

Molecular dynamics simulations of a fully hydrated dimyristoylphosphatidylcholine membrane in liquid-crystalline phase

Igor Z. Zubrzycki

Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Yan Xu

Department of Anesthesiology and Critical Care Medicine and Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Marcela Madrid

Pittsburgh Supercomputing Center, Pittsburgh, Pennsylvania 15261

Pei Tang^{a)}

Department of Anesthesiology and Critical Care Medicine and Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

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Molecular dynamics (MD) simulations were performed to investigate the structure of a fully hydrated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayer in liquid-crystalline (fluid) phase at 30 °C. The bilayer consists of 200 DMPC lipid molecules with $n_w = 27.4$ water molecules per lipid. The membrane was built with reference to the coordinates of a previously published 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane patch. A four-step dynamic procedure (110 ps) with Berendsen pressure rescaling ($P=0$ and 1 bar), applied in all three directions, was used to rapidly prepare the bilayer. This system was then subjected to two separate constant pressure and temperature simulations at 1 bar and 30 °C for ~ 380 ps, using the Nosé–Hoover NPT method with periodical boundaries and Berendsen temperature and pressure rescaling method, respectively. The resultant bilayer has an area per lipid of 59.2 \AA^2 and a head-to-head thickness (D_{HH}) of 36.3 \AA . These values are in good agreement with the x-ray diffraction data of 59.7 \AA^2 and 34.4 \AA , respectively, for DMPC at 30 °C with n_w of 25.7 [H. I. Petrache, S. Tristram-Nagle, and J. F. Nagle, *Chem. Phys. Lipids* **95**, 83 (1998)]. The fractions of *trans* and *gauche* bonds in the hydrocarbon chains, averaged for the last 94 ps of simulation, are 81.7% and 18.3%, respectively, suggesting a fluid phase of the membrane. The electron density profile resembles closely that measured by x-ray diffraction. Water density profile suggests a significant penetration of water molecules into the bilayer head region to as deep as the carbonyl groups, with phosphate groups being strongly hydrated. © 2000 American Institute of Physics. [S0021-9606(00)50807-1]

INTRODUCTION

Computer-based molecular modeling of fully hydrated bilayers is of great biological and medical interest.^{1–5} For more than a decade, molecular simulations have proven to be an indispensable tool for better understanding of structures and dynamics of biological macromolecules. Recent advances in supercomputing have brought biomembranes into the realm of large-scale modeling.^{6–12} The fully hydrated phospholipid bilayers exist in several phases, including the crystalline (L_c) phase,^{13,14} the gel phase ($L_{\beta'}$) with disordered polar head groups but regularly packed, all-*trans* hydrocarbon chains,¹⁵ the rippled phase ($P_{\beta'}$) with hexagonally packed chains,¹⁶ and the liquid-crystalline phase (L_{α}) with disordered chains and loosely packed head groups.^{17,18} Of these, the liquid-crystalline phase, which is often referred to as fluid phase,¹⁷ is most biologically relevant.

Different approaches, including stochastic boundary conditions,^{10,19} periodic boundary conditions at constant volume,^{20–22} and periodic boundary conditions at constant pressure,^{23–26} have been used to simulate the hydrated bilayers. Because of technical problems and scientific issues yet to be resolved,²³ the choice of a particular method often results in certain errors associated with that method. For instance, stochastic boundary conditions may inhibit chain tilting of the boundary lipid and fail to simulate infinite bilayer systems, whereas periodic boundary conditions may introduce spurious collective motions or orientations. It has also been shown that prolonged lipid bilayer simulation with constant volume may lead to significant artifacts.²⁷ In simulation of the lipid bilayer with excessive water molecules, the method of constant pressure and temperature seems to be most appropriate.^{23,27,28}

Many membrane simulations start with lipid bilayers in the crystal phase.^{11,12,23,29} To correctly simulate the transition to the fluid phase, simulations of a few nanoseconds or

^{a)} Author to whom correspondence should be addressed. Phone: (412) 383-9798; Fax: (412) 648-9587; electronic mail: tang@smtp.anes.upmc.edu

longer are recommended.^{11,12,30} A different approach has been suggested to start from a library of lipids pre-equilibrated in a Marcelja field.³¹ Independent lipid configurations are then packed into an L_α phase bilayer assembly with packing parameters adjusted to experimental values.²⁰ This procedure can greatly reduce the computing time otherwise necessary to equilibrate the system.

