Mechanical surface tension governs membrane thermal fluctuations

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We use analytical considerations and computer simulations to show that the membrane spectrum of thermal fluctuations is governed by the mechanical and not the intrinsic tension. Our study highlights the fact that the commonly used quadratic approximation of the Helfrich effective Hamiltonian is not rotationally invariant. We demonstrate that this nonphysical feature leads to a calculated mechanical tension that differs dramatically from the correct mechanical tension. Specifically, our results suggest that the mechanical and intrinsic tensions vanish simultaneously, which contradicts recent theoretical predictions derived for the approximated Hamiltonian.

I. INTRODUCTION

Bilayer membranes are quasi-two-dimensional (2D) fluid sheets formed by spontaneous self-assembly of lipid molecules in water [1]. Their elasticity is traditionally studied in the framework of the Helfrich effective surface Hamiltonian for 2D manifolds with local principle curvatures c_1 and c_2 [2]

$$\mathcal{H}_0 = \int_A dS \left[\sigma_0 + \frac{1}{2}\kappa_0(c_1 + c_2 - 2c_0)^2 + \bar{\kappa}_0 c_1 c_2 \right], \quad (1)$$

where the integration is carried over the whole surface of the membrane. The Helfrich Hamiltonian involves four parameters: the spontaneous curvature c_0, the surface tension σ_0, the bending modulus κ_0, and the saddle-splay modulus $\bar{\kappa}_0$. For symmetric bilayer membranes, $c_0 = 0$. If, in addition, the discussion is limited to deformations that preserve the topology of the membrane, then (by virtue of the Gauss-Bonnet theorem) the total energy associated with the last term is a constant, and one arrives at the more simple form

$$\mathcal{H}_0 = \int_A dS \left[\sigma_0 + \frac{1}{2}\kappa_0(c_1 + c_2)^2 \right] = \sigma_0 A + \frac{1}{2}\kappa_0 J^2, \quad (2)$$

where A is the total area of the membrane and J, defined by $J^2 = \int dS(c_1 + c_2)^2$, is the integrated total curvature.

The surface tension appearing in Eq. (2) is known as the "intrinsic tension." It represents the elastic energy required to increase the surface of the membrane by a unit area, and can be identified with the derivative of the energy with respect to the area A, $\sigma_0 = (\partial\mathcal{H}/\partial A)$, at constant J. As this quantity is not directly measurable, its physical meaning is still a matter of a fierce debate. In molecular simulations, one can attach the membrane to a "frame" and measure the "mechanical (frame) tension," τ, which is the lateral force per unit length exerted on the boundaries of the membrane [3–6]. Experimentally, the mechanical tension is routinely measured by micropipette aspiration of vesicles [7,8]. Formally, the mechanical tension is obtained by taking the full derivative of the *free energy F* with respect to the frame (projected) area: $\tau = dF/dA_p$ [9]. From a comparison of the above definitions of σ_0 and τ, it becomes clear that the intrinsic and mechanical tensions are different quantities. Contrary the former, the latter is a thermodynamic quantity that also depends on the entropy of the membrane. Bilayer membranes usually exhibit relatively large thermal undulations at room temperature [10]

and, indeed, their mechanical tension also includes an entropic contribution due to the suppression of the amplitude of the undulations upon increasing the projected area.

The surface tension can be also measured indirectly by recording and analyzing the statistics of the membrane height fluctuations [11,12]. The analysis is based on the so-called Monge parametrization, where the surface of the fluctuating membrane is represented by a height function, $h(x,y)$, above the frame (x,y) plane. The Helfrich Hamiltonian does not have a simple form when expressed in terms of $h(x,y)$. However, for a nearly flat membrane, i.e., when the derivatives of h with respect to x and y are small ($|\partial_x h|$, $|\partial_y h| \ll 1$), one obtains the quadratic approximation

$$\mathcal{H}_2^M = \sigma_0 A_p + \int dx dy \left[\frac{\sigma_0}{2}(\nabla h)^2 + \frac{\kappa_0}{2}(\nabla^2 h)^2 \right]. \quad (3)$$

