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Dynamical Motions of Lipids and a Finite Size Effect in Simulations of Bilayers

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Abstract

Molecular dynamics (MD) simulations of dipalmitoylphosphatidylcholine (DPPC) bilayers composed of 72 and 288 lipids are used to examine system size dependence on dynamical properties associated with the particle-mesh Ewald (PME) treatment of electrostatic interactions. The lateral diffusion constant, D_{ℓ} , is 2.92×10^{-7} and 0.95×10^{-7} cm²/s for 72 and 288 lipids, respectively. This dramatic finite size effect originates from the correlation length of lipid diffusion, which extends to next-nearest neighbors in the 288 lipid system. Consequently, diffusional events in smaller systems can propagate across the boundaries of the periodic box. The internal dynamics of lipids calculated from the PME simulations are independent of the system size. Specifically, reorientational correlation functions for the slowly relaxing phosphorous-glycerol hydrogen, phosphorous-nitrogen vectors, and more rapidly relaxing CH vectors in the aliphatic chains are equivalent for the 72 and 288 lipid simulations. A third MD simulation of a bilayer with 72 lipids using spherical force-shift electrostatic cutoffs resulted in interdigitated chains, thereby rendering this cutoff method inappropriate.

I. Introduction

Molecular dynamics (MD) simulations are limited in length and system size by computational resources. Calculated properties from MD simulations can depend on system size, and some vary more than others. The dependence of system size for lipid bilayers has been studied for equilibrium properties, including surface area per lipid,¹ surface tension,² electron density, and deuterium order parameters.³ Systematic studies of system size dependence of dynamical properties are more limited. de Vries et al.³ demonstrated that the correlation functions for several carbons on the sn-1 chain of dipalmitoylphosphatidylcholine (DPPC) are converged for a system size of 16 lipids per leaflet, but their 20ns simulations were not sufficiently long to satisfactorily compare correlation functions for the head group phosphorous-nitrogen (P-N) vector. Self diffusivities in homogeneous bulk systems have recently been shown to have a strong system size dependence.^{4,5} Lateral diffusion constants of lipids in bilayers have been evaluated at different system sizes by other groups (e.g., refs 6-8), but variations in cutoff methods and force fields confound comparisons.

Closely related to system size is the treatment of long-range electrostatics. Ewald or particle-mesh Ewald (PME)⁹ methods are widely used to treat long-range electrostatics in lipid bilayer simulations.^{8,10} Simple truncation of electrostatics results in artificial maxima and minima in radial distribution functions at the cutoff distance.¹⁰ Moreover, relaxation times from electrostatic truncation of the P-N vector and carbon vectors on the sn-1 chains differ dramatically⁸ from PME, and the lateral diffusion constant of DPPC converges slowly to the PME value with an increase in the electrostatic cutoff radius. However, Ewald methods may result in artifacts including long-range correlation¹¹ and anisotropy¹² from the artificially induced periodicity. Consequently, it is important to periodically revalidate the application of Ewald summation to bilayers.

This work focuses on dynamical quantities in PME simulations of lipid bilayers. Trajectories of DPPC bilayers containing 72 and 288 lipids are analyzed for system size dependence of the short- and long-time lateral diffusion constants, and the reorientational correlation functions of molecular vectors that probe internal and overall rotation. The 50ns trajectories (three replicates for the smaller system and one for the larger) are sufficient to obtain well converged results. Separately, a striking artifact from a 50ns simulation of the 72 lipid system with a force-shift spherical cutoff on the electrostatics is also reported.

By way of outline, the following section describes the simulation methodology, an assortment of methods pertaining to translational diffusion (determination of diffusion constants, jump model parameters, and the concertedness of translational jumps and their correlation lengths). Section III (Results and Discussion) is divided into two subsections. The first subsection compares lipid diffusion for the two system sizes used in this work and also to past simulations and experimental measurements. It then relates the enhanced DPPC diffusion calculated from the 72 lipid system to the lipid diffusion correlation length. The second subsection considers system size effects on bilayer structure and the reorientational dynamics of individual lipids. Section IV summarizes the work.