Given the currently available computing power, the requirement for long simulations often limits the number of lipid molecules and the water-to-lipid ratios in the calculation. Although large-scale membrane simulations, up to 1024 lipids over a period of 10 ns, have been reported recently using the united atom model,³² for all-atom simulations, typical numbers are 16–32 lipid molecules in each monolayer with n_w of 10–20. Such small patches of membrane bilayer are usually not adequate to be used for simulations of membrane-associated protein systems such as transmembrane ion channels. To simulate an ion channel, the distance from the channel to the edge of the membrane should be at least 15 Å for a 12 Å cutoff of electrostatic interaction. For instance, for the homopentameric channel formed by the $\alpha 1$ subunits of the human glycine receptors,³³ it can be estimated that a minimum bilayer patch of $70 \times 70 \text{ \AA}^2$, consisting of ~ 200 lipid molecules, is needed. To use all-atom simulation to prepare such a large membrane with full hydration of $n_w = 27$ water molecules per lipid will be time consuming. We show here that a large, fully hydrated, fluid-phase bilayer of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid can be rapidly prepared from a different fluid-phase membrane, in our case 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). The physical properties of the calculated membrane are in good agreement with the experimental data. The membrane is readily usable in further molecular simulations of membrane-associated proteins.

METHODS

The X-plor program³⁴ was used to set up the initial membrane. Instead of starting from a crystalline phase or random packing of duplicates of a single lipid, the initial configuration of the DMPC membrane was constructed using the coordinates of the POPC membrane in the L_α phase.¹⁰ Two and four methylene groups were deleted from the palmitoyl and oleoyl chains, respectively, and the double bond in the oleoyl chain was converted to a single bond with the addition of two hydrogen atoms. The monomeric DMPC molecule was built using the topology and parameter files of Charmm 22.^{35,36} The bilayer consisting of 200 DMPC molecules was sandwiched by 5483 molecules of TIP3 water.³⁷ The starting configuration is shown in the insert to Fig. 1.

The all-atom molecular dynamics (MD) simulations were carried out using the NAMD2 program³⁸ on the T3E parallel computer at the Pittsburgh Supercomputing Center. The progress of the simulations was monitored interactively using the visual molecular dynamics (VMD) program³⁹ on an Octane workstation (Silicon Graphic Inc.). The local interactions, including the bonded interactions and the short-range van der Waals and electrostatic interactions were calculated every time step. The long-range van der Waals and electrostatic interactions up to 14 Å were computed every 4

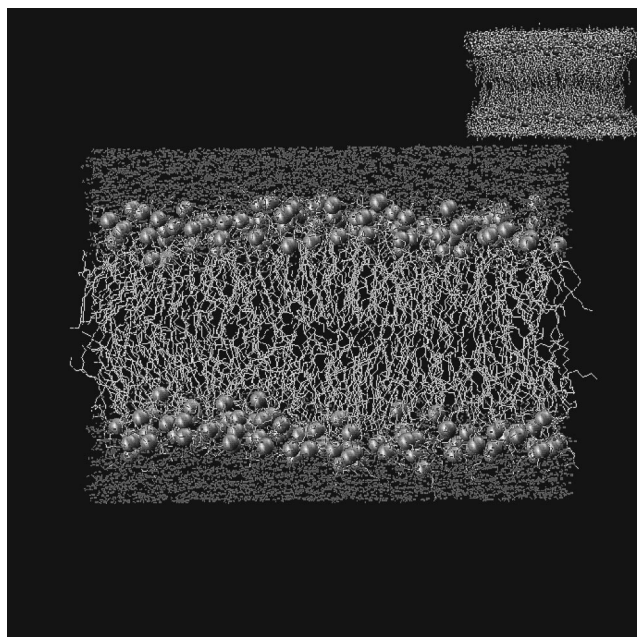


FIG. 1. The final structure of the DMPC membrane in the fluid phase. The membrane consists of 200 DMPC lipid molecules and 5483 TIP3 water molecules. Spheres, phosphate groups; lines, hydrocarbon chains; and small gray dots, water molecules. Insert, the starting membrane configuration rebuilt from the POPC membrane template.

time steps to reduce computational cost. A smooth splitting function at a switching distance of 7 Å was used to separate the short-range portion of the electrostatic interaction from the long-range component. The cutoff distance for the non-bonded interactions was 12 Å with the pair list distance extended to 14 Å.

