# Combined Monte Carlo and Molecular Dynamics Simulation of Hydrated Lipid-Cholesterol Lipid Bilayers at Low Cholesterol Concentration

S. W. Chiu,* Eric Jakobsson,* and H. Larry Scott†

*Department of Physics, Oklahoma State University, Stillwater, Oklahoma 74078; and †Department of Molecular and Integrative Physiology, Department of Biochemistry, UIUC Programs in Biophysics, Neuroscience, and Bioengineering, and Beckman Institute, University of Illinois, Urbana, Illinois 61801 USA

ABSTRACT We have applied a hybrid equilibration and sampling procedure for the atomic level simulation of a hydrated lipid bilayer to systems consisting of dipalmitoyl phosphatidylcholine (DPPC) and cholesterol, and palmitoyl-oleyl phosphatidylcholine (POPC) at low (~6%) cholesterol concentration. The procedure is applied to bilayers of 94 molecules of DPPC, 6 molecules of cholesterol, and 3205 water molecules, and to bilayers of 120 molecules of POPC, 8 molecules of cholesterol, and 4268 water molecules, at a temperature of 325 K. After equilibration, three separate 400-ps continuous molecular dynamics runs, separated by 10,000 configurational bias Monte Carlo steps, were carried out for each system. Properties of the systems were calculated and averaged over the three separate runs. Results of the simulations are presented and compared with experimental data and with other recent simulations of DPPC and cholesterol, and of pure DPPC, and pure POPC. Certain properties of the bilayers are indistinguishable from cholesterol-free bilayers, including lateral diffusion and electron density. Other properties, most notably the order parameter profile, show the effect of cholesterol even at low concentrations.

## INTRODUCTION

The ubiquity of cholesterol in vertebrate cell membranes underscores the biological importance of this sterol. Research over the past several decades has demonstrated that at least one of its roles in living cells is that of a fluidity regulator (Yeagle, 1993; Presti, 1995). Other biological roles for cholesterol involve regulation of membrane protein interaction and activity (Yeagle, 1993). Extensive experimental investigations over the years (Finegold, 1993; McMullen et al., 1994; Le Guerneve and Auger, 1995; McMullen and MacElhaney, 1995; Thewalt and Bloom, 1992; Vist and Davis, 1999) demonstrated that cholesterol strongly affects mechanical and thermodynamic properties of lipid bilayers, by (i) increasing the area per molecule in the gel phase of a lipid bilayer, and reducing the area per molecule in a fluid phase bilayer, (ii) broadening the main chain melting lipid phase transition until, above about 25–30% cholesterol, the transition is no longer observed, (iii) causing the ripple phase to disappear (Copeland and McConnell, 1980), and (iv) partitioning lipid bilayers into cholesterol-rich and cholesterol-poor domains (Slotte, 1995; Keller and McConnell, 1999; Radhakrishnan and McConnell, 1999a,b). Though the majority of experimental studies have involved dipalmitoyl or dimyristoyl phosphatidylcholine (DPPC or DMPC), there have also been studies of the effect of cholesterol on palmitoyl-oleyl phosphatidylcholine (POPC) (Seelig and Waespe-Šarčevič, 1978; Hyslop et al., 1990; Urbina et al., 1995; Morrison and Bloom, 1992; Lafleur et al., 1990; Kodati and Lafleur, 1993) and the sphingomyelin family of lipids (Brown, 1998). For POPC, Hyslop et al. (1990) utilized fluorescence depolarization studies to estimate that cholesterol molecules in a 1:1 POPC:cholesterol bilayer remain separated with a minimum separation distance of ~10 Å. They conclude that POPC and cholesterol exist at this concentration in an ordered lattice arrangement. Evidence that POPC and DPPC or DMPC interact differently with cholesterol and other sterols is also contained in studies of Urbina et al (1995). They have utilized NMR spectroscopy to comparatively study POPC, DPPC, and DMPC-cholesterol, ergosterol, and lanosterol bilayers at sterol concentrations between 10 and 50%. They conclude that the sterols have a less marked effect on POPC than DPPC. At 30% sterol concentration, ergosterol is more effective at inducing increased chain ordering in DMPC than is cholesterol, but cholesterol is more effective at increasing chain order in POPC than is ergosterol. Deuterium NMR studies of chain ordering in POPC-cholesterol mixtures have been carried out for a wide range of cholesterol concentrations by Lafleur and coworkers (Lafleur et al., 1990; Kodati and Lafleur, 1993). Using perdeuterated chains order parameter profiles were measured for cholesterol concentrations between 5% and 45%. They find that POPC-cholesterol mixtures, at temperatures above 0°C, exhibit a monotonic increase in order parameter with cholesterol concentration. At 0°C, the average molecular order parameter for POPC-cholesterol appears to be nearly the same for 30% and 45% cholesterol concentration. DPPC-cholesterol mixtures are reported to exhibit almost identical order parameter profiles for 33% and 50% choles-

*Received for publication 27 April 2000 and in final form 13 December 2000.*

Current address for H. L. S.: Department of Biological, Chemical, and Physical Sciences, Illinois Institute of Technology, Chicago, IL 60616.

Address reprint requests to Hugh L. Scott, Jr., Department of Biological, Chemical, and Physical Sciences, Illinois Institute of Technology, 3101 S. Dearborn, Chicago, IL 60616. Tel.: 312-567-3730; Fax: 312-567-3494; E-mail: scotth@iit@edu.



0006-3495/01/03/1104/11 $2.00

terol concentration at 52°C (Sankaram and Thompson, 1990). Diffusion constants were measured for cholesterol in POPC, di-oleylphosphatidylcholine (DOPC), and egg yolk lecithin by Lindblom et al. at low hydration (20% w/w). They found that, within error bars, the diffusion constants for these lipids did not change for cholesterol concentrations between 0 and 33%. This may have been in part a consequence of the lower hydration levels (Lindblom et al., 1981) Reduced hydration leads to a more ordered bilayer in the case of DOPC (Hristova and White, 1998), and probably for the other lipids as well.

Lattice-based and continuum-based theoretical models have been developed for lipid bilayers containing cholesterol by Ipsen et al. (1997) and by Scott (1991), but theoretical models are not yet refined enough to yield atomic level predictions. Both experimental and theoretical progress is hindered by a lack of understanding of the detailed nature of the interactions between lipids and cholesterol at the atomic level.

Simulation offers an alternative way to gain insights into complex systems at a microscopic level. The number of papers using molecular dynamics to study single-component lipid bilayers has grown substantially in recent years. (Chiu et al., 1995, 1999a,b; Pastor, 1994; Stouch, 1993; Damodaran and Merz, 1994; Huang et al., 1994; Feller et al., 1994, 1997; Tu et al., 1995; Venable et al., 1993; Egberts et al., 1994; Armen et al., 1998; Tieleman and Berendsen, 1996; Berger et al., 1997; Smondryev and Berkowitz, 1999a; Husslein et al., 1998; Pasenkiewiscz-Gierula et al., 1997). Several reviews of simulation methods and results have been published (Pastor et al., 1996; Merz and Roux, 1996; Tieleman et al., 1995; Jakobsson, 1997).

Early atomic level simulations of lipids and cholesterol were carried out using Monte Carlo (MC) (Scott, 1991), and molecular dynamics (MD) (Edholm and Nyberg, 1992; Robinson et al., 1995) methods. Improvements in models and in computing power have produced more details in recent simulations of lipid-cholesterol bilayers. The simulations of Tu et al. (1998), Smondryev and Berkowitz (1999b), and Pasenkiewiscz-Gierula et al. (2000) have begun to examine in detail interaction mechanisms between DPPC, cholesterol, and water. Working at different lipid: cholesterol ratios, all three groups have observed that cholesterol hydroxyl groups interact strongly with all other polar groups in the membrane, and with water, with about half of the cholesterols interacting with water and the other half interacting equally with DPPC carbonyl or phosphate groups.

