COMBINED MONTE CARLO AND MOLECULAR DYNAMICS SIMULATIONS OF CHOLESTEROL IN PHOSPHOLIPID BILAYERS

By

XUELI HOU

Bachelor of Science

Norther Jiaotong University

Beijing, P. R. China

1997

Submitted to the Falculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the degree of
MASTER OF SCIENCE
July, 1999

COMBINED MONTE CARLO AND MOLECULAR DYNAMICS SIMULATIONS OF CHOLESTEROL IN PHOSPHOLIPID BILAYERS

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to take this opportunity to express my sincere gratitude to my major advisor Dr. H. Larry Scott for his intelligent supervision, instructive guidance, patient help and warm encouragement through the whole course of my study and research. I also would like to give my sincere thanks to my other committee members Dr. Bruce Ackerson and Dr. Paul Westhaus for their invaluable assistance and encouragement.

At the mean time, I wish to show my special appreciation to See Wing Chiu, Eric Jakobsson and Shakar Subramaniam at University of Illinois, Urbana–Champaign for their valuable guidance , effective cooperation and great help.

I feel very indebted to my father Weiyi and mother Rongjie for their priceless love, understanding and strong support to my study.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

INTRODUCTION

Membranes

Brief introduction to membranes

Biological membranes are the boundaries which keep an organism isolated from its outer surroundings. The importance of membranes can never be over emphasized due to their crucial roles in cell function and life phenomena.

Membranes can fall into categories such as plasma membranes, intracellular membranes and viral membranes. Plasma membranes separate a cell's interior side from the outside. They regulate the material communication between intracellular and extracellular domains, and such control is vital for cell metabolism and other cell functions. Intracellular membranes envelop each individual cell compartment, distinguish them and provide connections between them. The viral membrane is another special kind of membrane which encloses genetic materials such as RNA or DNA (1).

Our understanding of membranes has evolved a long way since early this century. In 1925, Goeter and Grendl first postulated the lipid bilayer structure of membranes (4). As a matter of fact, this model is valid for all kinds of membranes and represents a basis for membrane research. Later on in 1960, sound experimental evidence for Goeter's postulate was provided by J.D.Robertson (5)by means of electron micrographs of the erythrocyte membrane. The discovery of lipid bilayers stimulated new research interest on membrane structure and models. The lipid-protein complexes concept proposed by Green for the incorporation of proteins into membranes (6) is an instructive application of the membrane model. In this model, proteins were buried into the lipid bilayer as an integral component of lipid bilayer. The main limits on such a model at that time was the lack of knowledge of the prominence of

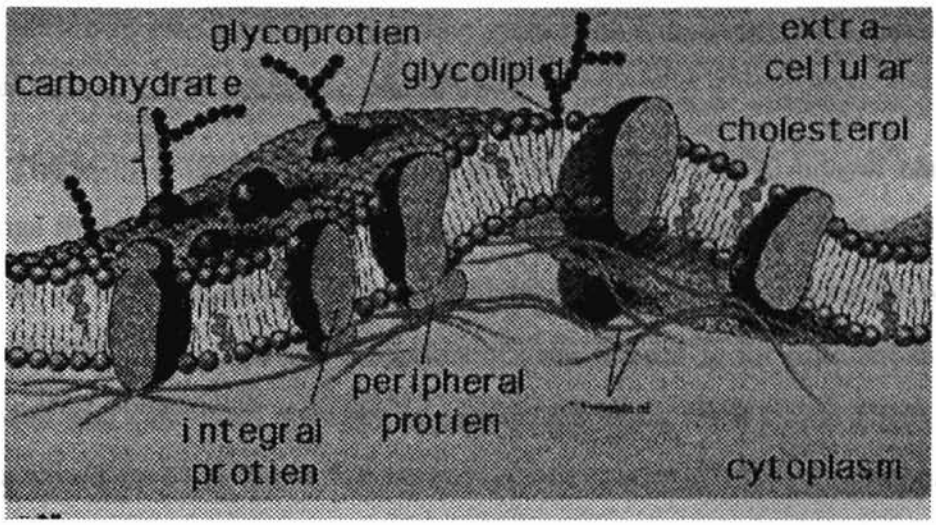

Figure 1: Picture of "Fluid Mosaic Model". As we can see, the membrane has a phospholipid bilayer. Proteins and cholesterols are located on the bilayer surface or in the bilayer. Refer to the website at: "http://academic.brooklyn.cuny.edu/biology/bio4fv/page/pm_mos.html" Permission received from Dr. James Nishiura.

fluid bilayers in membranes . Based on the previous work on membranes, S.J.Singer and G.Nicholson proposed the most widely used model – "fluid mosaic model" (3) in which lipid bilayers are regarded as the structural matrix for membranes. The typical characteristics of membrane in such a model is "fluidity" and dynamic. Within such a fluid structure, many other membrane constituents such as cholesterols, proteins and other membrane molecules, are incorporated into membranes and can diffuse laterally. (See figure 1)

Recent experimental results in membranes

With the help of advanced experimental techniques, such as X-Ray diffraction measurements, differential scanning calorimetry and Nuclear Magnetic Resonance (NMR), people have deeper insight into membrane structures and dynamics. In early 1980s, Brown and co-workers used NMR spectroscopy to measure the C-H relaxation times in lipid. Such measurements provide us with a general idea about the time scale of

motions within lipids (9). Moreover, time scale is an important issue considered in computer simulation of lipids and membranes.

In 1993, Nagle, et.al (24) estimated area per molecule of DOPC bilayer can span a large range from 58 A^2 to 71 A^2 . X-Ray structure determination on fully hydrated fluid phase DPPC (Dipalmitoyl Phosphatidylcholine)bilayer was done by Nagle, et, al. in 1996 (23). They reported the ratios of form factors f_h for different degrees of dehydration and concluded that the maximum thickness for a stable DPPC bilayer structure would be 54 A under full hydration(24). Later in 1998, S.Tristram-Nagle, et al. conducted study on DOPC bilayers structure changes due to different relative humidity. The area per DOPC molecule measured at full hydration is 72.2 A (24). It turned out much larger than the value measured at low hydration (66%) done by Weiner and White in 1992 . The determination of of the area can lead to the availability of a number of other structural parameters ,such as the number of water molecules, the corresponding water spacing, bilayer thickness and so forth. A discussion on all the other structural parameters is given by Nagle, et al. in (24). These data are highly informative for theoretical modeling and simulations on phospholipid bilayer.

Lipids

In membranes, the lipid bilayer has a highly significant role because it is the structural "backbone". (2) Phopholipids, which form a double layer of lipid molecules in water, have receive the most attraction and are best understood at this time. Lipid bilayers are shown in Figure 2.

As we can see, the bilayer consists of two leaflets of monolayer lipid molecules.The molecular matrix has hydrophilic (water soluble) headgroups as the exterior interface

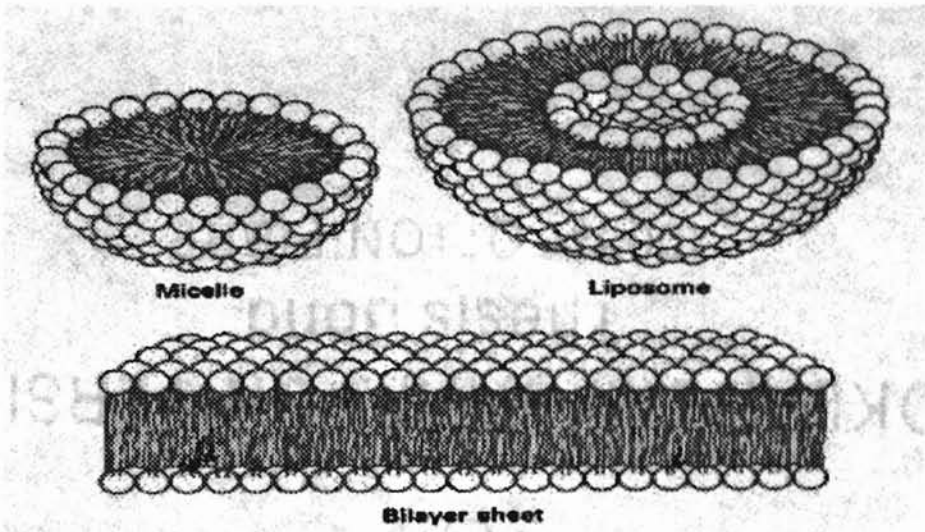

Figure 2: The different kinds of membranes formed by phospholipid bilayer. The common feature among them is the enclosure of hydrophobic chains. Refer to the website at:
"http://academic.brooklyn.cuny.edu/biology/bio4fv/page/phosphb.htm"
Get permission from Dr. James Nishiura.

with water solvent, and all the hydrophobic hydrocarbon chains pointing inwards to form the hydrophobic center of bilayers. In an aqueous phase which is the common living environment for biological cells, such an orientation of lipid molecules excludes water from the hydrophobic hydrocarbon chains so as to minimize the system's free energy. In excess water, all of the molecules in the bilayer close in on themselves and form the so called spherical vesicle . In this way, a biological membrane acts as a barrier between an interior and an exterior region.