The initial bilayer system was subjected to 5000 steps of energy minimization using a simple quenching scheme. The minimized membrane bilayer was then subjected to a 110-ps preparation using the Berendsen constant pressure and temperature procedure.²⁶ The pressure was applied in all three directions by using a uniform dimension rescaling factor. Thereafter, the Berendsen and Nosé–Hoover constant pressure and temperature (NPT) procedures^{40,41} were performed separately for 380 ps for data collection.

Four distinct steps were taken in the 110 ps preparation. First, a 25 ps MD simulation was performed at 310 K for 50 000 steps, with a time step of 0.5 fs. The constant temperature was maintained by velocity rescaling every 50 fs. The Berendsen pressure of 1 bar was applied in 1 ps intervals with a relaxation time constant of 1 ps. The SHAKE routine was applied to all hydrogen atoms with a tolerance of 0.001 Å. Second, a periodic boundary with the unit cell dimension equaling the dimension of the membrane at the end of the first 25 ps preparation ($88.7 \times 99.8 \times 71.0 \text{ \AA}^3$) was imposed. A 20 ps Berendsen constant pressure and temperature run was performed, with a time step of 1 fs, velocity rescaling to 305 K every 50 fs, and the pressure coupling to 1 bar every 0.1 ps (relaxation time=1 ps). The SHAKE routine was used to restrain all hydrogen bonds to a tolerance of 10^{-5} Å. Third, the dimension of the periodic box was enlarged in the directions perpendicular to the normal of the bilayer such that the area per lipid doubled the experimental value of 59.7

$\text{\AA}^2/\text{lipid}$.⁴² This procedure was introduced to convert a rectangular membrane patch into a square one. A periodic boundary with a unit cell dimension of $109.3 \times 109.3 \times 62.7 \text{\AA}^3$ was imposed for a constant volume and temperature MD simulation. The time step was set to 0.5 fs and the pressure was recoupled to 0 bar in all three directions in 50 fs intervals, with a 1 ps relaxation time constant. The SHAKE routine was used with a tolerance of 10^{-5}\AA . The changes in the membrane dimension were monitored continuously to collect structures with satisfactory dimensional parameters. This procedure caused nonequilibrium repetitive pulsation of the membrane, resulting in changes in lipid packing. The gap between the two leaflets of the bilayer due to the deletion of the methylene groups disappeared in this step. The structure at 15 ps of this step was chosen to enter the final preparation. In the final preparation step, the dimension of the periodic box was changed to $85.0 \times 85.0 \times 62.7 \text{\AA}^3$. The Berendsen pressure was kept constant at 0 bar with a coupling constant of 4 ps to reduce the short-term fluctuations of the system. The temperature was maintained at 305 K by velocity rescaling every 25 fs. The simulation was carried out for 50 ps with a time step of 0.5 fs.

The prepared membrane was then submitted to two separate MD simulations for data collection. The Nosé–Hoover continuous NPT dynamics^{40,41} were carried out for 380 ps with an initial flexible periodic box of $78.0 \times 78.0 \times 64.0 \text{\AA}^3$. The VerletI integrator was used with a time step of 1 fs. The target temperature and pressure were set to 305 K and 1 bar, respectively, with an oscillation period of 20 fs and a relaxation time constant of 1 ps. The Berendsen constant pressure and temperature procedure²⁶ was performed also for 380 ps in 1 fs time steps but without the periodic boundaries. The simulation temperature was 305 K by velocity rescaling every 50 fs. The pressure was controlled by dimensional rescaling every 50 fs with a relaxation time of 1 ps. For both runs, the SHAKE routine was applied to all hydrogen atoms with a tolerance of 10^{-5}\AA . The structural parameters were sampled every 2 ps. The simulation results were analyzed using the CHARMM Program (Version C27b1).⁴³

