Examining the phase transition behavior of amphiphilic lipids in solution using statistical temperature molecular dynamics and replica-exchange Wang-Landau methods

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Two different techniques – replica-exchange Wang-Landau (REWL) and statistical temperature molecular dynamics (STMD) – were applied to systematically study the phase transition behavior of self-assembling lipids as a function of temperature using an off-lattice lipid model. Both methods allow the direct calculation of the density of states with improved efficiency compared to the original Wang-Landau method. A 3-segment model of amphiphilic lipids solvated in water has been studied with varied particle interaction energies (ϵ) and lipid concentrations. The phase behavior of the lipid molecules with respect to bilayer formation has been characterized through the calculation of the heat capacity as a function of temperature, in addition to various order parameters and general visual inspection. The simulations conducted by both methods can go to very low temperatures with the whole system exhibiting well-ordered structures. With optimized parameters, several bilayer phases are observed within the temperature range studied, including gel phase bilayers with frozen water, mixed water (i.e., frozen and liquid water), and liquid water, and a more fluid bilayer with liquid water. The results obtained from both methods, STMD and REWL, are consistently in excellent agreement with each other, thereby validating both the methods and the results. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4816520>]

I. INTRODUCTION

Amphiphilic molecules consisting of a hydrophilic head group and hydrophobic tail groups can self-assemble into a wide variety of structures, such as bilayers, micelles, and vesicles, when mixed with a suitable solvent.^{1,2} Such self-assembly behavior is critical in many industrial and biological processes, including the lipid membranes that form the basis of almost all forms of life.³ As a result, the self-assembly of these systems has been extensively studied, both computationally and experimentally.^{4–6} Generally speaking, amphiphilic systems have rough energy landscapes, exhibiting many minima close to each other separated by large energy barriers.⁷ These features can cause problems for the numerical exploration of their phase behavior as a function of temperature using standard molecular dynamics (MD) or Monte Carlo (MC) methods, for example, since some energy states may not be visited due to the energy barriers. Thus, conventional MD or MC simulations can become locked into a particular region of phase space (i.e., sample only small regions), and the results may depend on the initial conditions and thermodynamic pathway of the simulation.⁸

The time needed to ensure convergence of conventional simulations is often prohibitively long and thus various methods have been proposed to improve the rough energy ex-

ploration by going beyond standard MD or MC methods.^{8,9} Among them, the Wang-Landau (WL) method,⁹ which performs a random walk over the whole energy range, allows sampling of the conformations at all relevant temperatures in a single simulation. The basic idea of the WL algorithm^{9,10} is to achieve a flat energy distribution with a non-Boltzmann sampling weight proportional to the reciprocal of the density of states, $1/g(E)$, which forces the system to visit otherwise rarely sampled energy regions. The initially unknown $g(E)$ is determined by an iterative process. Every time an energy E is visited, $g(E)$ is modified instantaneously at that point and eventually converges towards the true $g(E)$ after several rounds of iteration. Compared to other flat-histogram methods, this dynamic update of $g(E)$ on the fly can provide quicker exploration of phase space,¹¹ and once the $g(E)$ is known, all thermodynamic and structural quantities can be calculated at any temperature. The WL method has been applied to study a number of different systems, focusing primarily on simple models for polymers and proteins.^{9,12–15} When applying the WL algorithm to more complex systems, convergence problems^{11,16} can be encountered due to the extensive nature of the energy and discrete representation of the density of states on an energy grid, which results in the number of energy bins required increasing substantially with system size.

To improve the sampling efficiency, a parallel replica-exchange framework for the WL sampling scheme can be

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employed.¹⁷ In this approach individual simulations with overlapping energy distributions are carried out and replicas are allowed to move between the individual simulations.^{18,19} A different approach, termed statistical-temperature molecular dynamics (STMD), was recently proposed by Kim *et al.*,^{11,16} with the objective of generating a deterministic trajectory by MD sampling and to augment the capabilities of the WL method to complicated systems beyond the scope of effective MC moves. The STMD algorithm implements the idea of WL sampling into MD simulation using the generalized ensemble via the statistical temperature. This is achieved by relying on the thermodynamic relationship between the statistical temperature $T(E)$ and the density of states $g(E)$; STMD achieves a flat energy distribution by dynamic modification of $T(E)$. By updating the *intensive* variable $T(E)$, a continuum description of $g(E)$ can be obtained through analytical integration of $T(E)$; therefore, moderately larger bins, and hence fewer bins, can be used to accelerate the simulation convergence, while still maintaining the statistical accuracy.¹¹

Although many MC and MD simulations have been performed to study micelle formation from the self-assembly of amphiphiles, limited studies have been performed on the systematic examination of bilayer phase behavior as a function of temperature.^{1,20–26} Of those studies, conventional MD or MC methods, along with temperature quenching, have been used as the main simulation techniques to induce phase transitions. In addition, these studies all use visual inspection or calculation of order parameters to support the structural phase transition as a function of the system temperature; however, more recently, the calculation of the heat capacity (C_v) has been used as a more direct signature of the phase transition. Rodgers *et al.*²⁰ reported the first attempt to characterize the phase transition of a solvated amphiphilic model by monitoring changes in enthalpy with a computational calorimetry approach developed based on a dissipative particle dynamics (DPD) model. Almost simultaneously, Nagai *et al.*²¹ reported the application of replica-exchange MD (REMD) to an explicit atom simulation of a small dipalmitoylphosphatidylcholine (DPPC) bilayer system using the coarse-grained (CG) Martini force field, with enthalpy tracking for the calculation of heat capacity. These studies represent promising initial quantitative heat capacity investigations of the structural phase transition behavior. However, although the simple DPD model given by Rodgers *et al.*²⁰ describes structurally all the phases of a DPPC bilayer (subgel, gel, ripple, and fluid), the enthalpy change between certain phase transitions was either underestimated or no observable enthalpy changes were detected between some of the structures. In the study by Nagai *et al.*,²¹ reference temperatures at 127 points between 283 and 390 K are needed to implement valid swapping of structures (replicas are required to be separated by small temperature differences). As the number of degrees of freedom of the system increases, the required number of replicas also greatly increases, and so only a small system of 32 lipids was studied, with only preliminary results presented for a larger 128-lipid system. In addition, the hysteresis between heating and cooling processes is still quite substantial in the systems studied above and may mask the underlying equilibrium phases.

