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Headgroup Conformations of Phospholipids from Molecular Dynamics Simulation: Sampling Challenges and Comparison to Experiment

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Abstract The preferred conformations of the glycerol region of a phospholipid have been explored using replica exchange molecular dynamics (MD) simulations and compared with the results of standard MD approaches and with experiment. We found that due to isomerization rates in key torsions that are slow on the timescale of atomistic MD simulations, standard MD is not able to produce accurate equilibrium conformer distributions from reasonable trajectory lengths (e.g., on the 100 ns) timescale. Replica exchange MD, however, results in quite efficient sampling due to the rapid increase in isomerization rate with temperature. The equilibrium distributions obtained from replica exchange MD have been compared with the results of experimental nuclear magnetic resonance observations. This comparison suggests that the sampling approach demonstrated here is a valuable tool that can be used in evaluating force fields for molecular simulation of lipids.

Keywords Molecular dynamics · Model membrane · Replica exchange · NMR study · Artificial bilayer membrane

Introduction

Phospholipids constitute an important class of biomolecules, providing the basis for cellular membranes and creating nanoscopic interfaces with extreme variations in polarity over a length scale of angstroms. The backbone of the phospholipid is a substituted glycerol with the hydrophilic phosphate headgroup connected to carbon atom C1 via a phosphate ester bond and two hydrophobic fatty acid chains connected at C2 and C3 via ester bonds (see Fig. 1). Situated in a unique region of astounding chemical heterogeneity (Wiener and White 1992), the conformational preferences of the glycerol region of the phospholipid determine important details of headgroup and tail orientations, influencing properties such as the dipole orientation and dipole potential (Gawrisch et al. 1992) and the packing of the aliphatic chains that are critical determinants of membrane structure and function (Israelachvili 1977). Hauser et al. (1980, 1988) have described how the values of the θ_3/θ_4 torsion angles (see Fig. 1 for definitions of these angles) determine the relative orientation of the acyl chains and compared the torsional states of a variety of phospholipids as crystalline solids, as fluid phase (L_α) bilayers and as individual monomers dissolved in a range of solvents. While the crystal structure indicates a single conformation (θ_3 = trans, θ_4 = gauche+), under physiological temperature, in both the monomeric and bilayer forms, several conformations are observed by nuclear magnetic resonance (NMR). Hauser et al. labeled the global free energy minimum conformer observed in the crystal structures "A" and identified by NMR analysis the "B" conformer (θ_3 = gauche+, θ_4 = gauche–) and the "C" conformer (θ_3 = gauche-, θ_4 = trans). The relative population of these rotational isomers depends somewhat on acyl chain composition, aggregation state and solvent polarity; but for glycerophosphatidylcholines the A conformer always predominates (50–60%) and the C conformer constitutes only a small fraction (1–10%).

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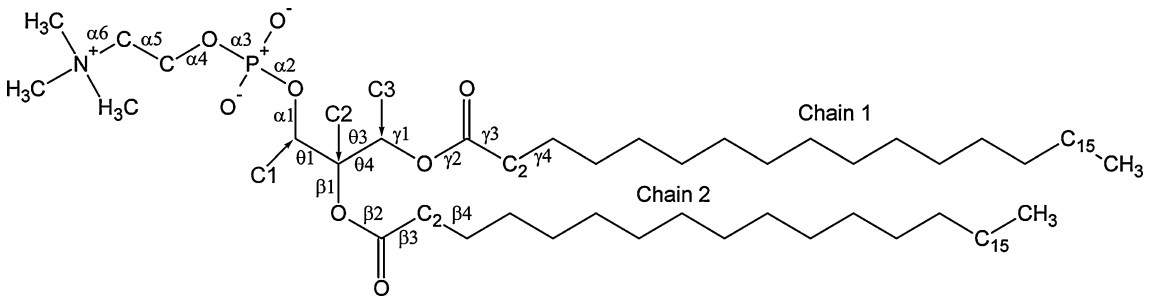

Fig. 1 Structure of DPPC using the torsion angle notation of Sundaralingam (1972)