Two significant problems in simulations of systems as complex as lipid bilayers are: reaching equilibrium in finite computational time scales, and sampling a sufficiently large region of the configuration space of the system. These problems are more difficult if the system to be simulated is heterogeneous in composition, due to the increased complexity of the configuration space and energy landscape. A new approach to equilibrating and sampling lipid membranes has recently been developed by our group (Chiu et al., 1999a; Scott et al., 1998). The procedure involves successive 20-ps MD runs at constant temperature and pressure (or surface tension), followed by 10,000 steps of configurational bias Monte Carlo (CBMC; Seipmann and Frenkel, 1992) on the lipids only, at constant volume and temperature. CBMC moves are followed by re-insertion of the water molecules, energy minimization to remove any bad lipid-water contacts caused during the CBMC, and the next round of MD, and so on. We have applied the method to simulations of DPPC, POPC and di-oleylphosphatidylcholine (DOPC) bilayers (Chiu et al., 1999a,b), and have shown that the MD-CBMC simulation equilibrate much more rapidly in CPU time than does a simple MD simulation. The MD-CBMC procedure is expected to be even more advantageous in the equilibration process in heterogeneous environments such as a mixed bilayer of lipids plus cholesterol. Earlier Monte Carlo studies of simple models of cholesterol plus hydrocarbon chains (Scott, 1991) showed that chains that were near neighbors to one or more cholesterol molecules suffered rejection rates of over 99% due to the presence of the rigid sterol fused rings. The CBMC procedure promises to improve on this situation by a more thorough sampling of the available free volume for a lipid chain during a trial move.

The purpose of this paper is to describe the results of application of the MD-CBMC method to two fully hydrated bilayers consisting of lower concentrations of cholesterol than have previously been studied, for two different types of phospholipid. By simulating bilayers of DPPC-cholesterol, and POPC-cholesterol containing about 6% cholesterol we hope to gain insights into the interactions between individual lipid and cholesterol molecules, without the complication of direct cholesterol-cholesterol interactions. By considering POPC-cholesterol mixtures, we examine the effect of an unsaturated hydrocarbon chain on these interactions. In the following sections we describe the details of the simulations and present the results.

## SIMULATION METHODS AND PROCEDURES

We constructed bilayer patches of DPPC-cholesterol and POPC-cholesterol from previously equilibrated DPPC and POPC bilayers by replacing selected lipid molecules with cholesterol molecules. Bilayer 1 contained 94 DPPC molecules, 6 cholesterol molecules, and 3205 water molecules. Bilayer 2 contained 120 POPC molecules, 8 cholesterol molecules, and 4268 water molecules. Initially cholesterols were placed in the lipid matrix in an arrangement which roughly maximized the separation between cholesterols both within each leaflet and between leaflets. The bilayers were initially energy minimized to remove bad steric contacts. Then over 30 cycles consisting of 20 ps of MD followed by 10000 CBMC steps were run on each system using a temperature of 425 K. The MD was run under constraints of isotropic constant pressure (1 atm) and temperature using the GROMOS simulation code (BIOMOS b.v, Laboratory of Physical Chemistry, ETH Zentrum UniversitStstrasse 6, CH-8092 Zurich, or see http://igc.ethz.ch/gromos/). For the CBMC trial moves one of the three separate chains (sn-1, sn-2, or polar group) was selected at random on a randomly

selected molecule for re-growth. A bond was selected at random on the chosen chain, and regrowth was attempted from that point to the end of the chain. No attempts were made to regrow entire lipid molecules, and the CBMC procedure was confined to lipids only. The CBMC procedure consisted of 120 sampling trial moves at each chain position. During CBMC no lateral diffusive moves were done, as the procedure was intended for lipid chain conformational equilibration only. In earlier work we have described our CBMC procedure fully (Scott et al., 1998). Although water molecules were ignored during CBMC, this causes minimal problems. Only accepted head group CBMC moves can result in potential bad steric contacts with waters. In general head group CBMC moves are accepted at a lower rate than chain moves. In a CBMC run of 10,000 steps the number of accepted head group moves is only around 6 (for this reason our future CBMC runs will eliminate headgroup trial moves altogether), and a subset of these give rise to to high-energy contacts with at most 0.5% of the water molecules. Energy minimization is able to remove these contacts in a few steps and we have found in our earlier simulations that only a few ps of MD are needed to equilibrate the water around the repositioned head groups, as measured by water dipole vector orientations, surface potential profiles, or correlation function calculations involving water molecules.

After the systems reached equilibrium, as judged by a lack of drift of the potential energy and area per molecule of each system with time, five or more MD/CBMC cycles were run at the lower temperature, 325 K. Upon completion of this step, we began a set of three continuous MD runs of 400 ps in duration for each bilayer. The continuous MD simulations were done in an NPAT ensemble, in which the area per molecule was constrained to fluctuate around 59.4 $Å^2$ (DPPC-cholesterol) and, 62.7 $Å^2$ (POPC-cholesterol). These areas were chosen after the MD/CBMC cycles at 325K, and represent values at which the areas for each bilayer, segmental order parameters, and potential energy all remained stable throughout these cycles. There are no experimental guidelines for the choice of area per molecule for the given systems, so we relied upon the simulation reaching a steady state. The areas used are each about 5% lower that those we used for simulations of fluid phase DPPC (62.1 $Å^2$/mol) and POPC (66.4 $Å^2$/mol) at the same temperature, using the same force field parameters as we used in pure lipid simulations of DPPC, POPC, and DOPC. (63, 64) By comparison, in monolayer studies at an applied lateral pressure of 30 dyn/cm the measured area of a POPC-chol film declined from 63 $Å^2$/mol to 58 $Å^2$/mol upon addition of 10% cholesterol, and the measured area of a DMPC-chol film declined from 58$Å^2$/mol to 52 $Å^2$/mol upon addition of 10% cholesterol (Smaby et al., 1997).

A pressure of 1 atm was applied normal to the bilayer, and the temperature was constrained to fluctuate around 325K. Between continuous MD runs, 10,000 CBMC steps were run for each system. All boundary constraints utilized the weak coupling method with a coupling constant of 0.2 ps for temperature, 4.0 ps for pressure scaling, and 2.0 ps for area scaling. Temperature coupling was applied separately to lipid and water components. Discarding the first 10 ps to allow for equilibration after CBMC, this procedure was repeated to give a total of three separate continuous trajectories which were then used for averaging for each system.

Interaction parameters for water, cholesterol, and lipid head groups, were the same as those we used earlier (Chiu et al., 1999a,b), namely the GROMOS96 set. United atom models omitting explicit hydrogens were used for lipid molecules. Explicit hydrogen atoms were added to cholesterol ring atoms to allow for the inclusion of the pi-electron effect electrostatic interactions between ring atoms and atoms on neighboring lipid molecules. Appendix A lists partial charges and 6–12 parameters used for cholesterol atoms. For computing torsion angle motions around lipid and cholesterol chain saturated bonds, third neighbor 6–12 interactions were replaced with the dihedral potential function due to Ryckaert and Bellmans (1978). For the hydrocarbon chain interactions between atoms on different chains, we utilized a modified set of parameters developed by our group by fitting to densities of a series of alkanes, and, for the C = C double bond, to 5-decene (Chiu et al., 1999c). Group-based spherical cutoffs of 20 Å for both electrostatics and 6–12 forces were used. Neutral charge groups were employed. In the CBMC runs, electrostatic interactions were modulated by the continuum dielectric constant of water. We have discussed the rationale for these choices in an earlier paper (Chiu et al., 1999a).

