Role of molecular tilt in thermal fluctuations of lipid membranes

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Long-wavelength thermal fluctuations of lipid membranes are adequately described by the Helfrich elastic model. On the other hand, fluctuations of wavelengths comparable with bilayer thickness exhibit significant deviations from the prediction of the elastic model and are typically assumed to be dominated by microscopic surface tension due to protrusion of lipid molecules into the solvent. We present evidence that the short-wavelength modes of a lipid membrane are dominated by fluctuations of the tilt of lipid molecules with respect to the membrane normal rather than the microscopic surface tension. We obtain an expression for the spectral intensity of the thermal membrane fluctuations by appealing to the Hamm-Kozlov model, which accounts for both membrane bending and lipid tilt contributions to the total membrane energy but neglects the contributions of the microscopic surface tension. The tilt and the bending fluctuations obtained from our coarse-grained molecular dynamics simulations of a dipalmitoylphosphatidylcholine lipid bilayer show good agreement with the theory. Furthermore, the obtained tilt and bending moduli are in close agreement with experimentally determined values. The magnitude of the microscopic protrusion tension estimated from our simulations is significantly smaller than that of the tilt modulus. These results indicate that the membrane fluctuations can be adequately described by a macroscopic elastic model down to scales of interlipid distance provided one accounts for the tilt fluctuations.

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I. INTRODUCTION

Cells undergo many processes involving membrane configuration change. Examples of such processes include chemotaxis, wherein the plasma membrane deforms to produce protrusions [1], and endocytosis (exocytosis), wherein the membrane is remodeled to form a near-spherical invagination (projection) [2,3]. In order to predict membrane configurations, one must fully understand the chemical composition of the membrane and the corresponding elastic properties. While the chemistry can be very complex and often a subject of debate, the elastic properties can be assessed by investigations of model systems, such as lipid bilayers.

In his pioneering work on equilibrium configuration of homogeneous bilayers, Helfrich [4] assumed that the dominant contribution to the membrane energy is associated with bending of the membrane. Under this assumption, he showed that the free energy F_H of a deformed membrane is given by

$$F_H = \int \left[\frac{\kappa}{2}(J - J_s)^2 + \bar{\kappa} K \right] dA, \tag{1}$$

where J and K are the mean and Gaussian curvatures of the membrane, respectively, J_s is the spontaneous curvature, κ is the bending modulus, and $\bar{\kappa}$ is the Gaussian modulus. The equilibrium configuration of a membrane corresponds to the particular shape that minimizes the total energy. It turns out that the second (Gaussian) term is the same for all membrane configurations containing no tears or discontinuities. The configuration corresponding to the minimal energy is therefore completely determined by the first term (proportional to κ).

In particular, for a homogeneous lipid bilayer with vanishing spontaneous curvature J_s, the Helfrich model predicts that the magnitude of thermal fluctuations of a bending mode with wave number q is proportional to $q^{-\alpha}$ with $\alpha=4$. Several independent molecular dynamics (MD) simulations employing detailed atomistic [5,6] and coarse-grained models [7–10] have demonstrated that the bilayer thermal fluctuations follow this power law quite closely with $\alpha \approx 4$ for wavelengths almost as small as the membrane thickness.

For wavelengths smaller than the membrane thickness, it is observed [5–8] that the fluctuations of lipid membranes scale as $q^{-\beta}$ with $\beta \approx 2$. This scaling is the same as that for fluctuations driven by surface tension [11] and is usually attributed to the so-called *microscopic* or *protrusion* surface tension [12,13]. The microscopic tension is caused by contact between a polar solvent and hydrophobic tails of lipids protruding out of a bilayer.