Here, we have applied both the replica exchange WL (REWL) and STMD methods, which are very different techniques, to calculate the free energy and thermodynamic properties of self-assembling bilayers using an off-lattice lipid model. Since the systems and phenomena being studied are of a complex nature, they hold the potential for hidden algorithmic difficulties, which might not be revealed by using a single method. We report a series of density of states simulations by both the REWL and STMD methods to systematically study the phase transition behavior of self-assembling lipid molecules as a function of temperature based on the CG models of Goetz and Lipowsky²⁵ and Fujiwara *et al.*²⁶ From the density of states, we determine a number of thermodynamic properties (average energy and heat capacity) to characterize the phase transition, in addition to visual inspection and order parameter-based analysis. Together the analysis provides a more complete understanding of the lipid phase transitions.

II. SIMULATION MODELS AND METHODS

The amphiphilic molecules are modeled as short linear chains composed of a hydrophilic head bead and two hydrophobic tail beads (Fig. 1). The water molecules are represented as hydrophilic beads. The total number of particles in the system is fixed at $N = 1000$ and the number of amphiphilic molecules M is varied. The volume of the simulation box is chosen such that the number density, ρ , is maintained at $\rho = 0.8$.

All non-bonded particles of the same type as well as the solvent and hydrophilic head beads interact via the Lennard-Jones (LJ) potential:

$$U_{LJ}(r_{ij}) = 4\epsilon \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right]. \quad (1)$$

The interaction between tail beads and head or solution particles is purely repulsive:

$$U_{rep}(r_{ij}) = 4\epsilon_{rep} \left(\frac{\sigma_{rep}}{r_{ij}} \right)^9, \quad (2)$$

where r_{ij} is the distance between two particles i and j . The van der Waals radii, σ and σ_{rep} , are set to 1.0 and 1.05,

FIG. 1. Schematic of the model amphiphilic lipid and solvent molecules studied. P: polar head beads, H: hydrophobic tail beads, W: solution (water) beads.

respectively, and $\varepsilon = \varepsilon_{\text{rep}} = \varepsilon_{\text{w}} = 1.0$, following Fujiwara *et al.*²⁶ A model is also studied in which the interaction between water particles ε_{w} is reduced to 0.7. Bonds between monomers in the lipid molecules are finitely extendible, non-linear elastic, and modeled by the corresponding FENE and WCA potentials:^{27,28}

$$U_{\text{FENE}}(r_b) = -\frac{k}{2} R^2 \ln \left(1 - \left(\frac{r_b}{R} \right)^2 \right) + U_{\text{WCA}}(r_b) \quad (3)$$

with

$$U_{\text{WCA}}(r) = \begin{cases} 4\varepsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] + \varepsilon, & r < 2^{1/6}\sigma \\ 0, & r \geq 2^{1/6}\sigma \end{cases}. \quad (4)$$

A. The parallel Wang-Landau MC algorithm

In the Wang-Landau Monte Carlo method^{9,10} the conformational space at all relevant temperatures is sampled in a single simulation. This is achieved by creating a generalized ensemble in which the simulation eventually generates a chain of conformations with energies equally distributed over the whole energy range. As in every MC scheme, a conformational update is proposed in each step that is accepted with a certain probability P_{acc} depending on the total energy E (note reduced units are used and so E is the reduced dimensionless energy) of the actual (X) and proposed new state (Y) and the statistical ensemble. In this case,

$$P_{\text{acc}}(X \rightarrow Y) = \min \left[1, \frac{g(E(X))}{g(E(Y))} \cdot b \right], \quad (5)$$

where $g(E(X))$ is the estimator for the density of states, which is adapted during the sampling and eventually converges towards the real density of states,^{9,10} and b is a correction factor for any configurationally bias that might be introduced by the MC update moves.

To speed up the simulation, the global energy range is divided into individual energy windows²⁹ with significant energetic overlap and replica exchanges are allowed between neighboring energy windows, which are accepted with

$$P_{\text{acc}}(X_i Y_j \rightarrow Y_i X_j) = \min \left[1, \frac{g_i(E(X)) g_j(E(Y))}{g_i(E(Y)) g_j(E(X))} \right]. \quad (6)$$

Here, $g_i(E(X))$ is the current estimate of the density of states of walker i at the energy of its actual conformation X . More generally, we allow for multiple, individual Wang-Landau walkers in each energy window that merge their density of states

FIG. 2. Schematic representation of the division of the total energy range into nine individual energy windows with fixed mutual overlap of 75%. Three WL walkers are deployed in each window as indicated by the dotted lines. Arrows illustrate the replica-exchange communication scheme, i.e., walkers can exchange configurations with walkers from neighboring windows.

estimates at defined stages in the Wang-Landau run, resulting in a decrease in the statistical error. For a detailed introduction and discussion of the parallel Wang-Landau framework used, see Refs. 17 and 30.

The energy range splitting in combination with the replica exchange makes it possible to study large temperature ranges and, depending on the system size, to access lowest energy structures in the first place. For this study, individual energy windows are created with a mutual overlap of 75% and three WL walkers per window. This overlap leads to significant speed-ups compared to single processor Wang-Landau simulations but still allows for reasonable replica-exchange rates, which is discussed and shown in detail in Refs. 17 and 30. Figure 2 provides a schematic overview of the simulation setup, where we split up the energy range in nine windows.