Note that unlike Eq. (2), the integral in Eq. (3) runs over the frame area rather than over the area of the manifold. The quadratic approximation can be diagonalized by introducing the Fourier transformation: $h_q = (1/A_p) \int dx dy \, h(x,y) \exp(-i\vec{q} \cdot \vec{r})$. In Fourier space the Hamiltonian reads

$$\mathcal{H}_2^M = \sigma_0 A_p + \frac{A_p}{2} \sum_{\vec{q}} (\sigma_0 q^2 + \kappa_0 q^4)|h_q|^2, \quad (4)$$

and by invoking the equipartition theorem, we find the mean square amplitude of mode $\vec{q}$ ("spectral intensity"):

$$\langle |h_q|^2 \rangle = \frac{k_B T}{A_p(\sigma_0 q^2 + \kappa_0 q^4)}. \quad (5)$$

From the last result, it seems as if σ_0 can be extracted from the fluctuation spectrum of the membrane. Results of both fully atomistic and coarse-grained simulations [13–17] show that spectral intensity can indeed be fitted to the form

$$\langle |h_q|^2 \rangle = \frac{k_B T}{A_p(rq^2 + O(q^4))}. \quad (6)$$

However, the derivation of Eq. (5) is based on the approximated Hamiltonian $\mathcal{H}_2^M$ and, therefore, it is not *a priori* clear why the so-called "q^2-coefficient" appearing in Eq. (6) $r = \sigma_0$. In fact, some theoretical studies have argued that the q^2-coefficient is actually equal to the mechanical tension τ [18–20]. This conclusion has been rejected more recently in

favor of the more common interpretation that $r = \sigma_0$ [21,22]. Membrane simulations carried at fixed mechanical tension (usually performed for $\tau = 0$) tend to agree with the result that $r = \tau$ [4,13,15,16,23], but simulations that show the opposite $r \neq \tau$ also exist [21,24].

Which of the above two results is correct? Theoretical arguments [18,19] and computer simulations [20] show that $r = \tau$. In this paper we demonstrate that the incorrect form $r = \sigma_0$ arises from the use of the truncated Hamiltonian Eq. (3) that breaks rotational invariance.

II. WHAT DOES THE INTRINSIC TENSION REPRESENT?

Much of the confusion associated with the physical meaning of surface tension in membranes is related to fact that the concept of surface tension has been originally defined for an interface between bulk phases (e.g., between water and oil) [25]. In its original context, the surface tension represents the access free energy ΔF per unit area A of the interface between the bulk phases

$$\gamma = \frac{\Delta F}{A}. \tag{7}$$

When the concept of surface tension is introduced into the theory of bilayer membranes, its meaning is distorted due to the following two major differences between membranes and interfaces of bulk phases:

(1) In the case of bulk phases, it is often assumed that the interface between them is flat. There is usually very little interest in the thermal roughness of the interface, unless it is very soft. In contrast, bilayer membranes are treated as highly fluctuating surfaces whose elastic response is very much influenced by the entropy associated with the thermal fluctuations.

(2) The changes in the areas of both systems arise from very different origins. In the case of, say, a water-oil interface, the changes in the interfacial area are *not* produced by elastic deformations (dilation/compression) that modify the molecular densities of the bulk phases. Instead, they result from transfer of molecules between the bulk phases and the interface occurring, for instance, when the shape of the container is changed. The surface tension γ is essentially a chemical potential which is directly related to the exchange parameter between the coexisting phases [26]. The case of bilayer membranes is completely different. Here, there is an exchange of water between the bulk fluid and interfacial region, but almost no exchange of *lipid* material because the concentration of free lipids in the embedding solution is extremely low (10^{-6} to 10^{-10} M [1]). In other words, there is no reservoir of lipids outside of the bilayer and, therefore, a change in the bilayer area results in a change in the area density of the lipids. The membrane surface tension measures the response to this elastic deformation, including the indirect contribution due to the exchange of water between the bilayer and solution resulting from the deformation (which has influence on the effective elastic moduli).