In order to estimate the effects of the protrusion tension, Lipowsky and Grotehans [12,13] approximated the lipid molecules by rigid rods perpendicular to the bilayer surface and proposed the following model for the energy of the protrusion tension:

$$F_P = \sum_{\langle ij \rangle} \frac{a_0 \gamma}{n} |h_i - h_j|. \tag{2}$$

Here, the summation is performed over the pairs of nearest neighbors $\langle ij \rangle$, n is the number of the nearest neighbors of each lipid molecule, a_0 is the circumference of the lipid cross section, γ is the free energy per unit area of the interface between the nonpolar part of a lipid molecule and the polar solvent, and h_i is the magnitude of the protrusion of the ith molecule. It is shown that this model belongs to the same universality class as the model for the macroscopic surface tension [12,13],

$$F_P = \frac{\gamma_p}{2} \int [\nabla h(x,y)]^2 dxdy. \tag{3}$$

Here, the function $z=h(x,y)$ specifies the membrane surface, $\gamma_p = c_\gamma (a_0 \gamma)^2 / k_B T$ is the protrusion tension coefficient, k_B is the Boltzmann constant, T is the temperature, and c_γ is a constant, $c_\gamma \approx 0.067$.

Model (3) suggests that the protrusion modes correspond to the q^{-2} scaling of the spectral intensity of the membrane bending modes. However, a direct combination of the Helfrich model for the bending fluctuations and the Lipowsky-Grotehans model for the protrusion tension contradicts the observed scaling of the membrane fluctuations. In fact, assuming that the total membrane energy is a sum of the Helfrich free energy F_H given by Eq. (1) and the protrusion tension free energy F_P given by Eq. (3) leads to the following expression for the membrane bending fluctuations:

$$\langle |\hat{h}(\mathbf{q})|^2 \rangle = \frac{k_B T}{\kappa |\mathbf{q}|^4 + \gamma_p |\mathbf{q}|^2}, \tag{4}$$

where $\hat{h}(\mathbf{q})$ is the Fourier transform of the membrane surface $h(x,y)$. This equation predicts that, for small q, the fluctuations will be dominated by the q^{-2} scaling and, for large q, they will be dominated by the q^{-4} scaling, which is the opposite of what is observed in MD simulations.

In order to circumvent this discrepancy, two empirical models have been proposed, both of which assume that the protrusion effects do not contribute to long-wavelength membrane fluctuations and the bending modes do not contribute to the short-wavelength fluctuations. In one of these models, it is assumed that the membrane bending is given by [5,6]

$$\langle |\hat{h}(\mathbf{q})|^2 \rangle = \begin{cases} \dfrac{k_B T}{\kappa |\mathbf{q}|^4}, & q < q_0, \\ \dfrac{k_B T}{\gamma_p |\mathbf{q}|^2}, & q > q_0. \end{cases} \tag{5}$$

The cutoff wave number q_0 is typically chosen to correspond to the bilayer thickness h_0.

An alternative to the above model is the following expression which yields a smooth transition between the bending and the protrusion fluctuations modes [7]:

$$\langle |\hat{h}(\mathbf{q})|^2 \rangle = k_B T \left(\frac{1}{\kappa |\mathbf{q}|^4} + \frac{1}{\gamma_p |\mathbf{q}|^2} \right). \tag{6}$$

However, both models (5) and (6) are obtained empirically and the underlying assumption of the failure of the Helfrich elastic model at short wavelengths is not rigorously justified.

The goal of the current work is to demonstrate that both long- and short-wavelength membrane fluctuations can be adequately described by an elastic model provided one accounts for the *tilt* fluctuations of lipid molecules. We show that the tilt fluctuations (neglected in the models discussed above) are dominant at short wavelengths and that the q^{-2} scaling of the membrane fluctuations is caused almost exclusively by the lipid tilting. To this end, we consider an extension of the Helfrich model proposed by Hamm and Kozlov [14]. The Hamm-Kozlov (HK) model explicitly accounts for the bending and tilt contributions to the membrane energy but neglects the surface free energy between lipid tails and solvent, thereby neglecting the protrusion tension. We demonstrate that the empirical equation (6) is a natural consequence of the HK model and that the second term of this equation corresponds to the tilt fluctuations instead of the commonly assumed protrusion tension. The model predictions are verified by coarse-grained MD simulations of a lipid bilayer. Although the HK model does not explicitly account for the protrusion tension, we use the results of our MD simulations to demonstrate that the contribution of the latter to the membrane dynamics is significantly smaller than that of the tilt fluctuations. Finally, we demonstrate that application of model (5) to the analysis of the membrane fluctuations leads to an underestimate of the bending modulus κ.

