Determination of Phase Transition Temperatures for Atomistic Models of Lipids from Temperature-Dependent Stripe Domain Growth Kinetics

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Received: May 18, 2010; Revised Manuscript Received: June 29, 2010

Many lipid bilayers undergo a reversible order−disorder transition between the gel and liquid crystalline (LC) phases at a main phase transition temperature T_m that is an important characteristic property of the lipid. Although T_m should serve as a useful standard for validation and calibration of simulation models of lipid bilayers, its evaluation within simulations is difficult due to the slow kinetics of the gel−LC transition, especially near T_m. A stripe growth strategy for calculating T_m, which aims to bypass the slowest steps in this transition, has been applied to dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine bilayers represented with a commonly used united-atom force field. The strategy consists of initial preparation of a bilayer containing gel and LC domains arranged as parallel stripes, observation of the direction and rate of domain growth over a range of temperatures, and fitting rates to an Arrhenius-like equation for their temperature dependence that crosses zero at T_m. Calculated T_m's for both lipids are 5−6 degrees lower than their experimental values, in much closer agreement with experiment than suggested by recent simulations that simulate heating and cooling of bilayer patches. The stripe growth method also yields rates of phase front propagation that are in order-of-magnitude agreement with experimental estimates, as well as insight into glycerol backbone disordering at the LC−gel interface.

Introduction

Much has been learned about the phase behavior of lipids, the primary component of biological membranes, in the past few decades. One contribution of interest in this field is the hypothesis that ordered lipid domains called rafts are central to many biochemical processes.[1−5] Computer simulation has been an important tool in the investigation of lipid bilayers, providing details that are generally inaccessible to experiments. Most force fields used in the study of lipid bilayers were originally developed for the fluid liquid crystalline (LC) phase but have proven to be transferable to the more ordered gel phase[6,7] as well as intermediate phases,[8] reproducing structural features with generally good success. It is more difficult to validate the ability of a force field to reproduce the thermodynamics of the ordering process, the main transition temperature T_m being the most relevant quantity, because transitions between the two phases are slow on the simulation time scale, particularly near T_m. Insight about how well force fields agree with experimental phase behavior of single-component lipid systems is desirable for the interpretation of simulation data pertaining to more complex ordered phases such as the cholesterol-containing liquid ordered structure.[9−11]

Two recent attempts at calculating T_m have been published, both using the same popular united-atom force field of Berger et al.[12] Leekumjorn and Sum simulated annealing a fully hydrated DPPC bilayer from 250 to 350 K and back to 250 K.[13] Plotting the area per lipid as a function of temperature revealed a loop of hysteresis with a crossover between two linear regimes, most evidently in the heating trajectory. The intersection of the linear portions was identified as the transition temperature, found to be 10 °C below the experimental main phase transition temperature ($T_{m,exp}$). Qin et al. used similar annealing runs plus long trajectories over a range of fixed temperature points to estimate a T_m of 299 K for DSPC, almost 30° below that of experiment.[14] The very significant degree of hysteresis in the annealing runs makes the identification of a transition temperature quite difficult and potentially dependent on the rate of heating and cooling. Part of the reason for this hysteresis is the interfacial free-energy barrier that must be surmounted in the nucleation of a new phase. A recent coarse-grained study suggested the gel−LC transition occurs in four distinct steps, (i) phase nucleation, where 20−80 lipids congeal and form a gel domain within the bulk LC, (ii) fast growth, where the number of lipids added to the gel domain increases rapidly, (iii) slow growth, in which gel domains start to percolate, and (iv), optimization, where trapped LC domains persist on the microsecond time scale and defects are eventually resolved.[15,16] The size and time limitations of atomistic studies suggest that, of all the above steps, step (ii), fast growth, is the most practical to study.

This report describes an approach to evaluating T_m within a molecular simulation of a lipid bilayer based on a strategy of isolating the fast growth stage. This simple method consists of preparing a system consisting of gel and fluid phases coexisting in adjacent stripes and observing the rate at which one phase or the other grows as a function of temperature. Our aim in using pre-existing stripes is to permit molecules to undergo phase changes without either creating a new interface, which is expected to involve a high free-energy barrier, or changing the length of existing interfaces, which would introduce a bias due to interfacial line tension. Results from this method for both DPPC and DSPC bilayers suggest transition temperatures that are ∼5−6 K lower than those of experiment. Possible sources of systematic error and reasons for the discrepancy with estimates taken from annealing trajectories will be discussed. Finally, we will comment on the structure of the interface and the rate of transition near the transition temperature.