In the recent past molecular dynamics (MD) computer simulations have emerged as a powerful tool for providing structural, dynamic and thermodynamic descriptions of lipid bilayer membranes at the atomic level (Brandt and Edholm 2009; Lyubartsev and Rabinovich 2011; Marrink et al. 2009). These calculations have been found to complement experimental techniques such as X-ray scattering and NMR and are finding widespread use in membrane biophysics research programs. There are, however, many details that must be considered in carrying out such computer simulations, such as the time and length scales of the processes under study, the statistical mechanical ensemble to be employed, the algorithm used to integrate the equations of motion and the form of the energy function and its parameterization. A key quantity that must be assessed in evaluating such simulations is the degree to which the results can be said to be converged; i.e., the observed conformations correspond to the equilibrium distribution and the number of transitions between states is sufficiently large that reliable statistics on the dynamics of fluctuations are obtained (Grossfield and Zuckerman 2009). For degrees of freedom with long correlation times, it may be difficult to determine accurate equilibrium distributions and it may be unclear whether discrepancies with experiment arise from a systematic error, e.g., deficiencies in the potential energy function, or from statistical error. A powerful algorithm for accelerating the sampling in MD simulations is the replica exchange (REX) technique. In its most frequent implementation, a series of MD simulations (system replicas) are run in parallel at different temperatures. The temperature range typically begins at or near the temperature of primary interest and increases to a significantly higher temperature, where rates for crossing energetic barriers are significantly higher. A Monte Carlo procedure is applied periodically to swap configurations among the various temperatures; thus, each replica spends time at higher temperatures, accelerating processes that have long correlation times at the temperature of interest. For a detailed description of REX MD, the reader is referred to Sugita and Okamoto (1999).

Here, we describe the application of REX MD to a dipalmitoylphosphatidylcholine (DPPC) monomer in aqueous solution. While many simulations of DPPC as a bilayer or monolayer have been reported in the literature, few have examined the monomeric form. We chose this system composition because it more closely matches the conditions of the NMR experiments we seek to connect to and because it allows extremely efficient sampling using the REX MD technique. Our results, however, have important implications for simulations of the phospholipid bilayers that are our ultimate goal.

Procedure

The program CHARMM (Brooks et al. 1983) was employed for all simulations, using the CHARMM c32b2 all-atom force field (Feller and MacKerell 2000; Schlenkrich et al. 1996). A single DPPC molecule was built with all bond lengths, angles and dihedrals generated from internal coordinates. In that process the dihedral angles θ_3 and θ_4 were generated as 180° and 60°, respectively. After hydration with 898 TIP3P water molecules, the system consisted of 2,725 atoms in cubic periodic boundary conditions with a side length that fluctuated about a value of 30.1 Å during the conventional simulation. Constant pressure (1 atm) was maintained using the Langevin piston (Feller et al. 1995) algorithm, while constant temperature (300 K) was maintained via a Hoover (1985) thermostat. All bonds to hydrogen atoms were fixed at their equilibrium lengths using the SHAKE (Ryckaert et al. 1977) procedure.

The conventional MD simulation was equilibrated for 1.2 ns and subsequently run for 110 ns with a 1-fs time step in the NPT ensemble. The coordinates produced after 3.9 ns of this simulation were used as a starting configuration of a REX MD simulation that ran for 35.7 ns with a 1-fs time step. In this simulation, 20 replicas of the system were simulated at constant volume at the following temperatures: 300, 306, 312, 320, 328, 336, 346, 356, 366, 378, 390, 402, 416, 430, 444, 460, 476, 492, 510, and 528 K. Systems at neighboring temperature baths were allowed to swap in 250-fs intervals of MD simulation. With this temperature spacing the swap probability was approximately 10%.

Results

Figure 2 shows the time evolution of the θ_4 torsion angle over more than 100 ns of standard MD simulation. The majority of the simulation time is spent in conformation A. The number of isomerizations is small ($\sim$0.8/ns), with many producing only short periods in conformations B and C. The B conformation in particular is rarely observed in the simulation, making up only $\sim$1% of the states, much less than observed experimentally. The single transition to the B state observed in over 100 ns of simulation powerfully demonstrates the challenge in sampling lipid headgroup conformations. While the lifetimes of rotational isomers along the acyl chain are in the range of tens to hundreds of picoseconds (Venable et al. 1993), the dramatically longer lifetimes observed in the headgroup lead to a situation where lipid simulations begun from arbitrary initial conditions may not achieve an equilibrium distribution on the timescale of current MD simulations. While it might be argued that in a typical lipid bilayer simulation with $\sim$100 lipid molecules taken from preequilibrated libraries the equilibrium distribution may still be obtained when averaging over all molecules, this will not be the case for an inhomogenous membrane such as one containing a transmembrane protein or membrane-bound peptide where the distribution of glycerol backbone dihedrals might be shifted.