RESULTS AND DISCUSSION

The structure of the DMPC membrane after 110 ps preparation and 380 ps Nosé–Hoover NPT procedure is shown in Fig. 1. Similar results were also obtained with the Berendsen method. The insert to Fig. 1 shows the starting configuration, displaying the artificially packed water layers and a large gap between the two monolayers due to the deletion of methylene groups from the POPC template. Using the short procedures described above, we were able to obtain a DMPC membrane with structural parameters in good agreement with experimental results. Figure 2 depicts the area per lipid and the fraction of the *trans* and *gauche* bonds in the hydrocarbon chains as a function of simulation time. Clearly, the simulation has converged. The time-averaged head-to-head thickness (D_{HH} , measured between the averaged levels of the phosphate groups) and the area per lipid for the last 94 ps simulations are listed in Table I to compare with the x-ray diffraction results.⁴² The profiles of electron density along the bilayer normal axis, averaged for the last

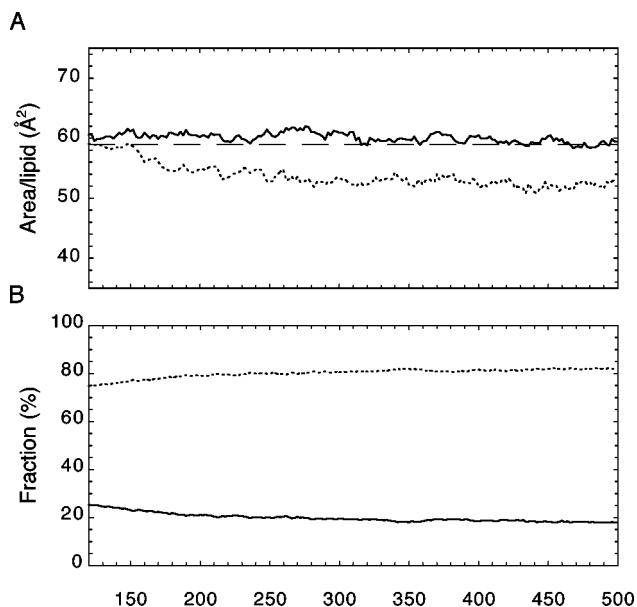


FIG. 2. (A) The time course of area per lipid for the last 380 ps of Berendsen (dotted lines) and Nosé–Hoover (solid lines) NPT simulations, respectively. The dashed line indicates the experimental value. (B) The time course of the fraction of *trans* (dotted line) and *gauche* (solid line) bonds in the hydrocarbon chains during the last 380 ps of the Nosé–Hoover NPT simulations.

94 ps of the Nosé–Hoover NPT simulation, are plotted in Fig. 3. To calculate the electron density, the heavy atoms (C, O, N, and P) were weighted by their atomic number minus the signed partial charges of the group. The total electron-density profile, which is the sum of all components, is in good agreement with the electron density measured by x-ray diffraction.⁴²

For membrane simulations starting with the known x-ray crystalline structures, reminiscence of primary ordering in the head group positions can still be seen in some final structures even with simulations up to several hundred picoseconds at elevated temperatures. Our approach suggests that a fluid-phase membrane can be obtained by starting with the reasonably randomized coordinates of a different membrane, so that significant amount of computational cost can be saved. During the thermal equilibration of the membrane, which generally takes 50–100 ps, the bilayer thickness may vary several angstroms. It is also known that the torsion isomerization of the hydrocarbon chains requires time on the order of 100 ps. Careful examination of the energy and temperature profiles during the preparation periods of this study indicated that the periodic recoupling of the pressure, especially during the constant volume and temperature run, effec-

TABLE I. Comparison of simulated DMPC membrane parameters with x-ray diffraction results.

	Experiment ^a	Berendsen method	Nosé–Hoover method
Waters per lipid (n_w)	25.7	27.4	27.4
Area per lipid (\AA^2)	59.7	52.1	59.2
P – P thickness D_{HH} (\AA)	34.4	37.3	36.3

^aReference 42.

