FLUCTUATIONS IN LIPID BILAYERS: ARE THEY UNDERSTOOD?

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We review recent computer simulation studies of undulating lipid bilayers. Theoretical interpretations of such fluctuating membranes are most commonly based on generalized Helfrich-type elastic models, with additional contributions of local "protrusions" and/or density fluctuations. Such models provide an excellent basis for describing the fluctuations of tensionless bilayers in the fluid $L_\alpha$ phase at a quantitative level.

However, this description is found to fail for membranes in the gel phase and for membranes subject to high tensions. The fluctuations of tilted gel membranes ($L_{\beta'}$ phase) show a signature of the modulated ripple structure $P_{\beta'}$, which is a nearby phase observed in the pretransition regime between the $L_\alpha$ and $L_{\beta'}$ state. This complicates a quantitative analysis on mesoscopic length scales.

In the latter context, we also address the general problem of the relation between frame tension and the fluctuation tension, which has been discussed somewhat controversially in recent years. Simulations of very simple model membranes with fixed area show that the fluctuations should be controlled by the frame tension, and not by the internal tension.

I. INTRODUCTION

Lipid bilayers in aqueous solution have been studied intensely for many decades as simple model systems for biological membranes,[1–4], both experimentally[5–7] and by computer simulations (see Refs. 8–16 for recent reviews). The physical and thermodynamical properties of such bilayers are similar in many respect for a broad variety of lipids[2, 5–7]. For example, phase diagrams of single-component lipid bilayers have a generic structure. At high temperatures, the bilayers are in a "fluid" state ($L_\alpha$ phase) where the hydrophobic tails of the lipids are disordered and the lipids are mobile. At lower temperatures, they undergo a transition into a stiffer and more ordered "gel" phase with ordered tails, reduced lipid mobility, and hexatic positional order. The detailed structure of that state depends on the type of the hydrophilic head groups, and most notably, on their size compared to the hydrophobic tails. If the heads are small compared to the tails – e.g., for ethanolamines[5] – the tails point on average in the direction of the membrane normal ($L_\beta$ phase). If the heads are large – e.g., in the common class of phosphatidylcholines[6] – the chains adjust to the packing mismatch by collective tilting ($L_{\beta'}$ phase). In some cases where the coupling between them is weak, e.g., ether-linked phosphatidylcholines[6], they may even give up the bilayer structure and assemble into a straight interdigitated phase ($L_{\beta INT}$ phase). The transition between the $L_\alpha$ phase and the $L_{\beta'}$ phase or the $L_{\beta INT}$ phase proceeds via an intermediate modulated "ripple" phase ($P_{\beta'}$). In nature, most biomembranes seem to be maintained in a fluid state. Nevertheless, it is remarkable that the transition between the fluid and the gel state, the so-called main transition, occurs at temperatures well above room temperature for some of the most common lipid molecules (e.g., ∼41°C for the phospholipid dipalmitoylphosphatidycholine (DPPC)[6]). One can speculate that nature might use the vicinity of such a phase transition to tune central properties of the membrane such as its permeability[17, 18].

One striking property of lipid bilayers is their softness and the ensuing large thermal undulations. Membrane fluctuations have attracted persistent interest over the years, going back to a seminal paper by Helfrich[19] in 1973 up to today. They affect the interactions of membranes with other objects, e.g., in the context of adhesion, lipid-mediated protein interactions and lateral protein diffusion. Moreover, the detailed analysis of fluctuations allows one to extract elastic constants and establish a connection between experiments or molecular models and mesoscale continuum theories of membranes. Such an analysis requires a detailed knowledge of the relation between the membrane fluctuations and the membrane elasticity, and it thus relies strongly on excellent theoretical descriptions.
In the present paper, we review simulation results for fluctuating lipid bilayers, with a strong focus on own work. We first give a very brief and incomplete overview over the history and the current state of membrane fluctuation theories for planar membranes. Then, in Section III, we focus on fluid membranes, construct a general form of membrane Hamiltonian for coupled monolayers and address in particular the issue of membrane tension. In Section IV A, we discuss own simulation results with a generic coarse-grained simulation model. We have studied fluctuating membranes in the liquid phase and the gel phase, and also under tension. The theoretical description is far from satisfactory in many respects, and further refinements are necessary.

II. FLUCTUATIONS OF FLAT MEMBRANES: A BRIEF OVERVIEW

The oldest and simplest Ansatz for the curvature elastic free energy density per unit area of a fluid membrane is the famous Helfrich Hamiltonian

\[ \mathcal{H} = \int dA \left\{ \frac{k_c}{2} (H - 2c_0)^2 + \bar{\kappa} K \right\}. \]  
(1)

Here \( \int dA \) is a surface integral running over the membrane area \( A \), \( H \) the total curvature at a given membrane position, \( i.e., \) the sum of the inverse curvature radii, and \( K \) is the Gaussian curvature, \( i.e., \) their product. The elastic parameters \( k_c, \bar{\kappa}, \) and \( c_0 \) are the bending rigidity, the Gaussian rigidity, and the spontaneous curvature of the membrane. For symmetric bilayers, \( c_0 \) is zero. The integral over \( K \) on closed surfaces contributes a constant which only depends on the topology, according to the Gauss-Bonnet theorem. Hence it can be omitted if the topology is fixed. Deuling and Helfrich later introduced a generalized Hamiltonian which includes a "surface tension" term \( \Gamma \int dA \) that couples directly to the membrane surface in a Lagrange multiplier sense

\[ \mathcal{H} = \int dA \left\{ \Gamma + \frac{k_c}{2} (H - 2c_0)^2 + \bar{\kappa} K \right\}. \]  
(2)