## RESULTS

Figs. 1 and 2 show the order parameter profiles calculated for bilayers 1 and 2. Also shown are simulation data for pure DPPC and POPC bilayers. Examination of the two sets of simulation profiles shows that the ordering effect of cholesterol is evident even at very low cholesterol concentrations. For DPPC bilayers, the order parameters are increased in absolute value compared to the respective pure lipid bilayers for carbons 2 through 8 on both the sn-1 and sn-2 chains. The errors in the simulated values of the order parameters, estimated as standard deviations over the three production runs, are about $\pm 0.05$ for these carbons. For carbons below carbon 8, the fluctuations in the calculated average order parameters are between $\pm 0.05$ and $\pm 0.1$, and overlap with simulation data for pure DPPC. For the DPPC-cholesterol system the difference in order parameters is around 10% for the first seven carbons, but the difference decreases until at the C-15 position the order parameters are nearly identical for both systems. For the POPC-cholesterol system the situation is more complex. The saturated chain of POPC is basically unaffected by cholesterol, while the unsaturated chain has order parameters increased by about 10% for bonds 1–8 (up to the double bond), and then the affect is negligible. The implication is that cholesterol is able to increase the order only in the portion of the sn-2 chain of POPC above the double bond, at low cholesterol concentration. Fig. 3 compares simulation results with experimental data (Lafleur et al., 1990). In this figure, the ratio of C-D order parameter for the POPC-cholesterol mixture to that of pure POPC is plotted as a function of carbon number. Experimental data for perdeuterated chains do not contain the signature dip in the order parameter of carbon 10 of the oleyl chain. For this reason, we have used only the palmitoyl chain data for the comparison. To two significant figures experiment and simulation agree very well for carbons 2 through 9. For the tail carbons, the simulation data show a larger effect of cholesterol on $S(n)$ than does experiment at 5% cholesterol concentration. Interestingly, the simulation profile resembles in overall shape experimental profiles at higher chol:POPC ratios reported by Lafleur et al. (1990).

In Fig. 4 we show the distribution of molecular tilt angles for cholesterol for bilayers 1 and 2, using the line between the hydroxyl oxygen and the tail methyl carbon (whole molecule). The distribution for the DPPC-cholesterol bilayer is centered at about 25°, whereas the distribution for the POPC-cholesterol bilayer is centered at about 32°. For comparison with cholesterol tilt at higher cholesterol concentrations, the cholesterol whole-molecule tilt distribution was found to peak at 12° for a 1–1 DPPC-cholesterol system

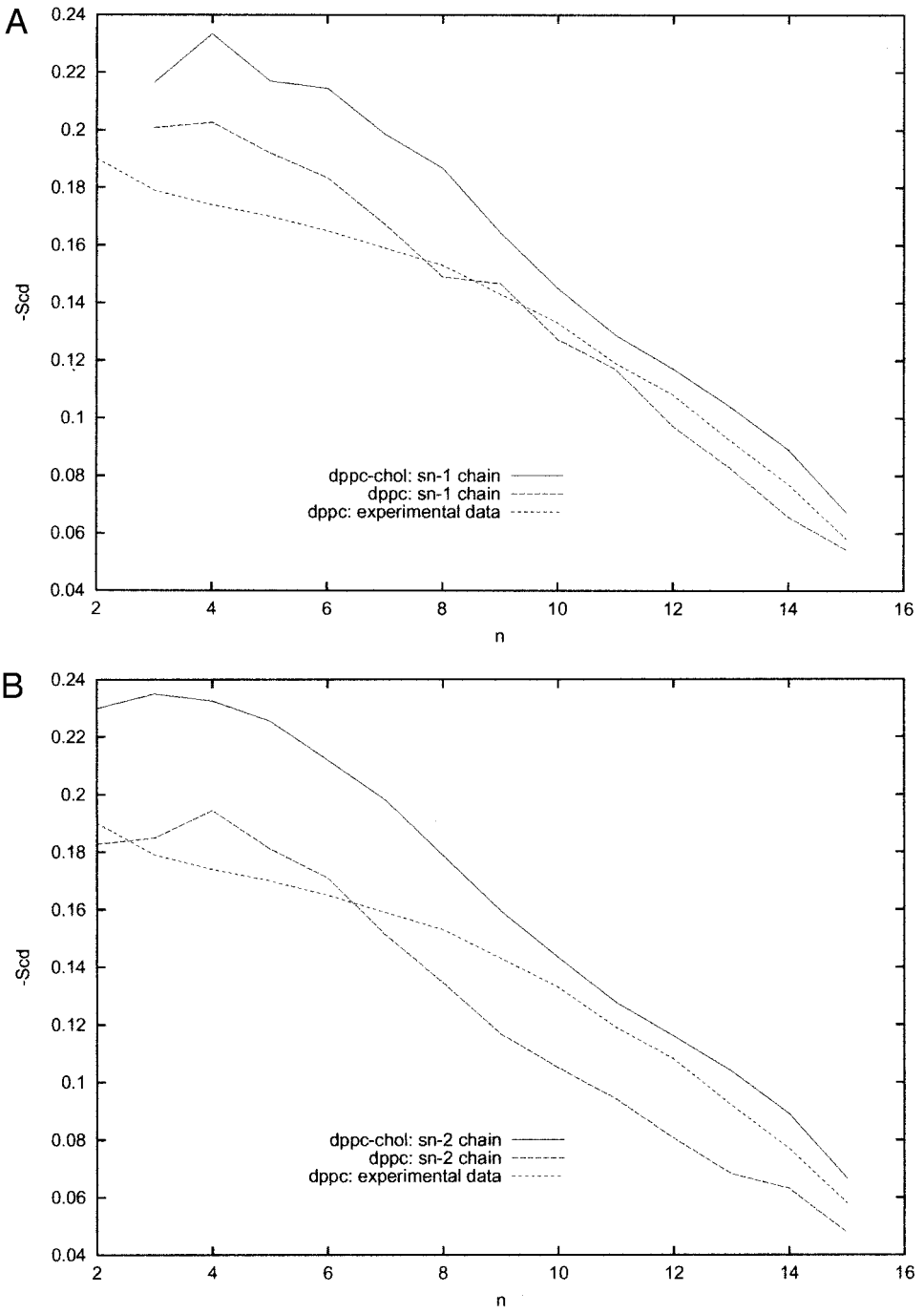


FIGURE 1 Plot of order parameters for the DPPC-cholesterol bilayer, calculated from averages over the three continuous MD runs as described in the text. (*A*) sn-1 chain. (*B*) sn-2 chain. Experimental profile is averaged over sn-1 and sn-2 chains for pure DPPC (McCabe et al., 1994).

(Chiu, Jakobsson, and Scott, unpublished results; Smondryev and Berkowitz, 1999a).

Since the introduction of cholesterol into a bilayer displaces electron-rich phosphate groups, it is of interest to calculate electron density profiles from simulation data. Fig. 5 shows electron density profiles calculated for the POPC bilayer with and without cholesterol. It is apparent from the figure that at this low cholesterol concentration the effect is minimal. The peak-peak spacing is 34 Å for both systems, but the average peak height is slightly lower for the 5% cholesterol bilayer due to fewer phosphate groups. The DPPC-cholesterol bilayer showed very similar results.