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10.1021/jp104529m
Published on Web 08/06/2010

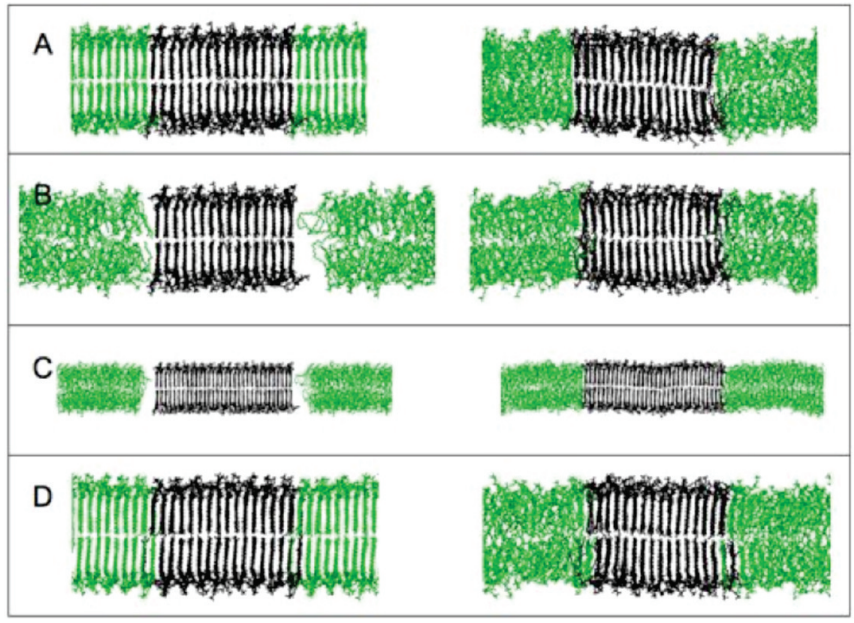

Figure 1. Constructs before and after equilibration. Systems were prepared from all-gel (A, D) or pre-equilibrated gel and LC (B, C). Panel A shows a 256 lipid DPPC system, (B) a 256 lipid DPPC system, (C) a 512 lipid DPPC system, and (D) a 256 lipid DSPC system. All panels show the system before equilibration (left) and after (right).

Methods

Simulations. ***Two-Phase System Construction.*** The two-phase (stripe) systems were based on gel structures constructed as follows: two lipids were patterned after the two unique lipids in the crystal structure of DMPC,[17] with two methylene carbons added per tail using Spartan molecular modeling software (Wave Function, Inc.). The remainder of the system preparation was done with GROMACS 3.2.[18−20] The molecules were oriented in the simulation box with the *z*-axis corresponding to the bilayer normal and the primary axis of the acyl tails in the *yz*-plane. The two lipids were rotated 180° about the *y*-axis and translated in the *x*-axis to make the lipid tails' primary axes collinear. The four resulting lipids were copied and translated in the *x*- and *y*-axes to achieve hexagonal packing of the lipid tails with an area per lipid of 44.7 Å^2. For the DPPC gel, the 8 lipid configuration was then quadrupled in *x*, hydrated with 25 SPC waters per lipid, minimized over 100 steps via the method of steepest descents, and incubated with molecular dynamics (MD) for 10 ns at 273 K. The resulting configuration was doubled once again in *x* and *y*, and this 128 lipid system was run for another 10 ns at 293 K. The area per lipid evolved to 52 Å^2, consistent with values previously reported.[7,14,21] The DSPC gel was constructed in almost exactly the same way. The 8 lipid configuration was doubled twice in *y* and once in *x* to make a 64 lipid configuration, which was minimized and run at 313 K for 10 ns, then doubled for 128 lipids and 3600 water molecules, and run for another 20 ns, again at 313 K. The mean area per lipid was 52.2 and 52.6 Å^2 for DPPC and DSPC gel constructs, and tilt angles were 41.9 and 42.4°, respectively; these are in fair agreement with experimental values, that is, 47 Å^2 and 32°.[22]