From Eq. (2), one can derive that flat fluid membranes at \( \Gamma = 0 \) should have soft long-wavelength modes that diverge as \( q^{-4} \) with the wavevector \( q \). With few exceptions (see section III for a more detailed discussion), most researchers have assumed that such membranes must be "tensionless" in the sense that they do not experience mechanical stress \( \ref{eq:1} \). Experimentally, this is not a common situation: When membranes form closed vesicles, tension usually builds up due to osmotic pressure differences between the inside and the outside of the vesicle. Tension also arises in suspended membranes clamped to a flat frame. Thus the "tensionless" case is mostly of theoretical interest. In computer simulations, it can be implemented in a straightforward manner by employing simulation methods where the shape of the simulation box is allowed to fluctuate\( \ref{eq:2} \), or by varying the system dimensions until the surface tension as evaluated from the integrated stress profile across the membrane vanishes\( \ref{eq:3} \). The \( q^{-4} \)-spectrum was first verified in computer simulations of a coarse-grained bilayer model by Goetz, Gompper, and Lipowsky\( \ref{eq:4} \), and later confirmed by numerous later studies of coarse-grained as well as atomistic models\( \ref{eq:5} \). Coupled Helfrich Hamiltonians were also found to provide a suitable framework for a quantitative theoretical description of fluctuating membrane stacks\( \ref{eq:6} \).

While the Helfrich Hamiltonian captures nicely the long-wavelength fluctuations of membranes, it is not designed for describing the structure and fluctuations on small wavelengths, \( i.e., \) of the order of the membrane thickness. Several extensions have been proposed to improve the description of membranes on these molecular scales within continuum approaches. Lipowsky and coworkers\( \ref{eq:7} \) introduced the notion of "protrusions", \( i.e., \) independent fluctuations of single lipids that supposedly govern the fluctuation spectrum of membranes on the molecular level. Recently, Brandt et al.\( \ref{eq:8} \) suggested that these protrusions correspond to lipid density fluctuations within the membrane, and corroborated this picture with atomistic and coarse-grained simulations (see also Section IV B). One way to analyze the molecular scale fluctuations of membranes in more detail, pioneered by Lindahl and Edholm, is to consider separately the height and thickness fluctuations\( \ref{eq:9} \). Thickness distortions have traditionally played a role in elastic theories for membrane mediated interactions between inclusions\( \ref{eq:10} \). Extending these approaches to the fluctuation problem, Brannigan and Brown have proposed a continuum model which allows to study height and thickness fluctuations and relate them to protein-protein interactions in one
unified framework\textsuperscript{29, 41}. The Helfrich Hamiltonian has also been extended to include internal degrees of freedom, such as local tilt\textsuperscript{42, 43}.

Most studies in the past have focused on fluid membranes. In the gel phase, the situation is complicated by the fact that the lipids have positional order. Already in 1987, Nelson and Peliti\textsuperscript{44} have analyzed by analytical theory the fluctuations of crystalline and hexatic membranes, which can sustain lateral elastic shear stress to some extent. They argued that crystalline membranes should stiffen due to fluctuations on larger length scales, such that the renormalized effective bending rigidity becomes q-dependent and scales as $k_c(q) \propto 1/q$. Their calculation was later refined by Le Doussal and Radzihovsky\textsuperscript{45}, who obtained $k_c(q) \propto q^{-0.821}$ within a self-consistent screening approximation. For hexatic membranes, the strict positional order is destroyed due to the presence of free dislocations, but lipids still have long range ‘bond-orientational’ order, i.e., the vectors connecting nearest neighbor lipids have well-defined average orientations. According to Nelson and Peliti, the bending stiffness then still increases for large length scales, but only logarithmically, $k_c(q) \sim \sqrt{-\ln(q_\xi)}$, where $\xi_T$ is a translational correlation length. Another factor that must be considered in the gel $L_\gamma' \text{ phase is the collective tilt of the molecules.}$ According to Park, however, the qualitative behavior of the bending rigidity does not differ for tilted\textsuperscript{46} and untilted\textsuperscript{47} hexatic membranes in the so-called "strong coupling limit". One might note that the bending rigidity should also slightly be renormalized in fluid membranes by fluctuations - in this case, they soften them\textsuperscript{48}. However, this only becomes relevant on length scales where the membranes exhibit large deviations from planar.