Fig. 6 is a representative plot that shows the mean-square deviation of cholesterol molecules in the plane of the bilayer, in the DPPC-cholesterol bilayer, from their positions at the beginning of the simulation as a function of time. We have done mean-square deviation calculations for DPPC and POPC molecules, and for cholesterol molecules in both systems. Data were calculated using the position of the center of mass of the DPPC molecules, and for the oxygen

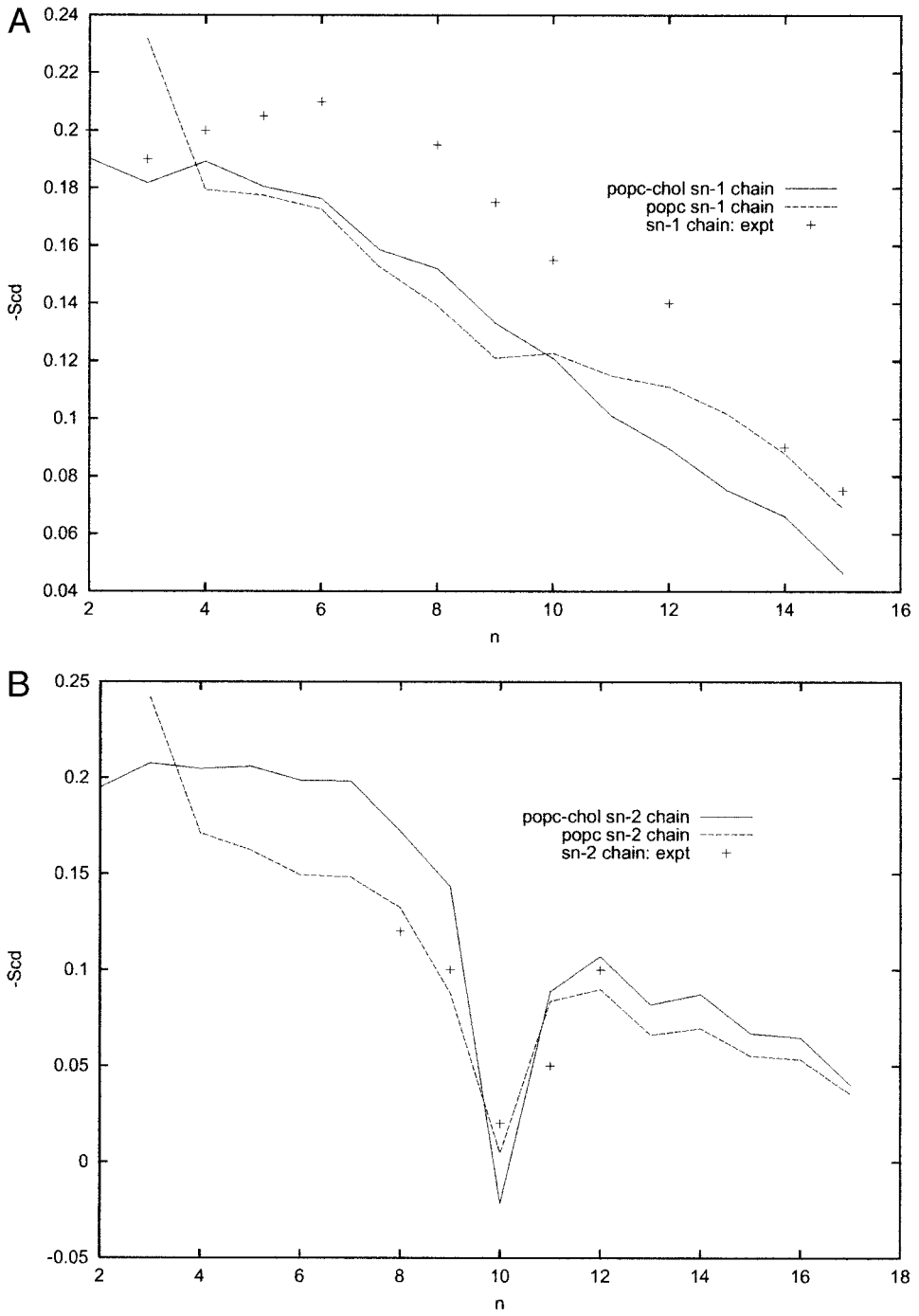


FIGURE 2 Plot of order parameters for the POPC-cholesterol bilayer, calculated from averages over the three continuous MD runs as described in the text. (*A*) sn-1 chain. (*B*) sn-2 chain. Experimental data are for pure POPC, from Seelig and Waespe-Šarčevič (1978).

atom of the cholesterols, and were collected over the last 800 ps of the simulation. The diffusion constants are estimated over the simulation time scales based on "rattling in a cage" motion, as there is insufficient time for large scale lateral hopping. This approximation leads to an incorrect diffusion constant for motion perpendicular to the bilayer plane, so these data are not shown. In-plane diffusion constants calculated from the slope of the linear region of the data are given in Table 1. For both DPPC and POPC bilayers, the diffusion constants of cholesterol molecules are lower than those of the lipid molecules. For comparison, the experimental value of the diffusion constant for a pure DPPC bilayer is also about $10^{-10}$ $m^2/s$, whereas the diffusion constant for a DPPC bilayer containing 40% cholesterol is about $10^{-12}$ $m^2/s$ (Gliss et al., 1999). The data show that lateral mobility within a lipid bilayer containing a low concentration of cholesterol is not measurably different from that of a pure lipid bilayer. The experimental data of

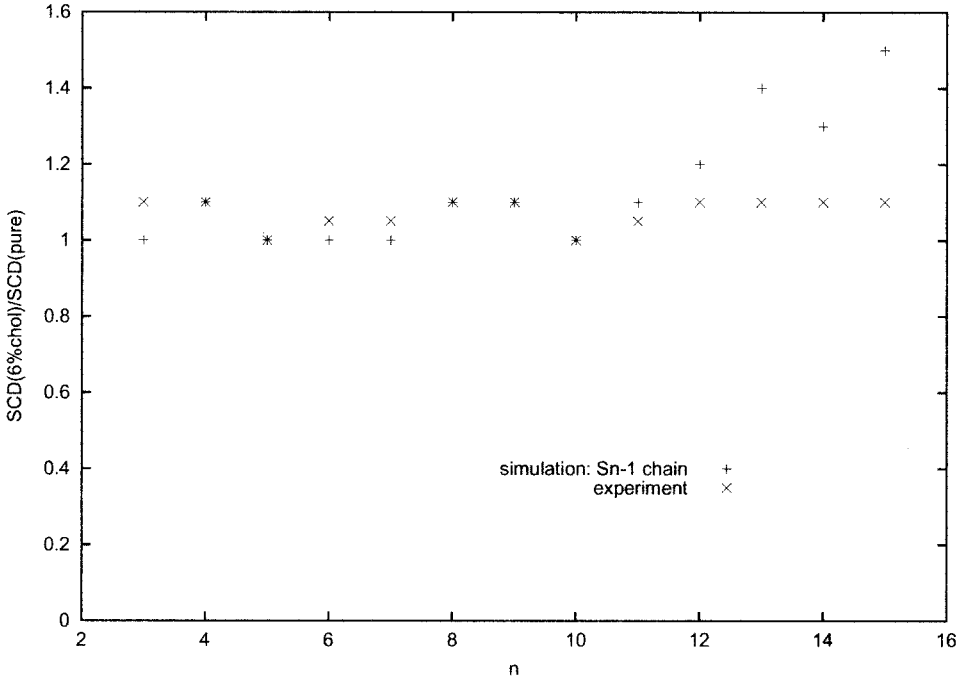


FIGURE 3 Plot of ratio order parameters for the POPC-cholesterol bilayer to order parameters for pure POPC. Simulation data are for Sn-1 chain only. Experimental data are for perdeuterated chains, from Lafleur et al. (1990).

Lindblom et al. (1981) also suggest little or no variation of lipid lateral mobility with cholesterol concentration. It should be noted that the measurements of Lindblom et al. were made on bilayers at low hydration, so that measured diffusion constants ($\approx 6 \pm 2 \times 10^{-12}$ m$^2$/s) are about 1.5 orders of magnitude lower than values for other lipids at full hydration. To our knowledge there are no published data for diffusion constants of POPC, with or without cholesterol, in excess water. There is some anisotropy in the in-plane cholesterol mean-square deviation data for smaller time lag (not shown) in the POPC bilayer,. This is likely due to the more rigid and anisotropic environment of the POPC bilayer due to the double bonds on the sn-2 chains of the lipids. Some residual anisotropy may remain in the POPC system.

