Simulations of edge behavior in a mixed-lipid bilayer: Fluctuation analysis

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Coarse-grained molecular dynamics simulations of lipid bilayer ribbons consisting of a mixture of lipids of different tail lengths have been performed to gain insight into bicelle mixtures. The line tension of the bilayer edge decreases as the mole fraction of short-chain lipids in the system is increased, dropping below zero between 30% and 35%. The mole fraction of short-chain lipids in the ribbon interior is lower than the total mole fraction, as the short-chain lipids segregate towards the edge, but continues to rise even after the line tension vanishes, in contrast to predictions of a two-component two-phase model. The fluctuations of the bilayer edge in both high and low line tension regimes have been analyzed to extract information about the factors that influence the length and shape of the edge. At high line tension the wavelength-dependent in-plane fluctuations of the edge are predicted quantitatively using a simple analytical model using only the line tension as input. Where line tension is vanishing, the fluctuations can be modeled as arising from a combination of harmonic fluctuations around a minimum energy contour length and an in-plane bending elasticity. The estimated value of the in-plane bending modulus is of order $10^{-29}$ J m, placing the intrinsic persistence length for the edge near the bilayer thickness of 4 nm.

INTRODUCTION

Mixtures of short-chain phospholipids [dihexanoylphosphatidylcholine; systematic name: 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC)] and long-chain phospholipids [dimyristoylphosphatidylcholine; systematic name: 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)] have been the focus of considerable interest recently.Sanders and Schwonek1 in the early 1990s introduced a mixed lipid system (DMPC/DHPC) called bicelles for use in NMR studies of biomembrane-associated macromolecules. Tjandra and Bax2 developed a method to use the same bicelle mixture for NMR analysis of large soluble biomolecules, taking advantage of the slight alignment of the molecules imparted by the lipid aggregates in their nematic liquid crystalline phase. Despite the wide applications of bicelle systems in NMR experiments, questions persist about their thermodynamic behavior and microscopic morphology is still yet to be determined. Early publications3 proposed that mixed DMPC/DHPC form discoidal structures that can easily be described by an “ideal-disk” model. This model assumes that the long-chain lipids (DMPC) exclusively occupy the center region of the disk and short-chain lipids (DHPC) occupy the rim of the disk. Recently, van Dam et al.4 and Nieh et al.5 have experimentally demonstrated that DMPC/DHPC mixtures undergo a series of different phases and morphologies over a wide range of temperature $T$, total lipid concentration $c_{lp}$, and long-tail:short-tail lipid number ratio $q$. In addition to disks, cylindrical micelles or ribbons, porous sheets and vesicles, and branched network structures have been proposed.

Understanding the morphological transformation in mixed lipid systems requires some physical properties as input. The aggregate structure in a system that may have disk, ribbon, and pore morphologies is sensitive to the free energy on the length and curvature of the bilayer edge. It is commonly known that line tension, or excess free energy per unit length, is sufficiently high in most pure lipid systems to drive the formation of vesicles from disks and to close pores. However, as these systems are believed to have stable edges, it is reasonable to assume that the line tension is greatly reduced or eliminated by the presence of the shorter-chain component; at vanishing line tension, subtle effects such as the bending elasticity of the edge may become important. By simulating the edge of a mixed bilayer with vanishing line tension and analyzing the shape fluctuations of the edge, we aim to learn more about these effects that may play important roles in determining morphology in bicelle systems.

Molecular dynamics simulation has been widely used in understanding structures and dynamics of self-assembled lipid systems. However, studying mixed lipid system using full atomistic simulations has limitations on time scale and length scale. The diffusion of lipids within bilayer to achieve segregation of different lipids to different environments requires longer time scale than practical with current computational power. The discoidal bicelles observed in experiment are on the order of 20 nm in diameter (containing around 1000 lipids), which approaches the upper bound of practicality. To solve this dilemma, coarse-grained models, where a group of atoms is simplified and parametrized by one interacting site, have been developed by various groups.6,7

Full atomistic8 and coarse-grained9 simulations on lipid bilayer edge have been done in our earlier work. A ribbon geometry was used in these simulations due to its simplicity to analyze the structure and energetics of the edge under periodic boundary conditions. Using a coarse-grained (CG)
model of a bicellelike mixture, we observed the domination of long-tail lipids in the bulk of the ribbon (i.e., the flat bilayer environment) and an enhancement of short-tail lipids at the edge region. Further analysis revealed that next to the bilayer rim, the long-tail lipid concentration and the bilayer thickness both reach a maximum before returning to their bulk values in the ribbon center. Line tension can be calculated from the anisotropy of pressure between the main axis along the ribbon edge and the perpendicular axes. The coarse-grained model in the previous work leads to systematically higher line tension than atomistic models calculated for lipid ribbons, pores, and experiments. More importantly, even in the limit of a system composed purely of the shorter-chain component, the simulations indicated the bilayer structure to be stable, in qualitative disagreement with theoretical predictions.