II. THEORY OF TILT AND BENDING FLUCTUATIONS

The tilt of a lipid molecule with respect to a lipid monolayer surface is defined using the *tilt vector* $\mathbf{t}$ which quantifies the deviation of the director $\mathbf{n}$ of the molecule from the normal $\mathbf{N}$ to the monolayer surface [14],

$$\mathbf{t} = \frac{\mathbf{n}}{\mathbf{n} \cdot \mathbf{N}} - \mathbf{N}. \tag{7}$$

Here, the vectors $\mathbf{N}$ and $\mathbf{n}$ are of unit length. Both the tilt and the bending of a lipid monolayer are captured by the effective total curvature $\tilde{J}$, defined as the divergence of the director $\mathbf{n}$ along the monolayer surface—i.e., $\tilde{J} = \nabla \cdot \mathbf{n}$. In the case of a vanishing tilt, the director $\mathbf{n}$ of the lipids coincides with $\mathbf{N}$ and the effective total curvature becomes identical to the mean curvature of a monolayer, $J = \nabla \cdot \mathbf{N}$ [15]. The explicit inclusion of the tilt in the model allows one to account for the changes in the splay energy when the curvature of the monolayer surface remains constant, but the lipids are tilted with respect to the normal to the surface.

Within the framework of the HK model, the free energy of each of the lipid monolayers comprising a homogeneous bilayer of vanishing spontaneous total curvature is given by

$$F_{HK} = \frac{1}{2} \int [\kappa \tilde{J}^2(x,y) + \kappa_\theta t^2(x,y)] dxdy. \tag{8}$$

Here, the bilayer is assumed to be parallel to the x-y plane, κ is the bending (splay) modulus of the monolayer, and κ_θ is the tilt modulus. When the fluctuations of the bilayer are sufficiently small, the effective total curvature is approximated to the leading order by

$$\tilde{J}(x,y) = \nabla^2 h(x,y) + \nabla \cdot \mathbf{t}(x,y), \tag{9}$$

where the function $z=h(x,y)$ specifies the monolayer surface.

Further analysis of the membrane fluctuation magnitude is performed in Fourier space. It is convenient to define the vector $\mathbf{f}(\mathbf{q}) \equiv (\hat{h}(\mathbf{q}), \hat{t}_x(\mathbf{q}), \hat{t}_y(\mathbf{q}))$, where $\hat{h}(\mathbf{q})$, $\hat{t}_x(\mathbf{q})$, and $\hat{t}_y(\mathbf{q})$ are the Fourier transforms of the monolayer surface $h(x,y)$ and of the x and y components of the tilt vector $\mathbf{t}(x,y)$, re-

spectively. Then the monolayer energy, Eq. (8), can be rewritten as

$$F_{HK}(\mathbf{f}) = \frac{1}{2}\sum_{\mathbf{q}} \mathbf{f}(\mathbf{q}) \cdot \mathbf{A}(\mathbf{q})\mathbf{f}(\mathbf{q}), \tag{10}$$

with the matrix $\mathbf{A}(\mathbf{q})$ given by

$$\mathbf{A}(\mathbf{q}) = \begin{pmatrix} \kappa q^4 & -i\kappa q^2 q_x & -i\kappa q^2 q_y \\ i\kappa q^2 q_x & (\kappa q_x^2 + \kappa_\theta) & \kappa q_x q_y \\ i\kappa q^2 q_y & \kappa q_x q_y & (\kappa q_y^2 + \kappa_\theta) \end{pmatrix}. \tag{11}$$

Since the matrix $\mathbf{A}(\mathbf{q})$ is self-adjoint for each value of the wave vector $\mathbf{q}$, the inner products in Eq. (10) can be rewritten using the normal coordinates:

$$F_{HK}(\mathbf{g}) = \frac{1}{2}\sum_{\mathbf{q}} \sum_{j=1}^{3} \lambda_{\mathbf{q},j} |\mathbf{g}(\mathbf{q})|^2. \tag{12}$$