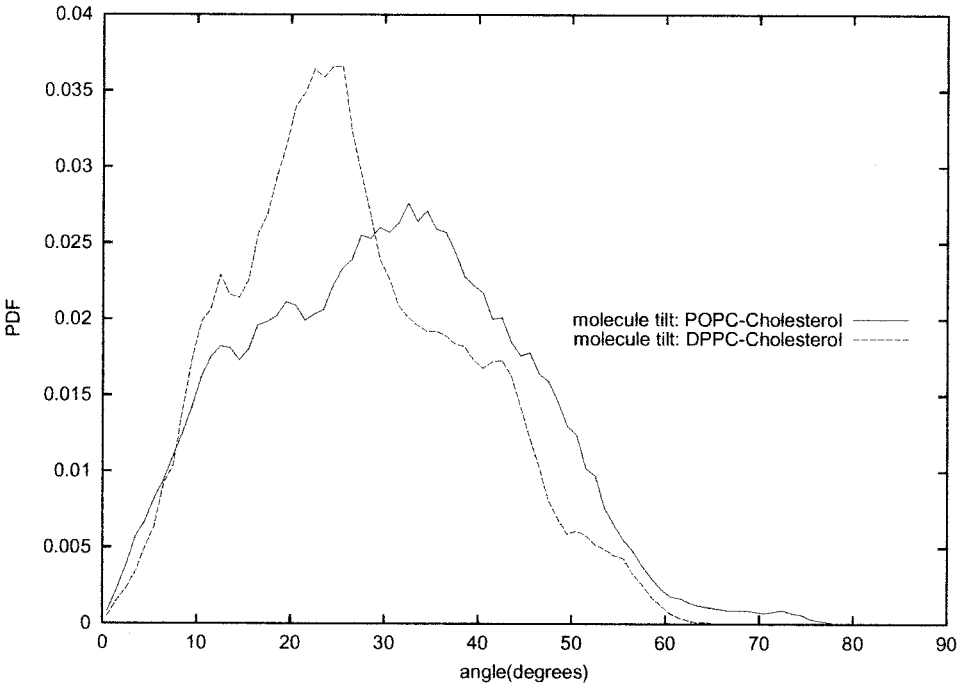


FIGURE 4 Plot of distribution of tilt angle for cholesterol molecules for DPPC-cholesterol and POPC-cholesterol systems. Whole molecule tilt is defined as the angle between the bilayer normal and the vector joining hydroxyl hydrogen to the last methyelenic carbon of the tail.

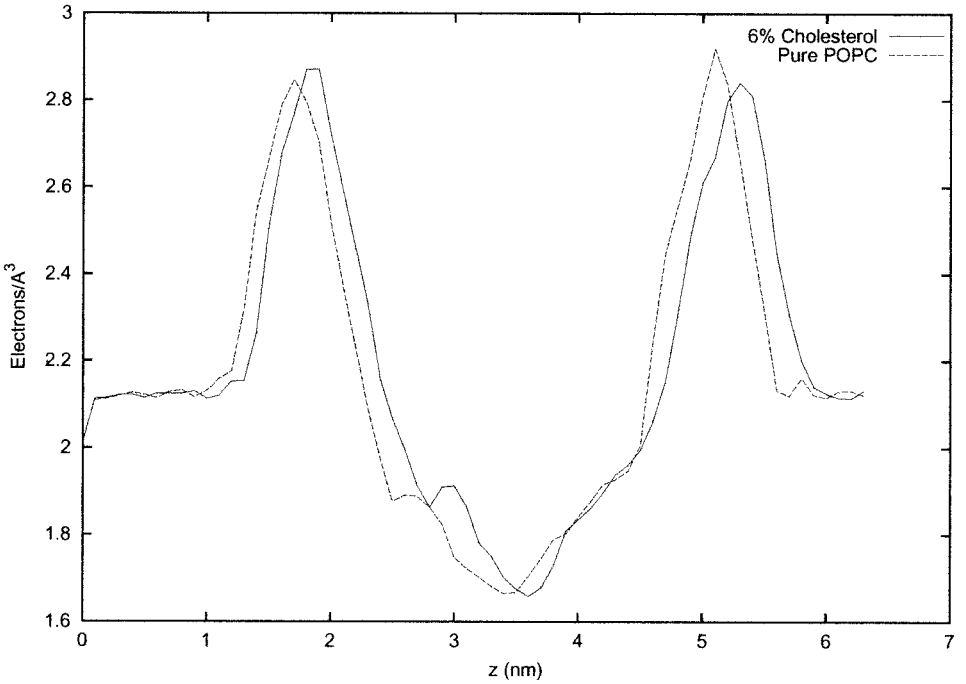


FIGURE 5 Electron density profiles for a pure POPC bilayer and the 6% cholesterol:POPC bilayer.

This system was originally "melted" from an ordered structure (Chiu et al., 1999b) and has now been subject to over 3 ns of simulation, but it is possible that a remnant orientational anisotropy has persisted.

Figs. 7 and 8 show snapshots of individual cholesterol molecules and their closest neighboring lipid molecules. DPPC chains are generally forced into straight, ordered conformations if they are close to a cholesterol molecule. POPC chains tend to be less affected by cholesterol, because the rigid double bond gives them less ability to conform to the shape of the cholesterol molecule.

## DISCUSSION

The systems simulated consist of small numbers (6 or 8) of isolated cholesterols in lipid bilayers. We have therefore

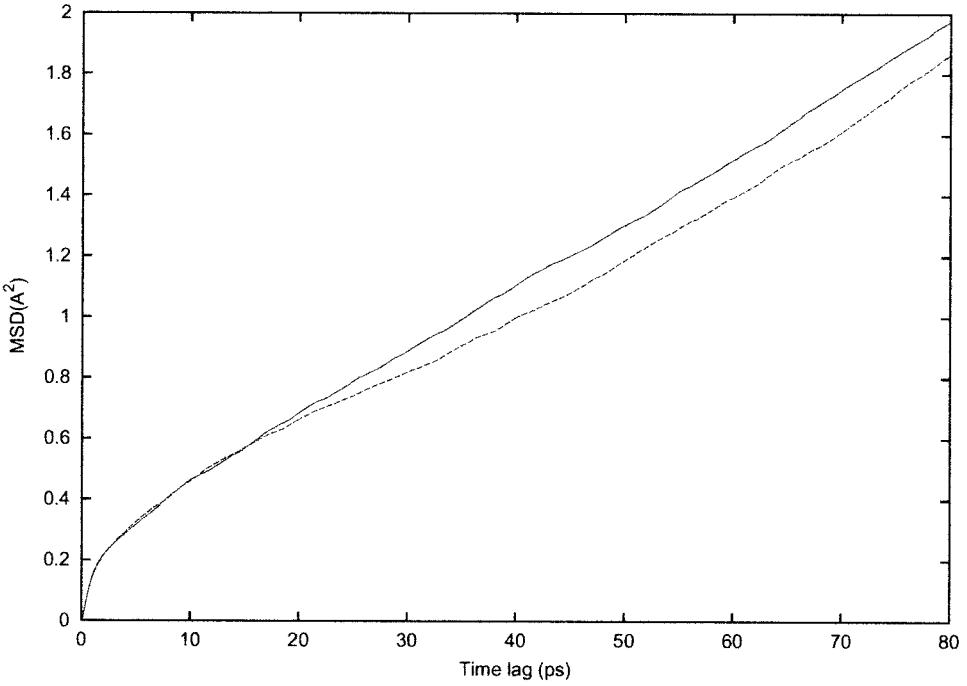


FIGURE 6 Plot of the mean-square displacements of oxygen atoms on cholesterol molecules for the DPPC-cholesterol system.

**TABLE 1 Calculated bilayer plane lateral diffusion constants ($m^2/s$)**

| System | Molecule | $D_{xy}$ |
|---|---|---|
| DPPC-cholesterol | DPPC | $3.4 \pm 0.9 \times 10^{-10}$ |
| POPC-cholesterol | POPC | $4.2 \pm 1.0 \times 10^{-10}$ |
| DPPC-cholesterol | Cholesterol | $2.1 \pm 0.9 \times 10^{-10}$ |
| POPC-cholesterol | Cholesterol | $1.4 \pm 1.0 \times 10^{-10}$ |

examined every cholesterol molecule and its neighboring lipids for differences in intermolecular contact between cholesterol and DPPC versus POPC. This is a small sample, but we have observed consistent patterns of interaction, which we discuss here. This is an example of the potential insights one can obtain from simulation. The calculated global properties of these bilayers are in good agreement with experimental data, so it is highly appropriate to examine the detailed interactions in the simulation that caused these properties.