Most earlier theoretical investigations of membranes involved the assumption that the area per lipid a is constant. This assumption relies on the observation that the energy cost involved in density fluctuations is much larger than the

energy scale associated with curvature fluctuations. Further assuming that the lipids are insoluble in water (and, therefore, they all reside on the membrane where their number N is constant) implies that the total area of the membrane $A = Na$ is constant. If that is the case, then why does one need to include this constant in the Helfrich Hamiltonian [first term on the right-hand side of Eq. (2)], and what does the coefficient σ_0 represent? The answer is simple. It is technically very hard to calculate analytically the partition function for a fluctuating manifold with a fixed area A. The first term in Eq. (2) is a Lagrange multiplier that fixes the mean area $\langle A \rangle$ of the membrane, and the value of σ_0 is set by the requirement that $A = \langle A \rangle = \partial F / \partial \sigma_0$. As usual, it is assumed that in the thermodynamic limit the relative fluctuations in the total area become negligible, and there is no distinction between $\langle A \rangle$ and A.

The renewed interest in the meaning of surface tension during the past decade is very much linked with the rapid development in computer modeling and simulations of bilayer membranes. In molecular simulations the number of lipids N is usually fixed, but the total area is not. Membranes are no longer treated as incompressible thin films, but rather as stretchable/compressible surfaces whose elastic response results from their intermolecular forces. With this point of view in mind, it is clear that the intrinsic tension σ_0 in the Helfrich Hamiltonian represents an elastic coefficient which, potentially, may be related to the mechanical tension τ. There is, of course, no reason to expect that the elastic energy E of the membrane is linear in A [27]. A quadratic elastic function $E = 1/2 K_A (A - A_0)^2$ seems like a more appropriate form, where K_A is the stretching/compression modulus and A_0 is the relaxed area of the membrane [28] (also known as Schulman's area [29]). Expressing the total area as the sum of the projected and undulations areas $A = A_p + \delta A$, and assuming that $\delta A \ll |A_p - A_0|$, yields the linear approximation $E \simeq 1/2 K_A (A_p - A_0)^2 + K_A (A_p - A_0) \delta A$ from which one identifies that $\sigma_0 = K_A (A_p - A_0)$. The assumption that $\delta A \ll |A_p - A_0|$ becomes increasingly accurate at high tensions; but, nevertheless, the linear relationship between E and A is used in Eq. (2) over the entire range of intrinsic tensions.

III. THE IMPORTANCE OF ROTATIONAL INVARIANCE

The detailed derivation of the equality $r = \tau$ appears in Ref. [19] and will not be repeated here. One aspect of the derivation, which is the fact that the equality relies on the rotational invariance of the membrane, must however be emphasized. The derivation consists of two steps. In the first step, it is demonstrated that $r = \mu$, where μ is the *out-of-plane* simple shear modulus. The shear modulus μ is the force per unit length required to introduce the deformation depicted in Fig. 1(b) from the initial reference state depicted in Fig. 1(a). This deformation is achieved by applying opposite tangential forces on boundaries of the membrane. The shear modulus is *not* related to the deformation of a specific configuration, but rather to the deformation of a fluctuating membrane. It is obtained by taking the derivative of the free energy with respect to A, the area of the *mean membrane profile* (i.e.,
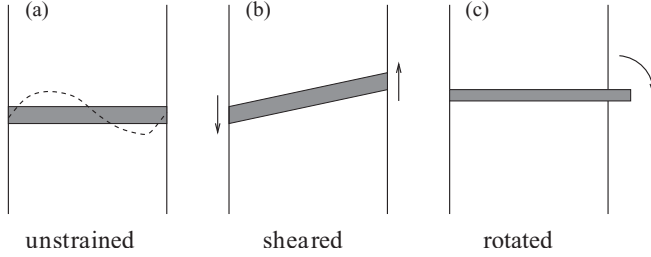
FIG. 1. (a) The unstrained reference state of a thin film. (b) The film subjected to a simple shear deformation. (c) The film subjected to a pure shear deformation. The two deformed states have the same elastic free energy since they can be transformed into each other by rotation. The volume of the film (indicated by the gray shaded area) is the same at all three states. The dashed line in (a) serves as a reminder that the membrane is fluctuating around its mean profile.