II. Methodology

A. Simulation details

The CHARMM program¹³ is used in the simulations with 36 lipids per leaflet (72 total). The NAMD program¹⁴ is employed for simulations with 144 lipids per leaflet (288 total) because of its efficient parallelization for large systems. The force fields used in the simulations are the revised CHARMM27 (C27r)⁴ for lipids and the modified TIP3P water model.¹⁵ The lipid bilayers are fully hydrated (30.4 waters per lipid) and contain 15,927 and 63,708 atoms for the 72 and 288 lipid systems, respectively. The initial coordinates for the system with 72 lipids were obtained using a similar procedure to that reported previously by Feller et al.,¹⁶ but with unrealistic lipid head group conformations removed and the head group dihedrals highly restrained during minimization. The large DPPC bilayer is a quadruple replicate of the 72 lipid system with the coordinates from 5.5ns of a previous production run.⁴ The initial atomic coordinates for the simulation with force-switching of electrostatics are obtained from the end of a 72 lipid Particle mesh Ewald⁹ production run (50ns).

Two sets of MD simulations with 72 DPPC molecules were run: (1) Particle mesh Ewald for electrostatic interactions beyond 10 Å ($\kappa = 0.34 \text{ Å}^{-1}$ and a fast-Fourier grid density of about 1 Å^{-1}); and (2) Force-switching for electrostatic interactions over 8 to 10 Å.¹³ These systems are denoted 72 PME and 72 FSW, respectively. Three independent runs from previous simulations⁴ were extended to 50ns beyond 3ns of equilibration for 72 PME. A single 50ns trajectory was generated for 72 FSW, based on the 5.5ns time point of a previous simulation.⁴ The leapfrog Verlet algorithm was used with a time step of 1fs. These simulations were run in tetragonal periodic boundary conditions in the constant particle number, pressure, surface area, and temperature ensemble (NPAT). The cross-sectional area in these simulations was set to 64 Å^2 per lipid¹⁷ and the temperature to 323.15 K. The equivalence of NPAT and NPT ensembles for calculations of lateral displacements¹⁸ and area compressibility¹⁹ has been demonstrated, and implies that the results presented here should be independent of ensemble. Lennard-Jones (LJ) interactions were forced-shifted to zero between 8 to 10 Å. The pressure-based long-range correction²⁰ to LJ interactions with a long-range cutoff of 30 Å was applied to 72 PME, while 72 FSW was simulated at a constant normal pressure of 1 atm to maintain consistency with the absence of long range electrostatic interactions in this system. All hydrogen atoms were constrained using the SHAKE algorithm.²¹ The extended system formalism was used to maintain the temperature via the Hoover thermostat²² with a thermostat

coupling constant of 20,000 kcal mol⁻¹ ps⁻², and the normal pressure was maintained with a barostat²³ with a piston mass of 2000 amu.

The system of 288 DPPC molecules was simulated with NAMD¹⁴ using similar algorithms to those described above, i.e., PME and a long-range correction in pressure. Currently, NAMD only supports temperature control with Langevin dynamics, temperature coupling, or temperature rescaling. To minimize the perturbation on the lipid bilayer by these methods, the temperature was rescaled every 10ps for the first 2ns in equilibration and then unscaled through the final 1ns of equilibration and during production. Consequently, the ensemble for this system is NPAH (H, constant enthalpy). There was a minimal temperature drift of less than 1.3 K for 50ns.

B. Translational diffusion

The system size dependence on the lateral diffusion of lipids is a main focus of this work. To obtain the lateral diffusion constant, D_ℓ , the lateral mean-squared displacement of the lipid center of mass (COM) is calculated,

$$MSD \equiv \left\langle \left(\mathbf{r}(t) - \mathbf{r}(0) \right)^2 \right\rangle = \frac{1}{N_L} \sum_i \left(x_i(t) - x_i(0) \right)^2 + \left(y_i(t) - y_i(0) \right)^2 \quad (1)$$

where i is the individual lipid and N_L is the number of lipids. The long-time slope of the lateral mean squared displacement versus time equals $4D_\ell$. A weighted least squared fit is used to obtain D_ℓ with weights obtained from averages of 8 subgroups of molecules per trajectory. Patra et al.⁸ have suggested that the COM drift of the each leaflet should be removed from the individual lipid motion when calculating the diffusion constant. While this method can be useful for removing artifacts in some simulations, it also removes realistic displacements of lipid (the COM of the *entire* system must be constrained to remain fixed, but not the individual components). Diffusion constants with and without the monolayer COM displacement removed are calculated in order to evaluate the system size dependence of the removal.