At the atomic level, the simulations show significant structural differences in the interactions of a saturated-chain phospholipid and a mono-unsaturated-chain phospholipid with cholesterol. The single factor that drives the different behavior is the double bond at C9-C10 of the sn-2 chain of POPC. The rigid bond forces a kink into this chain, which hinders close packing between the chain and a neighboring cholesterol molecule. This hindrance also affects the ability of the saturated sn-1 chain of POPC to effectively pack around a cholesterol molecule, and the result is that order parameters for POPC are not significantly different at low concentration from those in a pure POPC bilayer. By contrast, the saturated DPPC chains are able to "wet" the surfaces of the cholesterol molecule, as indicated in Fig. 6.

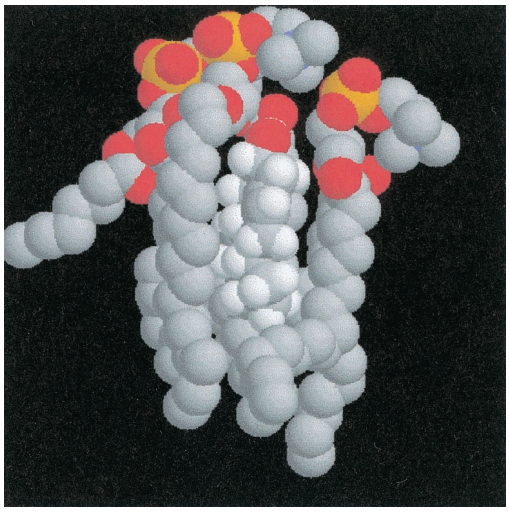

FIGURE 7 Snapshot of a cholesterol molecule and three neighboring DPPC molecules. The cholesterol hydroxyl is hydrogen bonded to a DPPC carbonyl oxygen, and two hydrocarbon chains from different lipids are in nearly all-trans conformations.

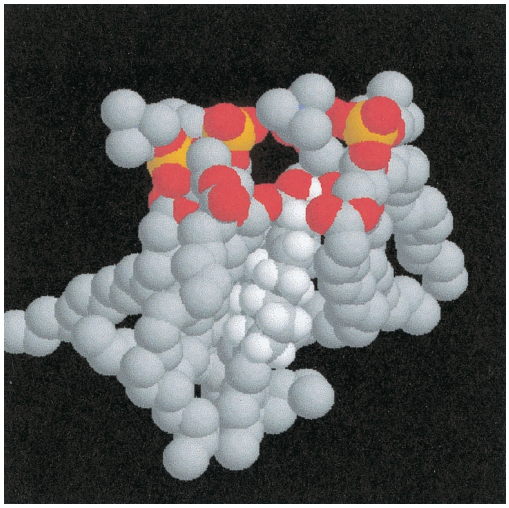

FIGURE 8 Snapshot of a cholesterol molecule and two neighboring POPC molecules. The cholesterol hydroxyl is hydrogen bonded to a POPC carbonyl oxygen. The inability of the POPC chains to pack closely against the cholesterol in spite of the close contact due to the hydrogen bond is apparent.

The result is that even at 6% cholesterol concentration, the order parameter profile for DPPC differs from that of a pure DPPC bilayer, whereas the profile for the 6% POPC-cholesterol bilayer is nearly identical to that for a pure POPC bilayer. Differences in the POPC and DPPC systems are also clear from the cholesterol tilt distributions. The broader distribution of cholesterol molecular tilt angles in POPC compared to DPPC are a consequence of the greater amount of disorder in the POPC bilayer. At low concentrations of cholesterol, the tilt distributions for both bilayers is representative of cholesterols unhindered by the presence of other near neighbor cholesterols. This situation persists from low (6%) cholesterol concentrations to an upper limit of about 33% cholesterol concentrations. Above this concentration, most cholesterols will have at least one near neighbor cholesterol. In a POPC bilayer, the most probable unhindered cholesterol tilt angle is about 8° greater than in a DPPC bilayer.

Wetting provides a mechanism by which cholesterol can disrupt the main DPPC phase transition, through steric hindrance to isomeric lipid chain disordering. If one cholesterol molecule is able to prevent two neighboring saturated lipid chains from undergoing rotameric conformational changes, then there should be no lipid chain melting phase transition at 1:1 lipid:cholesterol concentrations. If one cholesterol molecule is able to prevent $2n$ neighboring lipid chains from undergoing rotameric conformational changes, then there should be no lipid chain melting phase transition at $n$:1 lipid:cholesterol concentrations. If $n = 1$, the steric hindrance must have a range greater than one lipid chain cross-section. Experimental data that show the disappearance of the main lipid phase transition at a 2:1 lipid:

**TABLE A1 6–12 Parameters for interactions between atoms on different DPPC chains and on all cholesterol atoms**

| Atom type | $\sigma$ (nm) | $\varepsilon$ (kJ/m) | Q (e) |
|---|---|---|---|
| Lipid Chain $CH_2$ | 0.400 | 0.380 | 0.0 |
| Lipid Chain $CH_3$ | 0.351 | 0.570 | 0.0 |
| Cholesterol O | 0.287 | 1.01 | −0.76 |
| Cholesterol H1 | 0.237 | 0.118 | +0.40 |
| Cholesterol C3 | 0.336 | 0.406 | +0.21 |
| Cholesterol H6 | 0.237 | 0.118 | +0.18 |
| Cholesterol C4 | 0.336 | 0.406 | −0.36 |
| Cholesterol H7 | 0.237 | 0.118 | +0.18 |
| Cholesterol H8 | 0.237 | 0.118 | +0.18 |
| Cholesterol C5 | 0.336 | 0.406 | +0.06 |
| Cholesterol C6 | 0.336 | 0.406 | −0.25 |
| Cholesterol H9 | 0.237 | 0.118 | +0.16 |
| Cholesterol C7 | 0.336 | 0.406 | −0.31 |
| Cholesterol H10 | 0.237 | 0.118 | +0.17 |
| Cholesterol C8 | 0.336 | 0.406 | −0.16 |
| Cholesterol H12 | 0.237 | 0.118 | +0.16 |
| Cholesterol C9 | 0.336 | 0.406 | −0.16 |
| Cholesterol H13 | 0.237 | 0.118 | +0.16 |
| Cholesterol C10 | 0.336 | 0.406 | 0.0 |
| Cholesterol C19 | 0.351 | 0.570 | 0.0 |
| Cholesterol C1 | 0.336 | 0.406 | −0.34 |
| Cholesterol H2 | 0.237 | 0.118 | +0.17 |
| Cholesterol H3 | 0.237 | 0.118 | +0.17 |
| Cholesterol C2 | 0.336 | 0.406 | −0.34 |
| Cholesterol H4 | 0.237 | 0.118 | +0.17 |
| Cholesterol H5 | 0.237 | 0.118 | +0.17 |
| Cholesterol C14 | 0.336 | 0.406 | −0.16 |
| Cholesterol H18 | 0.237 | 0.118 | +0.16 |
| Cholesterol C15 | 0.336 | 0.406 | −0.32 |
| Cholesterol H19 | 0.237 | 0.118 | +0.16 |
| Cholesterol H20 | 0.237 | 0.118 | +0.16 |
| Cholesterol C16 | 0.336 | 0.406 | −0.34 |
| Cholesterol H21 | 0.237 | 0.118 | +0.17 |
| Cholesterol H22 | 0.237 | 0.118 | +0.17 |
| Cholesterol C17 | 0.336 | 0.406 | −0.15 |
| Cholesterol H23 | 0.237 | 0.118 | +0.15 |
| Cholesterol C13 | 0.336 | 0.406 | −0.03 |
| Cholesterol C12 | 0.336 | 0.406 | −0.29 |
| Cholesterol H16 | 0.237 | 0.118 | +0.16 |
| Cholesterol H17 | 0.237 | 0.118 | +0.16 |
| Cholesterol C11 | 0.336 | 0.406 | −0.34 |
| Cholesterol H14 | 0.237 | 0.118 | +0.17 |
| Cholesterol H15 | 0.237 | 0.118 | +0.17 |
| Cholesterol C18 | 0.336 | 0.406 | 0.0 |
| Cholesterol C20 | 0.336 | 0.406 | 0.0 |
| Cholesterol C21–25 | 0.400 | 0.380 | 0.0 |
| Cholesterol C26–27 | 0.351 | 0.570 | 0.0 |