Two methods were used to prepare two-phase systems. In the first, referred to below as the m method, the pre-equilibrated gel was incubated with half of the lipids (divided along one axis) set at 293 K and the other half (and solvent) set at 353 K for 10 ns (see Figure 1A). In the second, referred to below as the p method, two 128 lipid pre-equilibrated bilayers, gel and LC, were combined side-by-side within a simulation box, with adequate space to avoid overlaps, and run for 10 ns (see Figure 1B). The temperatures of the gel, the LC, and solvent were set to 293, 353, and 353 K, respectively, as before. Areas per lipid and the fraction of tail dihedrals in the trans conformation reached plateau values within 10 ns (data not shown). The stripe DSPC system was constructed as described above, except that the gel and LC/solvent were run at 303 and 363 K, respectively (see Figure 1D). In all cases, sufficient solvent molecules (25 waters per lipid head group) were included to bring the simulations well within the fully hydrated regime; properties of DPPC gel and LC phases are reported to be hydration-independent above 11 and 20 waters/lipid, respectively, both according to experiment[23] and simulation.[24]

All MD simulations were run with GROMACS 3.3 and 3.4[18−20] and the following parameters. The Berendsen barostat[25] was used for anisotropic pressure coupling, with box dimensions and angles free to adapt independently to the stresses in the system. The reference pressures were 1 bar, with all compressibilities set to 4.5×10^{-5} bar^{-1} and a time constant of 2 ps. Langevin dynamics were used for the thermostat,[26] with a time constant of 0.2 ps. Interaction cutoffs (neighbor lists, van der Waals radii, and Coulombic cutoffs) were all set to 1.0 nm. Electrostatics were calculated with Particle Mesh Ewald sums.[27] A time step of 2 fs was used. Both the DPPC and DSPC systems were run at a range of temperatures listed in Table 1.

DSPC Cooling. A previously equilibrated system containing 128 DSPC lipids and 3200 water molecules in the fluid phase[7] was cooled from 333 K, above the experimental main phase transition temperature of 328 K, down below 300 K at various rates, 1, 0.5, 0.2, and 0.1 K/ns.

Analysis. To calculate phase amounts, a lipid was classified as belonging to the gel phase if it, and at least four of its nearest neighbors, had a maximum of two gauche tail dihedrals. The nearest-neighbor criterion was used to eliminate error due to transient ordering of a lipid, which can happen in the LC phase. The lipid gel fraction was plotted against simulation time. A linear regression of these curves gives a line whose slope is the rate of change; since the simulation boxes have two-phase interfaces, the rate of interface translation is the rate of change of phase composition multiplied by the width of the simulation box (in nm) divided by 2. These curves were divided into

TABLE 1: Description of Systems Simulated[a]

lipid	temperature (K)	number of lipids-construction method	simulation length (ns)
DPPC	293	256-m	100
DPPC	298	256-m	100
DPPC	300.5	256-m	100
DPPC	303	256-m; 256-p; 512-p	160; 100; 20
DPPC	308	256-m; 256-p; 512-p	100; 100; 20
DPPC	310.5	256-m	100
DPPC	313	256-m	100
DSPC	313	256-m	100
DSPC	318	256-m	100
DSPC	320.5	256-m	100
DSPC	323	256-m	100
DSPC	325.5	256-m	100
DSPC	328	256-m	100

[a] The range of temperatures for each lipid system was simulated is shown (second column). The number of lipids and method of construction for systems starting as all-gel and creating a LC stripe by melting half of the lipids, m, and for the systems made by flanking pre-equilibrated gels with LCs, p, are given.