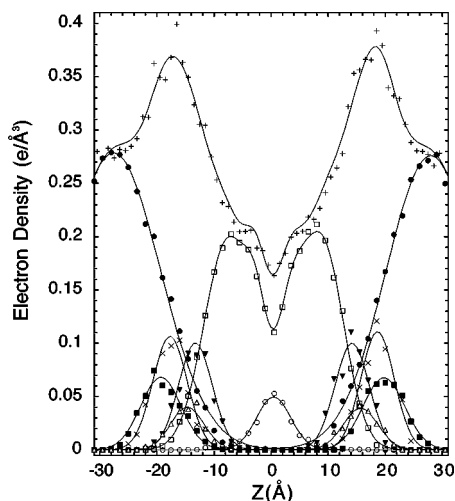


FIG. 3. The electron density profiles averaged over the last 94 ps of the Nosé-Hoover NPT simulations along the normal axis of the simulated DMPC membrane. (Legend: \circ , terminal methyl group; \square , long-chain methylene group; \blacktriangledown , carbonyl group; \triangle , glyceryl groups; \times , phosphate groups; \blacksquare , choline groups; and \bullet , water molecules.)

tively caused pulsatile nonequilibrium changes in energy, allowing for rapid sampling of the configuration space without inducing uncontrollable expansion of the system. This procedure also appears very effective in removing the artificial gap between the leaflets of the bilayer caused by the deletion of methylene groups. Stepped increase in the frequency of pressure recoupling gradually leads the system to the normal NPT procedure, in which pressure and temperature are tightly coupled to the external bath. We found that after about 20 ps entering the final Berendsen or Nosé-Hoover NPT simulation, the total energy becomes smooth and constant. Figure 2 indicates that the system is converged in the final ~ 100 ps of our ~ 0.5 ns simulations.

The boundary effect was observed in the simulations of a small patch of simplified membrane composed of 16 decanoate molecules per monolayer.⁴⁴ This was not observed when the number of decanoate molecules per monolayer was increased to 64.⁴⁴ Simulations of larger membrane size with complete lipid molecules were found to result in large discrepancies between the experimental values and simulated structure.¹⁰ Rescaling of coordinates to increase the surface area per lipid has been proposed in order to obtain correct structural parameters for large-scale simulations. Our results suggest that the discrepancies may be caused by the choice of simulation methods, rather than the size of the simulated system. In the isolated membrane patch with Berendsen pressure applied in all three directions, the lipids are more packed, leading to a smaller area per lipid than the experimental value (Table I). In contrast, the Nosé-Hoover NPT simulation resulted in an area per lipid value very close to the experimental data. It should be noted, however, that the initial unit cell dimension must be set correctly in the Nosé-Hoover method in order for the simulation results to conform to the experimental data. In the preliminary studies, we also noticed that the Nosé-Hoover method had more stringent requirement for the integrator. Using a naïve integrator, the simulation showed significantly more fluctuation in the re-

sults than with the Verlet integrator using the same time steps.

The analysis of the water distribution along the bilayer normal (Fig. 3) indicates an extensive water penetration into the head group region of the lipid. This is in agreement with the thorough theoretical investigation of water transport through a lipid membrane.⁴⁵ The thickness of completely dehydrated region, defined by nearly zero water density, is about 18 Å. A significant amount of water density, about 0.22 g/cm³, can be found at the peak distribution of the carbonyl groups. The water density quickly increases to about 0.52 g/cm³ for a significant hydration of the phosphate groups. At the membrane surface, the peak water density is about 1.05 g/cm³. Deep penetration of water into the membrane interfacial region may be an important contributing factor for the rapid dynamics of the lipid head groups and the vertical movement of lipid molecules up to 15 Å as observed by neutron and x-ray diffraction in the fluid-phase membranes.⁴⁶⁻⁴⁸ It should be noted that for a fully hydrated membrane in the gel phase, water penetration is significantly shallower.¹²

In conclusion, a procedure for rapid preparation of fully hydrated fluid membrane for all-atom MD simulation is presented. The structural parameters of the simulated DMPC membrane are in good agreement with the experimental results. The membrane is readily usable for simulations of membrane-associated proteins. The pdb file of the membrane will be available by contacting the corresponding author.

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