cholesterol ratio suggest that the range is, in fact, 2 lipid chain cross-sections, or about 8–10 Å. Our simulations suggest that this argument applies only to saturated chains. This would imply that cholesterol does not entirely remove the lipid phase transition for lipids with at least one unsaturated chain, even at relatively high cholesterol concentrations. Because the phase transition temperatures of lipids with unsaturated bonds in the hydrocarbon chains are very low, the effect of cholesterol on phase properties of these systems has not been systematically studied experimentally.

In contrast to order parameter data, electron density and diffusion data suggest that these properties in both POPC- and DPPC-cholesterol bilayers are not measurably different from their pure lipid counterparts at the lipid:cholesterol ratios studied here. At the low concentrations we considered, lipid chain ordering in the saturated chains of the DPPC system is the one structural and dynamical property most sensitive to the presence of cholesterol molecules. The inability of POPC to pack tightly around a cholesterol stands out as the single clear difference in lipid-cholesterol interactions at low concentrations. This differential interaction could, in mixtures of DPPC and POPC, lead to phase separation. The fact that the lateral diffusion is about the same for cholesterol in DPPC and POPC in spite of the difference in sterol-hydrocarbon chain interactions suggests that most of the frictional resistance to lateral diffusion lies in the polar region of the membrane, from carbonyl groups outward.

The results presented here further demonstrate the utility of the CBMC-MD simulation method. The rigid ring portion of the cholesterol molecule hinders reorientational and torsional motions of neighboring lipid chains. The use of a smart MC algorithm can allow lipid chains to jump over steric barriers, which may be problematic for MD simulations. The use of CBMC (or any other efficient Monte Carlo procedure that drives the system toward thermal equilibrium or otherwise moves the system to a different region of phase space) has the effects of lengthening the time scale of the simulation and of allowing more thorough sampling of the configuration space of the bilayer.

Supported by National Institutes of Health grant GM54651 (to E. J. and H. L. S.) and National Science Foundation grant MCB 96–31050 (to E. J.).

## APPENDIX

The figure below shows the cholesterol molecular structure with atom numbers used in the simulations reported here. United atoms are enclosed by boxes. Table A1 lists 6–12 interaction parameters used between atoms on different molecules and partial charges for each atom.

## REFERENCES

Armen, R. S., O. D. Uitto, and S. E. Feller. 1998. Phospholipid component volumes: determination from bilayer structure calculations. *Biophys. J.* 75:734–744.

Berger, O., O. Edholm, and F. Jahnig. 1997. Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. *Biophys. J.* 72: 2002–2013.

Brown, R. E. 1998. Sphingolipid organization in membranes: what physical studies of model membranes reveal. *J. Cell Sci.* 111:1–9.

Chiu S.-W., M. Clark. S. Subramaniam, H. L. Scott, and E. Jakobsson. 1995. Incorporation of surface tension into molecular dynamics simulation of an interface: a fluid phase lipid bilayer membrane. *Biophys. J.* 69:1230–1245.

Chiu, S.-W., M. Clark, E. Jakobsson, S. Subramaniam, and H. L. Scott. 1999a. Application of a combined Monte Carlo and molecular dynamics

method to the simulation of a dipalmitoyl phosphatidylcholine lipid bilayer. *J. Comp. Chem.* 20:1153–1164.

Chiu, S.-W., E. Jakobsson, S. Subramaniam, and H. L. Scott. 1999b. Application of a combined Monte Carlo and molecular dynamics method to the simulation of palmitoyl-oleyl and di-oleyl phosphatidylcholine lipid bilayer. *Biophys. J.* 77:2462–2469.

Chiu, S.-W., M. Clark, S. Subramaniam, E. Jakobsson, and H. L. Scott. 1999c. Optimization of hydrocarbon chain interaction parameters: application to the simulation of lipid bilayers. *J. Phys. Chem. B* 103: 6323–6327.

Copeland, B. R., and H. M. McConnell. 1980. The rippled structure in bilayer membranes of phosphatidycholine and binary mixtures of phosphatidycholine and cholesterol. *Biochim. Biophys. Acta.* 599:95–109.

Damodaran, K. V., and K. M. Merz. 1994. A comparison of DMPC and DLPE-based lipid bilayers *Langmuir*. 9:1179–1183.

Edholm, O., and A. Nyberg. 1992. Cholesterol in model membranes: a molecular dynamics simulation. *Biophys. J.* 63:1081–1089.

Egberts, E., S. J. Marrink, and H. J. C. Berendsen. 1994. Molecular dynamics simulation of a phospholipid membrane. *Eur. Biophys. J.* 222:423–436.

Feller, S. E., Y. Zhang, and R. W. Pastor. 1994. Computer simulation of liquid/liquid interfaces II. Surface tension-area dependence of a bilayer and monolayer. *J. Chem. Phys.* 103:10267–10276.

Feller, S. E., D. Yin, R. W. Pastor, and A. D. MacKerrell, Jr. 1997. Molecular dynamics simulation of unsaturated lipid bilayers at low hydration: parameterization and comparison with diffraction studies. *Biophys. J.* 73:2269–2279.

Finegold, L., ed. 1993. Cholesterol in Membrane Models. CRC Press, Boca Raton, FL.

Gliss, C., O. Randel, H. Casalta, E. Sackmann, R. Zorn, and T. Bayerl. 1999. Anisotropic motion of cholesterol in oriented DPPC bilayers studied by quasielastic neutron scattering: the liquid ordered phase. *Biophys. J.* 77:331–340.

Huang, P., J. J. Perez, and G. H. Loew. 1994. Molecular dynamics simulations of phospholipid bilayers. *J. Biol. Struct. Dyn.* 11:927–956.

Hristova, K., and S. H. White. 1998. Determination of the hydrocarbon core structure of fluid dioleylphosphocholine bilayers by x-ray diffraction using specific bromination of the double bonds: effect of hydration. *Biophys. J.* 74:2419–2433.

Husslein, T., D. M. Newns, P. C. Pattnaik, Q. Zhong, P. B. Moore, and M. Klein. 1998. Constant pressure and temperature molecular dynamics simulation of the hydrated diphytanolphosphatidylcholine lipid bilayer. *J. Chem. Phys.* 109:2826–2832.

Hyslop, P. A., B. Morel, and R. D. Sauerheber. 1990. *Biochemistry.* 29:1025–1038.

Ipsen, J. H., G. Karlström, O. G. Mouritsen, H. Wennerstrom, and M. J. Zuckermann. 1987. Phase equilibria in the phosphatidylcholine-cholesterol system. *Biochim. Biophys. Acta.* 905:162–172.

Jakobsson, E. 1997. Computer simulation studies of biological membranes: progress, promise, and pitfalls. *Trends Biochem. Sci.* 9:339–354.

Keller, S. L., and H. M. McConnell. 1999. Stripe phases in lipid monolayers near a critical point. *Phys. Rev. Lett.* 82:1602–1605.

Kodati, V. R., and Lafleur, M. 1003. Comparison between orientational and conformational orders in fluid lipid bilayers. *Biophys. J.* 64: 163–170.