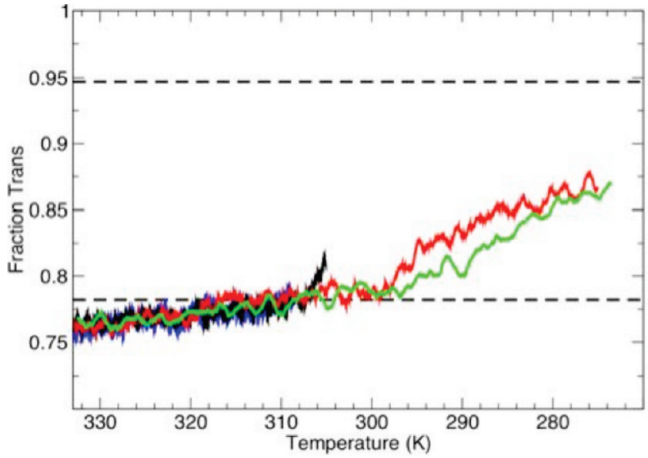

Figure 2. DSPC tail order versus temperature. A pre-equilibrated DSPC LC cooled at different rates; shown is the time evolution of fraction of tail dihedrals in the trans conformation for simulations run at 1 (green), 0.5 (red), 0.2 (black), and 0.1 K/ns (blue), all with 1 ns running averages. For comparison, 50 ns averages of gel (at 0.95) and LC (at 0.77), both run at 313 K, are shown by black dashed lines.

uncorrelated samples by the method described in Allen and Tildesley,[28] section 6.4, yielding segment lengths between 1 and 9 ns, and error bars represent the standard error of the slopes calculated over uncorrelated segments of the trajectory.

Results

Lipid Bilayer Annealing. In principle, a gradually cooled lipid bilayer should undergo a phase transition at a characteristic temperature T_m. At least two previous simulation studies have aimed to determine T_m through this approach.[13,14] In both cases, a pre-equilibrated LC system was cooled at 2.5 K/ns. To investigate the reproducibility of this method, DSPC was cooled from 333 K, 5 K above the experimental T_m ($T_{m,exp}$), at even slower rates: 1, 0.5, 0.2, and 0.1 K/ns. The fraction of acyl tail *trans*-dihedrals initially increased slowly with decreasing temperature (Figure 2). As in the previous studies, a change in slope was observed upon reaching a sufficiently low temperature. However, two observations indicated that the results were influenced strongly by the kinetics of forming a new phase; the temperature where the change in slope was observed was

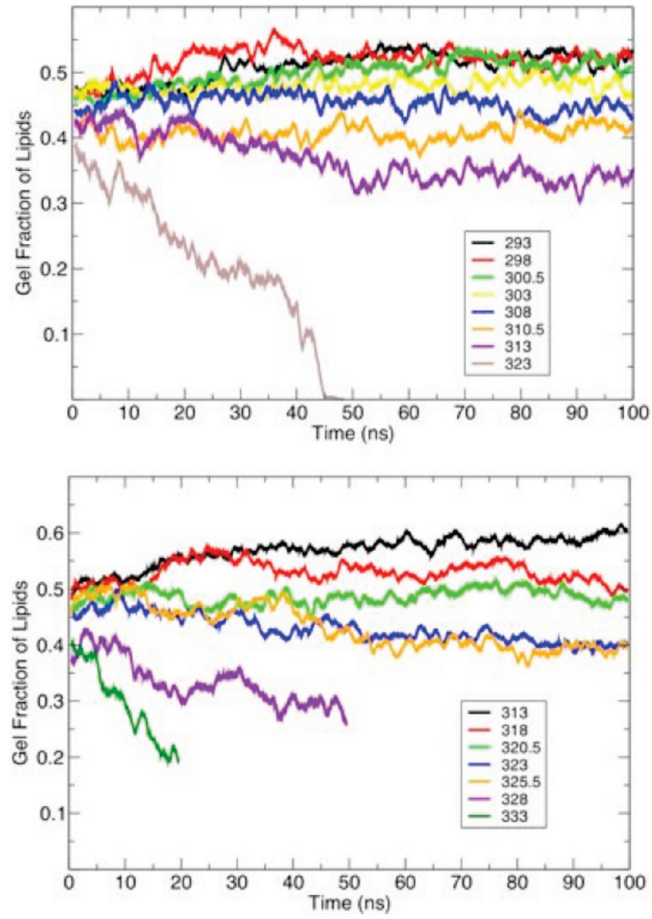

Figure 3. Gel fraction of PC lipids over a range of temperatures. The fraction of total lipids (DPPC, top panel; DSPC, bottom panel) qualifying as gels is plotted as a function of time. The 1 ns running averages are shown.

sensitive to the rate of cooling, and further cooling down to 273 K (for 1.0 and 0.5 K/ns trajectories) failed to produce a uniformly ordered gel phase.