The short-time diffusion constant, D_ℓ^{cage} , which reflects translational motions of the subnanosecond time scale,^{24,25} is not straightforward to calculate, and Eq. 1 cannot be used because the mean-squared displacement is non-linear in this region. Here the instantaneous slope of the MSD at $t=0$ was calculated for D_ℓ^{cage} from the forward numerical derivative from $t=0$ to 10 ps (the MSD was evaluated at 10ps intervals).

The diffusion constant for a lipid in a bilayer is defined in a jump model as follows,

$$D_\ell^{jump} = \frac{\lambda d^2}{4t_{run}} \quad (2)$$

where λ is the average number of jumps per unit time and d is the average distance between jump sites. Parameters λ and d were developed with the ART-2' nonhierarchical clustering algorithm based on a self-organizing neural net.²⁶ In this algorithm, a conformation, j , is described by a vector of N parameters, $\mathbf{x}_j$, where the vector of interest is simply the center of mass of a lipid. Each cluster k , C_k , is characterized by a cluster center, $\mathbf{c}_k$, that is the average conformation of its M members,

$$\mathbf{c}_k = \frac{1}{M} \sum_j \mathbf{x}_j \quad (3)$$

The coordinates are clustered based on the criteria that all conformations must be within a specified cutoff radius, r_c , from the cluster center based on the Euclidean distance,

$$d_{jk} = \left[(\mathbf{x}_j - \mathbf{c}_k) \cdot (\mathbf{x}_j - \mathbf{c}_k) \right]^{1/2} \quad (4)$$

The ART-2' algorithm compares $\mathbf{x}_j$ to $\mathbf{c}_k$ using Eq. 3 to determine if a conformation is in C_k if the distance is less than r_c . Otherwise the conformation seeds a new cluster and the cluster centers are recalculated for each new member of C_k . The assignment of clusters is repeated until the cluster membership remains the same (within a tolerance of 0.001) between sequential iterations. Based on visual inspection, $r_c = 7\text{\AA}$ provided the best discrimination of clusters. Because the clusters were developed from trajectories, the times, total number of transitions and thereby λ can be trivially calculated from the number of elements in each cluster. The cluster centers represent the location of jump sites, allowing evaluation of d .

The extend of concertedness (or correlation) in diffusion was evaluated using the analysis of Brown et al.²⁷ This method was originally developed to quantify concertedness in torsional transitions of the acyl chains of lipids, but is straightforward to extend to diffusion once the jump times are specified. Each time a lipid jumps, the neighboring lipids are surveyed to determine if they also jumped in a time window of 100ps (the approximate time it takes for a lipid to transverse between cages as estimated from the cluster analysis). Such transitions are denoted *followers*, and the number of followers is N^f . Windows of 50, 200, and 500ps were also evaluated to assess the robustness of the approach. Conceptually, N^f can be divided into the number of *independent followers* (those which would occur by chance in the absence of physical correlation), N^{if} , and *concerted followers* N^{cf} (those associated with correlation); i.e.,

$$N^f = N^{if} + N^{cf} \quad (5)$$

An *individual* follower transition cannot be determined as independent or concerted. Nevertheless, assuming independent Poisson statistics, N^{if} can be estimated as,

$$N^{if} = \lambda_w (N - 1) n_s \quad (6)$$

where λ_w is probability that an individual lipid will jump in the specified window size, N is the total number of jumps, and n_s is the number of lipids of the shell under consideration. $N-1$ enters into Eq. 6 because one of the N transitions has already taken place. λ_w was calculated here directly from the total number of jumps and lipids in each trajectory, and window size.

A measure of concertedness is then given by the probability π

$$\pi = \frac{N^{cf}}{N} \quad (7)$$

where N^{cf} is obtained from Eqs. 5 and 6. When jumps are independent, $\pi = 0$; a value substantially larger than 0 implies that the jumps are concerted. Values of π were calculated for follower transitions in the nearest neighbor or first shell ($r < 8\text{\AA}$), second shell ($8\text{\AA} \leq r < 16\text{\AA}$) and third shell ($16\text{\AA} \leq r < 24\text{\AA}$) of the leader. Correlation between leaflets was not evaluated.

A related measure of concertedness involves testing the null hypothesis: N^f could have arisen if the transitions were independent and described by a Poisson distribution with mean $\lambda = N^{if}$. The probability of incorrectly rejecting the null hypothesis is given by the *p-value* defined from the conditional probability of the Poisson distribution:

$$p = P(Y \geq N^f \mid \lambda = N^{if}) \quad (8)$$

A small value of p , typically < 0.05 or 0.1 , is commonly used as a cutoff for rejecting the null hypothesis. If p is below the cutoff, then there is good evidence of concerted behavior.