The time evolution of θ_4 in the lowest temperature replica of the REX MD simulation is plotted in Fig. 3. The dramatically shorter lifetimes arise primarily from swap moves between the $T = 300$ K bath and higher-temperature baths. While the long standard MD run retained

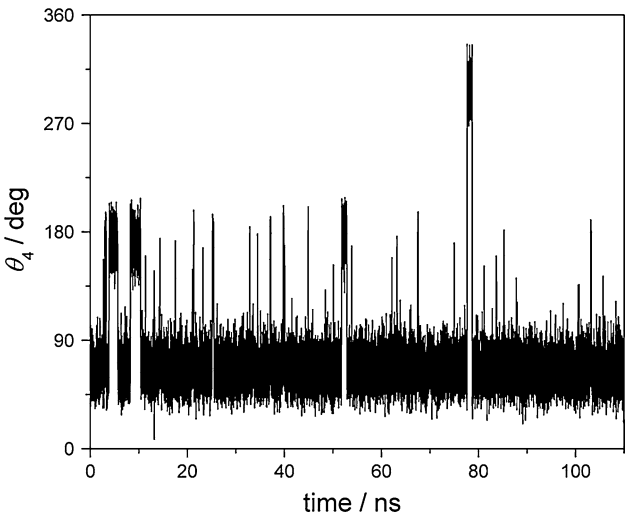

Fig. 2 Time evolution of the θ_4 torsion in the conventional MD simulation showing the small number of transitions observed in over 100 ns of simulation ($\sim$0.8/ns), with many having a lifetime of only a few picoseconds

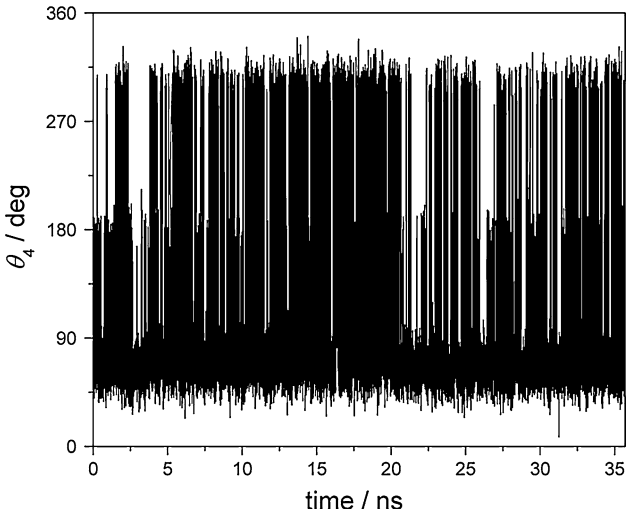

Fig. 3 Time evolution of the θ_4 torsion in the REX MD simulation at the lowest (300 K) temperature. The high rate of conformational transitions observed in the simulation at this temperature arises primarily from swaps with other temperature baths

significant memory of the initial condition, spending $\sim$93% in the A conformation that began the production run, new conformers emerge immediately in the REX MD run. By following the individual replicas, the isomerization rate occurring at each temperature, i.e., true transitions occurring at a given temperature rather than changes in a torsion arising from a swap move, was computed; and the results are plotted in Fig. 4. Over the temperature range investigated the transition rate increased by approximately 15-fold. The solid line in Fig. 5 gives the best fit of the observed rates to the Arrhenius equation, yielding activation energy of 4.5 kcal/mol. The small transition rate ($\sim$1/ns) in the low-temperature bath of the REX MD simulations is consistent with the observations from the standard MD run and reemphasizes the challenge in equilibrating the lipid headgroup structure.

While the slow dynamics of glycerol group isomerization provides a challenge to the successful implementation of atomistic MD simulation, it is not the case that the simulated dynamics are necessarily incorrect. In other words, the potential energy surface may be accurate even if it is not sufficiently well sampled in a finite-length simulation. The situation is analogous to the difficulties of folding a protein from the denatured state using atomic-level MD. Folding may be unsuccessful; i.e., the correct equilibrium structure is not obtained, even when the potential function is highly accurate, simply because insufficient time is available in the simulation. Errors are readily introduced into the interpretation of the simulation, however, when the distribution functions produced by the simulation are blindly assumed to represent the equilibrium distribution. For the glycerol θ_4 torsion, Fig. 6 shows the