To avoid the uncertainties associated with the annealing method, an independent method of obtaining T_m within a simulation model was applied. As described in the Methods section, the stripe growth method involves preparing a system composed of alternating stripes of gel and LC domains and observing the dynamics of domain growth in independent simulations over a range of temperatures.

Qualitative Analysis of the Gel−LC Transition. Two-phase simulations of both DPPC and DSPC run at several temperatures (Table 1) show that the speed and direction of the phase transition changes with temperature, as indicated by plots of gel fraction versus simulation time (Figure 3) and snapshots of simulation end points (Figure 4). The DSPC simulation at 313 K, for example, exhibits a visible widening of the gel stripe, while at the highest temperatures studied (328 and 333 K), the gel is plainly melting at the interface. At intermediate temperatures, the trend is not immediately obvious. By gross qualitative measures, the two-phase method appears to indicate a T_m between 313 and 328 K for the DSPC model, a range beginning 15 K below the experimental $T_{m,exp}$ and extending up to $T_{m,exp}$.

Visual inspection of stripe trajectories yields further insight into structural details of the phase transition. At all temperatures, changes in lipid order were localized at the gel−fluid interface. Even for DPPC at 313 K, where the gel phase melted completely, melting occurred from the boundary inward. This

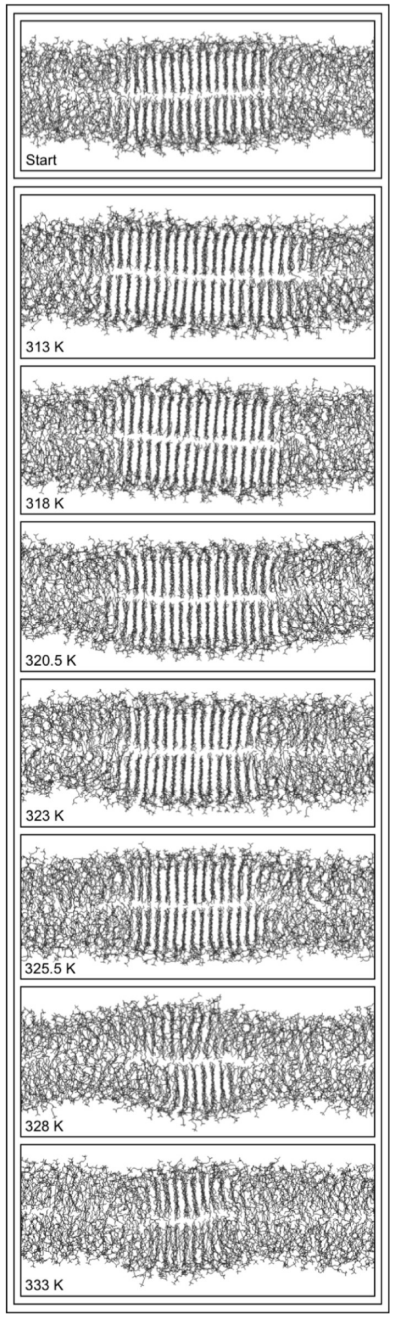

Figure 4. Snapshots of DSPC two-phase systems. Snapshots of all temperatures used for simulations are shown as labeled. The top panel is the system at $t = 0$ ns. The bottom panel shows the systems at 100 ns; (in descending order) 313, 318, 320.5, 323, 325.5, 328, and 333 K.

observation is consistent with the notion that phase nucleation is a rate-limiting step. Snapshots of a DSPC interface at 313 K (Figure 5) reveal that lipids that have transitioned from a LC to a gel at the interface exhibit decreased lateral orientational order, relative to those in the preprepared gel stripe. The snapshots are down the tails' primary axis to highlight their arrangement in a hexagonal array. As described in the Methods section, within the constructed gel phase, lipids are arranged in well-defined rows with their glycerol backbones parallel to the stripe dimension. In contrast, lipids that congeal at the interface do not conform to this arrangement. Although the tails still form a hexagonal array tilted parallel to the stripe dimension, the two tails of a given lipid may not lie in the same row and may not even occupy nearest-neighbor sites within the hexagonal lattice.