Here, $\lambda_{\mathbf{q},j}$ are the eigenvalues of matrix $\mathbf{A}(\mathbf{q})$ and the normal coordinates $\mathbf{g}(\mathbf{q})$ are defined by the equation

$$\mathbf{g}(\mathbf{q}) = \mathbf{U}^{-1}(\mathbf{q})\mathbf{f}(\mathbf{q}), \tag{13}$$

where $\mathbf{U}(\mathbf{q})$ is an orthogonal matrix whose columns are eigenvectors of the matrix $\mathbf{A}(\mathbf{q})$. Since the probability $P(\mathbf{g})$ of observing a monolayer in a configuration $\mathbf{g}$ is given by the Boltzmann distribution, $P(\mathbf{g}) \propto \exp(-F_{HK}(\mathbf{g})/k_B T)$, we obtain, using Eq. (12),

$$\langle g_j(\mathbf{q}) \rangle = 0, \quad \langle g_j(\mathbf{q}) g_{j'}(\mathbf{q}') \rangle = \delta_{j,j'} \delta_{\mathbf{q},-\mathbf{q}'} \frac{k_B T}{\lambda_j(\mathbf{q})}. \tag{14}$$

Substituting Eq. (14) into Eq. (13) yields

$$\langle \mathbf{f}(\mathbf{q}) \rangle = 0, \quad \langle \mathbf{f}(\mathbf{q}) \mathbf{f}(\mathbf{q}') \rangle = \delta_{\mathbf{q},-\mathbf{q}'} k_B T \mathbf{A}^{-1}(\mathbf{q}). \tag{15}$$

In particular, since

$$\mathbf{A}^{-1}(\mathbf{q}) = \begin{pmatrix} \left(\frac{1}{\kappa q^4} + \frac{1}{\kappa_\theta q^2}\right) & \frac{iq_x}{\kappa_\theta q^2} & \frac{iq_y}{\kappa_\theta q^2} \\ -\frac{iq_x}{\kappa_\theta q^2} & \frac{1}{\kappa_\theta} & 0 \\ -\frac{iq_y}{\kappa_\theta q^2} & 0 & \frac{1}{\kappa_\theta} \end{pmatrix}, \tag{16}$$

we obtain

$$\langle |\hat{t}_x(\mathbf{q})|^2 \rangle = \langle |\hat{t}_y(\mathbf{q})|^2 \rangle = \frac{k_B T}{\kappa_\theta}, \tag{17}$$

$$\langle |\hat{h}(\mathbf{q})|^2 \rangle = k_B T \left(\frac{1}{\kappa |\mathbf{q}|^4} + \frac{1}{\kappa_\theta |\mathbf{q}|^2} \right). \tag{18}$$

Thus, the magnitude of the fluctuations of $\hat{t}_x$ and $\hat{t}_y$ is independent of $\mathbf{q}$; i.e., the tilt fluctuations predicted by the HK model are independent for each lipid molecule. Moreover, the equation for the magnitude of the bending fluctuations coincides with the model (6) used in fitting MD simulations data [7]. Therefore, this model is justified provided it is recognized that the second term in Eq. (6) is due to the tilt fluctuations and not the protrusion tension.

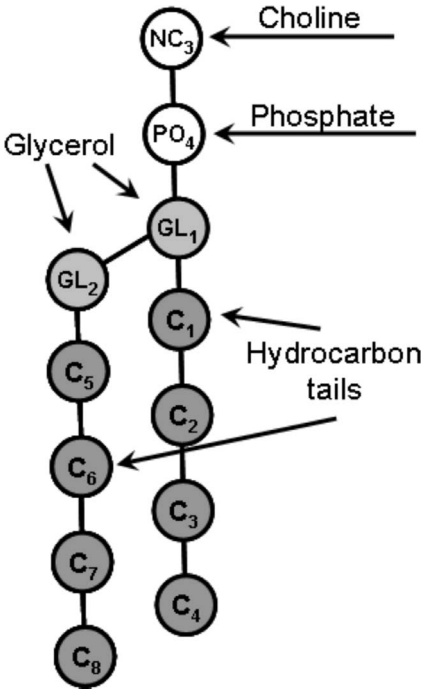

FIG. 1. Coarse-grained model of a DPPC lipid. The head group is modeled by two charged hydrophilic beads, representing the choline (NC_3) and the phosphate (PO_4) groups, the glycerol ester linkage is modeled by two beads of intermediate hydrophobicity, GL_1 and GL_2, and the lipid tails are modeled by chains of hydrophobic beads, C_i $(i=1,\ldots,8)$.