In this work we used a later version of the coarse-grained force field with a more realistic line tension than atomistic models calculated for lipid ribbons, pores, and experiments. In this study, we investigated edge shape fluctuations on the order of a few hundred nanoseconds. Binary mixtures of dipalmitylphosphatidylcholine; systematic name: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/systematic name: 1,2-dibutanyol-sn-glycero-3-phosphocholine (DBPC) systems, where DBPC has four methyl groups in each tail, in which composition varied from 100% to 60% of the long-chain DPPC component are modeled, including both systems with high and negligible line tensions. We compared the fluctuations in ribbon edge shape and contour length to simple statistical theory and to a simple Monte Carlo model. At low % DBPC, the spectrum of fluctuations could be predicted quantitatively using the independently obtained value of the line tension from pressure tensor calculation. For systems with low line tension, neither the fluctuations observed in the edge structure nor the partitioning of DBPC between the bilayer and its edge could be fully explained using simple models.

### METHODS

#### Coarse-grained model and force field

We used the CG model and force field developed by Marrink et al. to calculate lipid-water systems in molecular dynamics (MD) simulations. To represent phospholipids in this study, this model maps every four methylene/methyl groups in the two tails onto a single apolar particle, maps the glycerol ester linkage onto two nonpolar particles, and maps the PC headgroup onto a positively and a negatively charged particle for choline and phosphate, respectively. Four water molecules are represented by a single united atom with a corresponding mass of 72 amu. The force field parameters are chosen to well reproduce structural properties of lipid bilayers while using cheap short-ranged treatment for electrostatic interactions. In this study, the CG forcefield and topology (version 1.4) are from the website of Marrink et al. (http://md.chem.rug.nl/~marrink/coarsegrain.html). Following a later publication by Marrink and Mark, the phosphate-water Lennard-Jones (LJ) interaction energy was increased from 5.0 to 5.8 kJ/mol to improve the line tension calculation.

#### System construction and initialization

As the model can only represent tail chains in multiples of four carbons, we used DPPC and DBPC as our component in the binary mixture. These lipids have the same headgroup with a decreasing number of methyl/methylene groups on the tails; the 16 carbons of the DPPC tails are represented by 4 CG sites, while the 4 carbons of the DBPC tails are represented by a single CG site.

Our initial systems were derived from 128 DPPC lipid bilayers provided by the website of Marrink et al. To form a ribbon-shaped configuration, this bilayer patch was first replicated laterally. After that, two strips of lipids near the box boundary were removed along the one lateral axis (we hereafter denoted it as the z axis). In case of binary mixture, a certain number of short-tail lipids (DBPC) were obtained by chopping terminal CG particles from each tail of randomly chosen DPPC lipids. The vacancy due to the removal was filled thereafter with randomly spaced water particles which have a minimum distance of 0.4 nm among them so that the system density is close to 1 g/cm³. Energy minimization was performed using steepest descent algorithm before the systems were sent to production run. Table I summarizes systems in the CG MD runs in this study.

#### Molecular dynamics algorithm and technical details

Following the protocol of Marrink et al., we used an integration time step of 20 fs. The time scale of the calculations is on the order of hundreds of nanoseconds. Periodic boundary conditions are implemented in three-dimensions. We added a shift function to Coulombic force when the interaction distance falls between 0 and 1.2 nm so that the resulting force smoothly decays to zero in that range. van der Waals interactions were treated likewise except that the shift function was turned on between 0.9 and 1.2 nm. We used the grid-type neighbor searching algorithm: atoms in the neighboring grid were updated every ten time steps. Experimentally DPPC bilayer shows a fluidlike phase above 315 K. To simulate the DPPC bilayer in this phase, the system temperature is therefore coupled to 323 K for different lipid types and water separately. Berendsen algorithm was used for the temperature coupling with a time constant of 1 ps. We used a semi-isotropic pressure coupling scheme: the box dimensions in the z axis, parallel to the edge, are fixed (zero compressibility), whereas the box dimensions in the other two dimensions are jointly scaled to an external pressure at 1 bar with compressibility set to 5.0×10⁻⁵ bar⁻¹, through the Berendsen algorithm. According to this pressure coupling scheme, the line tension of the ribbon edge can be expressed as

$$\Lambda = \frac{1}{2} \left( L_x L_y \left[ \frac{1}{2} (P_{xx} + P_{yy}) - P_{zz} \right] \right),$$

where the $P_{xx}$, $P_{yy}$, and $P_{zz}$ are the diagonal elements of pressure tensor. $L_x$ and $L_y$ are box sizes in the x and y directions.
modified GROMACS utility programs, perl, and linux shell were used to carry out the simulations. Analysis tools include visualization.