III. Results and Discussion

A. Translational Diffusion

The uncorrected MSD versus time from 0 to 100ps is non-linear (Fig. 1, bottom) with a higher slope compared to the linear behavior at $t > 1$ ns (Fig. 1, top). This is consistent with the fast diffusion region over short distances and times observed in incoherent quasielastic neutron scattering (QENS).^{24,28} This motion reflects rapid fluctuations of the lipids as they “rattle in a cage” of their nearest neighbors,²⁴ and is described by the short-time diffusion constant D_ℓ^{cage} . It is distinct from long-time lateral diffusion, which entails hopping *between* cages and is described by D_ℓ .

Table 1 lists the values of D_ℓ^{cage} from the MD simulations. Results for both the PME simulations are in quite good agreement with experiment and with the simulated values of Lindahl and Edholm⁷ and Patra et al.⁸ The long-time diffusion constants are an order of magnitude smaller than D_ℓ^{cage} . These will be considered in detail for the PME systems after a brief discussion of the results from the force-shift simulation.

Both D_ℓ^{cage} and D_ℓ for the 72 FSW are substantially smaller than those from the PME simulations (Fig. 1 and Table 1). The reason for this is clear from Fig. 2, which shows the final (50 ns) snapshot of each system. The PME bilayers (left and middle panels) are in the state of disorder expected for the liquid crystalline phase. In contrast, the aliphatic chains in the 72 FSW system are mostly fully extended and interdigitated (Fig. 2, right). The observed restricted translation would be expected for such a system. Nucleation of this configuration began at approximately 10ns, and condensation proceeded rapidly. It is a consequence of unbalanced electrostatic forces and the constant area constraint. Had the system been simulated at constant surface tension, the area would have most likely rapidly decreased thereby preserving a bilayer structure, albeit a highly ordered one. Previous NPT simulations with a larger electrostatic cutoff (twin range of 10/18 Å) result in a DPPC liquid crystalline bilayer²⁹ also point to the short electrostatic cutoff as the underlying cause for the observed interdigitation. The present structure is similar to the minor domain (*m*) of the DPPC ripple phase as determined from experiment by Nagle and co-workers³⁰ and later supported via simulation by de Vries et al.³¹ However, it is an unphysical artifact under the present conditions (the ripple phase for DPPC is stable between of 308 to 315K³²), and further analysis is not included.

Turning to the long-time translational diffusion of the 288 lipid PME system, $D_\ell = 0.95 \times 10^{-7}$ cm²/s. This is reasonably close to results from fluorescence recovery²⁵ and magic angle scattering NMR experiments³³ (Table 1). It is also close to the diffusion constants evaluated from two GROMACS-based simulations: 1.2×10^{-7} cm²/s by Lindahl and Edholm⁷ (with twin range spherical cutoffs, 64 lipids), and 1.3×10^{-7} cm²/s by Patra et al.⁸ (with PME, 128 lipids). The latter authors also showed an over ten-fold dependence of D_ℓ on the spherical cutoff value for the 128 lipid system, but did not examine other system sizes.

In contrast to D_ℓ^{cage} , long-time lateral diffusion depends on the system size, i.e., $D_\ell = 2.9 \times 10^{-7}$ cm²/s for 72 PME, over three times larger than 288 PME. An analysis of this substantial finite size effect begins with the COM trajectories of individual lipids. Figure 3 shows one such trajectory from a 72 PME system. Fluctuations of the COM in relatively localized regions are punctuated by transitions to other regions, in what appears to be a combination of rattling in a cage and jumping between cages. The cluster analysis described in the Methods yields the 5 clusters for this lipid (depicted as circles in Fig. 3). The average distance between clusters, d , for all lipids is 9.05 and 8.23 Å for 72 PME and 288 PME,

respectively. Given that the surface area per lipid is $64 \text{ \AA}^2$, these values of d and the general features of Fig. 3 suggest that a jump model is suitable for the present analysis.