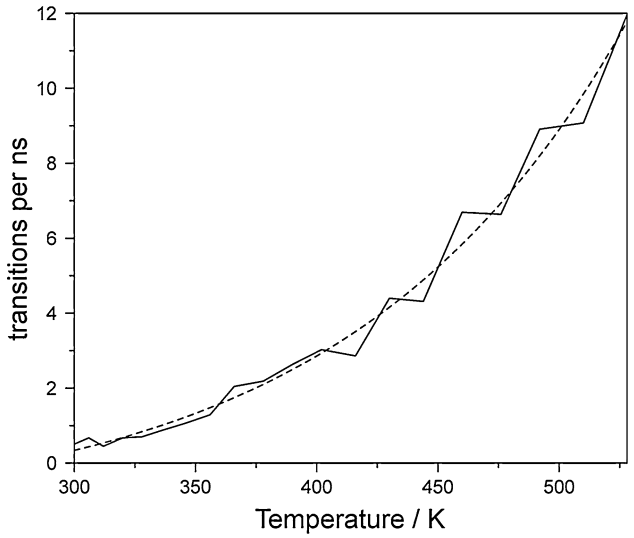

Fig. 4 Transition rates for the θ_4 torsion in the REX MD simulation as a function of temperature showing greater than order of magnitude acceleration in conformational transitions. The transition rates reported here are actual conformational changes, i.e., not changes arising from swaps between baths

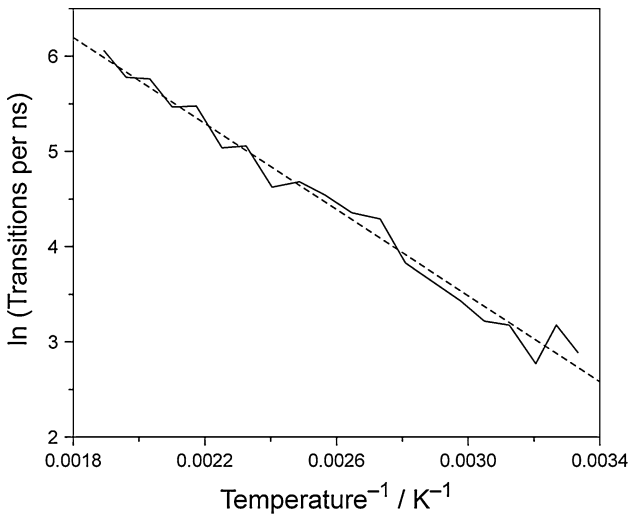

Fig. 5 Arrhenius plot of natural log of transition rates versus inverse Kelvin temperature (using data from Fig. 4), showing activation energy of $\sim$4.5 kcal/mol

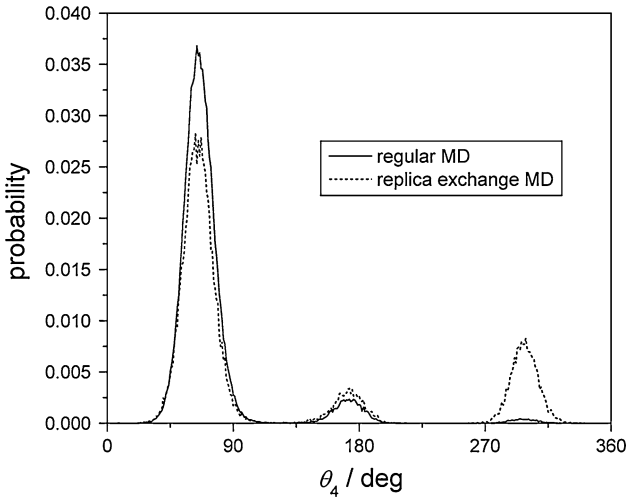

Fig. 6 Probability distribution for the θ_4 torsion observed in the REX MD and conventional MD simulations at 300 K, showing significant differences in the conformer distributions, particularly the frequency of observing the B conformation