For conformational updates a combination of local displacements of single particles in Cartesian coordinates and reptation moves for the amphiphilic molecules are used. As illustrated in Fig. 3, in the reptation move a molecule and one of its ends are selected at random. Then, from all the neighboring solution particles (i.e., particles within the maximal allowed bond length R , Eq. (3)), one is chosen randomly and connected to the molecule, while the bond to the monomer at the other end is broken (Fig. 3(b)). Finally, the types of the monomers are restored to create a valid conformation (Fig. 3(c)). Clearly, this move introduces a bias, as there is, in general, a different number of potential neighbors for the forward and backward moves. To fulfill detailed balance, this bias is corrected by an appropriate weight factor b in the acceptance probability as shown by Eq. (5).

During one Monte Carlo sweep N individual updates are made, of which $3M/10$ are reptation moves.

B. Statistical temperature molecular dynamics algorithm

STMD achieves a flat energy distribution by the systematic refinement of the statistical temperature $T(E)$,¹⁶ which is realized by the thermodynamic relationship between $T(E)$ and $g(E)$ via³¹

$$\frac{1}{T(E)} = \left(\frac{\partial S}{\partial E} \right)_{N,V} = \frac{\partial \ln g(E)}{\partial E}. \quad (7)$$

FIG. 3. Illustration of the applied reptation move for conformational update during the Wang-Landau sampling. (a) The initial state and (c) the final one. Note that the Cartesian coordinates do not change during the trial move.

By using the finite difference approximation with Eq. (7) and combining it with the $g(E)$ update scheme (converted to entropy S , $S'_j = S_j + \ln f$, where f is the modification factor), a dynamic update of the statistical temperature is realized by

$$T'_{j\pm 1} = \frac{T_{j\pm 1}}{1 \mp T_{j\pm 1} \times (\ln f / 2\Delta E)}, \quad (8)$$

where $\Delta E = E_{j+1} - E_j$ ($E_j = \text{nint}[E/\Delta E]\Delta E$) (nint being a function returning the nearest integer value) is the energy bin size, i.e., the update changes temperature estimates only at the discrete values of $E_{j\pm 1}$. For a detailed derivation of the above formulations, the reader is referred to the original Refs. 11 and 16. The temperature is updated in a range $T_l < T_j < T_h$ and at the boundary or beyond set to $T_j = T_l$ or T_h as appropriate, where T_l and T_h are the lower and upper boundaries, respectively.

To implement the variable $T(E)$ into a MD simulation and realize a random walk in energy space, a generalized ensemble technique⁸ is employed in which the energy state is weighted by a non-Boltzmann factor. In the generalized ensemble, the sampling of configurations is based on the weight

$$w(E) = e^{-S(E)}, \quad (9)$$

which leads to the effective potential in the canonical ensemble as

$$V_{eff} = T_0 S(E), \quad (10)$$

and then the force term $\tilde{f}$ can be solved by the derivative over position $\mathbf{q}$:

$$\begin{aligned} \tilde{f} &= -\frac{\partial V_{eff}}{\partial \mathbf{q}} \\ &= -T_0 \frac{\partial S(E(\mathbf{q}))}{\partial \mathbf{q}} = -T_0 \frac{\partial S(E(\mathbf{q}))}{\partial E} \frac{\partial E(\mathbf{q})}{\partial \mathbf{q}} \\ &= \frac{T_0}{T(E)} \mathbf{f}. \end{aligned} \quad (11)$$

Thus, the sampling weight $e^{-S(E)}$ is realized in a MD simulation with the Nose-Hoover thermostat by maintaining the

kinetic energy at the reference temperature $T_0 = T_h$ and constantly adapting the forces with the energy dependent scaling factor.

To implement STMD, all T_j are set to a constant value at the start of the simulation (usually T_h). After an energy bin E_j is visited, the actual $T_{j\pm 1}$ are updated by Eq. (8). When the simulation terminates with convergent temperature, Eq. (7) can be integrated to determine the $g(E)$. The fact that T_j is only defined on the grid points E_j and the sharp change of the integrand at low temperatures makes it difficult to apply a generic numerical integration, thus a linear interpolation scheme¹⁶ is used to connect successive bins. For a value of the potential energy E between E_j and E_{j+1} ,

$$T(E) = T_j + \lambda_j(E - E_j), \quad (12)$$

where $\lambda_j = (T_{j+1} - T_j)/\Delta E$ is the slope of the linear segment connecting the two neighboring bins. The continuum $g(E)$ estimate is then calculated by analytical integration at the end of MD simulations:

$$\begin{aligned} \ln[g(E)] &= \int_{E_l}^E \frac{1}{T(E')} dE' \\ &= \sum_{j=l+1}^{i^*} \frac{1}{\lambda_{j-1}} \ln \left[1 + \frac{\lambda_{j-1}(E_j - E_{j-1})}{T_{j-1}} \right] \\ &\quad + \frac{1}{\lambda_{i^*}} \ln \left[1 + \frac{\lambda_{i^*}(E - E_{i^*})}{T_{i^*}} \right] \\ &= \sum_{j=l+1}^{i^*} L_j(E_j) + L_{i^*+1}(E), \end{aligned} \quad (13)$$

where $i^* = i - 1$ for $\bar{E}_{i-1} \leq E \leq E_i$ ($\bar{E}_i = (E_i + E_{i+1})/2$), $i^* = i$ for $E_i \leq E \leq \bar{E}_i$, and $L_j = \frac{1}{\lambda_{j-1}} \ln \left[1 + \frac{\lambda_{j-1}(E_j - E_{j-1})}{T_{j-1}} \right]$. By updating the intensive variables T_j located at discrete points, a continuum description of $g(E)$ can be obtained through analytical integration of $T(E)$; therefore, moderately larger and fewer bins can be used to accelerate the simulation convergence, which still maintains the statistical accuracy.¹¹

After obtaining $g(E)$, the partition function Q can be calculated at any given temperature and other thermodynamic properties determined. For example, the average energy $\langle E \rangle$

and heat capacity C_V can be calculated as

$$\langle E \rangle = \frac{\sum_E E g(E) e^{-E/T}}{Q}, \quad (14)$$

$$C_V = \frac{1}{T^2} [\langle E^2 \rangle - \langle E \rangle^2], \quad (15)$$

where E is the system potential energy and $\langle E^2 \rangle - \langle E \rangle^2$ is the fluctuation in the energy.

