The order-disorder transition in model lipid bilayers is a first-order hexatic to liquid phase transition

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We characterize the order–disorder transition in a model lipid bilayer using molecular dynamics simulations. We find that the ordered phase is hexatic. In particular, in-plane structures possess a finite concentration of 5–7 disclination pairs that diffuse throughout the plane of the bilayer, and further, in-plane structures exhibit long-range orientational order and short-range translational order. In contrast, the disordered phase is liquid. The transition between the two phases is first order. Specifically, it exhibits hysteresis, and coexistence exhibits an interface with capillary scaling. The location of the interface and its spatial fluctuations are analyzed with a spatial field constructed from a rotational-invariant for local 6-fold orientational order. As a result of finite interfacial tension, there necessarily exist associated forces of assembly between membrane-bound solutes that pre-melt the ordered phase.

lipid bilayers, phase transition, hexatic, line tension

Abbreviations: DPPC (dipalmitoyl phosphatidylcholine)

In this and the following paper [1], we detail the existence of a first-order order—disorder transition in model lipid bilayers, and we show how pre-melting of the ordered phase is responsible for a powerful membrane-mediated force between transmembrane proteins. Pure lipid bilayers primarily exist in two phases—an ordered, solid-like phase often referred to as the 'gel' phase (L_{β} or $L_{\beta'}$ phase), and a disordered, liquid-like phase (L_{α} phase) [2]. Here, we use molecular dynamics of the MARTINI model [3,4] to investigate the nature of the ordered phase and the transition to the disordered phase.

Prior work with the MARTINI model [3,5] on small sections of membrane showed hysteresis when transitioning between ordered and disordered structures. This form of collective behavior is suggestive of singular behavior, but the methods of analysis employed in the earlier works were insufficient to demonstrate the scaling inherent to a first-order transition and whether the ordered phase is a crystal or some other structure.

In following up on Refs. [3] and [5], we establish that a stable interface exists between the ordered and disordered phases at coexistence, and that the fluctuations of the interface are capillary-like [6, 7]. These findings establish that the transition between the ordered and disordered membranes is first order, which is consistent with experiments [8]. Further, we show that the spatial structure of the ordered phase exhibits long-range bond-orientational order and short-range translational order. These findings indicate that the ordered membrane is a hexatic phase, like those considered in melting of two-dimensional systems [9–11], but experiments have not yet determined whether ordered phases of membranes have this structure.

A molecular model that reproduces the phase behavior of a lipid bilayer is needed to understand lipid behavior around solutes such as proteins and cholesterol, and therefore to un-

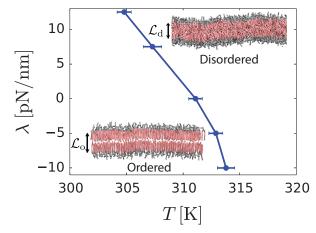


Fig. 1. Melting points of the ordered phase in terms of lateral pressure (λ) and temperature (T), estimated from 10 independent heating runs like those illustrated in Fig. 2. Insets are cross sections showing configurations of a bilayer with 3200 lipids in the ordered and disordered phases. The heads are colored gray while the tails are colored pink. Water particles are omitted for clarity. The hydrophobic thicknesses, \mathcal{L}_{O} and \mathcal{L}_{cl} , are the average vertical distances from the first tail particle of the upper monolayer to that of the lower monolayer.

derstand lipid-mediated interactions [1,12]. While atomisticlevel models [13,14] provide fine-scale structural details, they are far too computationally expensive to study the phase

Significance

Lipid bilayers exist in ordered and disordered states — so-called "gel" and "liquid" phases. First-order phase transitions between the two have long been known, but whether the ordered phase is crystal-like or hexatic has not been known. Here, using large scale molecular simulation, we demonstrate a first-order transition and predict that the ordered phase is hexatic, which can provide a basis for understanding mobility and organization of proteins in the ordered phase.