III. GENERAL CONSIDERATIONS FOR FLUID MEMBRANES

Returning to fluid membranes, we will now present a general framework for studying fluctuations of simple fluid membranes (without internal degrees of freedom) within a continuum theory. Following Brammigan and Brown\textsuperscript{29} and their predecessors\textsuperscript{36, 38, 40}, we describe planar membranes by a system of two coupled elastic surfaces, which correspond to the interfaces between the lipid region and the surrounding aqueous solvent. The membranes are taken to be almost flat and have no bubbles or overhangs, thus their surfaces can be parameterized by two unique functions $z_1, 2(x, y)$ of the projected coordinates $x$ and $y$. Equivalently, they can be characterized by their local height $H(x, y) = (z_1(x, y) + z_2(x, y))/2$ and thickness $2T(x, y) = (z_1(x, y) - z_2(x, y))$ and for simplicity, the coordinate system is chosen such that the mean height $\langle H \rangle$ is zero. We expand the Hamiltonian up to second order in the small parameters $H$ and $u = T - t_0$, where $2t_0$ is the equilibrium thickness of the membrane, keeping spatial derivatives up to second order. Based on a few elementary symmetry assumptions,

(i) Isotropy within the membrane plane

(ii) Translational invariance within the membrane and in the $z$ direction

(iii) Invariance with respect to flipping $H \rightarrow -H$ (i.e., the bilayer is symmetric.)

we can then construct the following very general Hamiltonian for the bilayer fluctuations of a membrane with projected area $A_p$,

$$\mathcal{H} = \frac{1}{2} \int_{A_p} dx \; dy \left\{ a_2 \langle \nabla H \rangle^2 + a_3 \langle \Delta H \rangle^2 + b_1 u^2 + b_2 \langle \nabla u \rangle^2 + b_3 \langle \Delta u \rangle^2 \right\} + \text{boundary terms}. \quad (3)$$

Other terms are either forbidden by symmetry (such as all cross-terms containing both $H$ and $u$) or can be incorporated into the boundary terms. For example, the curvature-like term $\Delta u$ gives a boundary term, and $u \Delta u$ can be converted into $\langle \nabla u \rangle^2$ (plus boundary terms) by means of a partial integration.

We emphasize that the derivation of Eq. (3) does not rely on any specific assumptions regarding the underlying physics. For example, nowhere was it necessary to require that we are dealing with bilayers consisting of well-separated monolayers, that lipids have fixed volume, etc. These assumptions come in when finding an interpretation for the elastic parameters $a_i$ and $b_i$. Brammigan and Brown\textsuperscript{29} have derived a Hamiltonian of the form (3) based on a physical picture where...
\( \mathcal{H} \) describes the bending modes of two coupled monolayers, each with area compressibility, spontaneous curvature, and bending rigidity, and subject to the constraint that the volume per lipid is constant. In this case, the tensionlike parameter \( a_2 = \Gamma \) vanishes for tensionless bilayers, the parameter \( b_2 \) can be associated with the area compressibility \( k_A \) via \( b_1 = k_A/t_0^2 \), the parameters \( a_3 \) and \( b_3 \) are identified with the bending rigidity \( k_c \) of the bilayer, \( a_3 = b_3 = k_c \), and the parameter \( b_2 \) is related to the spontaneous curvature \( c_0 \) of monolayers, \( b_2 = -4k_c\zeta/t_0 \), where \( \zeta = c_0 - \Sigma d c_0/\Sigma \) depends on \( c_0 \) and its derivative with respect to the lipid area, \( \Sigma \). In addition, Brannigan and Brown introduced additional protrusion fields \( \lambda_1, \lambda_2(x, y) \) following Lipowsky and Grotehans [39], which describe short-wavelength fluctuations on both surfaces and are taken to be independent of each other and of the bending degrees of freedom \( z_i(x, y) \). The total height and thickness are thus given by \( h(x, y) = H + (\lambda_1 + \lambda_2)/2 \) and \( t(x, y) = T + (\lambda_1 - \lambda_2)/2 \), and the fluctuation spectrum in Fourier space reads [29, 49]

\[
\langle |h_\mathbf{q}|^2 \rangle = \frac{k_B T}{\Gamma q^2 + k_c q^4} + \frac{k_B T}{2(k_\lambda + \gamma_\lambda q^2)} \tag{4}
\]

\[
\langle |t_\mathbf{q}|^2 \rangle = \frac{k_B T}{k_A/t_0^2 - 4k_c\zeta^2/t_0 + k_c q^4} + \frac{k_B T}{2(k_\lambda + \gamma_\lambda q^2)} \tag{5}
\]

where the parameters \( \gamma_\lambda \) and \( k_\lambda \) characterize the protrusion modes.

The parameter \( \Gamma \) deserves special attention [49]. It depends on the "frame tension" \( \Gamma_{\text{frame}} \), i.e., the lateral stress within the projected membrane plane (the \( (x, y) \) plane). The frame tension is a mechanical tension which serves to maintain the projected area \( A_p \) (corresponding to the "lateral tension" in Ref. [21]). The parameter \( \Gamma \) is an intrinsic coefficient characterizing the membrane fluctuations. Therefore, the relation between \( \Gamma \) and \( \Gamma_{\text{frame}} \) is not clear a priori.