III. MOLECULAR DYNAMICS SIMULATIONS

In order to verify the predictions of Eqs. (17) and (18) and to demonstrate that the contribution of the lipid tilt modes dominates over the protrusion modes at short wavelengths, we performed coarse-grained molecular dynamics (CGMD) simulations of a lipid bilayer. The spectral intensities of the tilt and the bending fluctuations measured in the simulations are fitted to Eqs. (17) and (18), thus producing two independent estimates of the tilt modulus κ_θ. The protrusion tension coefficient is estimated by computing the energy penalty of exposing nonpolar groups of lipid molecules to the polar solvent. Good agreement between the two estimates of the tilt modulus and a relatively small magnitude of the protrusion coefficient obtained from our simulations confirm the dominance of the tilt modes at short wavelengths.

A. Simulation details

As a model system, we consider a well-studied dipalmitoylphosphatidylcholine (DPPC) lipid bilayer in water. We use the coarse-grained model for water and lipids proposed by Marrink *et al.* [10]. In this model, a single uncharged polar bead represents a group of four water molecules. The coarse-grained model for a DPPC molecule is shown in Fig. 1. The DPPC head group consists of choline NC_3 (modeled as a positively charged polar bead) and a phosphate group PO_4 (modeled as a negatively charged polar bead). The two hydrocarbon tails of the lipid are modeled by two chains each consisting of four hydrophobic beads, C_i. The glycerol

ester linkage is modeled by two identical beads, GL_1 and GL_2, with hydrophobicity intermediate to that of the polar head and the hydrophobic tail groups.

Since this model is known to speed up the dynamics approximately by a factor of 4, all simulation times will be reported below as "real" time (i.e., the simulation time multiplied by a factor of 4). The simulations were performed using the GROMACS simulation package [16]. The temperature and the pressure were maintained at 323 K and 1 bar using the Berendsen coupling scheme [17]. All simulations were performed with periodic boundary conditions in all directions and anisotropic pressure coupling in order to maintain zero surface tension of the membrane.

The considered system consists of 3200 lipid molecules and 64 000 coarse-grained water beads (each representing four real water molecules). The system was created by first performing a simulation of self-assembly of a bilayer consisting of 200 lipids and 4000 coarse-grained water beads. The initial condition for this simulation was taken to be a random dispersion of DPPC molecules in water. The lipid molecules self-assembled into a bilayer within 20 ns, and the system was equilibrated for an additional 800 ns. The obtained equilibrated lipid bilayer consisting of 200 lipids was used to generate the 3200-lipid system by periodically repeating the 200-lipid system 4 times in both lateral directions. This large system was equilibrated for 2.64 μs followed by a production run of 1.0 μs.

B. Tilt and bending moduli

To relate the thermal fluctuations of each of the monolayers comprising the bilayer to the bending and tilt moduli, it is necessary to obtain the monolayer surface $h(x,y)$ and tilt $\mathbf{t}(x,y)$ as a function of the spatial coordinates x and y. We define the monolayer surface $h(x,y)$ as a surface passing through the bonds connecting the glycerol and the phosphate groups of each of the lipid molecules. In order to obtain the tilt vector of the lipids, it is necessary to obtain the normal $\mathbf{N}(x,y)$ to the monolayer surface and the lipid director vector $\mathbf{n}$. The derivatives of the monolayer surface that are required to obtain the normal vector $\mathbf{N}$ are obtained by differentiation of the Fourier series for the monolayer surface $h(x,y)$.

Since a lipid molecule has many degrees of freedom, there is no unique definition for its director $\mathbf{n}$. In the current work we have investigated four possible definitions of the director vectors. In all cases, the director is defined as a vector connecting two points representing the locations of the lipid tail and the head group. The tail location is defined to be either (i) the center of mass of all the tail beads or (ii) the average between the locations of the last beads of the two tails of a lipid molecule. The head-group location is defined as either (i) the center of mass of all head-group beads or (ii) as a midpoint of the bond connecting the phosphate and the glycerol groups of a lipid.