Here, we have implemented the STMD algorithm with modified source code within HOOMD-Blue,^{32,33} which is highly optimized to run MD simulation on graphical processing units (GPU). For the current lipid system, the simulations run ~ 4 -5 times faster on the GeForce GTX 480 GPUs than those on 8 core CPUs (2.4 GHz Opteron).

C. Methods for bilayer structure characterization

To quantify the structural properties and transitions within the self-assembled structures, several order parameters have been calculated. The ordering of the lipid molecules is quantified by using the nematic order parameter, S_2 ,³⁴ in which the average direction of each lipid is first quantified by calculating the moment of inertia tensor for each molecule:

$$I_{\alpha\beta} = \sum_{i=1}^{N_l} m_i (\hat{r}_i^2 \delta_{\alpha\beta} - r_{i\alpha} r_{i\beta}), \quad (16)$$

where $\hat{r}_i$ is the position vector of each particle i relative to the center of mass, and m_i is the mass of each particle i , $\delta_{\alpha\beta}$ is the Kronecker delta, N_l is the total number of particles in the lipid, and α and β are looping variables that correspond to the coordinate axes (i.e., x , y , z). The characteristic vector describing the lipid, $\hat{u}$, is the eigenvector associated with the smallest eigenvalue of the tensor, and is used to construct the nematic tensor

$$Q_{\alpha\beta} = \frac{1}{N} \sum_{i=1}^{N_l} \frac{3}{2} u_{i\alpha} u_{i\beta} - \frac{1}{2} \delta_{\alpha\beta}, \quad (17)$$

where N_L is the total number of lipids. The director of the system is the eigenvector associated with the largest eigenvalue, and the nematic order parameter, S_2 , corresponds to this largest eigenvalue. For a given simulation snapshot two Q tensors, one for each layer, were constructed and the two S_2 values were averaged. Using the same procedure, S_2 for the individual segments of the lipids has also been calculated. All results are calculated as block averages, where quantities are averaged for energy blocks of size $\Delta E = 32$.

To quantify the transition of the water to a crystalline (i.e., frozen) structure, techniques based upon shape matching are employed.^{35,36} Briefly, the global structure of the water particles is determined via super-position of the directions between a particle and its first neighbor shell (i.e., a bond-order diagram is constructed). Systems with a high degree of global ordering demonstrate strong correlations in terms of these “bond” directions. The bond-order diagram is transformed by taking the magnitude of the spherical harmonic transform for harmonics 4, 6, 8, 10, and 12, and concatenating these into a single vector, providing a characteristic “fingerprint” (i.e., shape-descriptor) of the system. To determine

the transition, the dot product normalized between 0 and 1 between this vector and the vector describing a low temperature, ordered crystalline state (determined visually) is calculated and then repeated using five different crystalline configurations as a reference. The order parameter is constructed such that an ideal match gives a value of unity.

III. RESULTS AND DISCUSSION

The self-assembly of amphiphilic lipids solvated in water at different concentrations has been simulated by both STMD and REWL MC in this work. As discussed earlier, the model studied is similar to that employed by Goetz and Lipowsky²⁵ and Fujiwara *et al.*,²⁶ however, previous work focused on the structural properties of the various aggregates, such as spherical micelles, cylindrical micelles, and bilayers, via the variation of the amphiphilic concentration and hydrophobic interactions using standard MC and MD simulations. Additionally, these previous studies relied only on visual inspection of simulation snapshots and calculated order parameters at discrete temperatures to characterize the different structures. In this work, we investigate the phase transition behavior of the bilayer structures through their thermodynamic properties as a function of temperature in addition to structural and visual analysis.

A. Exploring phase transition behavior of system with $M = 125$

In both the STMD and REWL simulations a wide temperature range from $T_l = 0.4$ to $T_h > 6$ was first tested, in order to cover a sufficient energy range corresponding to both fluid and gel regions. For the STMD runs, an energy bin size of $\Delta E = 32$ was used with an initial modification factor $\ln f = 0.00025$. The entire temperature range was split into three windows, $T \in [0.4, 1.0]$, $T \in [0.6, 4.0]$, and $T \in [3.5, 6.5]$, which were independently simulated with time steps of 0.01, 0.01, and 0.001, respectively. We found that it took approximately 4.7×10^6 , 4.2×10^7 , and 3.1×10^6 MD steps, respectively, for the three windows to reach the lowest temperatures for the first time and was then followed by uniform sampling of the temperature range. The total simulation time for each energy window, $T \in [0.4, 1.0]$, $T \in [0.6, 4.0]$, and $T \in [3.5, 6.5]$, was 4.0×10^9 , 2.0×10^8 , and 4.0×10^8 MD steps, respectively, with a final WL modification factor of $\ln f < 5 \times 10^{-7}$. Even though results from STMD and REWL agree over a wide range of temperature, for the results presented henceforth we focus on temperatures less than $T_h \lesssim 1$, as this range encompasses the bilayer phase transitions of interest.

The evolution of the potential energy E in the first two windows for the lipid system is shown in Fig. 4, each of which displays a walk with frequent sweeps in the energy range, which corresponds to the restricted temperature range applied. Once the simulations are converged, the density of states $g(E)$ is integrated from the convergent statistical temperature $T(E)$.

For the REWL simulations, preliminary scans were performed in the energy ranges $E \in [-5200, 3500]$ (which corresponds to a temperature range of approximately

FIG. 4. Energy trajectory from STMD simulation of $M = 125$ lipid system with $\varepsilon_w = 1.0$ for (a) $T \in [0.4, 1.0]$ and (b) $T \in [0.6, 4.0]$.