A single phospholipid is shown in Figure3 and 4. The two hydrocarbon chains are water insoluble (hydrophobic). Each of them contains at least 9 -CH2 units. In comparison , the polar headergroup part is negatively charged phosphate so that it is water soluble (hydrophilic). An understanding of the structure of a phospholipid molecule is important for simulation models and methods. More detailed information about lipid molecule will be discussed in later chapters.

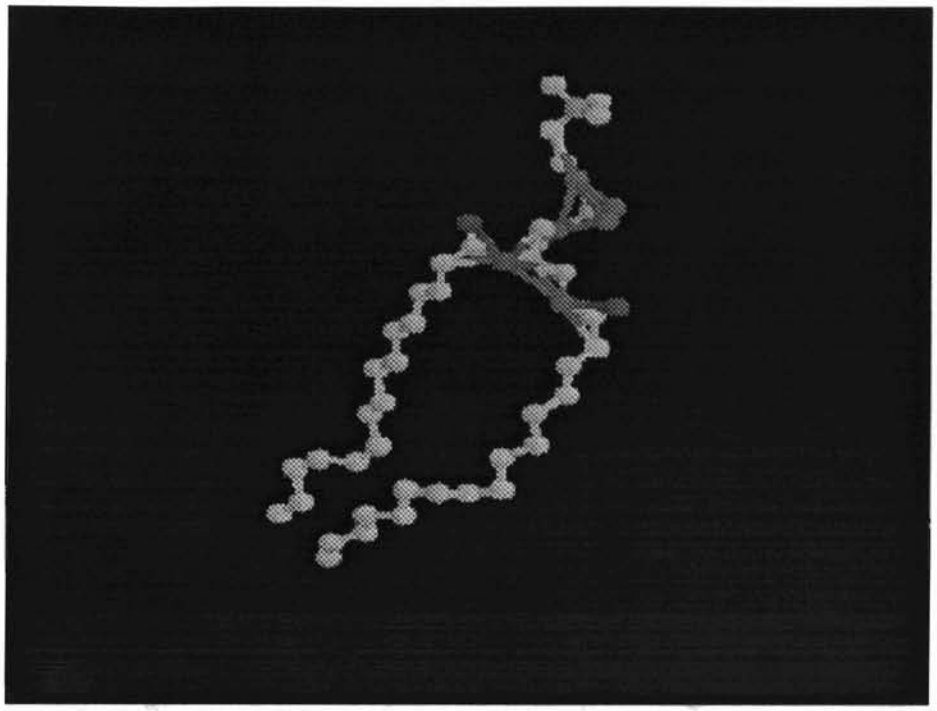

Figure 3: A single DPPC molecule.

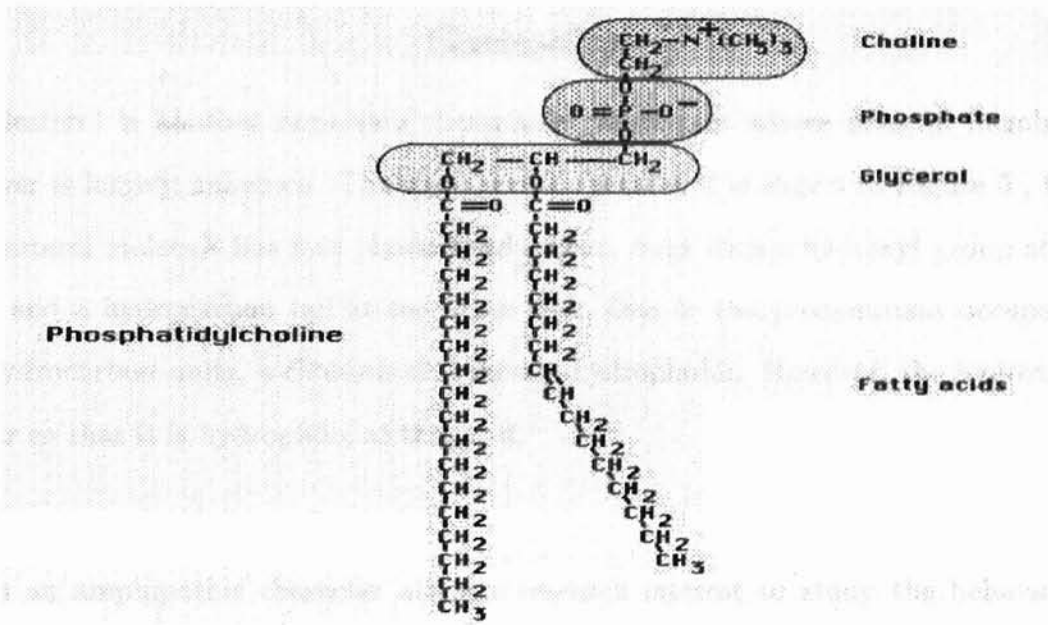

Figure 4: A diagram of a phospholipid structure. Refer to the website at:

Figure 5: "http://www.d.umn.edu/~sdowning/Membranes/phospholipidco mponents.html" Get permission from Dr.Steve Downing.

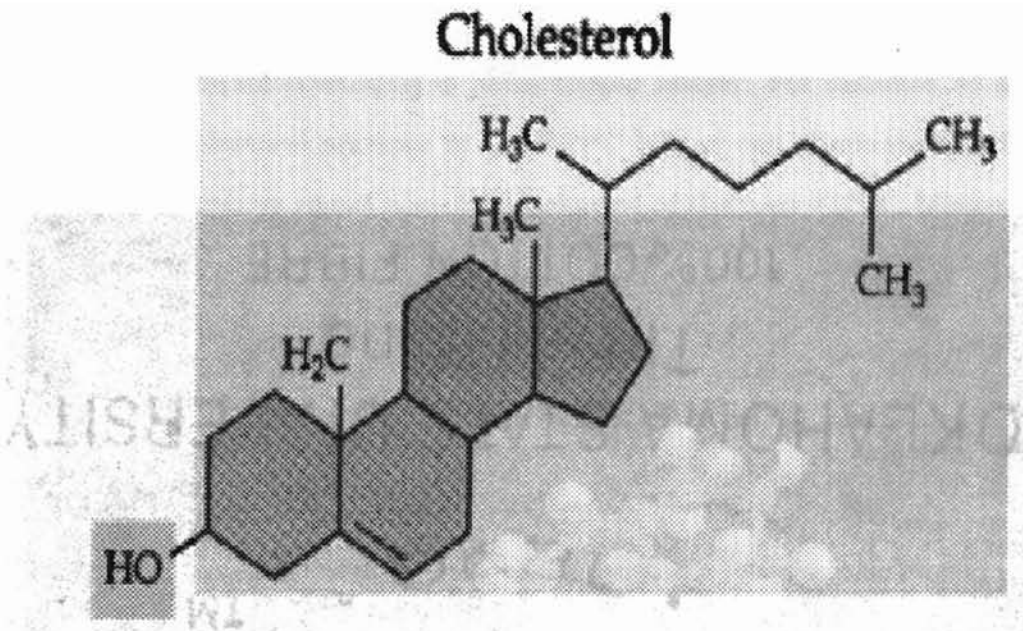

Figure 6: A diagram describing the structure of cholesterol molecule; Refer to website at: "http://academic.brooklyn.cuny.edu/biology/bio4fv/page/cholest.htm" Get permission fro Dr.James Nishiura.

Cholesterol

Cholesterol is another important membrane component whose roles in membrane bilayer is largely unknown. The structure of cholesterol is shown in Figure 5 , 6. A cholesterol molecule has four planar rigid carbon rings with a hydroxyl group at one end and a hydrocarbon tail at the other end. Due to the predominant occupation of hydrocarbon units, a cholesterol is mostly hydrophobic. However, the hydroxyl is polar so that it is hydrophilic at that end.