Reserved for Publication Footnotes

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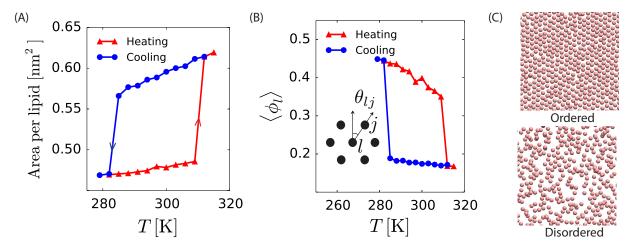


Fig. 2. Order parameter variation with temperature: (A) Variation in area per lipid with temperature during heating and cooling shows finite jumps and hysteresis. (B) The order parameter $\langle \phi_l \rangle$ also shows finite jumps as a function of temperature while heating and cooling. Both heating and cooling is performed at a rate of 3 K/ μ s. (C) Snapshots from the ordered phase at 279 K (top) and the disordered phase at 315 K (bottom). Both display the tail-ends of each lipid in one monolayer of the bilayer. The ordered phase exhibits a hexagonal packing, while the disordered phase exhibits a random arrangement of the tail-end particles. Empty regions in the disordered configuration are mostly filled by tail-ends from the other monolayer or by non tail-end particles, none of which are not shown in the snapshot.

behavior of a bilayer system with any reasonable degree of rigor. To enable access to sufficiently large length scales and time scales (admittedly at the cost of reduced detail), coarse-grained models have been constructed for lipid bilayers and studied using molecular simulations [3, 4, 15–17]. Some of these models exhibit a possibly solid-like ordered phase, and a liquid-like disordered phase.

We have chosen the MARTINI model because it successfully reproduces equilibrium bilayer properties such as the area per lipid and bending modulus [4]. Additionally it has been applied to studies of the plasma membrane [18], lipid rafts [19], organization of transmembrane proteins [20], and membrane tethers [21]. Although we choose dipalmitoyl phosphatidylcholine (DPPC) as a model lipid, we expect our results to hold for many other lipid bilayers or monolayers that exhibit ordered and disordered phases.

Order–disorder transition in a model lipid bilayer. Fig. 1 contrasts configurations and shows our estimated phase boundary between ordered and disordered phases in the DPPC MAR-TINI bilayer system. The ordered phase has regular tail packing compared to the disorganized tail arrangement of the disordered phase. A consequence of the regular tail packing is that hydrophobic thickness of the ordered phase, \mathcal{L}_{o} , is larger than that of the disordered phase, \mathcal{L}_{d} . Correspondingly, the area per lipid in the ordered phase is smaller than that in the disordered phase.

The lateral pressure–temperature, λ –T, phase diagram shown in Fig. 1 was estimated from heating runs of a small system — 128 lipids solvated by 2000 water particles. Fig. 2A shows the change in area per lipid with temperature while heating and cooling a bilayer. There are finite jumps in area per lipid as the system transitions between the two phases, suggesting a first-order phase transition. The hysteresis is indicative of the fact that ordering from the metastable disordered phase is much slower than disordering from the metastable ordered phase. Accordingly, contrasting melting and freezing from heating and cooling runs, respectively, the melting points from heating runs provide the more accurate estimates of the actual phase boundaries. The phase boundary graphed in Fig. 1 shows error estimates based upon the

statistics of several heating runs. Systematic errors due to small system size and heating rate have not been estimated.

A convenient visual representation to distinguish between the two phases is to view the end particles of each lipid chain in one of the two monolayers. (Which of the two chosen for this rendering is irrelevant.) This representation is shown in Fig. 2C for the ordered and disordered phases. These tail-end particles appear hexagonally-packed in the ordered phase and randomly arranged in the disordered phase.