the configuration defined by the mean height function around which the membrane thermally fluctuates),

$$\mu = \frac{\partial F}{\partial A}. \tag{8}$$

In the second step of the derivation, it is proved that $\mu = \tau$. This follows immediately from the invariance of the free energy with respect to rigid transformations. Upon rotation, the sheared membrane in Fig. 1(b) can be transformed into the deformed state shown in Fig. 1(c) where the mean profile lies in the (x, y) plane defined the undeformed state. The projected area A_p in Fig. 1(c) is equal to the area A in Fig. 1(b) and, assuming rotational invariance, the free-energy cost of both deformations is the same. Therefore, the mechanical tension, which represents the elastic response to the deformation depicted in Fig. 1(c)

$$\tau = \left. \frac{\partial F}{\partial A_p} \right|_V, \tag{9}$$

is equal to the shear modulus μ defined by Eq. (8). This completes the proof that $r = \tau$. Notice that in Eq. (9), the derivative with respect to A_p is taken at constant volume. This feature cannot be captured within the framework of the Helfrich model that treats the membrane as a 2D manifold with no 3D volume. In Figs. 1(a)–1(c) we have intentionally drawn the membranes as thin films, which highlights a very important point. The two deformations (b) and (c) are both volume preserving. Deformation (b) is known as simple shear, while (c) is a pure shear deformation. The fact that $\mu = \tau$ stems from the equivalence of these two shear moduli.

Experimentally and computationally, there is no practical way to fix or even determine the volume of a bilayer at the molecular resolution. However, the membrane and the embedding fluid medium are placed in a "container" whose volume can be easily controlled. Equation (9) states that the frame tension of the bilayer can be measured by changing the cross-sectional area of the container while keeping its volume fixed. By considering such a deformation, one arrives at the following expression for τ:

$$\tau = L_z \left[P_z - \frac{P_x + P_y}{2} \right] = L_z [P_n - P_t], \tag{10}$$

where P_n and P_t are, respectively, the normal and transverse components of the pressure tensor (the negative Cauchy stress tensor) relative to the plane of the membrane. Equation (10) is known as the mechanical definition of the surface tension [25]. This tension is associated with the entire "interfacial region" of the system that includes both the bilayer of lipids as well as the hydration layers of structured water around the bilayer. The fluid bulk water has no elastic response to volume preserving deformations and, therefore, it make no contribution to τ.

So why does one gets $r = \sigma_0$ rather than $r = \tau$ when dealing with the quadratic approximation of the effective surface Hamiltonian, Eq. (3)? The answer is simple: The approximated Hamiltonian is not rotationally invariant. This striking fact (which has been discussed by Grinstein and Pelcovitz in the context of lamellar liquid crystalline phases [30]) can be demonstrated by considering a certain configuration parametrized by the height function $h(x, y)$ and evaluating the elastic energy cost corresponding to simple and pure shear deformations. For the simple shear deformation $h_\mu(x, y) = h(x, y) + \epsilon x$, and upon substituting $h_\mu(x, y)$ in Eq. (3) one gets

$$\mathcal{H}_2^M(h_\mu(x, y)) = \sigma_0 A_p + \int dx dy \left[\frac{\sigma_0}{2} (\nabla h_\mu)^2 + \frac{\kappa_0}{2} (\nabla^2 h_\mu)^2 \right]$$

$$= \mathcal{H}_2^M(h(x, y)) + \sigma_0 A_p \frac{\epsilon^2}{2}. \tag{11}$$

From this result one finds that

$$\mu = \frac{1}{A_p} \frac{d^2 \langle \mathcal{H}_2^M(h_\mu(x, y)) \rangle}{d\epsilon^2} = \sigma_0, \tag{12}$$

