
[28] S. I. Mukhin and B. B. Kheyfets, Phys. Rev. E **82**, 051901 (2010).
[29] S. I. Mukhin and B. B. Kheyfets, JETP Lett. **99**, 358 (2014).
[30] A. A. Drozdova and S. I. Mukhin, Biophys. J. **104**, 244a (2013).
[31] J. Gullingsrud and K. Schulten, Biophys. J. **85**, 2087 (2003).
[32] M. Bulacu, X. Périole, and S. J. Marrink, Biomacromolecules **13**, 196 (2011).
[33] O. H. S. Ollila, H. J. Risselada, M. Louhivuori, E. Lindahl, I. Vattulainen, and S. J. Marrink, Phys. Rev. Lett. **102**, 078101 (2009).
[34] T. W. Burkhardt, J. Phys. A: Math. Gen. **28**, L629 (1995).
[35] In the original version of the flexible strings model the kinetic energy was not used at all [27] and free energy was normalized by the free chain partition function Z_x (see Eq. (12) in [27]). However, it seems more natural to include kinetic energy just for correct dimension of Z_x. This question is also discussed after Eq. (12) in Ref. [28].
[36] L. D. Landau and E. M. Lifshits, *Course of Theoretical Physics. Theory of Elasticity*, Vol. 7 (Butterworth-Heinemann, London, 2008).
[37] L. Vermeer, B. de Groot, V. Réat, A. Milon, and J. Czaplicki, Eur. Biophys. J. **36**, 919 (2007).
[38] J. N. Israelachvili, *Intermolecular and Surface Forces*, 3rd ed. (Academic Press, San Diego, 2011).
[39] A_n is an internal effective parameter which describes the intensity of the excluded volume interaction. The string bending rigidity K_f is considered as parameter in our model. An expression relating K_f with a chain length L is therefore only used for the purpose of estimate and exploits analogy with the Flory's formula for polymer chains [66] and should not be considered as functional dependence.
[40] D. Marsh, Biochim. Biophys. Acta **1286**, 183 (1996).
[41] D. Marsh, Biochim. Biophys. Acta **1279**, 119 (1996).
[42] J. F. Nagle and S. Tristram-Nagle, Biochim. Biophys. Acta **1469**, 159 (2000).
[43] W. Helfrich and H. Rennschuh, J. Phys. Colloques **51**, C7-189 (1990).
[44] S. Ljunggren and J. C. Eriksson, Langmuir **8**, 1300 (1992).
[45] R. H. Templer, J. M. Seddon, and N. A. Warrender, Biophys. Chem. **49**, 1 (1994).
[46] S. May and A. Ben-Shaul, J. Chem. Phys. **103**, 3839 (1995).
[47] U. S. Schwarz and G. Gompper, Langmuir **17**, 2084 (2001).
[48] D. P. Siegel and M. M. Kozlov, Biophys. J. **87**, 366 (2004).
[49] I. Szleifer, D. Kramer, A. Ben-Shaul, D. Roux, and W. M. Gelbart, Phys. Rev. Lett. **60**, 1966 (1988).
[50] S. A. Safran, Adv. Phys. **48**, 395 (1999).
[51] W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans, Biophys. J. **79**, 328 (2000).
[52] H. Bermúdez, D. A. Hammer, and D. E. Discher, Langmuir **20**, 540 (2004).
[53] D. Boal, *Mechanics of the Cell*, 2nd ed. (Cambridge University Press, Cambridge, 2012).
[54] L. Fernandez-Puente, I. Bivas, M. D. Mitov, and P. Méléard, Europhys. Lett. **28**, 181 (1994).
[55] N. Kučerka, Y. Liu, N. Chu, H. I. Petrache, S. Tristram-Nagle, and J. F. Nagle, Biophys. J. **88**, 2626 (2005).
[56] M. Deserno, Macromol. Rapid Commun. **30**, 752 (2009).
[57] J. Gullingsrud and K. Schulten, Biophys. J. **86**, 3496 (2004).
[58] M. M. Kozlov, H. T. McMahon, and L. V. Chernomordik, Trends Biochem. Sci. **35**, 699 (2010).
[59] Y. Kozlovsky, A. Efrat, D. A. Siegel, and M. M. Kozlov, Biophys. J. **87**, 2508 (2004).
[60] S. Lorenzen, R. M. Servuss, and W. Helfrich, Biophys. J. **50**, 565 (1986).
[61] G. Brannigan and F. L. H. Brown, Biophys. J. **92**, 864 (2007).
[62] M. Hu, J. J. Briguglio, and M. Deserno, Biophys. J. **102**, 1403 (2012).
[63] B. Velický, S. Kirkpatrick, and H. Ehrenreich, Phys. Rev. **175**, 747 (1968).
[64] X. Wang and M. Deserno, J. Phys. Chem. B **120**, 6061 (2016).
[65] S. J. Marrink and A. E. Mark, J. Am. Chem. Soc. **125**, 11144 (2003).
[66] F. Gittes, B. Mickey, J. Nettleton, and J. Howard, J. Cell Biol. **120**, 923 (1993).