Lafleur, M., P. R. Cullis, and M. Bloom. 1990. Modulation of the orientational order profile of the lipid acyl chain in the $L\alpha$ phase. *Eur. Biophys. J.* 19:55–62.

Le Guerneve, C., and M. Auger. 1995. New approach to study fast and slow motions in lipid bilayers: application to dimyristoylphosphatidylcholine-cholesterol interactions. *Biophys. J.* 68:1952–1959.

Lindblom, G., L. B.-A. Johansson, and G. Arvidson. Effect of cholesterol in membranes: pulsed nuclear magnetic resonance measurements of lipid lateral diffusion. *Biochemistry.* 20:2204–2.

McCabe, N. A., G. L. Griffith, W. D. Ehringer, W. Stillwell, and S. Wassal. 1994. $^2$H NMR studies of isomeric $\omega$3 and $\omega$6 polyunsaturated phospholipid membranes. *Biochemistry.* 33:7203–7210.

McMullen, T. W. P., R. A. N. Lewis, and R. N. McElhaney. 1994. Comparative differential scanning calorimetric and FTIR and $^3$1P NMR spectroscopic studies of the effects of cholesterol and androstenol on the thermotropic phase behavior and organization of phosphatidylcholine bilayers. *Biophys. J.* 66:741–752.

McMullen, T. P. W., and R. N. McElhaney. 1995. New aspects of the interaction of cholesterol with diaplmitoylphosphatidylcholine bilayers as revealed by high-sensitivity differential scanning calorimetry. *Biochim. Biophys. Acta.* 1234:90–98.

Merz, K., and B. Roux, eds. 1996. Biological Membranes: A Molecular Perspective from Computation and Experiment. Birkhauser, Boston.

Morrison, C., and M. Bloom. 1994. Orientation dependence of $^2$H nuclear magnetic resonance spin-lattice relaxation in phospholipid and phospholipid:cholesterol systems. *J. Chem. Phys.* 101:749–763.

Pasenkiewiscz-Gierula, M., Y. Takoda, H. Miyagawa, K. Kitamura, and A. Kusumi. 1997. Hydrogen bonding of water to phosphatidylcholine in the membrane as studied by molecular dynamics simulation: location, geometry, and lipid-lipid bridging via hydrogen-bonded water. *J. Phys. Chem.* 101:3677–3691.

Pasenkiewiscz-Gierula, M., Y., T. Rog, K. Kitamura, and A. Kusumi. 2000. Cholesterol effects on phosphatidylcholine bilayer polar region: a molecular dynamics study. *Biophys. J.* 78:1376–1389.

Pastor, R. 1994. Molecular dynamics and Monte Carlo simulations or lipid bilayers. *Curr. Opin. Struct. Biol.* 4:443–464.

Pastor, R. W., and S. E. Feller. 1996. Time scales of lipid dynamics and molecular dynamics. *In* Biological Membranes: A Molecular Perspective from Computation and Experiment. K. M. Merz and B. Roux, eds. Birkhauser, Boston. 1–29.

Presti, F. T. 1985. The role of cholesterol in membrane fluidity. *In* Membrane Fluidity in Biology, Vol. 4: Cellular Aspects. R. C. Aloia and J. M. Boggs, eds. Academic Press, New York. 97–146.

Radhakrishnan, A., and H. M. McConnell. 1999a. Condensed complexes of cholesterol and phospholipid. *Biophys. J.* 77:1507–1517.

Radhakrishnan, A., and H. M. McConnell. 1999b. Cholesterol-phospholipid complexes in membranes. *J. Am. Chem. Soc.* 121: 486–487.

Robinson, A. J., W. G. Richards, P. J. Thomas, and M. M. Hann. 1995. Behavior of cholesterol and its effect on head group and chain conformations in lipid bilayers: a molecular dynamics study. *Biophys. J.* 68:164–170.

Ryckaert, J. P., and A. Bellmans. Molecular dynamics of liquid n-butane near its boiling point. *Chem. Phys. Lett.* 30:123–126.

Sankaram, M. B., and T. Thompson. 1990. Modulation of phospholipid acyl chain order by cholesterol: a solid-state $^2$H nuclear magnetic resonance study. *Biochemistry.* 26:10676–10684.

Scott, H. L. 1991. Lipid-cholesterol interactions: Monte Carlo simulations and theory. *Biophys. J.* 59:445–455.

Scott, H. L., E. Jakobsson, and S. Subramaniam. 1998. Simulation of lipid membranes with atomic resolution. *Comput. Phys.* 12:328–334.

Seelig, J., and N. Waespe-Šarčevič. Molecular order in cis and trans unsaturated phospholipid bilayers. *Biochemistry.* 17:3310–3315.

Seipmann, I., and D. Frenkel. 1992. Configurational bias Monte Carlo: a new sampling scheme for flexible chains. *Mol. Phys.* 75:59–70.

Slotte, P. 1995. Lateral domain heterogeneity in cholesterol/phosphatidylcholine monolayers as a function of cholesterol concentration and phosphatidylcholine chain length. *Biochim. Biophys. Acta.* 1238:118–126.

Smaby, J. M., M. M. Momsen, H. L. Brockman, and R. E. Brown. 1997. Phosphatidylcholine acyl unsaturation modulates the decrease in interfacial elasticity induced by cholesterol. *Biophys. J.* 73:1492–1505.

Smondryev, A. M., and M. Berkowitz. 1999a. United atom AMBER force field for phospholipid membranes. constant pressure molecular dynamics simulation of DPPC/water system. *J. Comput. Chem.* 20:531–545.

Smondryev, A. M., and M. Berkowitz. 1999b. Structure of DPPC/cholesterol bilayer at low and high cholesterol concentrations: molecular dynamics simulation. *Biophys. J.* 77:2075–2089.

Stouch, T. 1993. Lipid membrane structure and dynamics studied by all-atom molecular dynamics simulations of hydrated phospholipid bilayers. *Mol. Sim.* 10:317–335.

Thewalt, J. L., and M. Bloom. 1992. Phosphatidylcholine:cholesterol phase diagrams. *Biophys. J.* 63:1176–1181.

Tieleman, D. P., and H. J. C. Berendsen. 1996. Molecular dynamics simulations of a fully hydrated dipalmitoylphosphatidylcholine bilayer with different macroscopic boundary conditions and parameters. *J. Chem. Phys.* 105:4871–4880.

Tieleman, D. P., S. J. Marrink, and H. J. C. Berendsen. 1997. A computer perspective of membranes: molecular dynamics of lipid bilayer systems. *Biochim. Biophys. Acta.* 1331:235–270.

Tu, K., D. Tobias, and M. Klein. 1995. Constant pressure and temperature molecular dynamics simulation of a fully hydrated liquid crystal phase dipalmitoylphosphatidylcholine. *Biophys. J.* 69:2558–2562.

Tu, K., M. L. Klein, and D. J. Tobias. 1998. Constant pressure molecular dynamics investigation of cholesterol effects in a dipalmitoylphosphatidylcholine bilayer. *Biophys. J.* 75:2147–2156.

Urbina, J. A., S. Pekerar, H-B., J. Patterson, B. Montez, and E. Oldfield. 1995. Molecular order and dynamics of phosphatidylcholine bilayer membranes in the presence of cholesterol, ergosterol, and lanosterol: a comparative study using $^{2}$H-, $^{13}$C, and $^{31}$P-NMR spectroscopy. *Biochim. Biophys. Acta.* 1238:163–176.

Venable, R., B. Zhang, B. Hardy, and R. Pastor. 1993. Molecular dynamics simulations of a lipid bilayer and of hexadecane: an investigation of membrane fluidity. *Science.* 262:223–226.

Vist, M. R., and J. H. Davis. 1990. Phase equilibria of cholesterol/dipalmitoyl phosphatidylcholine mixtures: $^{2}$H nuclear magnetic resonance and differential scanning calorimetry. *Biochemistry.* 29:451–464.

Yeagle, P. L. 1993. The Membranes of Cells. Academic Press, San Diego.