and, thus, $r = \mu = \sigma_0$. This conclusion that $r = \sigma_0$ is in agreement with what the equipartition theorem Eq. (5) predicts for the approximated Hamiltonian. The response to pure shear is determined by considering the transformation $h_\tau(x', y') = h(x(1 + \epsilon), y(1 + \epsilon))/(1 + \epsilon)^2$, with $0 < x' \leqslant L_x(1 + \epsilon)$, $0 < y' \leqslant L_y(1 + \epsilon)$. For this deformation, $A_p(\epsilon) = A_p(\epsilon = 0)(1 + \epsilon)^2$, $dx' dy' = (1 + \epsilon)^2 dx dy$, $\nabla h_\tau = \nabla h/(1 + \epsilon)^3$, and $\nabla^2 h_\tau = \nabla^2 h/(1 + \epsilon)^4$. When these relations are used in Eq. (3), we get

$$\mathcal{H}_2^M(h_\tau(x, y)) = (1 + \epsilon)^2 \sigma_0 A_p(\epsilon = 0)$$

$$+ \int dx' dy' \left[\frac{\sigma_0}{2} (\nabla h_\tau)^2 + \frac{\kappa_0}{2} (\nabla^2 h_\tau)^2 \right]$$

$$= \mathcal{H}_2^M(h(x, y)) + 2\epsilon \sigma_0 A_p(\epsilon = 0)$$

$$- \int dx dy [2\epsilon \sigma_0 (\nabla h)^2 + 3\epsilon \kappa_0 (\nabla^2 h)^2] + O(\epsilon^2). \tag{13}$$

From this result, one derives the mechanical tension, which is given by

$$\tau = \frac{1}{2A_p} \frac{d \langle \mathcal{H}_2^M(h_\tau(x, y)) \rangle}{d\epsilon} \bigg|_{\epsilon = 0}$$

$$= \sigma_0 - \frac{1}{A_p} \left\langle \int dx dy \left[\sigma_0 (\nabla h)^2 + \frac{3}{2} \kappa_0 (\nabla^2 h)^2 \right] \right\rangle. \tag{14}$$

The second term on the right-hand side of Eq. (14) is the entropic part of the mechanical tension, evaluated within the framework of the approximated quadratic Hamiltonian. This entropic contribution to τ is not reflected in the fluctuation

spectrum of the quadratic Hamiltonian, which has the nonphysical feature of not being rotationally invariant.

IV. COMPUTER SIMULATIONS

To summarize our discussion: We argue that $r = \tau$ is correct, and that it is the use of the not rotationally invariant quadratic Hamiltonian Eq. (3) that leads to the spurious result $r = \sigma_0 \neq \tau$. This conclusion can be tested by performing Monte Carlo simulations of the one-dimensional (1D) analogs of Eqs. (2) and (3). Within these two models, the membrane is represented by a string of $N = 1024$ points, the positions of which in 2D space are given by $\{\vec{r}_i = (x_i, h_i)\}$. In both cases, the simulations are performed at a constant projected length L_p with periodic boundary conditions. Denoting by $\vec{b}_i = \vec{r}_{i+1} - \vec{r}_i$ the distance vector ("bond") between adjacent points, the 1D analog of the rotationally invariant Helfrich effective Hamiltonian is given by [31]

$$\mathcal{H}_1 = \sigma_0 \sum_i |\vec{b}_i| + \kappa_0 \sum_i \left[1 - \frac{\vec{b}_i \cdot \vec{b}_{i-1}}{|\vec{b}_i||\vec{b}_{i-1}|} \right]. \quad (15)$$