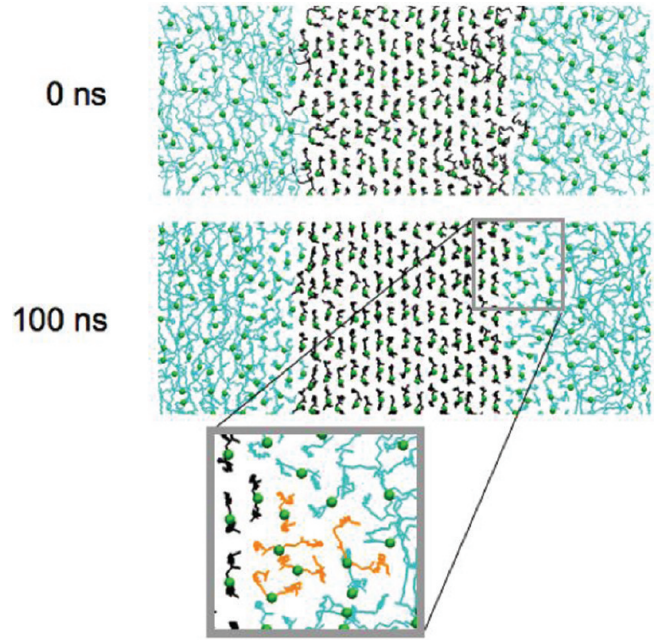

Figure 5. Gel−LC interface below T_m. The glycerol backbone and acyl tails of a DSPC system simulated at 313 K are shown at $t = 0$ (top) and 100 ns (bottom). For clarity, the system is doubled in the dimension parallel to the interface, only one leaflet is shown, the head groups are hidden, and the leaflet is rotated so the view is down the axis of the gel tails. Molecules are colored for their starting phase; gels are black, and LCs are cyan. To underscore molecular orientation, carbon #13 (of the glycerol backbone) is depicted by a green sphere. The magnified field shows five gel lipids that started as LCs recolored in orange to highlight their disordered tail packing.

Figure 5 displays this arrangement in the magnified field from the 100 ns time frame at the bottom. For example, of the five lipids colored orange, the top left lipid appears to have congealed following the same pattern of the original gel. However, the one at the bottom left is turned 60° in the plane perpendicular to the tails. Its two tails occupy separate rows. Two other lipids, the second from the top on the left and the rightmost, deviate even more from the original packing pattern; both of them have non-nearest-neighbor tails. Similar disordering at the interface is also seen during melting. Such disorder in glycerol backbone orientations has been inferred from X-ray and neutron scattering data[29,30] and therefore may be a characteristic of the equilibrium bulk structure and not just at the interface.

Quantitative Measurement of Domain Growth. The average rate of expansion or retraction of the gel phase edges for DPPC and DSPC at each temperature, as described in the Methods section, was estimated by linear regression from the data in Figure 3 and is plotted in Figure 6. We find that an equation with three adjustable parameters

$$\text{rate} = A'' \cdot (T_m - T) \cdot \exp(-E_a/RT) \qquad (1)$$

provides reasonable fits to the data. This equation makes phenomenological sense if we consider the conversion between phases to be an activated process with activation energy E_a and a driving force proportional to the difference in free energies between the phases and proportional to T_m - T close to the transition temperature. In contrast with both experimental and simulated domain growth kinetics that include a nucleation step followed by growth of compact domains,[15,16] line tension does not appear as a parameter in the current model because stripe