Such an amphipathic character attracts research interest to study the behavior of cholesterol when inserted into lipid bilayers with water (See Figure 7). A number of experiments have shown that cholesterol has a wide variety of effects on the physical properties of lipid membranes (1).

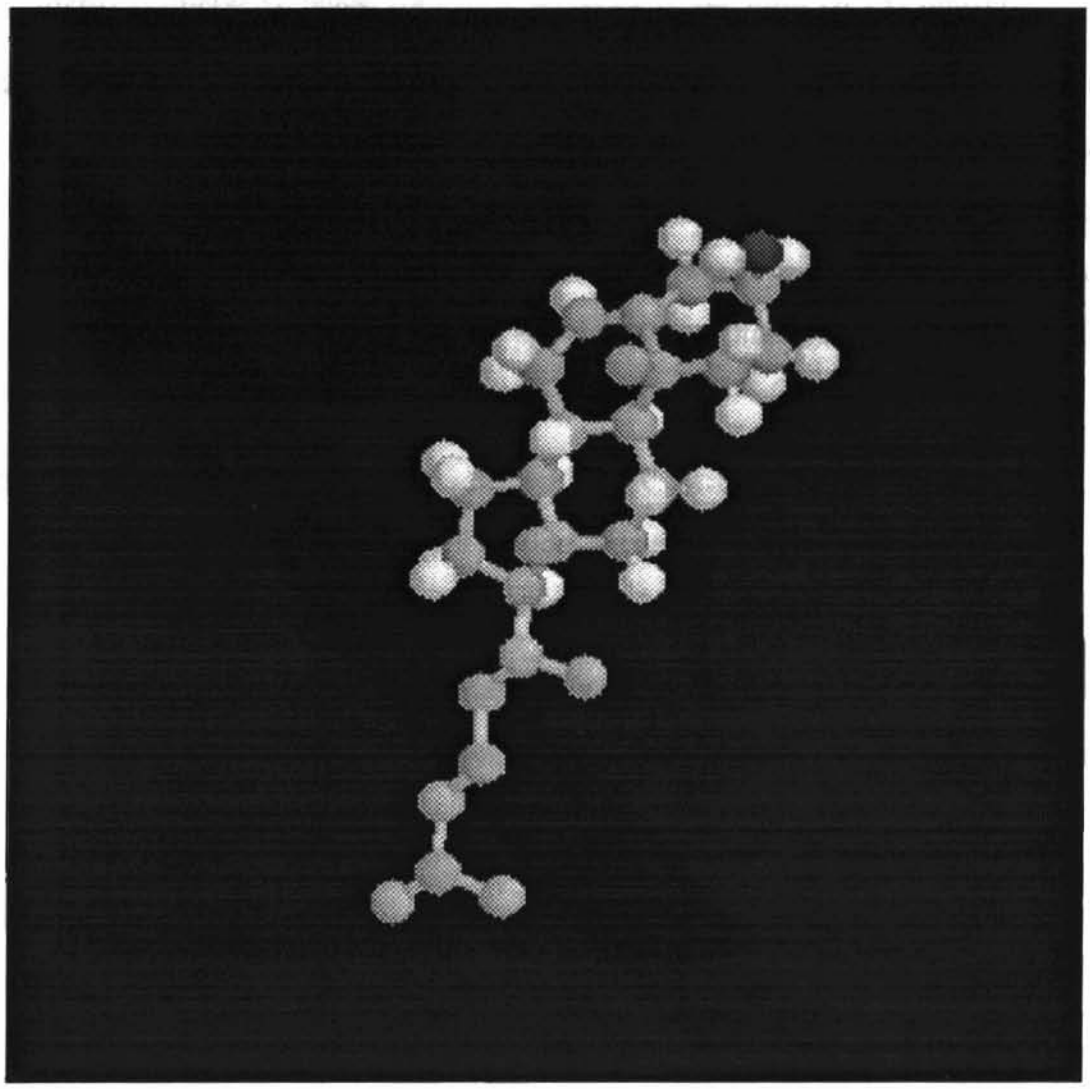

Figure 7: A real ball-stick 3-Dimensional model of a cholesterol used in our simulation.

1. One of the best known effect is the change in the behavior of the phase tansition from gel to liquid crystalline in phospholipid bilayer. For instance, at about 20-25 mol% cholesterol presence in the DPPC bilayer can eliminate the sharp and highly cooperative phase transition behavior of the pure DPPC bilayer.

2. Effects on membrane ordering is also well studied. Cholesterol increases the number of degrees of freedom of motion in the part of the hydrocarbon chain nearest to the glycerol, while it has little effect on the part of the hydrocarbon chain near the bilayer center. Such an effect would in turn cause more trans configurations in the upper part of a hydrocarbon chain. The macroscopic effect then would be an increase in bilayer thickness.

3. The area per molecule is also affected by the introduction of cholesterol. Since cholesterol disrupts the the interaction between phospholipid headgroups, this would cause the decrease of the surface area per molecule.

4. Other effects on phospholipid bilayer caused by cholesterol include a decrease of membrane permeability and a decrease in lateral diffusion.

All of these experimental observations of cholesterol effects on phospholipid bilayer stimulate strong interest in computer simulation on the complex membranes with cholesterol inserted. Gabdoulline et.al studied the influence of cholesterol on 16 DMPC with 16 cholesterol molecules . Chiu and Scott, et.al have been engaged in the simulation on 64 DPPC bilayer with the ratio 1:1 to cholesterol molecules, plus realistic number of water molecules. Our current research is about insertion of 6 cholesterol insertion into phospholipid bilayer and compute the free energy by employing thermodynamic integration.

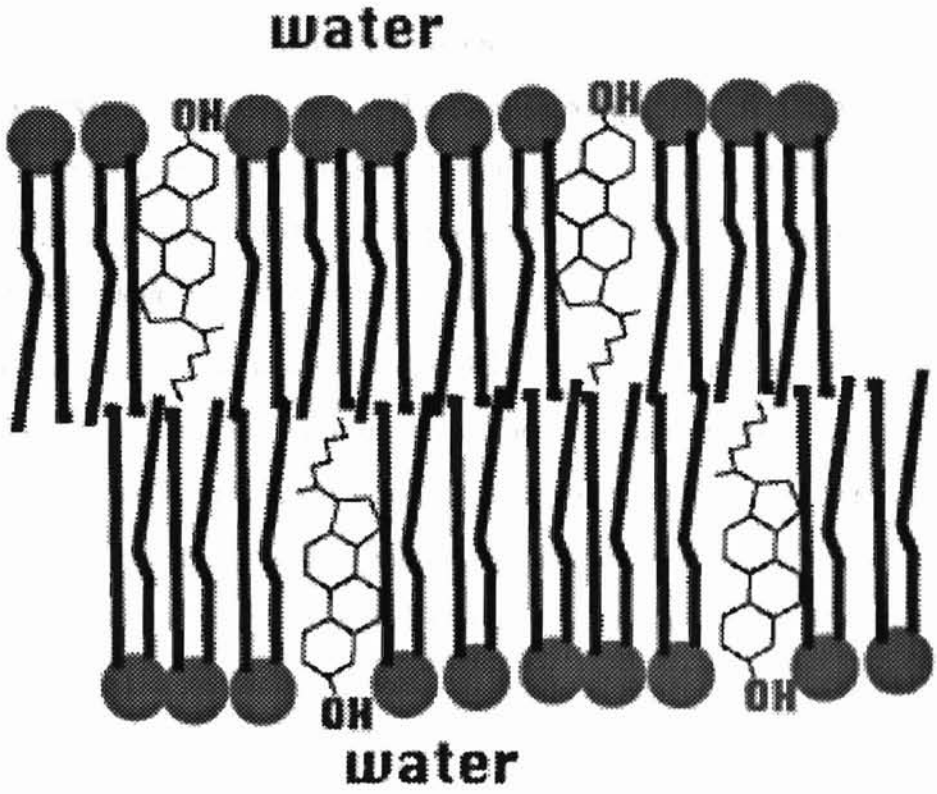

Figure 8: A diagram showing the insertion of cholesterols into lipid at an aqueous phase. Please refer to the website at:
"http://academic.brooklyn.cuny.edu/biology/bio4fv/page/cholest.htm" Permission received from Dr.James Nishiura.