The problem already arises when considering single fluctuating surfaces (no protrusions) governed by the Helfrich Hamiltonian [8]. For that case, it has been discussed intensely and somewhat controversially in the past [50–64]. For single almost flat surfaces, the linearized Helfrich Hamiltonian reads [65]

\[
\mathcal{H} = \frac{1}{2} \int_{A_p} dx \, dy \left\{ \frac{\Gamma}{(\nabla h)^2} + k_c (\Delta h)^2 \right\}, \tag{6}
\]

(the contribution of the Gaussian curvature has been omitted), and the corresponding fluctuation spectrum in Fourier space takes the form

\[
\langle |h_\mathbf{q}|^2 \rangle = \frac{k_B T}{\Gamma q^2 + k_c q^4}. \tag{7}
\]

Assuming that the number of degrees of freedom (the number of lipids) is fixed, the free energy \( F \) of the linearized Hamiltonian (6) can be evaluated exactly as a function of \( \Gamma \) and the projected area \( A_p \). The frame tension can then be evaluated as the derivative \( \Gamma_{\text{frame}} = \partial F/\partial A_p \), giving [55]

\[
\Gamma_{\text{frame}} = \Gamma \left( 1 + \frac{k_B T}{8\pi k_c} \ln \left( 1 + \frac{4\pi k_c N}{\Gamma A_p} \right) \right) - \frac{k_B T N}{2A_p}, \tag{8}
\]

which clearly differs from \( \Gamma \). Based on this observation, some authors [58–60] have recently argued that the tensionlike parameter in the fluctuation spectrum should differ from the frame tension – more precisely, they claimed that the "fluctuation tension" \( \Gamma_{\text{fluc}} \), defined as the coefficient which drives the \( q^2 \)-contribution to the inverse fluctuation spectrum in an equation of the form (cf. Eq. 7)

\[
\langle |h_\mathbf{q}|^2 \rangle = \frac{k_B T}{\Gamma_{\text{fluc}} q^2 + \mathcal{O}(q^4)} \tag{9}
\]

differs from the frame tension \( \Gamma_{\text{frame}} \). Within the linear theory (6), one of course has \( \Gamma_{\text{fluc}} = \Gamma \), but this is not true in general.

The problem with this argument is that the Hamiltonian (6) is not exact – it is just the linearized version of Eq. (2). Nonlinear contributions renormalize \( \Gamma_{\text{frame}} \) and, even more importantly, \( \Gamma_{\text{fluc}} \). Cai et al. [52] have examined the fluctuations of membranes fixed area per lipid in the grand
canonical ensemble $\langle \mu, A_p, T \rangle$, where the number of lipids is allowed to fluctuate. (In this case the frame tension is related to the grandcanonical potential via $\Omega(\mu, A_p, T) = \Gamma_{\text{frame}} A_p$.) They argued on very general grounds that the requirement of gauge invariance – invariance with respect to a rotation of the "projected plane" – implies $\Gamma_{\text{frame}} = \Gamma_{\text{fluc}}$ in the thermodynamic limit. Farago and Pincus[56, 62] presented a similar argument for the case of membranes with fixed projected area and fixed number of lipids ($(N, A_p, T)$ ensemble).

Now the existence of the thermodynamic limit must be questioned for "flat" membranes in the floppy low-tension regime, because they bend around on length scales larger than the persistence length. Therefore, infinitely large membranes at low tension are no longer planar. Furthermore, infinitely large membranes at finite tension can always lower their free energy by forming a pore, therefore they are not at thermodynamic equilibrium in the "thermodynamic limit"[22]. If there is no thermodynamic limit, different ensembles are no longer equivalent, and results for the $(\mu, A_p, T)$ ensemble or the $(N, A_p, T)$ ensemble are not necessarily valid for the $(N, \Gamma_{\text{frame}}, T)$ ensemble, which is practically more relevant and often implemented in simulations. To clarify the situation, the present author has carried out high-precision computer simulations of a one-dimensional infinitely thin "membrane", i.e., a stiff one-dimensional line, in the $(N, \Gamma_{\text{frame}}, T)$ ensemble for frame tensions $\Gamma_{\text{frame}}$ and bending rigidities $k_c$ spanning several orders of magnitude[61]. It turned out that the fluctuations could be described very accurately by analytical predictions of the linearized theory (6), if the tension parameter $\Gamma$ is replaced by a renormalized tension parameter $\Gamma_{\text{fluc}}$. This is demonstrated in Fig.1 for the example of the squared amplitude of fluctuations $w^2 = \langle h^2 \rangle - \langle h \rangle^2$.

The numerical results thus show that the fluctuations are indeed driven by the frame tension. They were confirmed by subsequent simulations of Farago, also of one dimensional membranes, which emphasize the role of the gauge invariance[62]: For Hamiltonians which are not gauge invariant, $\Gamma_{\text{frame}} = \Gamma_{\text{fluc}}$ is no longer valid.

Summarizing and concluding, Eq. (6) can be used to calculate fluctuation spectra of single membranes, if it is interpreted as an effective Hamiltonian with renormalized coefficients, $\Gamma \rightarrow \Gamma_{\text{fluc}}$ and $k_c \rightarrow k_{c,\text{fluc}}$. In particular, the fluctuation tension renormalizes to the frame tension, $\Gamma_{\text{fluc}} = \Gamma_{\text{frame}}$. The bending rigidity should be renormalized as well[48, 56, 60], but in practice, the renormalization effect is negligible (as mentioned in Sec. III $k_c$ softens slightly on large length scales). This conclusion should also hold for more sophisticated elastic membrane models such as the coupled surface models, Eqs. (3) and (4). Thus we conclude that the parameter $\Gamma$ should be
identified with the frame tension.