The spectral intensities of the tilt fluctuations are shown in Fig. 2 for the four different definitions of the director vector $\mathbf{n}$ discussed above. It is evident that the spectral dependence of the tilt fluctuations is relatively insensitive to the precise definition of the tilt vector. The largest deviation

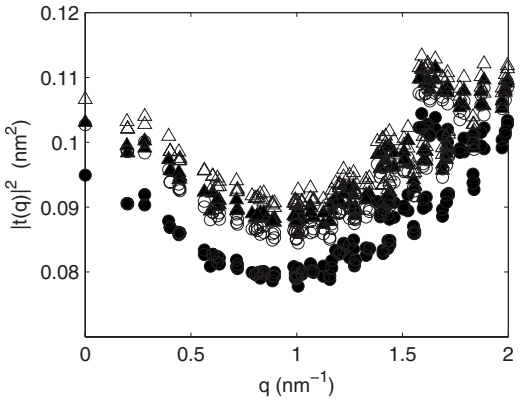

FIG. 2. Spectral intensity of tilt fluctuations for a DPPC bilayer obtained with the four different definitions of the molecular director vector $\mathbf{n}$ (see text). The solid (open) symbols show results obtained with the first (second) definition of the head group, and the circles (triangles) show the results obtained with the first (second) definition of the tail group.

from other director definitions is exhibited by the result shown by the solid circles in Fig. 2. This corresponds to the director with head- and tail-group locations defined as the centers of mass of the respective groups. The smaller magnitude of the tilt fluctuations observed in this case is probably due to averaging out of small fluctuations of beads within the tail and head groups.

We also observe a small systematic deviation of the magnitude of the tilt fluctuations from a constant predicted by Eq. (17). A possible reason for this discrepancy is the fact that the HK model neglects entropic contributions to the tilt energy as discussed in Ref. [18]. In addition, the protrusion tension neglected in this model may also contribute to this weak dependence of the tilt fluctuations on the wavelength.

However, to a good degree of approximation, the tilt fluctuations are independent of the wave vector and for the purposes of the current work we will use Eq. (17) to estimate the tilt modulus. Depending on the specific definition of the molecular director vector, the obtained tilt modulus is in the range between κ_θ=45.0 mN/m and 50.2 mN/m. This value is in good agreement with the theoretical estimate, $\kappa_\theta \approx 50$ mN/m, of Hamm and Kozlov [14] based on a crude molecular model and with their estimate $\kappa_\theta \approx 40$ mN/m based on an analysis of experimental data for DOPE phospholipid phases.

The spectral intensity of the surface fluctuations is shown in Fig. 3. The data are well approximated by the model (18), and a least-squares fit yields the following values of the elastic parameters: $\kappa=2.1\times10^{-19}$ J and κ_θ=50.9 mN/m. The obtained value of the bending modulus is in good agreement with the experimental estimates for similar lipids, from $\kappa=0.6\times10^{-19}$ J to $\kappa=1.2\times10^{-19}$ J [19]. Furthermore, the value of the tilt modulus κ_θ obtained from the surface fluctuations is in good agreement with the values independently estimated from the tilt fluctuations. The latter values are somewhat smaller, which can be explained by additional effects contributing to the membrane surface fluctuations, such as the protrusion tension neglected in the HK model.

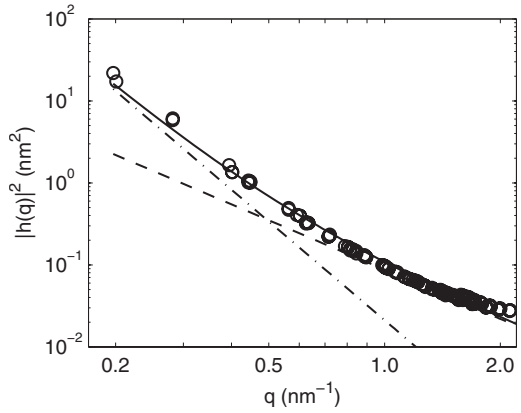

FIG. 3. Spectral intensity of a DPPC bilayer surface fluctuations (circles). The solid line shows a fit by Eq. (18). Dash-dotted and dashed lines show contributions of the first and second terms of Eq. (18), respectively.