Spatial spectral analysis

During the course of the simulations, the ribbons were free to rotate around the $z$ axis and to drift translationally in the $x$-$y$ plane. Therefore before the analysis of the edge fluctuations, ribbon coordinates were first translated so that the ribbons’ center of mass is the Cartesian origin and then were rotated around the $z$ axis to the point that the longer primary axis of inertia of the ribbon (defined by the phosphorus atoms projected onto $x$-$y$ plane) lies parallel to the $x$ axis and the shorter one parallel to the $y$ axis. Hereafter in this paper, $y$ denotes the direction of bilayer normal; $x$ denotes the direction orthogonal to both bilayer normal and the edge direction, the $z$ axis.

To describe the in-plane shape undulations of the edge, we segmented the ribbon into small equal slices along the $z$ dimension. The position of the lipid is defined by its phosphate position in the headgroup which fall into each slice. The two “edge points,” representing the edge positions in each slice, are defined as the two points with maximum and minimum lateral displacements to the edge axis. By connecting the edge points of both edges by line segment, one can extract the shape contour of the edge and contour length is the summation of those line segment lengths. A Fourier transform of the shape fluctuation is performed to yield the mode intensity as a function of mode number.

In some cases, a very small number (less than 10) of short-tail lipids in the ribbon binary mixtures diffused into the solvents for a few nanoseconds and then united with the ribbon again. These lipids were excluded from the shape fluctuation analysis for clarity.

THEORY OF BILAYER EDGE SHAPE FLUCTUATION

Bilayer ribbon

Although the bilayer is a two-dimensional surface, it is convenient to assume a flat bilayer, project the edge onto a plane, and expand the free energy in terms of the length and curvature of the edge in the plane. The lipid ribbon edge can hence be geometrically treated as a one-dimensional object and its thermal fluctuations reflect the material properties of the edge. Our analysis of these fluctuations is based on a large body of work characterizing fluctuations of two-dimensional objects, i.e., bilayer and interface surfaces. We use the Monge representation $h(z)$ to give the displacement of the edge from a flat reference axis $z$. The free energy associated with the ribbon edge is shown as follows:

$$F = \int_0^L f dl = F_0 + \int_0^L \left\{ \Lambda \left( 1 + \frac{1}{2} (\nabla h)^2 \right) + \frac{1}{2} \kappa (\nabla^2 h)^2 \right\} dz. \tag{2}$$

The first term in the integrand is the line energy where $\Lambda$ is the line tension, free energy per unit edge length. The second term is the bending energy where $\kappa$ is the in-plane bending modulus of the edge. In Eq. (2) we have used the approximation which is valid at small edge fluctuation,

$$dl = \sqrt{1 + (\nabla h)^2} dz \approx \left( 1 + \frac{1}{2} (\nabla h)^2 \right) dz. \tag{3}$$

The integral is from 0 to the box size in the $z$ dimension, $L$, and all the constant terms are included in $F_0$. The Monge representation of $h(z)$ can be expanded as a summation of Fourier series,

### Table I. Summary of MD simulation runs. Box size is the averaged value over the runs.

<table>
<thead>
<tr>
<th>(a)</th>
<th>DPPC No.</th>
<th>DBPC No.</th>
<th>Time (ns)</th>
<th>Box size $x$, $y$, $z$ (nm)</th>
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<tbody>
<tr>
<td>966</td>
<td>0</td>
<td></td>
<td>200</td>
<td>18.7,8.5,20.4</td>
</tr>
<tr>
<td>1432</td>
<td>0</td>
<td></td>
<td>200</td>
<td>21.6,8.4,30.0</td>
</tr>
<tr>
<td>1205</td>
<td>0</td>
<td></td>
<td>100</td>
<td>21.9,8.2,25.0</td>
</tr>
<tr>
<td>716</td>
<td>0</td>
<td></td>
<td>100</td>
<td>21.2,8.5,15.0</td>
</tr>
<tr>
<td>870</td>
<td>588</td>
<td></td>
<td>200</td>
<td>18.6,8.0,30.0 (0–100 ns)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21.9,12.1,30.0 (100–200 ns)</td>
</tr>
<tr>
<td>722</td>
<td>481</td>
<td></td>
<td>100</td>
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<tr>
<td>430</td>
<td></td>
<td></td>
<td>200</td>
<td>20.8,8.1,15.0</td>
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</table>