Figure 4 plots the jump times for one of the 72 PME systems (top) and 288 PME (bottom). The 72 PME system contains periods of relative quiet and bursts of highly correlated transitions; some sets of transitions appear to be coupled between leaflets. The 288 PME system does not exhibit such obvious trends, though some degree of correlated motion cannot be ruled out. Diffusion constants estimated from the jump model, D_ℓ^{jump} , show a substantial system size effect (Table 1). D_ℓ^{jump} also differs significantly from D_ℓ for 72 PME, implying that the jump rate in this system is not well described by an independent Poisson distribution. D_ℓ^{jump} is slightly lower than D_ℓ for 288 PME, but the difference is not statistically significant.

Figure 5 compares the probability distribution, $P(N_j)$, for number of lipid jumps (N_j) per leaflet in two ns intervals observed in the simulations and a Poisson distribution with the same mean. The average number of jumps per 2ns block, λ , is larger for 288 PME as expected from the system size. However, the increase in λ is only two-fold because the diffusion is slower than 72 PME. The probability distribution for 72 PME (top panel of Fig. 5) appears to be bimodal. This is consistent with the correlated bursts of activity shown in Fig. 4, and qualitatively different from a Poisson distribution. $P(N_j)$ for the 288 PME is closer to a Poisson distribution than 72 PME (Fig. 5, bottom panel) although is somewhat broader, implying some correlation in lipid jumps even for this system.

Correlation, or concertedness, in the jumps was analyzed by the approach of Brown et al.²⁷ described in the Section II-B. Table 2 lists the relevant statistics for the 100ps window size. The individual 72 PME systems show substantial evidence of concertedness in the first and second shells ($p \leq 0.1$ in all three cases), and run1 even shows potential correlation to the third shell. Similar results were observed for the 50 and 200ps time windows, but no concertedness is evident for the 500ps time window. Pooling the 100ps time window results of the three simulations yields $p \approx 10^{-6}$ for the first and second shells, and $p = 0.024$ for the third shell. Hence, there is little doubt that concertedness extends to the second shell and it likely extends to the third shell for systems of 72 lipids.

Results for 288 PME are somewhat less clear, partly because of sample size. There is no strong evidence for concertedness with the first shell ($p = 0.31$), but correlation with the second shell is high ($p = 0.005$). Correlation with the third and fourth shells is negligible (data not shown). Similar results are obtained with the 50 and 200ps time windows; the 500ps window yields no correlation for any of the shells. It is not clear at this juncture whether the lack of correlation for the first shell arises from incomplete sampling, limitations in the jump model, or, more deeply, to the mechanism of lipid diffusion. Nevertheless, the presence of correlation with the second shell is the central result of the study. If diffusion exhibits a natural correlation with the second shell (a length of $16 \text{ \AA}$), it becomes likely that the effects of a transition of a lipid in a system of 36 lipids/leaflet will propagate across the $50 \text{ \AA}$ /side box length, and thereby artificially increase the diffusion constant. Because the 288 PME system is nearly $200 \text{ \AA}$ /side, such artifacts are unlikely.

The finite size effect reported here is opposite of that in pure bulk simulations, where the self-diffusion constant increases with system size.^{4,5} Yeh and Hummer⁵ determined that the effect in homogeneous systems is hydrodynamic in origin. In bilayers, the present finite size effect is shorter range and predominantly collisional. It is possible that a longer-range hydrodynamic effect will be observed in simulations of larger bilayers.

Recent simulations of a 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE) lipid bilayer of 72 lipids showed hydrogen bond networks that span the simulation box size.³⁴ In contrast to the present DPPC simulations, the calculated D_ℓ is lower than experiment by a factor of about three. While the time spent in the clusters is small, such the hydrogen bond networks might be expected to retard diffusion. Simulations SOPE bilayer with larger sizes are needed to determine whether such large hydrogen bond networks are partly finite artifacts and are the cause of the large underestimate of D_ℓ .

Lastly, if the COM displacement of each monolayer is removed from the lipid COM displacement, the lateral diffusion constant (D_ℓ^{mono-}) is reduced by more than factor of 4 for 72 PME, but only 6% for 288 PME (Table 1). The origin of this finite size is two-fold. First, as the size of the bilayer increases, the effect of lipid diffusion on the COM of each monolayer decreases; i.e., $D_\ell^{mono-} \rightarrow D_\ell$. Second, correlated motion leads to substantial unidirectional displacements for 72 PME. Removal of the monolayer COM removes some of these, further reduces D_ℓ^{mono-} , and the errors partially cancel. In general, monolayer COM displacement should not be removed when evaluating diffusion constants of lipids in bilayers.