IV. FLUCTUATIONS IN A GENERIC COARSE-GRAINED MODEL FOR LIPID BILAYERS

The general theoretical considerations presented in the previous sections can best be tested by comparison with explicit molecular simulations of bilayers. In this section, we discuss a series of simulations of bilayers in different states, fluid and gel, and under tension, in a simple lipid bilayer model\[67–69\]. This model has been constructed in the spirit of coarse-grained approaches[8, 14] from a successful model for Langmuir monolayers[70–75] and has been shown to reproduce the most important membrane phases and phase transitions of DPPC[68], \ie, the transitions between the $L\alpha$-phase, the modulated $P\beta'$ phase, and the $L\beta'$-phase. It has also been used to test predictions of the elastic theory regarding membrane-protein and membrane-mediated protein-protein interactions[30, 76]. Here, we will focus on the undulations.

A. The Model

In our model[68, 69], each "lipid" consists of a chain of seven beads with one head bead of diameter $\sigma_h$ followed by six tail beads of diameter $\sigma_t$. Non-bonded beads interact, via a truncated and lifted Lennard-Jones potential:

$$V_{\text{bead}}(r) = \begin{cases} V_{\text{LJ}}(r/\sigma) - V_{\text{LJ}}(r_c/\sigma) & \text{if } r < r_c \\ 0 & \text{otherwise} \end{cases} (10)$$

with

$$V_{\text{LJ}}(x) = \epsilon \left( x^{-12} - 2x^{-6} \right) (11)$$

where $\sigma$ is the mean diameter of the two interacting beads, $\sigma_{ij} = (\sigma_i + \sigma_j)/2$ ($i, j = h$ or $t$). Head-head and head-tail interactions are purely repulsive ($r_c = \sigma$), while tail-tail interactions also have an attractive contribution ($r_c = 2\sigma$). Bonded beads are connected by FENE (Finitely Extensible Nonlinear Elastic) springs with the spring potential

$$V_{\text{FENE}}(r) = -\frac{1}{2} \epsilon_{\text{FENE}} \left( \Delta r_{\text{max}} \right)^2 \log \left( 1 - \left( \frac{r - r_0}{\Delta r_{\text{max}}} \right)^2 \right), (12)$$

where $r_0$ is the equilibrium distance, $\Delta r_{\text{max}}$ the maximal deviation, and $\epsilon_{\text{FENE}}$ the FENE spring constant. In addition, a bond-angle potential applies,

$$V_{\text{BA}}(\theta) = \epsilon_{\text{BA}} (1 - \cos(\theta)). (13)$$

The aqueous environment of the membrane is modeled with “phantom” solvent beads[67], which interact with lipids like head beads ($\sigma_s = \sigma_h$), but have no interactions with each other.

Systems of up to 7200 lipids were studied using Monte Carlo simulations at constant pressure, temperature, and surface tension with periodic boundary conditions. Here the pressure couples to the total volume $V$ of the simulation box, and the surface tension couples to the total area $A$. The simulation box is a parallelepiped and all side lengths and angles fluctuate independently during the simulation. Run lengths were up to 8 million Monte Carlo steps, where one Monte Carlo step corresponds to one Monte Carlo move per bead, and moves that change the shape of the simulation box were attempted every 50th Monte Carlo step. The code was parallelized using a domain decomposition scheme described in Ref. 69. To study the fluctuations of the membrane, we have determined the mean head positions $z_1(x, y)$, $z_2(x, y)$ of the two monolayers on a grid $(x, y)$, and derived the height and thickness profiles $h(x, y) = (z_1 + z_2)/2$ and $t(x, y) = (z_1 - z_2)/2$. The spectra were Fourier transformed according to

$$f_{q_x, q_y} = \frac{L_x L_y}{N_x N_y} \sum_{x, y} f(x, y) e^{-i(q_x x + q_y y)} (14)$$
FIG. 2: Slices through configurations of our model lipid bilayers (a) in the fluid phase $L_\alpha$ ($T = 1.3\epsilon/k_B$), (b) the gel phase $L_\beta'$ ($T = 1\epsilon/k_B$), and (c) the asymmetric ripple phase $P_\beta'$ ($T = 1.18\epsilon/k_B$)
FIG. 3: Radially averaged Fourier spectrum of the height (full circles) and thickness (open squares) fluctuations of tensionless membranes in the fluid state ($T = 1.3\epsilon$). Dashed lines show the best fit to the elastic theory, Eq. (4) and (5). After Ref. 30.

around $10\sigma_t$. At high $q$, the thickness fluctuation spectrum follows closely the height fluctuation spectrum [29]. This is consistent with Eqs. (4) and (5) and a direct consequence of the fact that the protrusion fluctuations of the two monolayers are independent of each other and of the bending fluctuations. Our simulation data can be fitted very nicely with the prediction of the elastic theory, Eqs. (4) and (5) with $\Gamma = 0$.