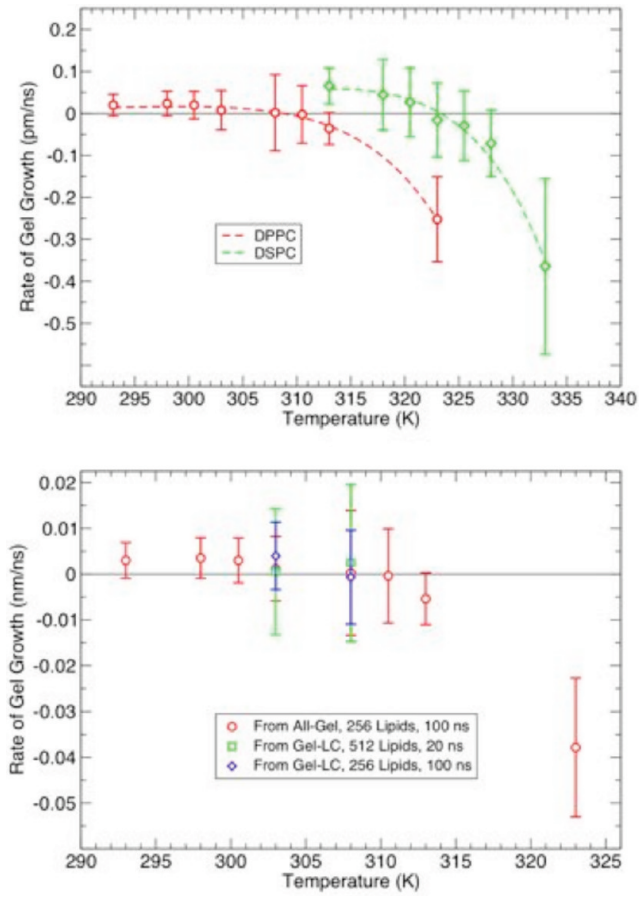

Figure 6. Main phase transition rates versus temperature. The rates of transition of DPPC (red) and DSPC are shown in red and green, respectively (top panel). Open circles are the rates, and the hashed line is the best fit. The rates of main simulations of DPPC are compared with the DPPC controls (bottom panel).

TABLE 2: Best Fit Parameters[a]

	A'' (nm·ns^{-1}·K^{-1})	T_m (K)	E_a (kJ/mol)
DPPC	1.99×10^9	308.5	68.4
DSPC	2.60×10^{10}	323.4	75.6

[a] The rates of phase transition for DPPC and DSPC systems were fit to eq 1, giving values shown here.

broadening does not change the length of the interface. A least-squares fit of the data to eq 1 (shown in Figure 6) yields estimates of A'', E_a, and T_m (see Table 2). Apparent activation energies are near 70 kJ/mol in both systems. Interconversion between gel and LC conformations involves a large conformational change to the lipid tails. With the torsional barrier of about 10 kJ/mol between gauche and anti structures for each tail dihedral, this activation energy could be interpreted as an indication that the transition requires cooperative rearrangements involving seven torsional angles within the tails.

The transition temperatures calculated from fits to eq 1, 308 K for DPPC and 323 K for DSPC, are closer to their experimental transition temperatures than the previous studies have suggested. Leekumjorn and Sum estimated the T_m of DPPC using this force field to be 10 K less than the experimental T_m,[13] and Qin et al.[14] estimated the T_m of DSPC (with the same force field) to be 29 K less than the experimental T_m. The present study suggests that the simulation model may underestimate the T_m by as little as 6 K for DPPC and 5 K for DSPC.

While the primary goal of this study was to estimate T_m, the actual values of the rates of domain growth are also of interest. An experimental study[15] indirectly established that within 5 K of T_m in DPPC, the interface travels at a rate proportional to $(T - T_m)$, with a constant equaling 0.002 nm·ns^{-1} K^{-1}; the corresponding constant from the present fit would be given by $A'' \exp(-E_a/RT_m) = 0.005$ nm·ns^{-1} K^{-1}, in fair qualitative agreement. A coarse-grained model study reported linear dependence of domain growth with a slightly higher coefficient, 0.007 nm·ns^{-1} K^{-1}.[16]

Discussion

A plausible explanation for why the stripe growth method yields a higher T_m than annealing methods lies in the stability of the gel phase. During relatively rapid heating/cooling loops, optimal packing of the gel lipids is probably not achieved, as is evident in the regions of disorder characteristic of the starting fluid phase (not shown) that persist even at temperatures well below the apparent T_m. Thus, the stabilities of the gels will tend to be underestimated by heating/cooling loops. In one study, an ordered phase formed with different orientations of lipid tails in the two layers during a deep quench to 250 K.[14] While this structure may represent the thermodynamic free-energy minimum well below T_m, if it is metastable close to T_m, it too will melt at a lower temperature than would a more stable ordered structure. Assuming that the high-temperature phase equilibrates more rapidly than the low-temperature phase, estimates to T_m in poorly equilibrated systems will generally be too low.