Computer Simulation of lipids

A review on computer simulation

Experimental research on lipid bilayers has yielded important data and information on lipid membranes. This wealth of new experimental data has allowed for the development of atomic level membrane simulation. The validity of simulation parameters, models and results largely depends on comparison with experimental data, macroscopic observation and conclusions. However, in comparison with atomic level simulations, the information generated from experiments is coarse-grained and it is very hard for experimental scientists to interpretate the data precisely without microscopic data on positions and dynamic motions. (8)

Computer simulation is a powerful tool which provides us with atomic level resolution on a biological system. The information yielded from a computer simulation always contain atomic level configuration and dynamic coordinates of all the atoms in the system. In our research, the information consists of trajectory files which contain 3 dimensional atomic coordinates and velocities. Simulation on a system is carried out according to the interaction potentials, thermodynamic properties, physical constraints and other factors which determine the behavior of a model system. All these data, as stated before, are evaluated by comparison with experiments. In turn, they are also applicable to explain the macroscopic properties of biological system and suggest some new ideas to experimental scientists.(2) Computer simulation has experienced a fast development in the past decades and much effort has been contributed to the optimization of simulation models, methods and parameters. Looking at a biological system, say lipid bilayer, from a computational simulation perspective, we see the enormous number of degrees of freedom within a simulated system. The internal motions of each lipid molecule involve dihedral rotation about atomic bonds

within tens of picoseconds, axial rotation of molecule itself with a time scale about a nanosecond, intramolecular interaction, etc. Lateral diffusion takes place at a time length of about a few hundred nanoseconds. Intermolecular motions or the whole system phase transition take much longer time up to the order of millisecond or longer. For more detailed reviews, see (9), (10).

Simulations of a complex system have improved vastly reach the current state of art and are still under further improvement. Wiegel and his co-workers first started simulation on monolayer membrane using molecular dynamics in 1980,(12). Simulation on simple lipid bilayer with no water solvent was carried out (13). Afterwards, solvent was introduced into simulation models, which was a critical step towards the simulation of a realistic biological system (14) (15), since most biological membranes exist in aqueous phase. More realistic lipid membrane models should contain other membrane molecules such as sterols, proteins in addition to phopholipids. The incorporation of heterogenous molecules into lipid bilayer is interesting but challenging to molecular dynamics studies and simulations. A few simulations have been done on mixtures and most of them are dealing with mixture of cholesterol and lipids. The effect of cholesterol insertion is still not sufficiently understood from simulation studies. One of the main goal of our research is to study cholesterol insertion into DPPC bilayer and POPC (Palmitoyl-oleyl Phosphatidylcholine)bilayer in different ways. More details will be given in Chapter 2.

Simulation methods

As stated above, biological system is so complicated that appropriate simulation methods and algorithms should be selected.(2) Presently, the most widely adapted ones are Molecular Dynamics (MD) and Monte Carlo (MC). As we shall see below, both of them are used in our simulations.

Molecular Dynamics (MD)

Molecular Dynamics treats all atoms in a system classically. The simulation is carried out based on the interaction potentials within the system. Interactions in a system include:

1. Bonded interaction between adjacent atoms connected by chemical bonds which can be subdivided into covalent bond forces, covalent bond-angle forces, improper dihedral forces and dihedral torsion forces.(16)
2. Non-bonded interactions between non-connecting atoms which are within a certain range called cutoff radius. Non-bonded interactions involve electrostatic forces and Van der Waals forces .

With the understanding of all the component forces within a simulated system, a typical expression for the total potential energy can be written as:

$$V = \sum_{i<j} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} + \sum_{i<j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \sum_{i<j} \frac{B_{ij}}{r_{ij}^{6}}\right) + \sum_{bonds} \frac{1}{2} k_{ij}^{b} (r_{ij} - b_{ij}^{0})^2 +$$

$$\sum_{angles} \frac{1}{2} K_{ij}^{\theta} (\theta_{ijk} - \theta_{ijk}^{0})^2 + \sum_{dihedrals} k^{\phi} (1 + cos(n(\phi - \phi^{0})))$$

The first term involving charges reflects the electrostatic energy; The second term is a "6 - 12" term which describes the intermolecular interaction energy. A_{ij} and B_{ij} are called "Lennard-Jones" parameters. The last three terms represent the intramolecular interaction energies.

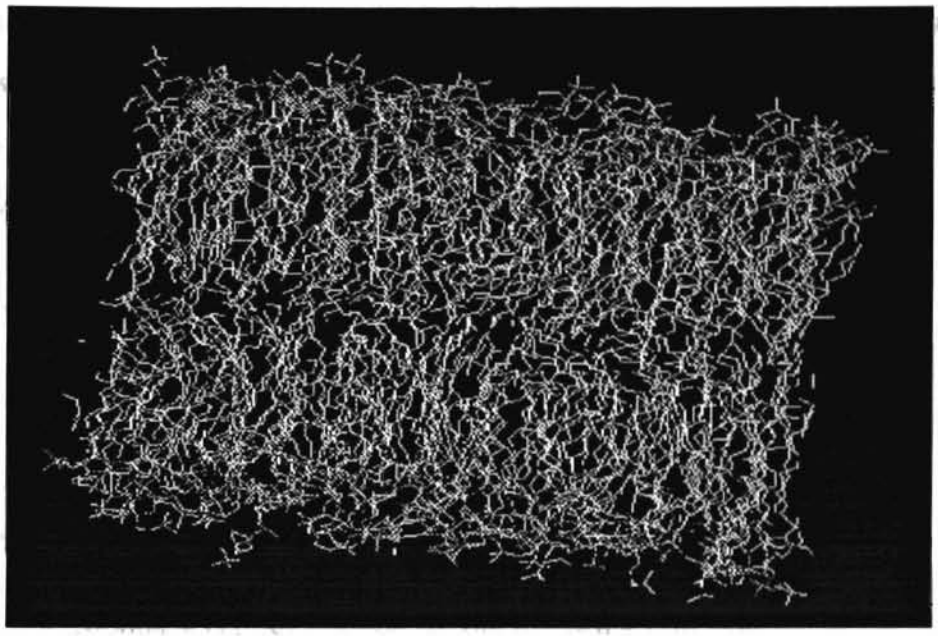

Figure 9: A disordered POPC bilayer resulting from MD simulation.

Given the energy formula, it is possible to set up Newton's equations of motions for all the atoms in a system. Theoretically, all those equations can be solved so that a complete set of dynamic data can be obtained. Typically, MD yields detailed trajectory files containing coordinates and velocities for all the atoms. A picture of POPC bilayer generated from MD simulation is shown in Figure 8. Energy of the system and other characteristic parameters can be obtained from the simulation . All these atomic level pictures of a simulated system are valuable and helpful. Up to now, many commercial molecular dynamics program packages are available. To name a few, GROMOS (87version and 96 version are both available) (16); OPLS(17) and CHARM (18)

MC and CBMC Monte Carlo method

Monte Carlo method uses an importance-weighted random walk to sample the phase space. Metropolis et.al came up with an efficient Monte Carlo scheme called "Metropo-

lis Monte Carlo algorithm". In such algorithm, we assume that in equilibrium, the rate at which a system leaves state O should be equal to the rate at which other states jump to state O. Then, a particle in the configuration space is selected at random and moved to a new position. This algorithm is carried out as the following:

1. calculate old energy U(old);
2. calculate new energy U(new);
3. If U(new) < U(old), the the trial is accepted. Otherwise, go to step (4) for metropolis acceptance.
4. The metropolis acceptance abide by the following criterion

$$\frac{P(o \to n)}{P(n \to o)} = exp(-[U(new) - U(old)]/K_B T)$$

When simulating a lipid bilayer with about 100 molecules, the Metropolis method is not efficient enough due to the high rejection probability. (19) Then an optimized method such as Configurational-bias MC (CBMC) method can be used (7, 26, 28) . In CBMC simulation algorithm, we use Rosenbluth weight to bias the moves so that the trial position and shape of the molecule to be moved have an enhanced probability to fit into the existing configuration. Detailed steps of CBMC method are listed below with POPC molecules as an example:

1. Pick a POPC molecule at random. Then do a random selection on either of the two chains or the headgroup.
2. Suppose we choose a chain, (headgroup is similar), we start with the first bond on the chain and make 120 trial positions for the atom i below the bond. Calculate the energy for each trial j. The energy E_j is the energy between the atom on

this new position and all the other atom on the chain and all the neighboring molecules. The weight for such a move at atom i is $w_i(n) = \sum_j exp(-\beta * E_j)$ where $\beta = \frac{1}{K_B T}$, K_B is Boltzmann's constant, and T is the absolute temperature.