An interesting alternative way of analyzing the data has very recently been introduced by Brandt et al. [31]. Instead of determining continuous surfaces $z_{1,2}(x, y)$ in real space by a binning procedure and then Fourier transforming these functions, they proposed to analyze directly the Fourier transform of the molecular positions, i.e., the quantities

$$\bar{z}_\alpha(q) = \frac{1}{N_\alpha} \sum_{l=1}^{N_\alpha} (z_l - \langle z \rangle) e^{i q \cdot r_l},$$

(15)

where the sum $l$ runs over the $N_\alpha$ lipids in monolayer $\alpha$ ($\alpha = 1, 2$), $r_l$ gives the positions of the head group of these lipids, and $\langle z \rangle = \frac{1}{N_\alpha} \sum_{l=1}^{N_\alpha} z_l$ gives the average height of the head groups within a monolayer. In the spirit of Section III, one can define a height and a thickness spectrum from the quantities $\bar{h} = \frac{1}{2}(\bar{z}_1 + \bar{z}_2)$ and $\bar{u} = \frac{1}{2}(\bar{z}_1 - \bar{z}_2)$. On molecular length scales ($q > 1\text{nm}^{-1}$), the two spectra are equal and proportional to the number density structure factor, $\langle |\bar{h}(q)|^2 \rangle = \langle |\bar{u}(q)|^2 \rangle = Cu^2 \langle |\rho(q)|^2 \rangle$, where the proportionality factor $C = (\sigma_{1,1}^2 + \sigma_{2,2}^2)/2$ is the mean width of the distributions of $z$-positions within the monolayers 1 and 2. After subtracting the contribution of density fluctuations, the remaining height spectrum $\langle |\bar{h}(q)|^2 \rangle - Cu^2 \langle |\rho(q)|^2 \rangle$ was found to follow closely a $q^{-4}$ behavior down to the noise level, and on length scales even beyond $q \sim 1\text{nm}^{-1}$. This was observed consistently both in coarse-grained and atomistic simulations [31, 37], and it strongly suggests that the ”protrusions” observed in the binning method are really a signature of density fluctuations.

The ”direct Fourier method” by Brandt et al. [31] provides new insights and is more elegant than the binning method, because it does not involve the somewhat arbitrary binning procedure. However, the comparison of the resulting spectra with theory is less straightforward than in the binning method, since the quantity $\bar{z}$ is the Fourier transform of a product, $z(x, y) \rho(x, y)$. In the remainder of the paper, we will only discuss results obtained with the binning method.
Coming back to our model, the fit to the elastic theory shown in Fig. 3 allows us to extract the elastic constants of our model membrane, most notably the bending stiffness \( k_c = 6.2 \pm 0.4 \) and the rescaled area compressibility \( k_A/\ell_0^2 = 1.3 \pm 0.3 \sigma_2^\ell \approx 3.6 \cdot 10^{-20} \text{J/} \text{nm}^4 \). As another elastic parameter, the spontaneous curvature of a monolayer can be obtained from the first moment of the pressure profile across the monolayer, giving \( c_0 = -0.05 \pm 0.02 \) \( \sigma^{-1} \approx -0.08 \text{nm}^{-1} \). These elastic constants have the same order of magnitude as those obtained from all-atom simulations of DPPC[23], \( k_c \sim 4 \cdot 10^{-20} \text{J}, k_A/\ell_0^2 \sim 1.1 \cdot 10^{-20} \text{J/} \text{nm}^4 \), and[30] \( c_0 \sim -0.04 \) \( \sim -0.05 \text{nm}^{-1} \), or from experimental estimates[51], \( k_c \sim 5 \cdot 10^{-20} \text{J}, k_A/\ell_0^2 \sim 0.1 \cdot 10^{-20} \text{J/} \text{nm}^4 \), \( c_0 \sim -0.04 \text{nm}^{-1} \). The agreement is remarkable, given that our model is highly simplified and has not been adjusted to reproduce the elastic properties of the bilayers. Our findings indicate that the elastic material parameters of membranes are largely generic and determined by global quantities such as the ratio of lipid area and squared membrane thickness (which is the quantity that was “adjusted” to map our model membranes on real lipid bilayers as discussed above).

Over all, we can conclude that the elastic theory gives an excellent description of the fluctuations of tensionless fluid membranes. We will now consider the less well-studied case of membranes subject to a frame tension \( \Gamma \). As pointed out earlier, the “tensionless” case is a rather academic one since most membranes in nature are slightly under stress. However, even such slightly stretched membranes can be considered quasi-tensionless in the “floppy” regime[22] where the stretching energy is small compared to the bending energy on the length scales \( \ell \) of interest, \( \Gamma/\ell^2 \ll k_c \).

For larger tensions and/or on larger length scales, \( \ell > \sqrt{k_c/\Gamma} \), the fluctuations are dominated by the tension and follow a \( \ell^{-2} \)-behavior. If the stretching energy becomes large on molecular length scales \( \ell_0 \), \( \Gamma/\ell^2 > k_BT \), the tension may also induce structural changes in the membrane[49]. Such strongly stretched membranes are only metastable and will eventually rupture, but they can exist on short time scales, e.g., under the influence of transverse ultrasonic pulses, and the effect of such pulses on membranes is of considerable medical interest[83]. Therefore, a number of simulation studies have also been devoted to membranes under tension[58, 59, 76, 84–89] or compressed membranes[90].