The quadratic approximation of this Hamiltonian is given by

$$\mathcal{H}_2 = \sigma_0 L_p + \frac{\sigma_0}{2l_p} \sum_i (h_{i+1} - h_i)^2$$
$$+ \frac{\kappa_0}{2l_p^2} \sum_i (h_{i+1} + h_{i-1} - 2h_i)^2, \quad (16)$$

where $l_p = L_P/N$. Notice that in the 1D models, the tension has units of a force (energy per unit length). A major difference between the two 1D models is related to the positions of the points. In the rotationally invariant case Eq. (15), the points are allowed to be anywhere in the available 2D space. At low tensions, this creates configurations in which the chain forms overhangs [see, e.g., Fig. 2(a)]. The existence of such configurations does not invalidate any of our discussion which only requires that the height function of the mean profile is a well-defined function. Within the quadratic approximation Eq. (16), the points are allowed to move only in the direction normal to the projected length [in the spirit of Eq. (3) in which the height is measured from the (x, y) plane]. Thus, the position of the ith point is given by $\vec{r}_i = (il_p, h_i)$ and, obviously, such moves do not generate any overhangs [see a typical configuration in Fig. 2(b)].

In the simulations, we vary σ_0 and measure both the mechanical tension τ and the q^2-coefficient r. For each value of σ_0, the simulations extended over 2–4×10^8 MC time units, where each time unit consists of N single-particle move attempts and one collective "mode excitation Monte Carlo"
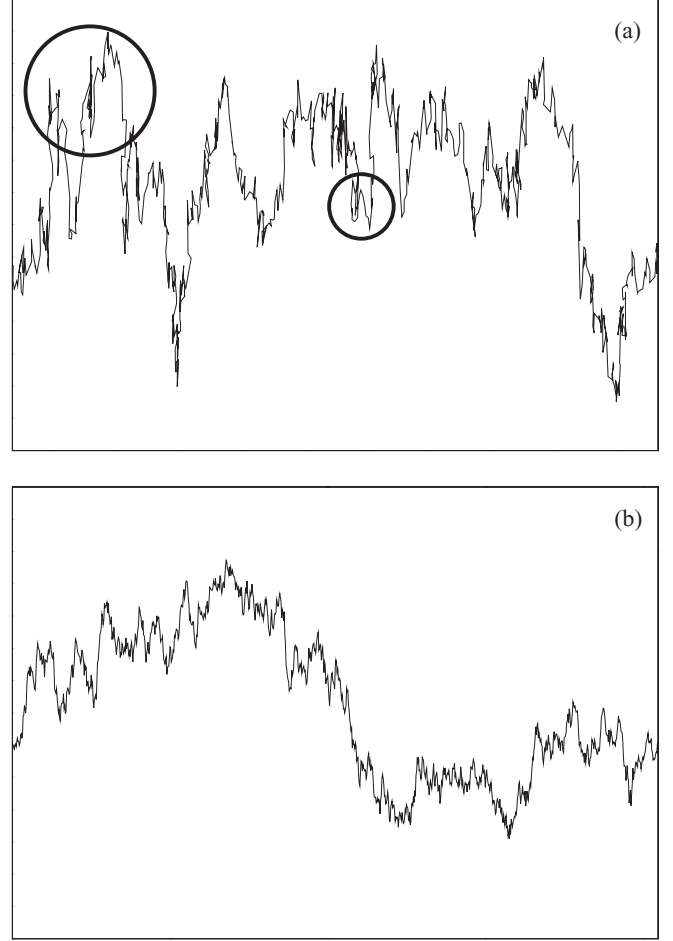

FIG. 2. Typical configurations of the chain in (a) the rotationally invariant Hamiltonian Eq. (15) and (b) its quadratic approximation Eq. (16). Both configurations have been obtained for $\sigma_0 = 0.75 \, k_B T/l_p$ and $\kappa_0 = 0$. In (a) the points of the chain are allowed to be anywhere in the available 2D space which creates overhangs such as those shown inside the bold circles. In (b) the points move only vertically to the line that connects the end points of the chain.