<table>
<thead>
<tr>
<th>(b)</th>
<th>DBPC%</th>
<th>Time (ns)</th>
<th>DPPC No.</th>
<th>DBPC No.</th>
<th>Box (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>300</td>
<td>870</td>
<td>96</td>
<td>25.6,8.0,20.2</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>300</td>
<td>822</td>
<td>144</td>
<td>24.9,8.0,20.4</td>
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<td>20</td>
<td>200</td>
<td>772</td>
<td>194</td>
<td>24.3,8.2,20.4</td>
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<td>300</td>
<td>724</td>
<td>242</td>
<td>24.8,8.1,20.0</td>
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<td>30</td>
<td>200</td>
<td>676</td>
<td>290</td>
<td>24.0,8.3,20.4</td>
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<tr>
<td>35</td>
<td>200</td>
<td>628</td>
<td>338</td>
<td>23.9,8.1,20.4</td>
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</tr>
<tr>
<td>37</td>
<td>100</td>
<td>604</td>
<td>362</td>
<td>23.6,8.2,20.4</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>580</td>
<td>386</td>
<td>23.5,8.2,20.4</td>
<td></td>
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</table>
where $u_q$ is the intensity of the mode with wave number $q$. $q$ spans in the range of $[-\infty, +\infty]$, with discrete values spaced by $2\pi/L$. Substitution of Eq. (4) into Eq. (2) yields

$$F = F_0 + \frac{1}{2} \Lambda \int_0^L \left( \sum_q u_q(iq)e^{iqz} \right) \left( \sum_q u_q(-iq)e^{-iqz} \right)dz \quad + \frac{1}{2} \kappa \int_0^L \left( \sum_q u_q(-q^2)e^{iqz} \right) \left( \sum_q u_q(q^2)e^{-iqz} \right)dz. \quad (5)$$

Note that the second and third terms in the equation above have a product of two summations in their integrand. Due to orthogonality, all the cross terms vanish when $|q| \neq |q'|$. It is easy to show that when $q = q'$, the term goes to zero since $\int_0^L e^{2iqz}dz = 0$. Therefore only two terms survive when $q$ and $q'$ only differ in sign ($q = -q'$). Finally, Eq. (4) can be rewritten as

$$F = F_0 + \frac{1}{2} \Lambda \left( \sum_{q>0} q^2|u_q|^2 + \sum_{q<0} q^2|u_q|^2 \right) \quad + \frac{1}{2} \kappa L \left( \sum_{q>0} q^4|u_q|^2 + \sum_{q<0} q^4|u_q|^2 \right). \quad (6)$$

The average configurational free energy in each harmonic term in this summation corresponds to $kT/2$ according to the equipartition theorem. As positive and negative $q$ values correspond to the conjugate term in Fourier series, their mode amplitude is equal as shown in the following:

$$\langle u_q^2 \rangle = \langle u_{-q}^2 \rangle = \frac{k_BT}{L} (\Lambda q^2 + \kappa q^4)^{-1}. \quad (7)$$

It is important to note that the wave amplitude scales as $q^{-2}$ when $\Lambda$ dominates and scales as $q^{-4}$ with vanishing line tension $\Lambda \approx 0$. We therefore calculate the correlation of two points’ position as a function of their distance,

$$C(\Delta z) = \langle h(z)h(z + \Delta z) \rangle = \left( \sum_q u_q e^{iqz} \cdot \sum_q u_{-q} e^{-iq(z+\Delta z)} \right), \quad (8)$$

where the brackets $\langle \rangle$ denote the statistical average of all the contour positions on the edge (i.e., integration over the z dimension). When $|q| \neq |q'|$, the cross terms on the right of Eq. (7) will vanish because of orthogonality. When $q = q'$, Eq. (7) can be rewritten as:

$$C(\Delta z) = \left( \sum_q u_q^2 e^{2iqz+i\Delta z} \right) \quad = \sum_q \frac{1}{L} \int_0^L u_q^2 e^{2iqz+i\Delta z}dz \quad = \sum_q \left\{ \langle u_q^2 \rangle e^{iqz} \frac{1}{L} \int_0^L e^{iz}dz \right\} = 0. \quad (9)$$