In our simulation model, the strongly-stretched regime is reached at frame tensions around \( \Gamma \sim 1/\sigma_2^\ell \). Fig. 4 shows the lipid area as a function of applied tension. One can clearly distinguish three regimes with different compressibilities, two stiffer ones at low and high \( \Gamma \) and a softer regime in between. A similar softening at reduced pressure one (in natural model units) was also observed in a different membrane model by Goetz and Lipowsky[21]. The softening is associated with structural rearrangements within the membrane such that the upper and lower monolayers become increasingly interdigitated (see snapshots in Fig. 4). At very high tensions, it becomes difficult to distinguish between the monolayers; the membrane rather has the structure of a single, disordered, interdigitated sheet. Nevertheless, the general considerations of Section 11[49] should still hold. Nowhere did they depend on the fact that one deals with well-separated monolayers. The same considerations can be made if \( z_1 \) and \( z_2 \) simply parametrize the two outer surfaces of the membrane. Therefore, Eqs. 4 and 5 should still apply, with the parameter \( \Gamma \) being the frame tension \( \Gamma \).

In the simulations, however, the elastic fit is only satisfactory at low to moderate tensions. In the strongly-stretched regime at \( \Gamma > 1/\sigma_2^\ell \), the fits fail to reproduce the low-\( q \) behavior of the height fluctuations; they consistently underestimate the height of the long-wavelength fluctuations. Good fits can be obtained if the parameter \( \Gamma \) is taken to be a fit parameter, but the fitted values \( \Gamma \) are then reduced by almost a factor of two, compared to the true frame tension. Thus membranes under strong tension are distinctly softer than predicted by theory on large length scales.

At this point, we recall the discussion of the relation between the frame tension \( \Gamma \) and the fluctuation tension \( \Gamma \) in Section 11[51] There, we had provided strong evidence for \( \Gamma = \Gamma \) from simulations of simple structureless one-dimensional membranes. If fluctuations do not renormalize \( \Gamma \) in one dimension, one would not expect them to do so in two dimensions, since the effect of fluctuations is usually smaller in higher dimensions.

Nevertheless, the effective fluctuation tension, \( \Gamma \equiv \Gamma \), in our molecular simulations is clearly smaller than the frame tension, \( \Gamma \), at high tensions \( \Gamma \). The main difference to the simple model discussed in Section 11 is that our molecular membranes have internal structure. As mentioned earlier, Imparato et al and Stecki have also reported discrepancies between \( \Gamma \) and \( \Gamma \) in molecular membrane simulations[57, 59]. In these studies, \( \Gamma \) was found to be larger than \( \Gamma \), which is opposite to our observation. Furthermore, they report discrepancies already in the zero tension regime, where we find \( \Gamma \approx \Gamma \approx 0 \) in agreement with most other simulation
FIG. 4: Top: area per lipid vs. tension in the fluid phase \( (T = 1.3\epsilon/k_B) \), together with linear fits to the regimes \( \Gamma_{\text{frame}} = 0 - 1\epsilon/\sigma_t^2 \), \( \Gamma_{\text{frame}} = 1 - 2\epsilon/\sigma_t^2 \), and \( \Gamma = 3 - 4\epsilon/\sigma_t^2 \). Inset focuses on the quasi-tensionless regime. Bottom: Slices through bilayer configurations in the fluid phase \( (T = 1.3\epsilon/k_B) \) at different tensions as indicated (in units of \( \epsilon/\sigma_t^2 \)). Chains pointing upward (from head to tail) are shown in red, chains pointing downward are shown in red. The size of the beads are not to scale. From Refs. 49, 91.

studies\(^{24–26, 28–31, 79}\) and also with the theoretical arguments of Cai et al \(^{52}\) and Farago \(^{56, 62}\). We note that the latter arguments are general enough that they should also apply to tensionless membranes with internal structure (subject to question marks regarding the thermodynamic limit as discussed in Section III). However, they cannot be applied to strongly stretched membranes, since the stretching breaks rotational symmetry.

So far, we have no explanation for our findings. We can only speculate that it must be a nonlinear effect, related to higher order terms in the Hamiltonian. For example, one can argue that there should be a higher order coupling between thickness and height fluctuations of the form \( \mathcal{H}' \sim -\Gamma_{\text{frame}} \int \! \! \! \int dy \left[ \nabla^2 u \right]^2 \), which might renormalize \( \Gamma \) to a value \( \Gamma_{\text{fluc}} \) that differs from \( \Gamma_{\text{frame}} \) at high tensions \(^{49}\). It is remarkable that we observe the deviations in a tension regime where the tension induces structural rearrangements in the membrane. The softening might be a signature of a nearby phase transition, or of an instability with respect to rupture. However, no sign of actual rupture events were observed in the membranes on the time scales of the simulations.