To quantify this impression, we build an order parameter from Halperin and Nelson's local rotational-invariant [9, 10],

$$\phi_l = \left| \frac{1}{6} \sum_{j \in \text{nn}(l)} \exp(6i\theta_{lj}) \right|^2,$$
 [1]

where θ_{lj} is the angle between an arbitrary axis and a vector connecting tail-end particle l to tail-end particle j. Here, the summation over $j \in \operatorname{nn}(l)$ is over the six nearest neighbors of particle l. (See inset in Fig. 2B.) The ensemble average, $\langle \phi_l \rangle$, is unity for a perfect hexagonal packing, and it is zero to the extent that hexagonal packing is entirely absent. The variation in $\langle \phi_l \rangle$ with temperature for a DPPC bilayer is shown in Fig. 2B. The finite jumps in $\langle \phi_l \rangle$ as a function of temperature, and the hysteresis, are again suggestive of a first-order phase transition.

Stable interface between the ordered and disordered phases exhibits capillary scaling. To establish whether the first-order-like behavior described above persists to larger scales and thus actually manifests a phase transition, we consider larger systems and the behavior of the interface that separates the ordered and disordered phases. Fig. 3A shows this coexistence for a system size of N=3900 lipids with an interface between the two phases. The interface, which spans the membrane, is equilibrated in the constant area ensemble. This ensemble can maintain an area per lipid that is intermediate between two phases and can therefore stabilize an interface if, in fact, two distinct phases do exist. At such conditions, a line tension can then be calculated from the power spectrum of the interfacial fluctuations.

To analyze interfacial fluctuations, we first identify the location of the interface at each instant. This location is found with a two-dimensional version of the three-dimensional construction described in [22]. Specifically, the interface is here

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defined as a line in the plane of the bilayer where the value of a coarse-grained order-parameter field is intermediate between those of the ordered and disordered phases. The field we use is

$$\bar{\phi}(\mathbf{r},t) = \sum_{l} f(\mathbf{r} - \mathbf{r}_{l}; \xi) \phi_{l},$$
 [2]

where \mathbf{r}_l is the position of the *l*th tail-end particle projected onto a plane parallel to that of the bilayer, and $f(\mathbf{r} - \mathbf{r}_l, \xi)$ is a coarse-grained delta-like function in the two-dimensional space,

$$f(\mathbf{r} - \mathbf{r}_l; \xi) = (1/2\pi\xi^2) \exp(-|\mathbf{r} - \mathbf{r}_l|^2/2\xi^2)$$
. [3]

Again, the tail-end particles are from the chains of only one of the two monolayers forming the bilayer. The field variable, \mathbf{r} , is a two-dimensional vector specifying a position in the plane of the bilayer. The coarse-graining width, ξ , is chosen to be the average separation between tail-end particles l and j when $\langle (\phi_l - \langle \phi_l \rangle)(\phi_j - \langle \phi_j \rangle) \rangle / \langle (\phi_l - \langle \phi_l \rangle)^2 \rangle$ in the ordered phase is 1/10. This choice yields a value of $\xi = 1.5$ nm.

For numerics, a square lattice tiles the average plane of the bilayer, and the coarse-grained field $\bar{\phi}(\mathbf{r})$ is evaluated at each lattice node. For convenience, the coarse-graining function is truncated and shifted to zero at 3ξ . The instantaneous order–disorder interface is identified by interpolating between these adjacent lattice nodes to find the set of points \mathbf{s} satisfying $\bar{\phi}(\mathbf{s},t)=(\phi_{\rm d}+\phi_{\rm o})/2$. Here $\phi_{\rm d}$ and $\phi_{\rm o}$ are $\langle\bar{\phi}(\mathbf{r})\rangle$ evaluated in the disordered and ordered phases, respectively.

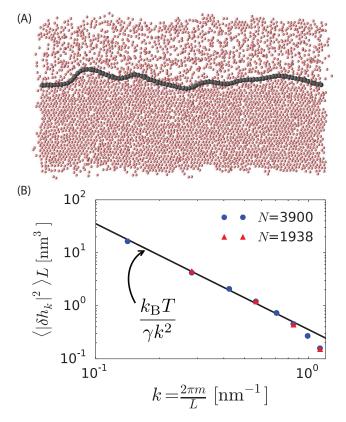


Fig. 3. Capillary fluctuations of the order–disorder interface: (A) Snapshot of a system showing coexistence between the ordered and disordered phases. The gray contour line indicates the location of the interface separating the ordered and disordered regions. The snapshot is a top view of the bilayer showing the tail-end particles of each lipid in one monolayer. (B) Fourier spectrum of the fluctuations of the instantaneous order–disorder interface. The line is the expected small-k scaling from capillarity theory.