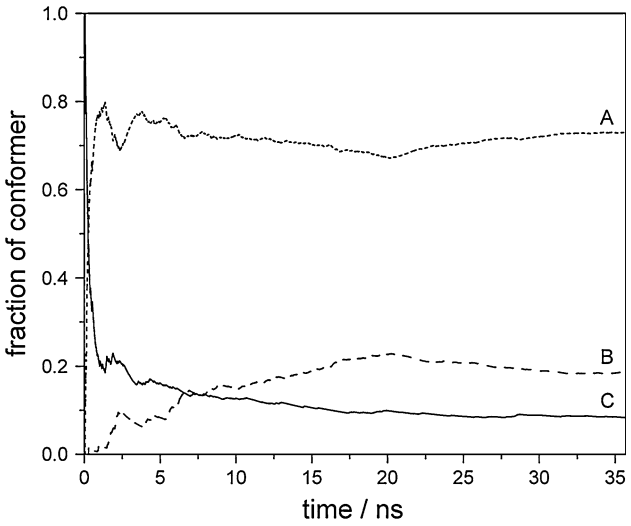

Fig. 7 Cumulative probability distribution of the θ_4 torsion in the REX MD simulation at the lowest (300 K) temperature. Compared to experiment, the A conformation is overrepresented in the simulation, while the B conformation is underrepresented (though much closer to experiment than was observed in the conventional MD calculation)

distribution of conformers observed in the REX MD and standard MD simulations. The plot shows that the calculated histograms differ significantly from one another with the relative populations in different orders, even though both simulations employed the identical potential energy function. Especially poorly sampled is the B conformation in the standard MD run, where it makes up only $\sim$1%, while the REX MD spends $\sim$18.5% in this state.

The results in Fig. 6 clearly show that a conventional simulation of 100 ns is insufficient to generate an equilibrium distribution of conformers, but it must also be noted that the REX MD simulations are more computationally expensive per unit of simulation time because they involve integrating the equations of motion of many systems. Thus, one must compare the computational cost of running an ensemble of, e.g., 20 exchanging replicas for a short period with the cost of running a single system for a long period. Figure 7 demonstrates the timescale required for convergence in the REX MD simulation. While the simulation time needed is relatively short, e.g., at approximately 25 ns reasonable convergence is obtained, this required 500 ns of total simulation time for the present case

of 20 replicas. The time series for the conventional simulation shown in Fig. 2 suggests that even after 500 ns of conventional MD the equilibrium distribution would not have converged. Another telling comparison is between the cumulative probability distribution from the REX MD simulation at 5 ns and the conventional result based on 100 ns. Even though every replica was started in the lowest probability conformation (*C*), in just a few nanoseconds a very reasonable distribution function is obtained. It also should be noted that scaling on a parallel computer is excellent with a REX simulation; thus, this approach can make good use of multiple processor systems. A potential downside for REX when simulating membrane systems is that the number of replicas must increase with system size in order to obtain reasonable swap probabilities; and given that membrane systems typically contain many atoms, this is a considerable challenge.

Conclusions

We have focused here on a single degree of freedom, namely, the θ_4 torsion angle found in the glycerol backbone region of a phospholipid, to illustrate the enormous challenge in determining the equilibrium conformer distribution in a molecule that possesses multiple rotational isomers having lifetimes that are long on the timescale of atomistic MD simulations. When compared to the other major classes of biomolecules, phospholipids are generally considered small and flexible because they consist of just a few monomeric units, e.g., a headgroup and two acyl chains, while proteins and DNA are orders of magnitude larger in terms of the number of monomers they contain. It has been shown that not only large proteins (Grossfield and Zuckerman 2009) but even small peptides (Vogel et al. 2010) pose considerable challenges for conformational sampling. Less appreciated is a property that the much smaller phospholipids nonetheless share with proteins and DNA, namely, that sampling their backbone conformations is a huge challenge for conventional MD simulations. One outcome of the present study is to demonstrate clearly and to quantify the difficulty in sampling lipid backbone conformations. This is important in particular for the setup of membrane simulations that most often rely on libraries of preequilibrated lipid structures (Jo et al. 2007). Our results demonstrate that equilibration simulations of tens of nanoseconds that were typical in the past are not sufficient for complete sampling of lipid backbone conformations. A membrane consisting of lipid structures from such relatively short preequilibrated lipid structures will at the beginning still lack a good distribution of these backbone conformations. Therefore, such libraries should be equilibrated either by very long classical simulations (possibly at high temperatures) or by approaches that improve sampling speed such as REX (Sugita and Okamoto 1999) or accelerated MD (Hamelberg et al. 2004) simulations. A second result is to show that simulations of monomeric phospholipids are potentially valuable in the refinement of potential energy parameters, i.e., force fields, for molecular simulations of lipid bilayer membranes. For example, it appears that the B conformation is less prevalent in our simulations than the NMR experiments (Hauser et al. 1988) indicate. This is indicative of inaccuracies in the torsional potential employed in this force field, and the most recent refinement of the CHARMM lipid force field (Klauda et al. 2010) alters the θ_4 torsional potential to make the B conformation more favorable. While their approach was to analyze NMR deuterium order parameters (and to carry out high-level quantum chemical computations on the relative gas-phase energies of model compounds containing this torsion), it is interesting to note that the analysis of monomeric lipids may have been a more efficient approach for this important element of the force field.