C. Gel phase

The situation becomes even worse in the gel phase. Fig. 6 shows the radially averaged thickness and height fluctuation spectra for tensionless membranes in the \( L_{\beta'} \) phase \(^{92} \) \( (T = 1\epsilon/k_B) \). At first sight, the qualitative features seem similar to those in the fluid phase, even though the amplitudes of the fluctuations are much smaller. As in the fluid phase, the height spectrum diverges at low \( q \), the thickness spectrum features a soft peristaltic mode at wavelengths around \( 10\sigma_t \), and both spectra are nearly identical at high \( q \). The fit to the elastic theory, Eqs. (4) and (5), is not as good as in the fluid state. Most notably, long-wavelength fluctuations are suppressed, compared to the theoretical prediction, and one never encounters a bending regime with \( \langle |h(q)|^2 \rangle \sim q^{-4} \). This is not surprising, given that one expects the shearing modes in gel membranes to renormalize the bending...
rigidity at small $q$, as discussed in the introduction. The in-plane shear modes introduce effective interactions between height fluctuation modes, which lead to a stiffening on large length scales \[44\]. However, even the strongest possible renormalization $k_c(q) \propto q$, corresponding to quasi-crystalline membranes\[44\], does not fully account for the discrepancy. It would result in a $q^{-3}$ divergence of the height fluctuations at small $q$, which is still too steep.

Despite these quantitative discrepancies, Fig. 6 suggests that the elastic theory describes the fluctuations of gel membranes reasonably well except for the large-wavelength height fluctuations. Unfortunately, the radial average shown in Fig. 6 hides the true extent of the problem. It becomes apparent when one inspects the fluctuations in tilt direction and perpendicular to the tilt direction separately, as shown in Fig. 7. The fluctuation spectra in these directions differ strongly from the radially averaged spectra, and they are not at all compatible with the elastic theory. The height fluctuations are mostly suppressed in the direction of tilt (with the exception of a broad peak around $q^2 \sim 0.15 \sigma_t^2$ to be discussed below), whereas they are relatively strong in the perpendicular direction. The thickness fluctuations show the opposite trend. Their spectrum mostly matches the radially averaged thickness spectrum in the direction of tilt, and it is almost entirely suppressed in the perpendicular direction.

Two features of these directionally resolved spectra are particularly remarkable. First, both the thickness and the height fluctuation spectra in the direction of tilt feature distinct maxima around $q^2 \sim 0.15 \sigma_t^2$ and $q^2 \sim 0.65 \sigma_t^2$, corresponding to soft modes with wavelength $\lambda \sim 8\sigma_t$ and $2\lambda \sim 16\sigma_t$. The wavelength $2\lambda$ coincides with the period of the ripple phase\[68\]. Thus the fluctuations in tilt direction carry the signature of the modulated phase which intrudes between the liquid and the gel state.

Second, the amplitudes of the height fluctuations in the perpendicular direction follow a $q^{-2}$
behavior over the whole $q$-range, suggesting that they are controlled by an interfacial tension rather than a bending rigidity. Such a behavior can be rationalized if one assumes that the shape fluctuations of the membrane are governed by "out-of-layer" fluctuations of the lipids (up- and down displacements without splaying) rather than bending modes which involve lipid splay\cite{92}. One would expect the out-of-layer fluctuations to eventually give way to bending-dominated fluctuations on very large length scales, but this is not observed in our simulations.

In sum, the elastic theory does not describe adequately the fluctuations in the gel phase, even though a naive inspection of the radially averaged fluctuation spectra suggests otherwise. Due to the ordered anisotropic structure of the gel membrane, the fluctuation spectra are highly anisotropic and they carry the signature of the ripple phase. On very large length scales, the fluctuation spectra will presumably adopt an asymptotic behavior which is compatible with the theoretical expectations outlined in the introduction. However, our findings suggest that the microscopic unit in such a description is not the molecular length scale, but rather a multiple of the period of the ripple phase, which is of order 10nm. Moreover, the anisotropy imprinted on the membrane shapes by the tilt order of the lipids may persist on length scales up to micrometers\cite{93}, and only on even larger length scales can then the membrane be treated as an isotropic polycrystalline sheet with multiple tilt directions. Based on these considerations, we expect that the asymptotic behavior will only be reached on length scales much larger than hundreds of nanometers, if not micrometers. Our simulation systems, which had linear dimensions of around $\sim 35$nm, were much too small to capture this limit. On these smaller length scales, the elastic behavior results from a complex interplay of contributions which cannot easily be told apart, and suitable continuum theories are still to be developed. Such continuum models might also contribute to a better understanding of the mechanisms driving the formation of the ripple phase.
FIG. 7: Fourier spectrum of the height (full circles) and thickness (open squares) fluctuations of the same membranes of Fig. 6 for wavevectors parallel (top) and perpendicular (bottom) to the tilt direction. The thin solid lines reproduce the thin solid lines from Fig. 6 (the fit of the radially averaged spectra to the elastic theory) for comparison. Vertical arrows in upper panel mark the positions of peaks in both the height and thickness fluctuation spectrum. Thick grey line in lower panel indicates $q^{-2}$-slope. After Ref. 92.

V. CONCLUSIONS

The analysis of membrane fluctuations provides an important link between molecular models and elastic continuum models. Despite the fact that fluctuation analyses have a long tradition, there are still amazingly many open questions. Membrane undulations can be described and understood at a quantitative level for fluid and tensionless (or floppy) membranes. In this case, the fluctuation analysis can be used to extract elastic parameters of the membranes, which can then be used to bridge between different representations of membranes in the context of multiscale modeling approaches. In all other cases – stretched membranes under tension, membranes in phases that are other than fluid – the connection between molecular model and an appropriate continuum theory does not yet succeed satisfactorily. Much work still remains to be done.
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