$T \in [0.5, 8.5]$) and $E \in [-5400, 0]$ ($T \in [0.4, 5]$). For these preliminary runs, a single walker per energy subinterval and the Wang-Landau flatness criterion proposed by Zhou and Bhatt³⁷ was used in order to obtain faster results. The energy bin size for these runs was $\Delta E = 5$. Replica exchanges were attempted every 10^5 MC sweeps. Figure 5 shows examples of two energy trajectories and the corresponding walks through energy windows for both of these runs. While the transition from completely mixed and disordered configurations into a fluid bilayer (Fig. 5(a)) does not present a significant obstacle for the algorithm, Fig. 5(b) illustrates that the fluid-gel bilayer-bilayer transition is hard to overcome. Following the preliminary scans, multiple independent simulation runs with three independent Wang-Landau walkers per energy subinterval were performed in the energy range $E \in [-5350, -3350]$, corresponding to the temperature range of the bilayer phases ($T \in [0.45, 1.4]$). Furthermore, the originally proposed, stricter 80%-flatness criterion^{9,10} and an energy bin size of $\Delta E = 1$ was used. Up to a maximum of 10^8 Monte Carlo sweeps (one sweep corresponds to one update of each particle in the system, on average) per walker were used to converge the estimator for the density of states, followed by a production run consisting of 3×10^7 MC sweeps for each walker. Error bars for all observables were obtained from 200 iterations of bootstrap resampling³⁸ of the multiple density of state pieces from the REWL runs. Confirming the quality of the WL weight determination, the estimated density of states at that point and the measured density of states in the production run were within mutual error bars.

From both methods, STMD and REWL, thermodynamic properties such as the average energy and heat capacity at constant volume were then calculated from the obtained density of states $g(E)$, with a temperature step < 0.001 , providing a systematic analysis of the phase transition behavior as summarized in Fig. 6.

We first note that excellent agreement is obtained for the density of states calculated from both the STMD and REWL methods. Second, we find that there are two jumps in the average energy between 0.5 and 0.6 in the temperature (Fig. 6(b)); we also note from Fig. 6(b) good agreement between the inverse reweighted average energy calculated from $g(E)$ and the convergent $T(E)$ obtained directly from STMD. The changes in the average energy are better marked quantitatively by the two peaks observed in the C_V curves shown in Fig. 6(c). The small energy jump at lower T manifests in a peak in C_V at $T = 0.513$ and the larger energy jump in the peak at a higher temperature of $T = 0.535$. These peaks in C_V indicate structural phase transitions in the bilayer system. Simulation snapshots are provided in Table I that demonstrate the three different bilayer phases observed; these snapshots are taken from STMD simulations; however, we note that equivalent configurations are observed in the REWL simulations. Note that at very high temperature $T \gg 1$, the lipid molecules aggregate forming various other structures, including micelles, which will not be discussed in this work.

The phase observed at low temperature ($T < 0.513$) represents a gel phase bilayer (denoted GB1) in which the lipid hydrophobic tails are highly ordered and show a tilt with

FIG. 5. Trajectories through energy space (top) and energy windows (bottom) from REWL preliminary scans for the same system studied in Fig. 4. (a) Sampling of energy range $E \in [-5200, 3500]$, covering the clustering of the lipid molecules and the bilayer formation. (b) Sampling of energy range $E \in [-5400, 0]$, focusing on the fluid-gel bilayer-bilayer transition. For each case, walks of two single replicas out of 39 (a) and 9 (b) are shown as examples.

FIG. 6. Density of states and thermodynamic properties of lipid system with $M = 125$ and $\varepsilon_w = 1.0$; comparison of data from STMD (symbols) and REWL (solid line) (a) logarithm of density of states, (b) energy-temperature relations, comparison between the convergent energy (dots) and reweighted energy (straight line). The inset shows the comparison between the reweighted energy from STMD and the mean energy calculated from REWL results. (c) Heat capacities calculated from STMD and REWL results. (d) Microcanonical analysis (see Appendix for details). As an example, $\beta = 1.950$ on left scale agrees with the peak temperature of $T = 0.513$ in (c) via the relation $\beta = 1/T$.

respect to the bilayer normal. At low temperatures the solvent is highly ordered and frozen (denoted FW). Upon heating, the frozen water first transforms to liquid water in two steps; first the pre-transition to an intermediate phase with mixed frozen

water and fluid water and then the main transition to fluid water. During this water pre-transition process, the bilayer also undergoes subtle changes, i.e., the bilayer denoted GB1 becomes more hydrated and increased disorder is seen in the

TABLE I. Simulation snapshots of the different bilayer structures seen for the $M = 125$ lipid system with $\varepsilon_w = 1.0$. From left to right the phases observed as temperature increases are denoted as follows: gel phase bilayer 1 with frozen water (GB1+FW); gel phase bilayer 2 with mixed water (i.e., frozen and liquid water, GB2+MW); gel phase bilayer 3 with liquid water (GB3+LW); and fluid bilayer with liquid water (FB+LW).

GB1+FW	GB2+MW	GB3+LW	FB+LW
		-	
		-	
		-	

TABLE II. Comparison of transition temperatures obtained from peak positions in the heat capacities (STMD and REWL^a) and by microcanonical analysis of the density of states obtained by REWL.^b It is known that for strong first-order like transitions in finite systems, canonical and microcanonical transition temperatures might^{41,42} differ, as can be seen for transition #2, for example. All other temperature values are in good agreement.

Energy constant	Transition	STMD	REWL ^a	REWL ^b
$\varepsilon_w = 1.0$	#1	0.513	0.5147 ± 0.0001	0.5128 ± 0.0009
	#2	0.535	0.5351 ± 0.0001	0.519 ± 0.006
$\varepsilon_w = 0.7$	#3	0.404	0.4032 ± 0.0001	0.401 ± 0.002
				0.408 ± 0.002
	#4	0.427	0.4273 ± 0.0001	0.4277 ± 0.0003
				0.433 ± 0.002
	#5	0.533	0.532 ± 0.001	0.533 ± 0.01

^aTransition temperatures obtained from peak positions in the heat capacities (STMD and REWL).