C. Protrusion tension

In order to assess the relative contributions of the tilt energy and the microscopic tension to the short-wavelength membrane fluctuations, we estimate the microscopic (protrusion) tension coefficient γ_p from the MD simulations. The contribution of the jth bead of a lipid to the protrusion tension is estimated as the energy penalty for exposing this bead to water. This energy penalty is approximated as the difference in the average potential energy of the bead when it is (i) located in a fluid consisting of unconnected beads of the same type j (called j-fluid in what follows) and (ii) near the interface between the j-fluid and water so that it is exposed to the same amount of water as the corresponding bead within a lipid bilayer. The average potential energies of the bead j in the bulk j-fluid and at the j-fluid–water interface are

$$\bar{U}_j^b = \int \rho_{jj}^b(r) U_{jj}(r) d^3\mathbf{r}, \tag{19}$$

$$\bar{U}_j^i = \int \rho_{jj}^i(r) U_{jj}(r) d^3\mathbf{r} + \int \rho_{jw}^i(r) U_{jw}(r) d^3\mathbf{r}, \tag{20}$$

respectively. Here, the superscripts b and i refer to quantities in the bulk j-fluid and at the interface, respectively, $\rho_{jk}(r)$ is the density of beads of type k at distance r away from the jth bead, and $U_{jk}(r)$ is the potential of interactions between beads of type j and k. We further assume that the total bead density around a bead is conserved upon the transfer of this bead from the bulk j-fluid to the interface—i.e., $\rho_{jj}^b(r) = \rho_{jj}^i(r) + \rho_{jw}^i(r)$. Therefore, the energy penalty of the transfer of the jth lipid bead to the interface is estimated as

$$\Delta\bar{U}_j = \bar{U}_j^i - \bar{U}_j^b = \int \rho_{jw}^i(r)[U_{jw}(r) - U_{jj}(r)] d^3\mathbf{r}, \tag{21}$$

and the corresponding contribution to the surface tension is $\gamma_{p,j} = \Delta\bar{U}_j / S_{\text{lip}}$, where $S_{\text{lip}} = 0.62$ nm^2/molecule is the average area per lipid.

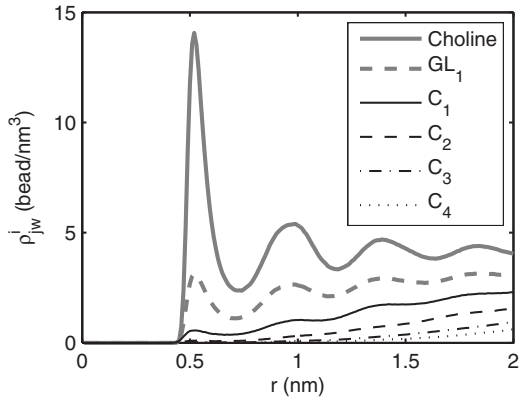

FIG. 4. Density $\rho_{jw}^i(r)$ of water beads located at distance r away from the jth bead of a DPPC lipid within the bilayer; j= choline, GL_1, C_1, C_2, C_3, and C_4 (see legend).

Representative plots of the density $\rho_{jw}^i(r)$ of water beads at distance r away from the jth lipid bead are shown in Fig. 4. The computed estimates of the contributions to the protrusion tension of the glycerol and the hydrocarbon groups are summarized in Table I. The total contribution to the tension is $\gamma_p \approx 16.12$ mN/m.

We expect that the obtained value is an overestimate of γ_p, since some of the energy penalties $\Delta\bar{U}_j$ computed above correspond to the *macroscopic* surface tension. This can be seen from the average number $\bar{N}_{jw}$ of water beads in the first solvation shell of the jth lipid bead shown in Table I. It is clear that the glycerol groups on average are exposed to at least one water bead. This implies that these groups are in constant contact with water even in the absence of protrusions. Therefore, most of the associated energy penalty $\Delta\bar{U}_{\text{GL}_j}$ contributes to the macroscopic surface tension rather than the microscopic protrusion tension. The protrusion tension is then dominated by the contribution of the hydrocarbon tails, $\gamma_{p,\text{C}} \approx 7.54$ mN/m. Nevertheless, even the overes-

TABLE I. Energy penalty per unit area, $\gamma_{p,j}$, of exposing the jth lipid bead to water and the average number $\bar{N}_{jw}$ of water molecules in the first solvation shell of the jth lipid bead.