Fig. 3A shows a snapshot of the instantaneous interface identified in this way. This free interface is stable for the length of simulations, 1.8 μs . For the thermodynamic state considered in that case, zero lateral pressure and 294 K, we have $\phi_{\rm d}=0.4\pm0.02\,{\rm nm}^{-2}$ and $\phi_{\rm o}=2.15\pm0.2\,{\rm nm}^{-2}.$

Fig. 3B shows the Fourier spectrum of the height fluctuations of this interface, $\langle |\delta h_k|^2 \rangle$. Two different system sizes are studied, with the larger system having approximately double the interface length of the smaller system. The Fourier component δh_k is related to the height fluctuation $\delta h(x)$ as $\delta h(x) = \sum_k \delta h_k \exp(ikx)$ where x is a point on the horizontal axis in Fig. 3A. Here, $0 \leqslant x \leqslant L$, and L is the box size. With periodic boundary conditions, $k = 2\pi m/L$, $m = 0, \pm 1, \pm 2, \cdots$. According to capillarity theory [7, 23], $\langle |\delta h_k|^2 \rangle \sim k_{\rm B}T/L\gamma k^2$ for small k, with $k_{\rm B}$ being the Boltzmann's constant. Given the proportionality with $1/k^2$ at small k (i.e., wavelengths larger than 10 nm), comparison of the proportionality constants from simulation and capillarity theory determines the interfacial line tension, yielding $\gamma = 11.5 \pm 0.46$ pN. (This value is significantly larger than the prior estimate of line tension for this model, 3 ± 2 pN [3]. That prior estimate was obtained from simulations of coarsening of the ordered phase.)

The stability of the interface and the quantitative consistency with capillary scaling provide strong evidence for the order–disorder transition being a first-order transition in the model we have simulated.

The ordered phase is a hexatic phase. The ordered phase shows hexagonal packing with a large number of unbound dislocations, each composed of 5–7 disclination pairs [9]. These pairs are shown in Fig. 4A, which renders a $25 \times 25 \,\mathrm{nm}^2$ bilayer in the ordered phase simulated at 294 K and zero lateral pressure. By showing three different configurations of the bilayer separated by 600 ps, we illustrate how the disclination pairs diffuse freely throughout the system. Given that a lipid bilayer is a quasi-two-dimensional system, it is possible that the ordered phase with its unbound dislocations could be in the socalled hexatic phase, an intermediate phase between the solid and liquid phases in two dimensional systems [9,10,24,27–30]. The hexatic phase, although first discovered in a purely twodimensional system, has also been observed in other quasitwo-dimensional systems such as colloidal suspensions [11,25] and lipid monolayers [26]. This phase is characterized by short-range translational order and long-range orientational order.

To test whether the ordered phase of the chosen model lipid bilayer system is a hexatic phase, we calculate appropriate translational and orientational correlation functions. As before, we project positions of tail-end particles from one of the monolayers onto the average plane of the bilayer. Translational order is examined by considering a projection of the pair correlation function along the sample orientation [35]. The pair correlation function is

$$g(\mathbf{r}) = \frac{1}{\rho} \left\langle \frac{1}{N} \sum_{l \neq j} \delta(\mathbf{r} - \mathbf{r}_l + \mathbf{r}_j) \right\rangle,$$
 [4]

where $\delta(\mathbf{r})$ is the delta function for \mathbf{r} in the plane of the bilayer, and ρ is the number of particles per unit area. Its projection, g(x,0), is $g(\mathbf{r})$ evaluated with $x=\mathbf{r}\cdot\hat{\mathbf{x}}$, where $\hat{\mathbf{x}}$ is the unit vector in the direction of the sample orientation. This orientation is the choice of Cartesian axis for which $\Psi=(1/N)\sum_l \Psi_l$ is maximal. Here, $\Psi_l=(1/6)\sum_{j\in \mathrm{nn}(l)} \exp(6i\theta_{jl})$, with the summation taken over the six nearest neighbors j of particle l.