B. Finite size effects on bilayer structure and lipid reorientation

The electron density of the lipid bilayer and deuterium order parameters of the lipids are equal for our 72 and 288 PME simulations. These results agree with de Vries et al.³, where structural properties converged at a simulation size of 72 lipids (36 per leaflet). Although these structural properties remain unchanged, the enhanced lipid diffusion calculated for the 72 PME may influence radial and angular distributions of the lipid charged atoms. Therefore, the two-dimensional radial distribution function, $g_{2D}(r)$, for the P and N atoms in DPPC for 72 and 288 PME is now considered. The $g_{2D}(r)$ averages out the bilayer normal axis and is a distribution function per leaflet. Figure 6 plots $g_{2D}(r)$ for P-N and N-N and demonstrates that the distributions are identical for both system sizes. The distribution of the relative orientation of the P-N dipole is also a measure of changes in angular distributions. Figure 7 shows the angular probability of all lipids within 7 Å of the reference lipid (averaged over all lipids). A cutoff value of 7 Å was chosen because it resulted in a distribution with the largest peak at 180°, i.e., neighboring dipoles are in opposite direction. The difference between 72 PME and 288 PME is negligible. These results demonstrate that even though the lateral diffusion shows system size dependence the head group radial and angular distribution functions do not.

The hopping rate in the 72 PME systems is approximately 0.1ns^{-1} (Table 2). Consequently, rotational motions of the lipid, which take place on the 1 to 10 ns time scale,^{35,36} could be perturbed by the finite size effect observed for lateral diffusion. To investigate this possibility, three vectors were considered: the principal axis (PA), phosphorous-nitrogen (P-N) vector, and phosphorous-hydrogen (P-H) vector with the neighboring hydrogen on glycerol. The P-N vector is a measure of head group motion, PA is associated with wobble,³⁵ and P-H with axial diffusion.³⁶ Figure 8 plots the reorientational correlation functions for these three vectors for 72 and 288 systems. There is a slight deviation for the $C_2(t)$ of the PA, but the three runs of 72 PME bracket the 288 PME result (only the average is shown). The fastest relaxing P-N vector shows negligible differences between the small and large systems.

Next, the fast torsional motions of the carbon-hydrogen vectors on the aliphatic chain are compared for the two systems. The reorientational correlation functions for the C2, C9, and C15 positions (C2 is closest to the headgroup) are shown in the bottom panel of Fig. 8. Similar to the above mentioned correlation functions, the decays are nearly identical for the 72 PME and 288 PME, with the expected decrease of relaxation times with increasing distance from the head group. The invariance of $C_2(t)$ to system sizes greater than or equal to 72 lipids allows

for force field parameterization on torsional changes to the potential function based on results of a smaller system of 72 lipids.

IV. Summary

Correlation of diffusional jumps of lipids extends to next nearest neighbors (16 Å) in a bilayer of 288 lipids simulated with particle mesh Ewald (PME). This explains the bursts of lipid jumps (Fig. 4) and three-fold increase in the lipid lateral diffusion constant D_ℓ in bilayers of 72 lipids (Table 1); i.e., the simulation box (50 Å/side) is not substantially larger than natural correlation length. D_ℓ for the 288 lipid system is in good agreement with experiment, as are the short-time lateral diffusion constants, D_ℓ^{cage} of both systems (Table 1). The preceding length-scale considerations argue against PME causing the finite size artifact. Rather, a 50 Å cell is simply too small to simulate lipid diffusion adequately.

A simulation of 72 lipids carried out with force switching on the electrostatic interactions yielded an unphysical interdigitated bilayer resembling the *m* domain of the ripple phase. This result further supports the use of PME, and highlights the sensitivity of phase behavior of lipids to long-range electrostatic forces.

Although lateral diffusion is dependent on system size, other properties calculated here are independent. A system size of 72 lipids is sufficiently large for calculating structural properties such as electron density and deuterium order parameters. The neighboring P-N dipole orientation, radial distribution functions, correlation functions for overall lipid rotation, and individual C-H isomerizations are also independent of system size.

In conclusion, MD simulations with 72 lipids and PME have converged structural properties and internal dynamical properties. Consequently, force field parameterization and limited-scope simulations can use these small system sizes with PME to rapidly obtain the desired properties. Larger simulation sizes are required to obtain accurate lateral diffusion constants. These results may also be important in simulations with membrane proteins, especially mechanosensitive channels,³⁷ where quiescent and hyperactive periods of motion for small systems could effect the gating of these proteins.