Note that in the above derivation, $qL$ are multiple of $2\pi$. As only terms where $q = q'$ give nonzero contribution, Eq. (7) can be rewritten as

$$C(\Delta z) = \sum_q u_q^2 \frac{2q_kT}{L} \cos q\Delta z. \quad (10)$$

Substituting $\langle u_q^2 \rangle$ by Eq. (6) yields

$$C(\Delta z) = \langle h(z)h(z + \Delta z) \rangle = \sum_{q>0} \frac{2k_BT \cos qz}{L(\Lambda q^2 + \kappa q^4)}. \quad (11)$$

Similar in Eq. (6), the theory predicts that the Fourier transform of the position correlation function shows $-2$ and $-4$ power law dependences on positive value of $q$ at high and low line tension regimes, respectively. Hereafter in this paper, $q$ refers to the absolute value of $q$ in Fourier (reciprocal) space.

**A Monte Carlo model to simulate bilayer edge fluctuations**

Because the theory described above is limited to the regime of small amplitude fluctuations, we also developed a Monte Carlo scheme to simulate the bilayer edge fluctuation shown in Fig. 1. Each point in the figure represents the position of the edge in each slice, similar in the definition of edge position in each slice from MD data. These “edge points” are equally spaced in the edge direction and are only allowed to move vertically. In each Monte Carlo (MC) step, several points along the edges are randomly chosen to move a displacement (both positive and negative) within a limit of 0.5 nm. The edge is represented by connecting the “edge points” all together. The edge length is therefore the summation of bond length. Periodic boundary condition is applied by connecting the both ends. To avoid very large bond length, we set a maximum bond length of 3 nm.

The Hamiltonian of this model is shown below.

$$H = \Lambda \sum_{i=1}^n l_i \quad \text{(for high } \Lambda \text{ systems)} \quad \quad = \sum_{i=1}^n \frac{1}{2} \theta_i^2 + \frac{1}{2} \kappa \left( \sum_{i=1}^n l_i - L_0 \right)^2 \quad \text{(for low } \Lambda \text{ systems).} \quad (12)$$

The term on the first line of Eq. (12) is the line energy term, namely, the line tension times the length of the edge. The first term on the second line is the bending energy term which accounts for the curvature of the edge. $\theta$ is the angle of neighboring three atoms. $\theta_i$ in the denominator of this term is defined as the mean bond length of the two bonds forming the angle. By including $\theta_i$ we properly weighted configura-
tions with different curvature radii. The second term for low line tension system assumes that the system has a preferred contour length of \( L_0 \) and there is a harmonic energy with the deviation from this preferred contour length. Parameter \( \gamma \) controls the strength of the harmonic term. After trying different combinations of these parameters, we found that neglecting the bending term and harmonic term in the Hamiltonian can well reproduce the fluctuation spectrum in high line tension systems. As a result, we split Eq. (12) for systems at high and low line tensions individually: it has one adjustable parameter \( \Lambda \) for high line tension system and has three adjustable parameters: \( \kappa \), \( \gamma \), and \( L_0 \) for low line tension systems. They will be used as input in MC simulation later in this paper.

RESULTS

Equilibrium dynamics

During the MD simulations of these ribbons, the edges of the ribbons underwent a variety of configurations. Snapshots from one of these simulations, composed of 65% DPPC and 35% DBPC, are shown in Fig. 2. In the mixed systems, the short-tail lipids (S) quickly established equilibrium between the edge region and bilayer bulk region at the beginning of the simulations. We see S lipids moved back and forth between the two regions until a stable state is reached. As have been seen in previous simulation, a preponderance of S lipids was observed in the edge region and majority of bulk region was occupied by long-tail lipids (L). In other words, we observe partial segregation of two lipid species with rapid equilibrium process of the S lipids taking place between the edge and the bulk region in the mean time.

To investigate whether the edges have propagated thoroughly in its configurational space within our simulation time, we calculate the ensemble average of correlation of edge point at time \( t \) and its position at a later time \( t+\Delta t \), \( \langle h(t; z) \cdot h(t+\Delta t; z) \rangle \), as shown in Fig. 3. Here the brackets mean averaging over each time steps along the trajectories and each edge point along the edge contour. Systems with the same lipid number ratio but different edge lengths are graphed. The top graph in Fig. 3 shows that in pure DPPC systems this correlation decays more slowly as the edge length increases. This is not surprising because the system with a longer edge contour comprises more modes and therefore requires more time to fully develop them, especially those with a longer wavelength. Consequently, we observed that a 40 nm curve does not reach zero within 40 ns, while 20 and 30 nm curves do so within 20 ns. For this reason, we confine our analysis to ribbons 30 nm and shorter.