(MEMC) move that accelerates the very slow relaxation dynamics of the 10 largest Fourier modes [32,33]. The introduction of MEMC moves is essential for the equilibration of the system. The mechanical tension is calculated using the 1D equivalent of Eq. (10), $\tau = f_n - f_t$, where f_n and f_t are the normal and transverse forces acting on the chain. Expressing these forces as thermal averages, one arrives at the following virial formulas. For the rotationally invariant Helfrich Hamiltonian $\mathcal{H}_1$

$$\tau = \left\langle \sum_i \left[\sigma_0 \frac{(x_{i+1} - x_i)^2 - (h_{i+1} - h_i)^2}{l_p |\vec{b}_i|} + \kappa_0 \frac{2}{l_p |\vec{b}_{i-1}||\vec{b}_i|} \right. \right.$$
$$\left. \left. \times \left(\frac{(h_i - h_{i-1})(h_{i+1} - h_i)(x_i - x_{i-1})^2 - (x_i - x_{i-1})(x_{i+1} - x_i)(h_i - h_{i-1})^2}{|\vec{b}_{i-1}|^2} + \frac{(x_i - x_{i-1})^2 + (h_i - h_{i-1})^2}{|\vec{b}_i|^2} \right) \right] \right\rangle. \quad (17)$$
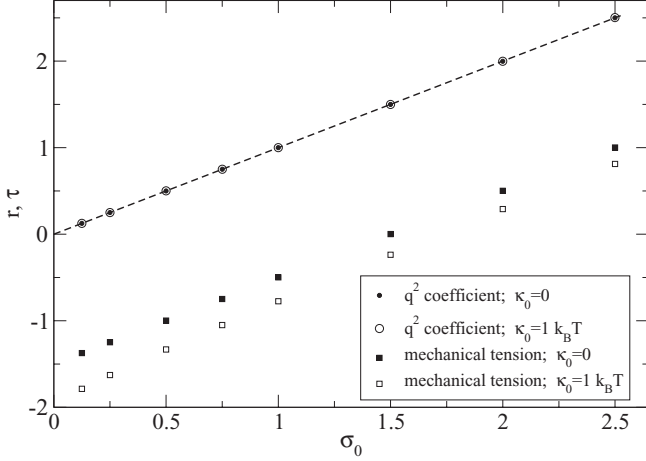
FIG. 3. Simulation results for Hamiltonian $\mathcal{H}_2$ [Eq. (16)]: The q^2-coefficient r (circles) and the mechanical tension τ (squares) as a function of the intrinsic tension σ_0. Results are shown for both $\kappa_0 = 0$ (solid symbols) and $\kappa_0 = 1 \, k_B T$ (open symbols). The dashed line is a guide to the eye for the relationship $r = \sigma_0$. Both axes are plotted in units of $k_B T / l_p$.
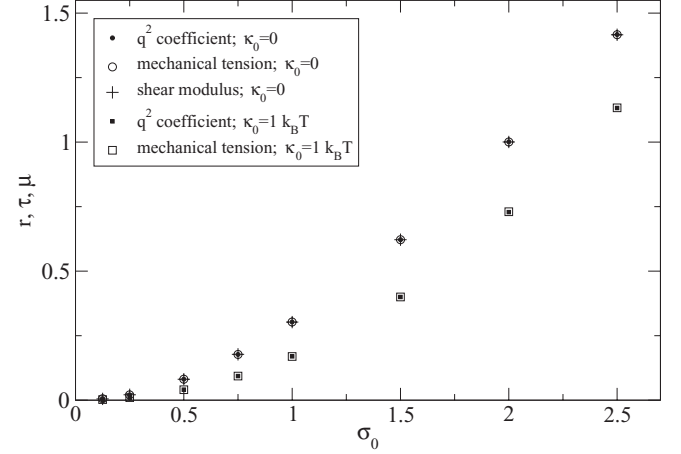

FIG. 4. Simulation results for Hamiltonian $\mathcal{H}_1$ [Eq. (15)]: The q^2-coefficient r (solid symbols) and the mechanical tension τ (open symbols) as a function of the intrinsic tension σ_0. Results are shown for both $\kappa_0 = 0$ (circles) and $\kappa_0 = 1 \, k_B T$ (squares). The pluses denote our results for the shear modulus μ for $\kappa_0 = 0$. Both axes are plotted in units of $k_B T / l_p$.