3. Accept a trail position as the new postion for this atom with the probability $exp(-\beta * E_j)/w_i(n)$.

4. Move on to the next atom i+1 and do the same way to generate a new position for atom i+1. Calculate its weight $w_{i+1}(n)$. Keep doing this until the end of the chain. Then the Rosenbluth weight of the whole new chain is $W(n) = \Pi_i w_i(n)$.

5. After getting the new weight,we go back to the old configuration of the chain and use the same method above to trace the old Rosenbluth Weight $W(o) = \Pi_i w_i(o)$. The only difference is that each trial position attempted for an atom is generated from the original position of the previous atom.

6. The new configuration of the chain shall be accepted with probability

$$p = min[W(n)/W(o), 1]$$

7. Once a new configuration is accepted, the new configuration will replace the old one for the whole chain. Otherwise, new configuration is rejected and old chain remains.

Daan Frenkel and Berend Smit discussed CBMC and applied it to complex molecules (19). H.L. Scott's research group has used CBMC on DPPC lipid bilayer to explore the feasibility and advantages of CBMC (7, 26) . The result shows that CBMC can greatly speed up the system to reach the equilibrium of the system. MD and MC are

both important methods used in simulations. Chiu et al. made comparison between them (7). More details about CBMC method will be discussed in Chapter 2.

Our research goals : Two Simulation Projects

1. We used a hybrid simulation method consisting of Molecular Dynamics (MD) and Configuration Bias Monte Carlo (CBMC) to explore the feasibility of Thermodynamic Integration for cholesterol insertion into DPPC bilayer . The simulation started with insertion of 6 cholesterol molecules into a bilayer of 100 molecules of dipalmitoyl phosphatidycholine (DPPC) and 3205 water molecules. We need to find out if such a direct insertion without taking out some DPPC molecules for space is feasible. Special attention should be given to the intermolecular parameters so as to eliminate the situation in which large interaction force might happen. Trial and error method is used in this case.

2. Six cholesterols are inserted into a POPC bilayer and the system was equilibrated . In this project, we first took out 6 POPC molecules and put 6 cholesterols into the spaces. In this way, we do not need to worry about the adjustment of intermolecular interaction parameters.

METHODOLOGY

Feasibility of Thermodynamic Integration

Thermodynamic Integration Theory

Free energy is an important quantity which contains , in principle , all of the thermodynamic information of a system. For a closed system with constant volume V, temperature T and particle number N, the Helmholtz free energy $F = E - TS$ is at a minimum once the system is equilibrated. E is the internal energy of the system, S is the entropy of the system. For a closed system with fixed temperature and pressure, the Gibbs free energy $G = F + PV$ reaches minimum once the system reaches equilibrium. This theory can be applied to the simulations of biological system to determine if a simulated system has reached equilibrium as long as we can determine the free energy .

However, only mechanical quantities can be directly determined from experiment. The free energy of a simulated system is not a mechanical quantity and can not be directly derived from the physical quantities measured experimentally or directly from simulations. Therefore, we need to find an indirect way to calculate the free energy of a system under simulation. It is known in thermodynamics that free energy has the following derivative relations with mechanical quantities:

$$\left(\frac{\partial F}{\partial V}\right)_{N,T} = -P$$

$$\left(\frac{\partial F/T}{\partial 1/T}\right)_{V,T} = E$$

Thus, the free energy of a system can be computed by doing a integral along a reversible path in the V-T plane. For free energy calculation in simulations, we can extend the thermodynamic variables to all the independent parameters used in free energy.

Coupling parameter approach

To better understand the role of cholesterol in biological membranes , it is important to calculate the free energy difference between a pure phospholipid bilayer and the same bilayer with cholesterol molecules inserted . Our research concentrated on DPPC bilayer . A coupling parameter approach may be used to compute the free energy difference between the two phases. In this way, a coupling parameter (λ) is used to scale the interaction potentials between molecules. The following formula states how λ is introduced into the potential energy:

$$V = \sum_{ij}\left(\frac{(\lambda C_{12_i}^{1/2})(\lambda C_{12_j}^{1/2})}{r^{12}} - \frac{(\lambda C_{6_i}^{1/2})(\lambda C_{6_j}^{1/2})}{r^{6}}\right) + \sum_{ij}\left(\frac{\lambda q_i \lambda q_j}{4\pi\epsilon r}\right)$$

For a system with a potential energy function that corresponds to a value of λ ranging from 0 to 1, the free energy becomes a function of λ based on statistical mechanics:

$$F(\lambda) = -K_B T ln Z(\lambda)$$

where $Z(\lambda)$ is the partition function of the system with N atoms. It is given by:

$$Z(\lambda) = \frac{1}{h^{3N} N!} \int exp[-\beta U(\lambda)] dr^N$$

The derivative of the free energy with respect to λ is given below (16):

$$(\frac{\partial F(\lambda)}{\partial \lambda}) = -K_B T \frac{\partial}{\partial \lambda} ln[Z(\lambda)] = -\frac{K_B T}{Z(\lambda)} \frac{\partial Z(\lambda)}{\partial \lambda}$$

$$= \frac{\int (\partial U(\lambda)/\partial \lambda) exp(-\beta U(\lambda)) dr^N}{\int exp[-\beta U(\lambda)] dr^N}$$

$$= \left\langle \frac{\partial U(\lambda)}{\partial \lambda} \right\rangle_\lambda$$

Therefore, the free energy is related to U(λ) by :

$$F'(\lambda) = < \frac{\partial U(\lambda)}{\partial \lambda} >_\lambda$$

Thus, the thermodynamic integration formula for F(λ) is:

$$\Delta F = \int_{\lambda old}^{\lambda new} < \frac{\partial U(\lambda)}{\partial \lambda} >_\lambda d\lambda$$

V(λ) is the potential energy of a system with the coupling parameter λ. For our simulation method, $\lambda_{old} = 0$ and $\lambda_{new} = 1$.

Simulation of DPPC bilayer with cholesterol

Initialization of the simulation

We started with a fluid phase DPPC bilayer. It was generated after a long CBMC and MD simulation by Chiu. et.al (7). This DPPC bilayer contained 100 DPPC molecules and 3205 water molecules. The simulation ensemble used by Chiu, et.,al was (N, p,

γ,T) which means constant pressure (p) which is perpendicular to the bilayer surface, constant lateral surface tension (γ), and constant temperature (T). Since we do not exactly know the surface tension of a DPPC bilayer with cholesterol embedded, we chose a different simulation ensemble, ie. (N,P,T) which ignores the surface tension and keeps constant pressure (P = 1 atmosphere) and constant temperature (T = 325 K).

Cholesterol insertion

For the simplicity, we purposely chose three widely separated postions in each leaflet of the bilayer so that cholesterol molecules at those postions should have little interaction between each other. Based on the information available from experimental measurements and observations, the appropriate insertion depth of cholesterol requires that the hydroxyl group in a cholesterol should approximately level with the ester carbonyl in its adjacent DPPC molecule (1).

Parameterization procedure

Direct insertion of cholesterols into lipid can introduce unphysically large intermolecular interaction forces due to close distances between atoms in cholesterol and DPPC molecules or even direct overlaps. For this reason, we should carefully adjust the coupling parameters for C-6 , C-12 and electrostatic partial charges of cholesterol in such way that the Van de Waals forces and electrostatic forces between a cholesterol molecule and the DPPC molecules in vicinity can be rescaled upward to reasonable values . Starting with the scaling factor $\lambda_1 = 0.05$, we planned to do a complete simulation on the system until it reaches an equilibrium state called state(1).

$$V = \sum_{ij}\left(\frac{(\lambda C_{12_i}^{1/2})(\lambda C_{12_j}^{1/2})}{r^{12}} - \frac{(\lambda C_{6_i}^{1/2})(\lambda C_{6_j}^{1/2})}{r^{6}}\right) + \sum_{ij}\left(\frac{\lambda q_i \lambda q_j}{4\pi\epsilon r}\right)$$

After the first equilibration, we need to change λ to a larger value $\lambda_2$and used state (1) as the initial configuration for the second complete simulation. After a certain amount of time, the system is supposed to reach its new equilibrium state called state(2). Then λ_3 , λ_4 , λ_5..... would be tried in the same manner up to $\lambda_f = 1$. At this moment, the interaction forces within the system are expected to be recovered to what they should be for a realistic system. Then a final equilibrium would be reached . This would be the equilibrium state for DPPC bilayer with cholesterol inserted.