Line tension

Equation (1) provided a convenient way to calculate the line tension from the pressure anisotropy: the difference of pressure tensor in the \( XY \) plane and \( Z \) axis must be compensated by a contraction force, which is defined as excess energy for a unit length. The calculation of error bars is based on the block averaging method. DPPC/DBPC systems reached the zero line tension region (<5 pN) with a number ratio of 1.5. The pure DPPC systems show a line tension around 50 pN. This value agrees well with atomistic simulations of DPPC ribbons, whereas it is higher than the experimental value (10–30 pN) of a similar lipid type.

Figure 4 shows the line tension dependencies on box \( z \) dimension and system composition. Although the line tension is strongly influenced by system composition, it fluctuates very little as system size expands. The line tension values set our systems into two categories: (1) high line tension region (~50 pN) and (2) low line tension region (<5 pN). To further investigate how line tension varies with composition, we gradually change the number ratio in DPPC/DBPC systems while keeping the total number of lipid constant. The result is shown in Fig. 5(a). The line tension smoothly decreases from around 46 to 20 pN until a sudden drop below 0 pN.

Partitioning of short-tail lipids

We calculate the DBPC percentage in the bulk region (a 4 nm wide strip in the center of the ribbon) for ribbons of increasing total DBPC concentration, as shown in Fig. 5(b).
The percentage of DBPC in the bulk is consistently lower than the total percentage of DBPC, consistent with the enhancement of DBPC at the edge. The rise in bulk DBPC is uninterrupted even as the line tension vanishes between 30% and 35% total DBPC.

**Edge contour length**

Figure 5(c) gives the mean edge contour length as a function of system DBPC percentage. The contour length basically remains unaltered until the percentage of DBPC reached 30% and starts to increase promptly with higher DBPC percentage, consistent with line tension drop in Fig. 5(a). The distribution also becomes broader in the low line tension regime.

**Position correlation function Fourier transform**

As discussed in the Methods section, to quantitatively analyze the in-plane fluctuations of the edge we represented the projection of each ribbon edge as a function \( h(z) \). We then calculated the position correlation function \( C(z) = \langle h(z)h(z+\Delta z) \rangle \), averaging over both edges and the full production run, from MD simulations at both high line tension region and low line tension region. Fourier transform of this function is plotted in Fig. 6. In Fig. 6(a), we observe that for all the four systems of different edge lengths the mode intensity behaves as \( q^{-2} \) at a small wave number, agreeing very well with theoretical prediction. To further test that, in Fig. 7 we fitted the mode intensity data from MD and MC simulations to theory [Eq. (9)] by inputting the line tension \( \Lambda_{MD} \) value calculated from pressure tensor [Eq. (1)] and neglecting the bending term (\( \kappa = 0 \)). The results show a very good agreement with theory at a wave number less than 0.1 nm\(^{-1}\). A plateau is seen for higher wave numbers. Figure 6(a) also shows that the modes with the same \( q \) have larger amplitudes at a smaller box boundary size along the edge, which is consistent with Eq. (4). In Fig. 6(b), the mode intensity behaves differently from what the theory predicts. The \( q^{-2} \) scaling no longer exists at small \( q \) regions but does not show \( q^{-4} \) scaling as predicted from theory when \( \Lambda = 0 \) [in the case of Fig. 6(b), \( \Lambda \) is close to zero] and if fluctuations were controlled by an in-plane bending rigidity \( \kappa \). These results indicate that at \( \Lambda = 0 \), the in-plane fluctuations of the edge are not controlled by a simple bending parameter alone. In contrast with Fig. 6(a), the mode intensity in Fig. 6(b) does not decrease with increasing ribbon length, so no free energy model based on a (positive) quadratic dependence on mode amplitude and a linear dependence on edge length will fit the data. We therefore turned to simple Monte Carlo simulation of a one-dimensional representation of the edge to test other free energy models.
tension values obtained from MD simulation pressure tensor calculations.

Fitting edge fluctuation at high line tension region (DPPC percentage $\geq$70%)

Results from above show that edge correlation function agrees very well with theoretical predictions at a large wavelength and high line tension. We therefore use the line tension, calculated from pressure tensor length and high line tension. We therefore use the line tension parameter fitting works very well until DPPC percent-

Fitting edge fluctuation at low line tension region (DPPC percentage $\leq$65%)

Figure 5(a) shows that line tension drops below zero as DPPC percentage in systems is equal or less than 65%. As this occurs, it is apparent through visual observation and through the sudden increase in edge contour length [Fig. 5(c)] that the edge enters a regime of large amplitude fluctuations, qualitatively different from the high line tension regime. In our Monte Carlo simulations, we set line tension equals zero and tune the $\kappa$, $\gamma$ and $L_0$ in Eq. (12). Fitting results show that including the harmonic term in Eq. (12) is essential to fit both Fourier transform of fluctuation correlation function and edge length distribution from MD simulations.