^bTransition temperatures obtained by microcanonical analysis of the density of states obtained by REWL.

lipid tails (denoted GB2). During the main transition, the gel bilayer phase concurrently with the water undergoes a transition to a fluid bilayer phase (denote FB) in which the tails are disordered without any tilt, concurrently with the water melting. This phase covers a wide temperature range for the current system and is physiologically generally the most important phase of the bilayer.

As both the water and bilayer phase transitions occur at temperatures very close to each other and to further demonstrate the extremely good agreement between the results obtained from the STMD and REWL methods, an additional

microcanonical analysis,^{39,40} which is based on the same relation used in STMD and given in Eq. (7), was carried out on the density of states obtained from REWL. The results are shown in Fig. 6(d) and compared to the analysis of the heat capacities (Fig. 6(c)). As can be seen from the figure, the peaks in γ corresponding to the phase transitions are well separated and the microcanonical temperature of the transition corresponding to the lower peak in the heat capacity from STMD at $T = 0.513$ is measured as $T = 1/\beta = 1/(1.950 \pm 0.003) = 0.513 \pm 0.001$. This essentially perfect agreement indicates once more that reliable results are obtained for the simulation of the self-assembly amphiphilic molecules. The complete comparison for all transition temperatures is discussed and presented separately in Table II.

B. Phase transition behavior at different ε

Using an equal strength for the interaction energy of the water and lipids (i.e., $\varepsilon_w = \varepsilon = 1.0$), the gel-fluid phase transition of the lipids and the frozen-liquid water transition occur almost concurrently in temperature. In order to examine the phase transitions of the lipids and solvent separately, and determine all of the phases that may be present during the self-assembly process, a lower energy constant for the water-water interaction has also been studied.

The thermodynamic data including the average energy and C_V obtained from simulations with $\varepsilon_w = 0.7\varepsilon$ are presented. In Fig. 7(a), the logarithm of the density of states calculated from both the STMD and REWL methods is presented and can be seen to agree well. From Fig. 7(b), good agreement between the reweighted average energy and convergent

FIG. 7. Density of states and thermodynamic properties of lipid system with $M = 125$ and $\varepsilon_w = 0.7$; comparison of data from STMD (symbols) and REWL (solid line) (a) logarithm of density of states, (b) energy-temperature relations, comparison between the convergent energy (dots) and reweighted energy (straight line). The inset shows the comparison between the statistical temperature from STMD and the mean energy calculated from REWL results. (c) Heat capacities calculated from STMD and REWL results. Inset: Comparison with data shown in Fig. 6 for $\varepsilon_w = 1.0$. (d) Microcanonical analysis. Values for β on left scale agree with peak temperatures in (c) via the relation $\beta = 1/T$. Generally, error bars are only shown every n th ($n > 20$) data point.

TABLE III. Simulation snapshots showing four different bilayer phases seen for the $M = 125$ lipid system with $\varepsilon_w = 0.7\varepsilon$.

$T(E)$ from STMD is again also observed, with a more gradual transition in temperature seen for $\varepsilon_w = 0.7$ than for $\varepsilon_w = 1.0$. Three energy changes are observed, each correlated to a transition temperature, which manifest in C_v peaks at $T = 0.404$, 0.427 , and 0.533 . These also agree very well with the transition temperatures obtained from the microcanonical analysis as reported in Fig. 7(d) and Table II. Furthermore, the microcanonical analysis reveals more clearly some pre-transitions that are not easily detected through analysis of the heat capacity peaks as they are represented as shoulders at slightly higher temperatures than the main transition peaks. To obtain the error bars in C_v from the STMD simulations, three independent simulations were run from different initial conditions.

Simulation snapshots are provided in Table III that demonstrate the different bilayer phases observed in the simulations. At low temperature, the system is again in the GB1+FW phase (cf. Table I) and as temperature increases the frozen water melts in the above-mentioned two steps via a pre-transition (exhibiting GB2+MW structure) and then the main transition (to GB3+LW phase). At a higher temperature still, the gel bilayer phase begins to change to the fluid bilayer phase (FB+LW phase).

From the comparison of the heat capacities obtained from the two sets of simulations performed for different water-water interactions (i.e., with $\varepsilon_w = 0.7$ and 1.0 , see Fig. 7(c) for a direct comparison), we can see that only two peaks are observed when $\varepsilon_w = 1.0$, since the gel-fluid phase transition of the lipids and the frozen-liquid water transitions occur almost concurrently. This also explains the large peak in C_v at $T = 0.535$ for $\varepsilon_w = 1.0$, which is generated from the energy changes in both the LJ potential energy between water particles and the lipid fluidization process. Thus, the utilization of a smaller ε_w for the water-water interaction separates the processes of the lipid gel-fluid transition and the solvent frozen-fluid transition, providing a clearer demonstration of the complicated phase behavior of this solvated lipid system.

To provide additional interpretation of the various phases and demonstrate that the peaks in C_v are indeed associated

with the structural transitions discussed above, several structural metrics have also been calculated for the system of $M = 125$ with $\varepsilon_w = 0.7$. In Fig. 8(a), the average nematic order parameter S_2 (see Sec. II) is plotted as a function of temperature. As can be seen from the figure, S_2 maps closely to the transitions observed in C_v at $T = 0.404$ and 0.427 . The most significant change (i.e., steepest) occurs at $T = 0.427$, resulting in a large increase in the orientational ordering of the lipids; this transition appears to finish by $T = 0.404$, where S_2 attains a near constant value with a small standard deviation (i.e., it is in the GB1 phase). Additionally, S_2 appears to first start increasing roughly around the transition marked by $T = 0.533$, marking the transition between the GB3 ($T < 0.533$) and the FB ($T > 0.533$) phases.