For the approximated quadratic Hamiltonian $\mathcal{H}_2$

$$\tau = \sigma_0 - \left\langle \sum_i \left[\frac{3\sigma_0}{2l_p^2} (h_{i+1} - h_i)^2 \right. \right.$$
$$\left. \left. + \frac{2\kappa_0}{l_p^3} (h_{i+1} + h_{i-1} - 2h_i)^2 \right] \right\rangle. \qquad (18)$$

The q^2-coefficient r is obtained by taking the Fourier transform of the the function h_i and fitting the measurements to Eq. (6). Our results for r are based on the analysis of the fluctuations of the 10 largest Fourier modes.

Our simulation results for the quadratic Hamiltonian $\mathcal{H}_2$ are summarized in Fig. 3. For both $\kappa_0 = 0$ and $\kappa_0 = 1 \, k_B T$ and for all values of σ_0, we find that, indeed, all our measurements of the q^2-coefficient agree with the predicted relationship $r = \sigma_0$ (which is denoted by the dashed line). As expected from Eq. (18), the mechanical tension τ is smaller than r and for small values of σ_0 even gets negative values. In comparison, the simulation results for the rotationally invariant Helfrich Hamiltonian $\mathcal{H}_1$ are shown in Fig. 4. In agreement with our expectations for this case, the various tensions satisfy the relationship that $r = \tau \neq \sigma_0$. To further demonstrate the validity of our discussion, we computed the simple shear modulus μ, which for Hamiltonian $\mathcal{H}_1$ is also expected to be equal to r and τ. The shear modulus μ can be computed using a rather cumbersome virial expression. For $\kappa_0 = 0$, the virial expression for μ takes the more simple form

$$\mu = \left\langle \sum_i \sigma_0 \frac{(x_{i+1} - x_i)^4}{l_p |\vec{b}_i|^3} - \frac{1}{l_p k_B T} \right.$$
$$\left. \times \left(\sum_i \sigma_0 \frac{(x_{i+1} - x_i)(h_{i+1} - h_i)}{|\vec{b}_i|} \right)^2 \right\rangle. \qquad (19)$$

Our results for μ agree perfectly with the results for τ and r.

V. CONCLUSIONS

We use computer simulations to demonstrate that the q^2-coefficient r is equal to the mechanical tension τ and not the intrinsic tension σ_0. Although this result was derived analytically a long time ago, it remained controversial and was disputed by several recent papers. In this paper we explain that the origin of the incorrect equality, $r = \sigma_0$, is the the use of the truncated Hamiltonian Eq. (3), which is not rotationally invariant. The recognition of the importance of rotational invariance calls for a reconsideration of some of the results derived through the truncated Hamiltonian. Specifically, in Refs. [21,22,28] the quadratic form has been used for a derivation of negative mechanical tension for positive intrinsic tension [see also Eq. (14) in the present paper]. Our simulations demonstrate that this result is achieved only with the faulty (nonphysical) quadratic Hamiltonian $\mathcal{H}_2$ (see Fig. 3). For the corresponding rotationally invariant Hamiltonian $\mathcal{H}_1$, the mechanical tension is always positive for $\sigma_0 > 0$ (see Fig. 4). Our computational results actually suggest that σ_0 and τ vanish simultaneously. As a last remark we note that our simulations yielding the result $r = \tau$ employ the Hamiltonian $\mathcal{H}_1$ with a constant intrinsic tension. As mentioned at the end of Sec. II, a stretching elasticity Hamiltonian would be more realistic for bilayer membranes. One should not however erroneously conclude that our discussion is limited to the constant σ_0 case. Our derivation in Ref. [19] of the equality $r = \tau$ is independent of the explicit form of the membrane Hamiltonian.

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[9] Later, it will become clear that one needs to take this derivative while keeping the volume of the system fixed. Mathematically, this implies that the surface in the Helfrich Hamiltonian should be treated as a thin film whose width becomes vanishingly small.

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