The stripe-growth method is itself subject to several potential sources of error in calculating T_m. It replaces the uncertainties of generating a gel phase through quenching with uncertainties associated with initial construction of the alternating stripe structure. For the gel phase domain in particular, choice of an initial configuration may bias the gel structure even over the course of a 100 ns simulation. An unequilibrated phase is by definition less than optimally stable; therefore, by assuming that the fluid phase is closer to equilibrium than the gel, the T_m evaluated by the present method will represent a lower bound to the T_m of a fully equilibrated gel. Even less certain is the choice of how to best prepare the gel−LC interface. In an attempt to control for the effects of how the initial interface is prepared, stripe systems were prepared in two different ways, first by melting half of the lipids in a pre-equilibrated gel (Figure 1A) and then by flanking pre-equilibrated gel with pre-equilibrated LCs (Figure 1B). The rates of phase change were the same within statistical error (Figure 6, bottom panel). Finite-size effects are also a concern in studies with interfaces. Specifically, how far do the effects of the interface extend into the bulk? And equivalently, are there lipids far enough from the interface that they exhibit true bulk properties? This is addressed by comparing the phase change rates of the constructs with different phase widths (Figure 6, bottom panel). No significant difference was identified between the small and large systems.

An open question about the current data is why the initial rates of gel phase growth or disappearance slow down over the course of the trajectory, resulting in a leveling off of most of the curves in Figure 3. This diminishment suggests that over time, either the driving force for further transitions goes down or the barrier goes up. The driving force for the conversion of one phase to another could change over the course of the trajectory due to finite-size effects; the rate may depend on the width of one phase or another. As stated above, the simulations on a system that is twice as wide do not seem to corroborate this; neither does the observation of diminishing rates for both gel growth and gel disappearance. Rather, we hypothesize that

the barrier for changing the position of the interfacial front increases over the course of the trajectory as the interface becomes increasingly stable through some slow relaxation process. In fact, the observed reordering of the interface (Figure 5), both during congealing and melting, is indicative of such a relaxation process. The gradual change in interfacial structure would reduce the rate of phase growth in both directions, affecting our estimates for the absolute rate of interfacial growth. The implications of such a change for the estimates of T_m are not clear. In principle, the direction of spontaneous phase transition is independent of the details of the interface; in practice, it is difficult to rule out the possibility that the restructuring of the interface may influence an apparent phase growth. The uncertainty about the structure of the interface and the computational expense of simulating long time intervals make this last concern the most difficult to address.

Conclusions

Determination of the main phase transition temperature of lipid bilayers simulated with atomistic force fields is an important step in force field validation.[13,14] In the present study, the rates of phase growth in coexisting gel and LC phases preformed into stripes have been investigated as a new way of obtaining the main phase transition temperature T_m. Our results indicate T_m values that are higher and in closer agreement with experiment than those obtained through annealing studies. The use of a linear phase interface allows the measurement of rates of domain growth, yielding an estimated activation barrier of 70 kJ/mol for the transition, with absolute rates of the same order of magnitude as those determined by experiment.[15] Inconsistency in rates over the course of 100 ns simulation trajectories may be related to structural evolution of the gel−LC interface from an initial state with aligned glycerol backbones to a rougher structure with orientational defects. Whether this disorder is localized at the interface and acts as a barrier to further growth or such disorder is in fact more characteristic of the bulk gel state than the ordered structure originally prepared remains a question for future study.

Acknowledgment. We thank Fuchang Yin and Ana West for many helpful discussions and Mr. Yin for his invaluable contributions of computational tools for analysis and system preparations. This material is based on work supported by the National Science Foundation under Grants No. CHE-0616383 and CHE-0911285.

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JP104529M