Additional insight into the transition at $T = 0.533$ can be gleaned by examining the value of S_2 for the individual segments of the lipid chain. In Fig. 8(b), S_2 calculated for “segment 1,” containing the head group and first bead of the tail, and “segment 2,” containing the two tail group beads is calculated. Overall, segment 1 has a more uniform alignment than segment 2 over the temperature range studied; however, S_2 is reduced in both cases as temperature increases, indicating more random configurations. The increased uniformity of segment 1 is likely related to the fact that it is located at the planar interface of the bilayer. Additionally, for segment 2, there is a clear transition in S_2 associated with $T = 0.533$, where S_2 begins to more rapidly increase as system temperature is reduced, consistent with visual inspection. Segment 1 shows a less obvious transition; as the system is cooled; orientational ordering of this first segment is actually reduced until the temperature is decreased below $T = 0.533$, where it then begins to trend upwards. This loss in ordering is associated with the formation of holes within the bilayers; the formation of the water filled holes induces curvature at the water-head group interface, reducing the overall global-order.

We observe that the normal to the bilayer does not change significantly during the simulation, which enables us to easily quantify the formation of the aforementioned holes. By projecting the lipids onto the plane parallel to the bilayer, a 2D histogram can be constructed; cells that do not contain lipid particles are considered to be holes. Figure 8(c) shows the fraction of the plane occupied by the bilayer, from which we can see that the key changes in fraction match closely to the transitions in C_v , where, in particular, there is a clear plateau of the average fraction in the region bounded by $0.427 < T < 0.533$. Even more telling is the standard deviation, where, from approximately $T = 0.6$ to $T = 0.5$, the error bars, corresponding to the standard deviation, grow significantly, reaching a peak at $T = 0.533$. For state points in this regime around $T > 0.533$, we see a mixture of systems with holes and those that span the box, while for $T < 0.533$ all systems appear to exhibit holes in the bilayer structure. The formation of holes results in a more compact bilayer sheet, as well as decreased hydration as T reduces to the point where water changes from liquid to frozen (i.e., the bilayer hole is not filled by water). This phenomenon results in decreased potential interactions between the head groups and water, which manifests itself in the heat capacity curve.

FIG. 8. Structural metrics, (a) global nematic order parameter S_2 ; (b) S_2 for the individual segments of the lipid chain; (c) the fraction of the plane occupied by the bilayer; (d) order parameter for crystallization of water particles. Note the structural transitions determined via C_V are plotted as dashed vertical lines for visual aid ($T = 0.404, 0.427, 0.533$).

Finally, the order parameter associated with the crystallization of water particles has been calculated showing a strong increase in the average order parameter starting at $T = 0.427$ and ending at $T = 0.404$ (Fig. 8(d)). This supports the results of the visual inspection that observed frozen water for $T < 0.404$, a mixture of frozen and liquid for $0.404 < T < 0.427$, and predominantly liquid water for $T > 0.427$.

To give an overall view of the bilayer structures discussed above, the features of the lipid system are summarized in Table IV.

C. Phase transition behavior at different lipid concentrations

A system with a lower lipid concentration of $M = 75$ ($\varepsilon_w = 0.7$) has also been studied to examine the effect of lipid concentration on the phase transition behavior. The calculated heat capacity for this system is provided in Fig. 9 and shows

TABLE IV. Feature summary of the lipid system at $M = 125$ with $\varepsilon_w = 0.7$.

T	0.35–0.404	0.404–0.427	0.427–0.533	$\gg 0.533$
Water	Frozen water	Mixture	Liquid water	
Lipids	GB1	GB2	GB3	Fluid bilayer
Holes	Yes, without water	Yes, filled with water	No	
Structural	Tail	Less order;	Less order;	Tail
feature	highly	more	curvature	random
	order	hydrated	at boundary	distributed

that the phase transition behavior is similar to the system with $M = 125$, i.e., three apparent phase transitions are observed but at shifted temperatures. Simulation snapshots for this system are also presented in Table V. At low temperatures when the water is frozen, the gel phase bilayer structure given in Table V is found to be quite similar to that seen for the system with $M = 125$; however, upon increasing the temperature, when the system is in the fluid phase (bilayer with liquid water, FB+LW), due to the smaller amphiphilic concentration, some head groups are found to wrap around the border of the bilayer which introduces curvature at the interface and prevents the lipid tails from interacting with the water particles. However, the large lipid aggregate will merge with itself at higher concentrations ($M = 125$) in one or two directions and form a stable bilayer.

FIG. 9. Heat capacity at $M = 125$ (black) and $M = 75$ (red dotted) lipid concentrations. $\varepsilon_w = 0.7\varepsilon$ in both cases.

TABLE V. Snapshots showing the four different bilayer phases seen simulations for $M = 75$ and $\varepsilon_w = 0.7\varepsilon$.

IV. CONCLUSIONS

The structural behavior of lipid bilayers formed by self-assembled amphiphilic molecules in solutions as a function of temperature has been examined systematically by the quantitative study of thermodynamic properties in addition to structural analysis. Simulations have been performed using the recently developed statistical temperature molecular dynamics and parallel replica-exchange Wang-Landau Monte Carlo methods, both of which allow the direct calculation of the density of states. We have demonstrated that these two very different methods provide essentially identical results when considering the density of states and key transitions in the heat capacity for the model lipid system studied, thereby validating both the methods and the results obtained, showing the applicability of these methods to determine the free energy of self-assembling lipid systems.

While both methods provide closely matching results for the systems studied herein, we note that there are considerations to take into account when selecting one method over the other. A particularly advantageous feature of STMD is that it does not require a reduction in the energy bin size as system size is increased, since the statistical temperature can be locally interpolated well by a linear function, making the results from STMD only weakly dependent on the energy bin size. However, to the best of our knowledge, no such interpolation scheme exists for the WL method. Therefore, REWL simulations use a much finer energy binning compared to STMD, making it more costly for all histogram bins to be filled for appropriate convergence. This can make STMD better suited for the examination of larger systems. However, as we observe in our calculations, the error bars associated with STMD tend to be larger than those obtained from REWL, which may be associated with the fact that STMD follows the “natural” dynamics dictated by the equations of motion which are absent in the MC moves underlying WL; as such, hysteresis effects around phase transitions may be more pronounced in STMD, influencing the ability to accurately resolve certain

behaviors/transitions. However, both STMD and REWL are important tools, enabling the accurate calculation of the density of states over a wide range of systems and state points.