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References

Brandt EG, Edholm O (2009) Dynamic structure factors from lipid membrane molecular dynamics simulations. Biophys J 96: 1828–1838

Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M (1983) CHARMM—a program for macromolecular energy, minimization, and dynamics calculations. J Comput Chem 4:187–217

Feller SE, MacKerell AD (2000) An improved empirical potential energy function for molecular simulations of phospholipids. J Phys Chem B 104:7510–7515

Feller SE, Zhang YH, Pastor RW, Brooks BR (1995) Constant-pressure molecular-dynamics simulation—the Langevin piston method. J Chem Phys 103:4613–4621

Gawrisch K, Ruston D, Zimmerberg J, Parsegian VA, Rand RP, Fuller N (1992) Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces. Biophys J 61:1213–1223

Grossfield A, Zuckerman DM (2009) Quantifying uncertainty and sampling quality in biomolecular simulations. In: Wheeler R, Spellmeyer D (eds) Annual reports in computational chemistry. Elsevier, Amsterdam, pp 23–48

Hamelberg D, Mongan J, McCammon JA (2004) Accelerated molecular dynamics: a promising and efficient simulation method for biomolecules. J Chem Phys 120:11919–11929

Hauser H, Guyer W, Pascher I, Skrabal P, Sundell S (1980) Polar group conformation of phosphatidylcholine. Effect of solvent and aggregation. Biochemistry 19:366–373

Hauser H, Pascher I, Sundell S (1988) Preferred conformation and dynamics of the glycerol backbone in phospholipids. An NMR and X-ray single-crystal analysis. Biochemistry 27:9166–9174

Hoover WG (1985) Canonical dynamics—equilibrium phase-space distributions. Phys Rev A 31:1695–1697

Israelachvili JN (1977) Refinement of fluid-mosaic model of membrane structure. Biochim Biophys Acta 469:221–225

Jo S, Kim T, Im W (2007) Automated builder and database of protein/membrane complexes for molecular dynamics simulations. PloS One 2:e880

Klauda JB, Venable RM, Freites JA, O'Connor JW, Tobias DJ, Mondragon-Ramirez C, Vorobyov I, MacKerell AD, Pastor RW (2010) Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. J Phys Chem B 114:7830–7843

Lyubartsev AP, Rabinovich AL (2011) Recent development in computer simulations of lipid bilayers. Soft Matter 7:25–39

Marrink SJ, de Vries AH, Tieleman D (2009) Lipids on the move: simulations of membrane pores, domains, stalks and curves. Biochim Biophys Acta 1788:149–168

Ryckaert JP, Ciccotti G, Berendsen HJC (1977) Numerical integration of Cartesian equations of motion of a system with constraints—molecular-dynamics of *n*-alkanes. J Comput Phys 23:327–341

Schlenkrich M, Brickmann J, MacKerell AD, Karplus M (1996) An empirical potential energy function for phospholipids: criteria for parameter optimization and applications. In: Merz KM, Roux B (eds) Biological membranes: a molecular perspective from computation and experiment. Birkhauser, Boston, pp 31–81

Sugita Y, Okamoto Y (1999) Replica-exchange molecular dynamics method for protein folding. Chem Phys Lett 314:141–151

Sundaralingam M (1972) Molecular structures and conformations of the phospholipids and sphingomyelins. Ann N Y Acad Sci 195: 324–355

Venable RM, Zhang YH, Hardy BJ, Pastor RW (1993) Molecular-dynamics simulations of a lipid bilayer and of hexadecane—an investigation of membrane fluidity. Science 262:223–226

Vogel A, Reuther G, Roark MB, Tan KT, Waldmann H, Feller SE, Huster D (2010) Backbone conformational flexibility of the lipid modified membrane anchor of the human N-Ras protein investigated by solid-state NMR and molecular dynamics simulation. Biochim Biophys Acta 1798:275–285

Wiener MC, White SH (1992) Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of X-ray and neutron-diffraction data. 3. Complete structure. Biophys J 61:434–447