This pair correlation function, g(x,0), has the same asymptotic decay as that of the large-distance translational

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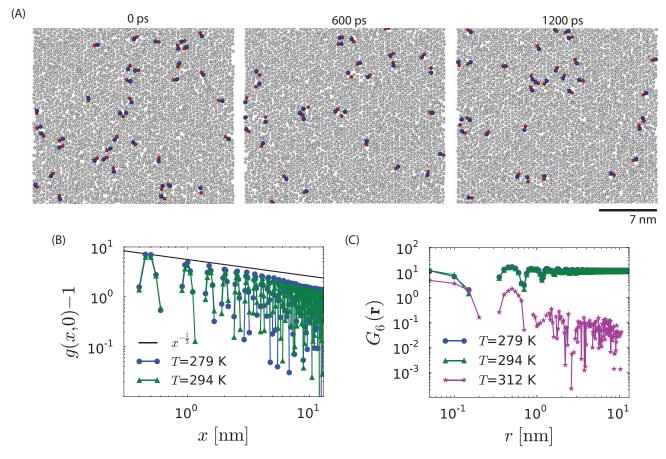


Fig. 4. Hexatic phase: (A) Dislocations (5–7 disclination pairs) in a $25 \times 25 \,\mathrm{nm}^2$ bilayer in the ordered phase. The tail-end particles of one monolayer are shown in gray. Particles with seven neighbors are highlighted in red, while particles with five neighbors are highlighted in blue. There exist several unbound dislocations as expected for a hexatic phase. These dislocations appear to diffuse freely throughout the system as seen from three different configurations separated by 600 ps. (B) One-dimensional pair correlation function g(x,0) showing decay faster than $x^{-1/3}$. (C) Orientational correlation function $G_6(\mathbf{r})$ showing long-range correlations.

correlation function defined by Halperin and Nelson [9]. In particular, a solid with long-range translational order will exhibit a power law decay of translational correlation, but our results in Fig. 4B show a more rapid decay, indicative of short-range translational order characteristic of a hexatic phase [9]. Our calculations are for a system of 3,200 lipids (solvated by 50,000 water particles), for temperatures $T=279\,\mathrm{K}$ and $294\,\mathrm{K}$. A decay proportional to $x^{-1/3}$ is the theoretical boundary below which a solid phase is no longer stable [31]. The decay of our computed g(x,0) is below that boundary for the system size we have considered.

For orientational correlations, we consider

$$\psi_6(\mathbf{r}) = \frac{1}{6} \sum_{l} \sum_{j \in \text{nn}(l)} \exp(6i\theta_{lj}) \, \delta(\mathbf{r} - \mathbf{r}_l).$$
 [5]

and compute

$$G_6(\mathbf{r}) = \langle \psi_6(\mathbf{r}) \, \psi_6^*(\mathbf{0}) \rangle$$
. [6]

For a hexatic phase, this correlation function can be either long-range or quasi-long-range (power-law decay) [24]. Our results, shown in Fig. 4C, tend to a constant value for the system sizes considered. Given the limitation in system size, we cannot differentiate between long-range and quasi-long-range orientational order. However, the decay is certainly not short-range as in a liquid. Above the melting temperature, both the translational as well as the orientational correlation functions

become short-range as seen in Fig. 4B and C, consistent with a liquid phase.

The dislocations highlighted in Fig. 4A and the correlation functions in Fig. 4B and C are calculated for the plane consisting of tail-end particles of one monolayer. We have also studied the structure and the correlation functions for planes corresponding to each particle along the length of the tail. We observe that the number of (bound and unbound) dislocations decreases as we move upwards in the lipid chain [32,33]. However, despite the disorder gradient, the translational and orientational correlations at every point along the lipid chains are consistent with the hexatic phase (see SI). The relative stability of the hexatic phase over a solid phase in this system can be interpreted in terms of the presence of thermally induced fluctuations in the midplane of the membrane [34]. These fluctuations can stabilize the unbinding of dislocations which destroys translational order.