Simulation procedures

Through the whole research project, MD part was carried out by using GROMOS96 simulation package (Gunsteren, et.al) . Metropolis Monte Carlo (MMC) and CBMC were used for MC simulation part.

For each simulation cycle , Energy Minimization (EM) was first run to obtain low-energy configuration of the system. Next was a 20 picosecond MD run followed by 5000 MMC and CBMC steps. This is one complete loop of combined MD and CBMC simulation. CBMC can efficiently make the system jump to a new configuration space. The next loop again started with Energy Minimization on the configuration generated from CBMC of the previous cycle. Then another 20 picosecond MD and 5000 CBMC steps followed .

Output trajectory and energy files and Energy Extraction programs (Chiu, et.al (7)) could be used to monitor the system. Once the equilibrium state was being reached, a 100 picosecond continuous MD was done while all the trajectory and energy files were saved for later data analysis.

Free energy calculation

As stated above , free energy can be calculated by evaluating the integral over the

average value of partial derivative of U(λ) with respect to λ. Therefore, we should first find the average value of $\frac{\partial U(\lambda)}{\partial \lambda}$. The total internal energy, as described in Chapter 1, contains both non-bonded and bonded energies. Since only non-bonded terms associated with cholesterol involve the intermolecular coupling parameter λ, the other terms will all vanish when $\frac{\partial U(\lambda)}{\partial \lambda}$ is computed. Hence, we ignore those unrelated terms and keep λ related terms when doing the following derivations:

$$V(\lambda) = \sum_{chol-chol} \left(\frac{\lambda^2\sqrt{C_{12_i}}\sqrt{C_{12_j}}}{r^{12}} + \frac{\lambda^2\sqrt{C_{6_i}}\sqrt{C}_{6_j}}{r^6} + \frac{\lambda^2 q_i q_j}{4\pi\epsilon r}\right)$$

$$+ \sum_{chol-dppc} \left(\frac{\lambda\sqrt{C_{12_i}}\sqrt{C_{12_j}}}{r^{12}} + \frac{\lambda\sqrt{C_{6_i}}\sqrt{C}_{6_j}}{r^6} + \frac{\lambda q_i q_j}{4\pi\epsilon r}\right)$$

$$+ \sum_{chol-H_2O} \left(\frac{\lambda\sqrt{C_{12_i}}\sqrt{C_{12_j}}}{r^{12}} + \frac{\lambda\sqrt{C_{6_i}}\sqrt{C}_{6_j}}{r^6} + \frac{\lambda q_i q_j}{4\pi\epsilon r}\right)$$

$$\left(\frac{\partial V}{\partial \lambda}\right) = \sum_{chol-chol} \left(\frac{2*\lambda\sqrt{C_{12_i}}\sqrt{C_{12_j}}}{r^{12}} + \frac{2*\lambda\sqrt{C_{6_i}}\sqrt{C}_{6_j}}{r^6} + \frac{2*\lambda q_i q_j}{4\pi\epsilon r}\right)$$

$$+ \sum_{chol-dppc} \left(\frac{\sqrt{C_{12_i}}\sqrt{C_{12_j}}}{r^{12}} + \frac{\sqrt{C_{6_i}}\sqrt{C}_{6_j}}{r^6} + \frac{q_i q_j}{4\pi\epsilon r}\right)$$

$$+ \sum_{chol-H_2O} \left(\frac{\sqrt{C_{12_i}}\sqrt{C_{12_j}}}{r^{12}} + \frac{\sqrt{C_{6_i}}\sqrt{C}_{6_j}}{r^6} + \frac{q_i q_j}{4\pi\epsilon r}\right)$$

The above is the analytic form of $(\frac{\partial V}{\partial \lambda})$ which can be used to calculate $< \frac{\partial U(\lambda)}{\partial \lambda} >_\lambda$ for each $\lambda$by using the N energy files stored in the last 100 picosecond of MD run :

$$< \frac{\partial U(\lambda)}{\partial \lambda} >_\lambda = \frac{\sum_{i=1}^{N} < \frac{\partial U_i(\lambda)}{\partial \lambda} >_\lambda}{N}$$

Once we get $< \frac{\partial U(\lambda)}{\partial \lambda_{jth}} >_\lambda$ for each λ, we can plot out $< \frac{\partial U(\lambda)}{\partial \lambda} >_\lambda$ vs. λ to compute the free energy difference.

Simulation of POPC bilayer with cholesterol

In comparison with the DPPC + Cholesterol case , we carried out a simulation of POPC bilayer containing 6 cholesterols.

Ensemble used in this project

An ordered 128 POPC plus 4628 H_2O bilayer was obtained from simulation of Chiu et.al (26). This bilayer was the result of combined MD and CBMC with constant normal pressure P (one atmosphere); constant temperature T (325 K) and constant lateral surface tension (46.0 dyne/cm) .

For the simulation in the mixed bilayer, we first used the exactly same ensemble described above. After running MD with the temperature 310K for 240 picoseconds, we raised the temperature to 350K and then 400K to heat the system . In this way, we speed up the thermal expansion process of the system. Once the bilayer expands to an equilibrium state at 400K, a "cooling down" procedure will be applied so as to bring the system back to an equilibrium state at the room temperature (310K).

Simulation on POPC bilayer with cholesterols

In this simulation, we substantially changed the cholesterol insertion procedure . Three POPC molecules located far apart in each of the two leaflets were removed

from the bilayer. Then, each vacancy was filled with a cholesterol molecule. Since a cholesterol is dimensionally smaller than a POPC molecule, the distance between a cholesterol and its surrounding POPC molecules is large enough to avoid any large interaction force. Thus, no adjustment of coupling parameters was needed.

After the insertion was done, we ran an energy minimization procedure to reach a low-energy configuration. Then 20 picosecond MD was run. After that, we modified the CBMC codes used in DPPC case to adapt it for POPC. Since the two chains in POPC molecules are not of the same length as those in DPPC molecules, we rewrote the CBMC program code and used the new version for our simulation. One complete cycle of combined simulation was quite similar to what we did in DPPC case . The results of the simulation will be discussed in the next chapter.

from the bilayer. Then, each vacancy was filled with a cholesterol molecule. Since a cholesterol is dimensionally smaller than a POPC molecule, the distance between a cholesterol and its surrounding POPC molecules is large enough to avoid any large interaction force. Thus, no adjustment of coupling parameters was needed.

After the insertion was done, we ran an energy minimization procedure to reach a low-energy configuration. Then 20 picosecond MD was run. After that, we modified the CBMC codes used in DPPC case to adapt it for POPC. Since the two chains in POPC molecules are not of the same length as those in DPPC molecules, we rewrote the CBMC program code and used the new version for our simulation. One complete cycle of combined simulation was quite similar to what we did in DPPC case . The results of the simulation will be discussed in the next chapter.

RESULTS AND DISCUSSIONS

Results on DPPC+CHOL simulations

Coupling Parameter

As we stated in the previous chapter, a critical step to use Thermodynamic Integration for the DPPC bilayer with the mixture of cholesterol is the choice of coupling parameter λ, which directly scales the interaction between molecules. An inappropriate choice of λ would cause the system to crash due to large interaction forces.

$\lambda = 0.1$was selected as the initial trial value for scaling the Van der Waals interactions and electrostatic forces in GROMOS96 . We modified the force field file for MD simulation as well as for CBMC. During the simulation, we kept tracking the change of the system while each simulation cycle ran. Each cycle contained Energy Minimization, 20 picosecond MD and 5000 CBMC steps . After about 3 cycles, we observed unusual behaviors of the cholesterol molecules. Compared with their initial positions in the bilayer (Figure 9) before simulation, at least one cholesterol molecule was seen to be "squeezed" out of the bilayer (Figure 10),which means the interaction forces between cholesterol molecules and neighboring DPPC molecules were so large that smaller cholesterol molecules were pushed out of the bilayer. Therefore, a smaller value of λ was tried .

A new trial of $\lambda = 0.05$ was selected . It turned out to be a good value to start with and avoided the "pump out" effect . Meanwhile, time step in MD runs was also an important factor to be considered. A large time step, say $dt = 0.05$ picosecond can speed up MD simulation but could cause catastrophic errors during the simulation under some circumstances (15). In the way of trial and error, we set the time step to be 0.005 picosecond.