Bead, j	$\gamma_{p,j}$ (mN/m)	$\bar{N}_{jw}$
Choline		8.040
Phosphate		6.731
GL_1	4.913	2.690
GL_2	3.672	1.905
C_1	3.026	0.735
C_2	0.877	0.154
C_3	0.300	0.044
C_4	0.190	0.036
C_5	2.109	0.457
C_6	0.633	0.103
C_7	0.233	0.036
C_8	0.170	0.033

timated value of γ_p, 16.12 mN/m, is significantly less than the value of the tilt coefficient κ_θ ($45.0\ \text{mN/m} \leq \kappa_\theta \leq 50.2\ \text{mN/m}$).

IV. DISCUSSION

Although in the current work we estimated individual contributions of the tilt fluctuations and the protrusion tension to the bending of the membrane, it is expected that these two effects act cooperatively. For example, one can imagine that tilting of a lipid will partially expose its hydrophobic tails to water, thus leading to a microscopic tension. The plausibility of coupling between the tilt and microscopic tension modes is supported by the fact that the estimated cumulative magnitude of the tilt modulus and the protrusion tension coefficient $(\kappa_\theta + \gamma_p)$ exceeds their estimated effect on the membrane bending fluctuations quantified by the value 50.9 mN/m of the prefactor to the q^{-2} term in Eq. (18). Therefore, the effect of the protrusion and the tilt is not simply additive and there is a correlation between these two types of motion.

In conclusion, we would like to discuss a practical implication of the tilt fluctuations for the analysis of MD simulations of lipid bilayers. As we discussed earlier, one of the common assumptions used in this analysis [5,6] is that the membrane bending fluctuations are described by model (5), which allows one to obtain the bending modulus κ by fitting the intensities of bending modes with wave numbers $q < q_0$ to the Helfrich model, Eq. (1). The cutoff wave number q_0 typically corresponds to the bilayer thickness h_0. This procedure is justified by an assumption that the protrusion effects do not contribute to long-wavelength membrane fluctuations. Although it may be true that the protrusion modes are negligible for the wavelengths longer than the membrane thickness, this assumption does not hold for the tilt modes. Indeed, as seen from Fig. 3, the second term of Eq. (18) makes a non-negligible contribution to surface modes with wave numbers as small as 0.3 nm^{-1} corresponding to modes more than 3 times longer than the bilayer thickness ($h_0 \approx 6$ nm). This observation implies that for the bending modes accessible to MD simulations (with wavelengths typically limited by $\approx 10 h_0$) one needs to account for the tilt modes in order to accurately estimate the bending modulus. In order to demonstrate this, we fit our data for the surface fluctuations to $1/\kappa q^\alpha$ for $q > q_0$ with $q_0 = 1\ \text{nm}^{-1}$ corresponding to wavelength comparable with the bilayer thickness. We obtain $\alpha = 3.3$ and $\kappa = 5.5 \times 10^{-20}$ J. Therefore, the scaling of the long-wavelength modes is not captured correctly, and the bending modulus is significantly underestimated if the tilt modes are neglected in fitting of MD data.

In summary, we have demonstrated that the tilt energy of the lipid molecules makes the dominant contribution to the bilayer dynamics at short wavelengths. The elastic HK model, which takes the tilt energy into account, yields a rigorous explanation of the scaling of the membrane bending fluctuations observed in MD simulations. The obtained results indicate that although there is some coupling between the lipid tilt and the microscopic tension effects, the tilt modes provide the dominant contribution to the bilayer fluctuations at short wavelengths. The influence of the tilt fluctuations remains significant for wavelengths several times larger than the bilayer thickness and therefore should be taken into account in analyses of MD simulations of membrane bending fluctuations. The dominance of the tilt energy at short wavelengths is also expected to play a significant role in interactions of lipid bilayers with inclusions, such as proteins, as well as in highly curved nonbilayer phases [15,20].

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