Taken together — the finite concentration of diffusing 5–7 disclination pairs, the long-range or quasi-long-range orientational order, and the relatively short-range translational order — indicate that the ordered phase of the model lipid bilayer we study is a hexatic phase.

Model dependence and relation to experiments. X-ray diffraction experiments have shown that an ordered hexatic phase exists in between the solid and disordered phases of lipid monolayers [26]. In the case of lipid bilayers, the X-

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ray scattering data found a finite in-plane correlation length in the ordered phase, about 20 nm [38], but the experiments were unable to distinguish between a hexatic phase and a solid phase with finite sized domains. Perhaps our prediction will motivate further experimental work to resolve this issue.

Concerning our finding of first-order character in the liquid–hexatic transition, recent simulations of the melting of hard-disk systems indicate that the hexatic to liquid transition is in fact a first-order phase transition [35], and other work on two dimensional systems shows that the order of the hexatic to liquid transition depends on the steepness of the repulsive interactions between particles [36]. Specifically, it was found that for soft disks with repulsive potentials r^{-n} , the transition is continuous for $n \lesssim 6$ while it is first-order for $n \gtrsim 6$. Therefore, the order of the hexatic-to-liquid phase transition is a system-dependent property. Indeed, a different model for lipid bilayers with soft repulsive interactions [37,39] was found by Rodgers et al. to change continuously between ordered and disordered structures. In that model, it is not even clear that a transition exists between distinct phases.

This system dependence is more than a theoretical curiosity. Experiments can vary the presence and order of a transition by varying membrane composition. For compositions where the transition remains first order, pre-melting of the ordered phase and related phenomena become relevant. In particular, large enough membrane-bound solutes, (e.g., transmembrane proteins) can form surfaces that disfavor the ordered phase even at thermodynamic conditions where the ordered phase is stable. At such conditions, a microscopic premelting layer with disordered membrane structure will then necessarily surround these solutes. Several such neighboring solutes will then assemble to minimize line tension.

This mechanism of self assembly is the subject of the subsequent paper [1]. Its strength will depend upon the temperature, lateral pressure and the composition of the membrane, all of which affect the nature of the order—disorder transition. Without its first-order character, solute-induced pre-melting layers will not exist, line tension will not exist, and this force of assembly will vanish. We think the possibility of moving between regimes with differing transition order implies an important degree of complexity and correlated behavior yet to be explored.

Materials and Methods

We use the MARTINI coarse-grained force field [4] to model a lipid bilayer system in which four carbon atoms (or equivalent) are approximated as one coarse-grained bead. This model has an explicit solvent with approximately four water molecules scaled to one solvent particle. Dipalmitoyl phosphatidylcholine (DPPC) is chosen as the model

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lipid species. The MARTINI model uses the Lennard-Jones potential for non-bonded interactions. The cut-off for these interactions is 1.2 nm. The GROMACS shifting function [42] is used in the range 0.99–1.2 nm. Bond and angle energies are modeled as harmonic potentials with associated force constants 1250 kJmol $^{-1}$ nm $^{-2}$ and $25\,\mathrm{kJmol}^{-1}$ rad $^{-2}$ respectively. We use a time step of $30\,\mathrm{fs}$, within the recommended range of 20–40 fs for this model. Simulations are performed using the GROMACS molecular dynamics package [43] in the ensemble with fixed numbers of particles, temperature, and stress tensor components for the system [4,44,45]. Thermostats and barostats were used to control temperature and pressure, and checks were performed to assure that different thermostats and barostats yielded similar results [46]. The compressibility is set to $3\times10^{-5}\,\mathrm{bar}^{-1}$. A lateral pressure of zero is maintained by choosing the diagonal components of the stress tensor to be 1 bar. The electrostatic interactions are shifted to zero in the range 0–1.2 nm. A dielectric constant of 15 is used for screening of electrostatic interactions.