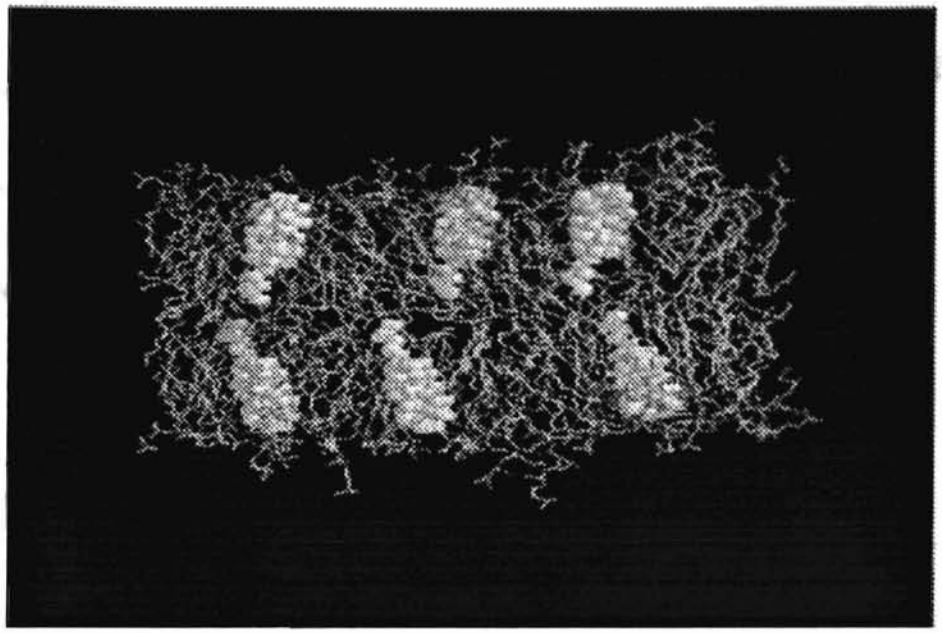

Figure 10: The initial configuration of the DPPC bilayer with 6 cholesterol molecules embedded.

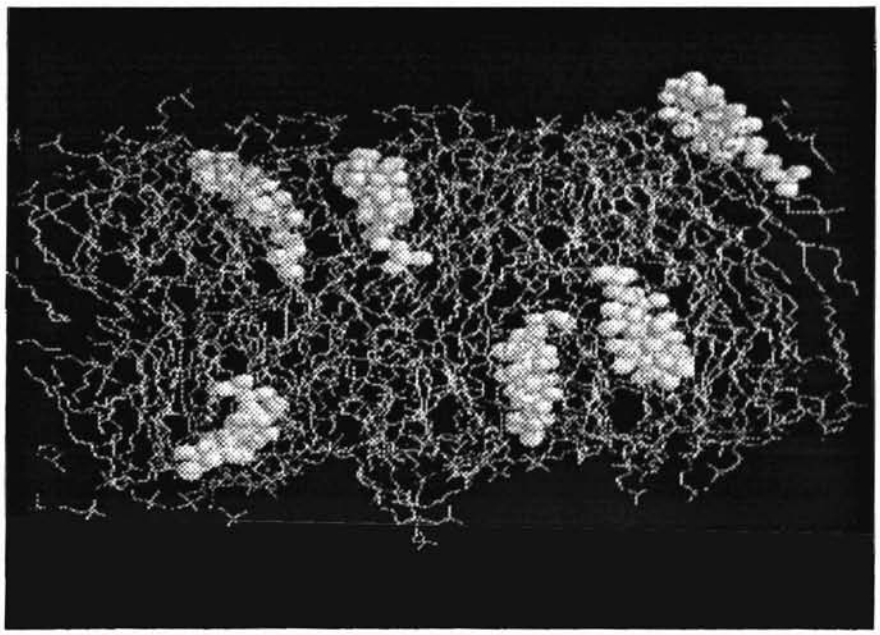

Figure 11: For $\lambda = 0.1$: The system after 200 ps MD run and 50000CBMC steps. Some cholesterol molecule is out of the bilayer due to large intermolecular forces.

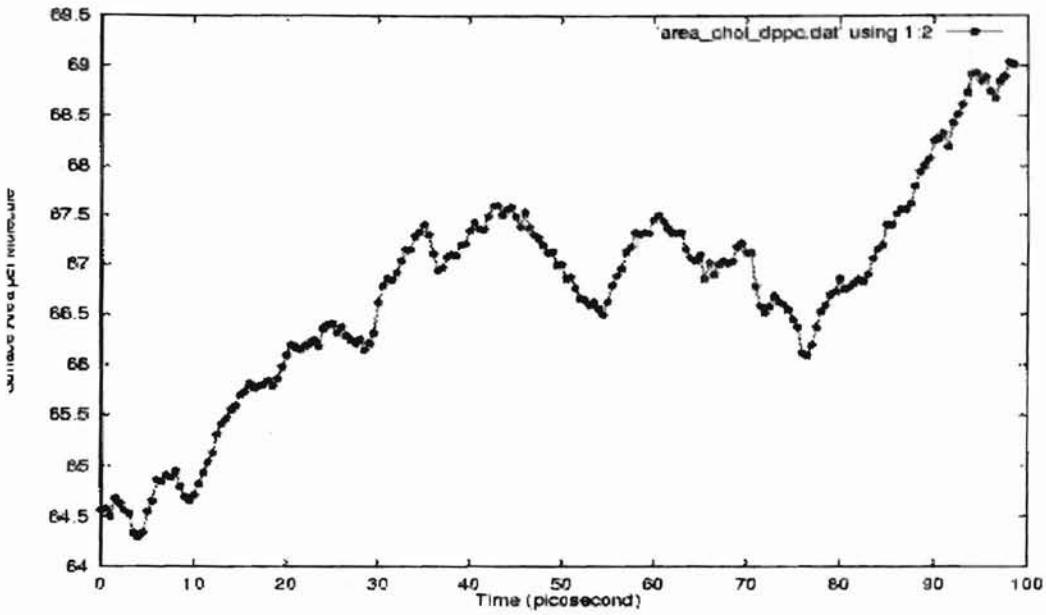

Figure 12: The plot of area per molecule vs. time shows an increasing tendency.

Energy and Area per molecule

Through the entire simulation, we monitored the system's energy and area per molecule to tell if the system was evolving in a reasonable way and approaching equilibrium. Figure 11 is a plot of area per molecule vs. time. The data were recorded in the last 100 ps MD run . In comparison with the area per molecule of pure DPPC bilayer (62 A^2 per molecule), the presence of cholesterol caused an increase in the area per molecule up to 69 A^2 per molecule.

Figure 12 is the plot of energy vs. time which was also generated from the last 100 ps MD run. The energy looks noisy with the fluctuation range to be about 2.6%. Such a large energy fluctuation implies that the system hasn't reached equilibrium yet. More MD run and CBMC steps should be done to the system . However, the simulation came to a stop because cholesterol molecules failed to be constrained to their initial positions and moved to the center of the bilayer (Figure 13). Such a configuration is

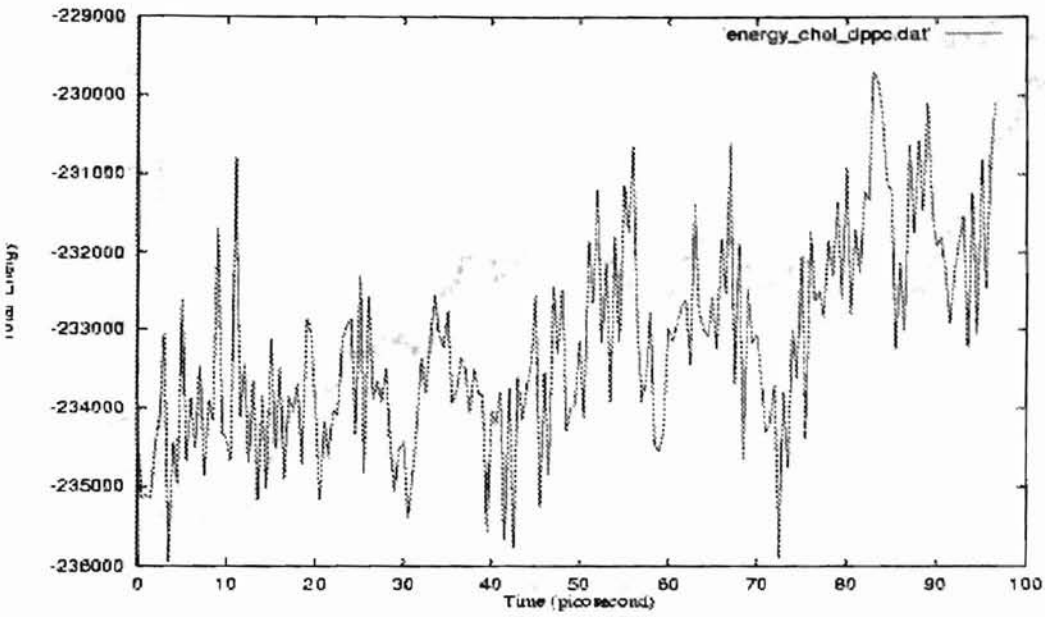

Figure 13: The potential energy vs. time shows the status of the system.

not physically reasonable and suggests the failure of the direct insertion method for cholesterol.