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Figure 1.

The lateral mean squared displacement of the lipids, $\langle l(t) - l(0) \rangle^2$, for 72 PME (an average of three independent simulations) and one 288 PME simulation for all 50ns (top) and for the interval 0-100ps (bottom). Standard errors are denoted with vertical bars.

Figure 2.
(color online) Final snapshot of one 72 PME (left), 288 PME (middle), and 72 FSW (right) simulation. The ends of the aliphatic chains are shown in large yellow spheres.

Figure 3.
(color online) The lateral trace (in 10ps intervals) of the center of mass (COM) of a lipid in one 72 PME simulation, with the five clusters with radius of 7 Å.

Figure 4.

Jump times (defined by the clustering algorithm and denoted by vertical tick marks) of all of the lipids in 72 PME (top) and 288 PME (bottom).

Figure 5.

The average probability of the number of jumps in a 2ns block (λ) ignoring the first and last block for 72 PME (top) and 288 PME (bottom), and the Poisson distribution for these values of λ (solid lines in each panel).

Figure 6. The 2-dimensional radial distribution functions, $g_{2D}(r)$, for one leaflet for P-P (top) and N-N (bottom).

Figure 7.

The probability of finding a neighbor with an angle θ defined by the angle formed between its P-N vector and those neighboring lipids less than 7 Å of separation between the phosphorous atoms.

Figure 8. (color online) Second rank reorientational correlation functions of the principal axis (PA), P-N, and the vector between the phosphate and adjacent glycerol hydrogen (P-H) (top), and C-H vectors of aliphatic carbons C2, C9, and C15 (bottom) for 72 PME and 288 PME.

Table 1

Lateral diffusion constants for DPPC: short-time cage diffusion, D_s^{cage} evaluated from the slope of the mean squared displacement (Eq. 1) between 0 and 10 ps; long-time lateral diffusion constant D_ℓ , evaluated over the range 1-50 ns; lateral diffusion with the monolayer COM displacement removed D_ℓ^{mono-} ; and D_ℓ^{jump} for a jump model, from Eq. 2. All diffusion constants have units of $10^{-7} \text{ cm}^2/\text{s}$ and, unless otherwise noted, are calculated at 323.15K.

Method	D_ℓ^{cage}	D_ℓ	D_ℓ^{mono-}	D_ℓ^{jump}
72 PME	14.7±0.04	2.92±0.42	0.66±0.11	2.08±0.13
72 FSW	4.81±0.62	0.13±0.01	--	--
288 PME	13.9±0.08	0.95±0.01	0.89±0.01	0.88±0.14
Experiment	12.0 (328 K) ²⁸		1.25 ²⁵ 1.52 ³³ (324 K)	

Statistics for concerted motion: N , the number of diffusional jumps for each trajectory based on a cluster analysis; N^f , the number of jumps following another jump within 100ps; N^{if} , the number of jumps that would be expected from independent Poisson statistics (Eq. 6); π , the probability of a concerted jump (Eq. 7); and the p -value, which is the probability that N^{if} would have been observed if the jumps were independent (Eq. 8). Lipids were divided into the first shell ($r < 8 \text{ \AA}$), second shell ($8 \text{ \AA} \leq r < 16 \text{ \AA}$) and third shell ($16 \text{ \AA} \leq r < 24 \text{ \AA}$), with n_s the average number of lipids in each shell.

Table 2

Simulation	Shell	n_s	N	N^f	π	p -value
72 PME run1 ($N=380$)	1	5.9	41	23.8	0.045	0.0005
	2	18.1	103	72.3	0.081	0.0003
	3	31.3	147	125.3	0.057	0.026
72 PME run2 ($N=355$)	1	5.5	36	19.4	0.047	0.0002
	2	18.4	74	64.2	0.028	0.10
	3	31.8	95	110.9	0.000	0.93
72 PME run3 ($N=359$)	1	5.7	27	20.4	0.018	0.063
	2	18.8	96	67.0	0.081	0.0003
	3	30.9	117	110.3	0.019	0.24
288 PME ($N=683$)	1	5.7	20	18.5	0.002	0.31
	2	18.4	80	59.5	0.030	0.005
	3	31.6	84	102.3	0.000	0.96