DISCUSSION

As in a previous study using a similar model, increasing the concentration of shorter-tailed lipid stabilized the bilayer edge, as indicated by a reduction in line tension. In the present work, the line tension is reduced to zero (in fact, a negative value) at moderate fraction of shorter-tailed lipids, leading to a great increase in shape fluctuations. In the positive line tension regime, the amplitudes of random thermal fluctuations are limited by the excess free energy of the increased contour length associated with these fluctuations. Both a simple expression [Eq. (11)] based on the equipartition theorem and a simple Monte Carlo model do a good job of predicting the long-wavelength amplitudes using the line tension, obtained independently from pressure tensor following Eq. (1), as the only input.

When line tension drops below zero, however, the behavior of the edge is more complex. If we assume $\Lambda = 0$, then the edge length could in principle diverge. Our original prediction was that the fluctuations would be limited by the in-plane bending elasticity of the edge; however, the $q^{-4}$ dependence predicted from Eq. (11) was not observed in the amplitude spectrum. We realized that the condition $\Lambda = 0$ does not mean a true absence of any contour length effects on energy within our small, fixed-composition system. The fluctuations of the edge are, in fact, limited by the availability of the short-tail lipid DBPC; increases to the edge contour length will lower the DBPC concentration in the edge and the bulk and reintroduce a line tension that will limit further increases. Reductions to the edge contour length will lead to an excess of DBPC that will lead to an effective line pressure. A harmonic dependence of free energy on edge length, to maintain the length in a range appropriate for the total system composition, would seem to be appropriate. We do not have an expression analogous to Eq. (11) for the fluctuation spectrum of such a system, so we resorted to MC simulation of a simplified edge model with a known potential to model the MD results. We obtained a good fit to the contour length distributions upon varying $\gamma$ and $L_0$ in Eq. (12), but found simultaneous agreement with both edge length distribution and fluctuation amplitude spectrum only when the in-plane bending modulus was included in the Hamiltonian. The harmonic model force constants obtained (II) were in the range of 3–10 pN/nm, indicating that the edge contour length can fluctuate by one or several nanometers before restoring forces reach the significant level of $\sim 10$ pN. The in plane bending elastic constant of the edge, $\kappa \sim 10^{-23}$ J m, corresponds to a persistence length of about 4 nm, of the

TABLE II. Parameters used in MC simulations to fit position correlation function of edge points and contour length distributions. * means the line tension values obtained from MD simulation pressure tensor calculations.

<table>
<thead>
<tr>
<th>DBPC%</th>
<th>$\Lambda$ (pN)</th>
<th>$\kappa \times 10^{-23}$ J m</th>
<th>$\gamma \times 10^{-2}$ J/m$^2$</th>
<th>$R_0$ (nm)</th>
</tr>
</thead>
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<tr>
<td>10</td>
<td>46.07</td>
<td>0.1</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td>15</td>
<td>41.71</td>
<td>0.1</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
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<td>31.49</td>
<td>0.1</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td>25</td>
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<td>30</td>
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<td>0.1</td>
<td>0.0</td>
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</tr>
<tr>
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<td>0.08</td>
<td>1.0</td>
<td>20.70</td>
</tr>
<tr>
<td>37</td>
<td>0.00</td>
<td>0.08</td>
<td>0.77</td>
<td>20.71</td>
</tr>
<tr>
<td>40</td>
<td>0.00</td>
<td>0.2</td>
<td>0.35</td>
<td>21.00</td>
</tr>
</tbody>
</table>
same order as the bilayer thickness. (Persistence length of the edge cannot be directly related to the decay length of orientational correlations, as is commonly done for polymers with free ends, because the geometry of the system ensures that the correlations reappear periodically.) Due to the multivariate nature of the fitting, we do not place any emphasis on the particular values obtained for \( \kappa \), but the general conclusion is that even at negligible line tension, the edge contour will tend to be smooth on a length scale comparable to bilayer thickness, but not exhibit any significant rigidity over a larger length scale. The fact that bending elasticity is not necessary to interpret the fluctuations of the edge at high line tension does not imply a lowering bending elasticity, rather that the stronger damping effect of the line tension obscures any influence the bending elasticity may have.