The flat interface is stabilized by juxtaposing an ordered bilayer equilibrated at 285 K and zero lateral pressure with a disordered bilayer equilibrated at the same conditions corresponding to the cooling and heating curves of the hysteresis loop in Fig. 2, respectively. The system thus constructed is equilibrated in the ensemble with fixed temperature, volume and numbers of particles. This ensemble allows for maintaining an area per lipid intermediate between the two phases, thus stabilizing the interface.

Although the MARTINI model is able to reproduce the order of the transition in DPPC bilayers, the structure of the ordered phase has one significant difference in that the model bilayers do not exhibit a tilted phase [3]. It is speculated that DPPC bilayers tilt because the head groups are large and cannot pack as closely as the tails. The tilted configuration allows tails to pack closely while accommodating the head groups. Tilt has been induced in the MARTINI model by decreasing the size of tail particles relative to head particles [3] and in a soft-sphere model by increasing repulsion between heads [37]. Using either strategy to induce tilt, it is likely that the nature of this transition continues to be first-order in the MARTINI model.

Another feature of a fully hydrated phosphatidylcholine bilayer, observed in cooling protocols of multilamellar vesicles and planar bilayers, is the appearance of the so-called ripple phase, where the surface of the bilayer appears corrugated [2]. This kind of structure is not observed in the MARTINI model. However, it is not known whether the ripple structure is a real thermodynamic phase or a metastable structure seen during heating or cooling. Also, both tilt as well as the rippled structure disappear upon addition of a small percentage of impurities such as cholesterol [40,41].

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Supporting Information

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Translational Correlations

In addition to the tail-end particles (C4), we also calculate pair correlation functions for non-tail-end particles C2 and C3 higher up on the lipid chains. The planes containing these particles show fewer unbound dislocations compared to the tail-end particles C4 as shown in Fig. S1. This shows that the amount of disorder decreases higher along the lipid chains, consistent with the disorder gradient observed in experiments [32] and explained by theory [33]. Despite the gradient in disorder, the one-dimensional pair correlation function g(x,0) seen in Fig. S2 decays faster than $x^{-1/3}$ for all three planes of particles, showing that the lipid bilayer is in the hexatic phase. Moreover, the dislocations in the C2 and C3 planes also diffuse freely in the time scale of the simulation, albeit slower than those in the C4 plane.

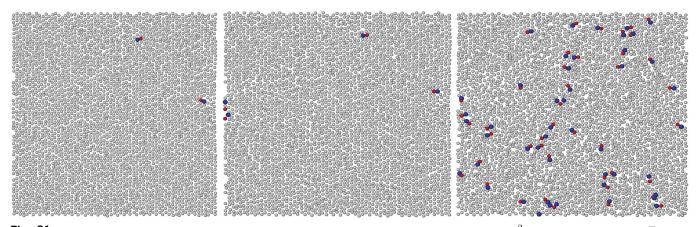


Fig. S1. 5–7 disclination pairs for planes containing C2 (left), C3 (center) and C4 (right) particles respectively, in a $25 \times 25 \,\mathrm{nm}^2$ bilayer in the ordered phase at T=294 K. The tail-end particles of one monolayer are shown in gray. Particles with seven neighbors are highlighted in red, while particles with five neighbors are highlighted in blue. There exist unbound dislocations as expected for a hexatic phase, and the number of dislocations increases towards the tail-end.

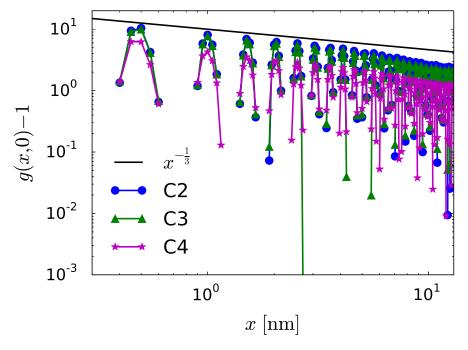


Fig. S2. Hexatic phase: Pair correlation function g(x,0) for tail particles C2, C3 and the tail-end particles C4 showing decay faster than $x^{-1/3}$ at T=294 K.

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