In general, the nature of the system and state points under consideration will play a key role in determining which method is most suited to a given application. Generally, the applicability is largely determined by the underlying algorithm of each method, i.e., MD versus MC, and the way in which parallelism is implemented in that method. For example, STMD relies on MD-based force calculations that, in this work, are implemented on massively parallel GPUs; this makes STMD well suited for the efficient examination of large systems, since the MD algorithm is known to scale well on massively multi-threaded GPUs, distributed CPU computing machines, and hybrid CPU-GPU systems.^{43,44} STMD may also lend itself more easily to the study of complex molecules, e.g., biopolymers, again as a result of the parallel nature of the underlying MD calculations but also since it avoids having to develop nonphysical effective MC trial moves to overcome steric hindrance.^{11,16,45} However, although the single-replica STMD used in this work is highly parallel with regards to the force computations, it may still experience problems associated with getting trapped in local energy minima at low temperature, making it prohibitively expensive to appropriately sample this regime. For example, even for the relatively small, coarse-grained system studied here, ~ 2 months of computation time was needed to study the lowest temperature window compared to ~ 5 days for the highest temperature window; however, we note that sufficient convergence was found over the entire range studied in this work. The ability to incorporate specialized MC moves into the WL method can be used to mitigate such problems, increasing sampling and avoid trapping (e.g., cluster moves and identity swaps). The REWL method is also less prone to such trapping issues at low temperatures as a result of the parallel sampling associated with the replica exchange MC moves; prior work has shown this approach to be superior over the original WL method without such exchanges¹⁷ and has additionally been demonstrated for statistical temperature MC⁴⁶ (note, statistical temperature MC is closely related to STMD). We note that the replica-exchange technique has been implemented for MD-based computations, specifically in the context of the STMD method, demonstrating increased performance as compared to a single-replica STMD.⁴⁷ However, in this work we chose to concentrate on the aforementioned parallelization direction and the efficient implementation on GPUs.

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FIG. 10. Illustration of the microcanonical analysis of phase transitions (see text for details). Data are the same as used for Fig. 6.

Advanced Computing Center for Research and Education (ACCRES) at Vanderbilt University.

APPENDIX: MICROCANONICAL ANALYSIS OF DATA USING MICROCANONICAL ENTROPY INFLECTION POINTS

Traditionally, phase transitions in thermodynamics and statistical mechanics are defined in the thermodynamic limit. However, in many areas of biological and chemical physics, the thermodynamic limit is not an applicable concept as one deals with specific, small, and non-extensive systems. Nonetheless, there is sufficient experimental evidence that it makes sense to apply the concepts of entropy and phase transitions to such systems as well.⁴⁰

We start off with the microcanonical entropy $S(E)$, which is proportional to the logarithm of the density of states:

$$S(E) \sim \ln g(E). \quad (\text{A1})$$

The microcanonical entropy is usually a concave function. In phase coexistence regions, however, the entropy becomes locally convex in an interval $E_1 < E < E_2$. To illustrate this effect, a concave hull $\mathcal{H}_s(E)$, which is the tangent to $S(E)$ in both points E_1 and E_2 , can be constructed.⁴⁸ The exponential of the difference $\exp(S(E) - \mathcal{H}_s(E)) =: P(E)$ is a bimodal distribution with peaks at E_1 and E_2 , which corresponds to the canonical energy distribution $\sim g(E) \cdot e^{-\beta_c E}$ at the transition temperature in a hypothetical thermodynamic limit. The energy difference $\Delta Q = E_2 - E_1 > 0$ can then be related to a latent heat. See Figs. 10(a) and 10(b) for illustration of the discussion so far.

Now, the inverse microcanonical temperature $\beta(E)$ (see Fig. 10(c)) is defined as the first derivative of the entropy:

$$\beta(E) = \left(\frac{\partial \ln g(E)}{\partial E} \right)_{N,V}. \quad (\text{A2})$$

The “convex intruder” in the entropy obviously causes a “back bending” in the microcanonical inverse temperature $\beta(E)$, which is hence not uniquely defined in that region. Furthermore, the second derivative of the entropy

$$\gamma(E) = \frac{d\beta(E)}{dE} = \left(\frac{\partial^2 \ln g(E)}{\partial E^2} \right)_{N,V} \quad (\text{A3})$$

is positive in the interval $E_1 < E < E_2$, see Fig. 10(d). We use the peak position of $\gamma(E)$ to define the transition energy E_c . The corresponding microcanonical transition temperature T_c would be determined via $T_c^{-1} = \beta_c = \beta(E_c)$ (see Fig. 10(c)). In analogy to the canonical ensemble (remember the bimodal distribution and $\Delta Q > 0$) we define such transitions to be of first order. (We want to stress that whenever we speak about “transitions” here, we do not refer to classical phase transitions defined in the thermodynamic limit, but to structural transitions in finite systems.) On the other hand, if $\beta(E)$ is a monotonic function in the vicinity of a transition, $\gamma(E_c)$ would be smaller than zero and the corresponding latent heat would vanish. We, hence, call such transitions second order (or continuous).

Summarizing, in the microcanonical analysis we detect transition points through peak positions E_c in the second derivative of the microcanonical entropy. If the corresponding peak values $\gamma(E_c) > 0$, we call it a first order transition, otherwise second order. The microcanonical transition temperatures are determined through the relation $\beta_c = \beta(E_c)$.

These transition temperatures would coincide with the canonical transition temperatures in a thermodynamic limit. However, for small systems and strong transitions, both temperature definitions can differ to a certain extent. For more details and complete discussions, see Refs. 39–41, 48, and 49.

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