**Partitioning of long- and short-tail lipids between the bulk and the edge**

Our MD simulations show that the edge of ribbon is predominately occupied by short-tail lipids and bulk region predominately occupied by long-tail lipids. If we can treat the edge and the bulk as two phases within a pseudobinary system, as suggested by Triba et al., then as long as both phases are stable and at equilibrium with each other (i.e., zero line tension) their compositions should not change: increasing the amount of short-chain component would increase the length of the edge without affecting the composition of the edge or the interior. In contrast to this appealing notion, the current results [Fig. 5(b)] show an uninterrupted increase in the bulk DBPC fraction even in the regime of low or vanishing line tension. The assumption behind the two-phase model is that the chemical potential of each component in each “phase” is a simple function of the composition of that phase. This assumption is not strictly true, and the significant deviation from the model’s prediction may arise from one of several phenomena.

One issue is that in order for the edge to increase its contour length in the box of fixed length, it has to curve. If the relative chemical potentials of the two lipid types are sensitive to the degree of curvature, then the composition of the interior at equilibrium with the edge may shift. More importantly, if the curvature increases the free energy of the edge, the resistance to bending may prevent the edge from expanding to its true equilibrium length and absorbing excess bulk DBPC. This is consistent with the observed negative line tension—the free energy of the system would decrease if the box were extended, allowing the edge to lengthen without bending. Although the simulation geometry is constrained in an unnatural way, experimental factors may also effectively constrain the in-plane edge curvature, as in the ideal bicelle disk model where composition determines disk radius.

Other possible mechanisms leading to the breakdown in the two-component two-phase model involve the coupling between edge and interior “phases.” If this coupling is significant, the chemical potentials of short and long lipids at the edge gain an additional dependence on the bulk composition. The minimum free energy of the system with coexisting phases can then no longer be obtained through a linear combination of phases with fixed composition. Such a coupling could arise from the influence of the interior composition on the bilayer thickness, which will in turn affect the stability and structure of the edge. If the edge could be made more stable by changing the thickness, as the edge becomes a greater fraction of the total system the composition of the bulk will change to better accommodate the edge. Bulk composition changes might influence the edge stability by changing bending rigidity as well as thickness. At high line tensions, the increase in edge length that will accompany most out-of-plane surface bending modes will suppress surface bending fluctuations of the ribbon. When line tension is no longer significant, fluctuations of the edge normal to the bilayer plane will incur an energy cost from the bending of the surface, which may explain why these out-of-plane fluctuations are weaker than in-plane fluctuations, as seen in Fig. 2.

**Protrusion contribution to fluctuation**

The plateau of fluctuation amplitudes at \( q > 0.3 \text{ nm}^{-1} \) in Figs. 6 and 7 is a deviation from the predictions of the model that treats the edge as a continuum structure with a line tension. As a small wavelength effect it is likely to come from molecular level fluctuations, analogous to the lipid protrusions from a bilayer sheet as addressed in previous papers. In those analyses, the effect of molecular protrusions on the mean square amplitude of fluctuations in Fourier space was found to be the addition of a term proportional to \((1+bq^2)^{-1}\). When \( bq^2 \) is not too large, a plateau like the one observed is expected. Fluctuations with \( q \)-independent amplitude could arise from random noise, which may be a contributor to the plateau value in the MD data and is the only explanation available for the plateau in the MC data, where molecular protrusion modes are not represented.

**CONCLUSIONS**

We have performed molecular dynamics simulations on mixed-lipid systems using a coarse-grained force field to investigate the behavior of a stable bilayer edge. The system edge behavior can be divided into two regimes based on its fluctuation property and line tension as follows.

1. **High line tension regime.** When DBPC% \( \leqslant 30\% \), although fluctuation of the edges has a trend to become larger, the amplitude of fluctuation remained small. Line tension calculated in this regime is \( \geqslant 24 \text{ pN} \).

2. **Low line tension regime.** When DBPC% is from 35% to 60%, line tension becomes negligible and edges show very strong fluctuations.

By fitting the MD data with a simple MC model, we discover that in regime (1) line tension is the dominating factor that determines the edge fluctuations. This result is confirmed by a theoretical model as well. However, in regime (2) the edge is controlled by a harmonic term that has a preferred edge length and by a bending term. The in-plane bending rigidity of the stable edge leads to an apparent persistence length of the order of the bilayer thickness. We observed that short-tail lipid concentration in the bulk of ribbon increases with the total short-tail lipid concentration in the
systems even in the low line tension regime, where a two-component two-phase model would predict a plateau. Several possible explanations for this effect are proposed, but in any case the results call into question of the common assumption that in experimental bicelle systems that bilayer edge and interior compositions are fixed over the range of compositions at a given temperature.

ACKNOWLEDGMENT

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