Although such an approach of direct insertion of cholesterol did not produce physically reasonable results , we can still make analysis on the data available to begin to understand the effects that cholesterol has on the phospholipid bilayer .

Order parameters

One of the major physical effect that cholesterol has on lipid bilayer is an increase of motional order of those lipid atoms adjacent to the cholesterol molecules. Motional order describes the number of degrees of motional freedom experienced by a molecule or its segment. Generally, a higher motional order corresponds to a more ordered system . The order parameter can be analytically described as

$$S = \frac{1}{2}(3 < cos^2\theta > -1)$$

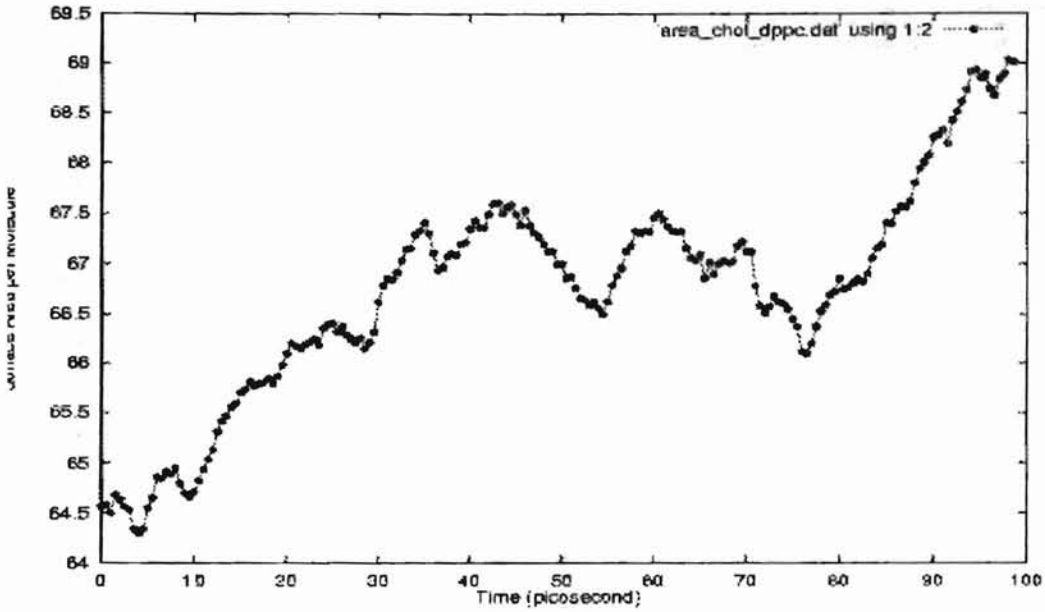

Figure 14: The plot of area per molecule vs. time shows an increasing tendency.

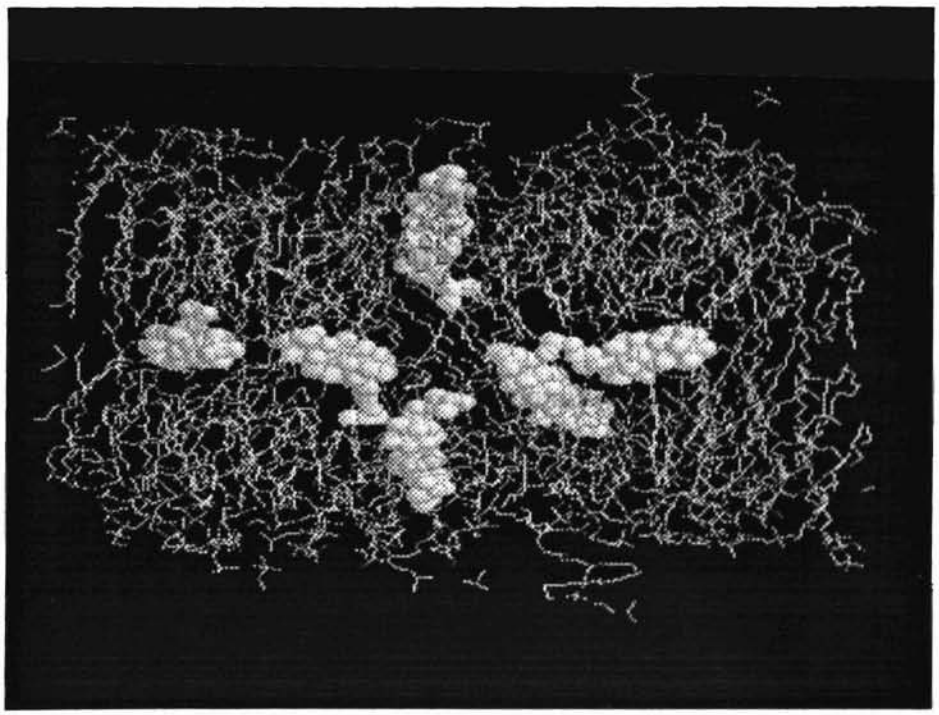

Figure 15: for $\lambda = 0.05$; The cholesterol molecules diffused to the center of the bilayer. This is unphysically reasonable. Thus, the direct insertion of cholesterol into the DPPC bilayer is not successful.

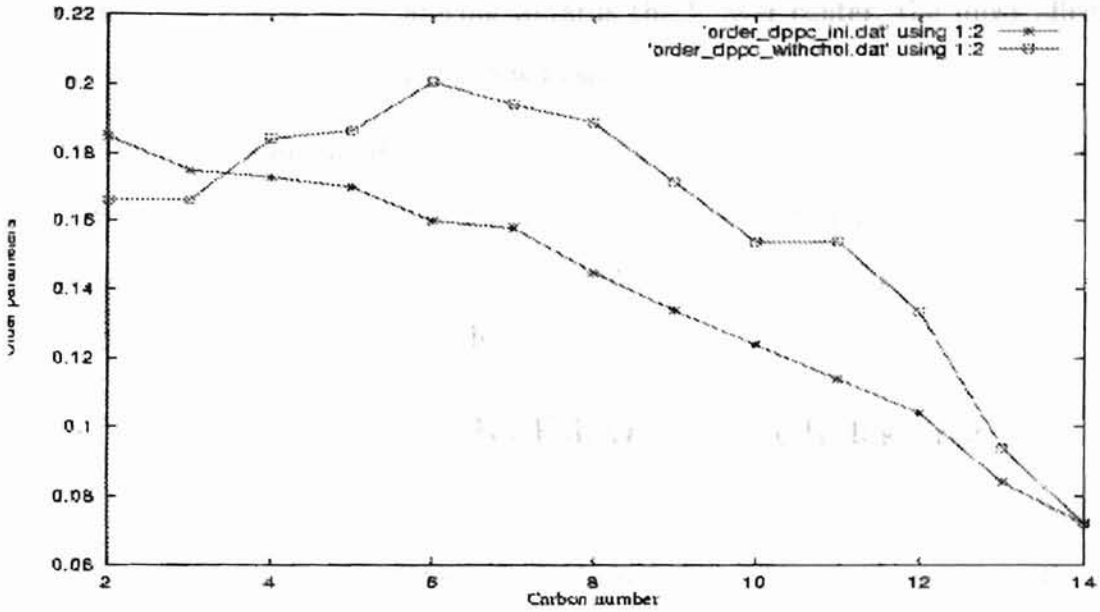

Figure 16: Order parameters compared between pure DPPC chains and mixed DPPC chains with cholesterol mixed in the bilayer.

where θ is the angle that a carbon - hydrogen bond makes with respect to the bilayer normal direction. The order parameters of carbon atoms in a chain reflect the average conformation of that chain . In our simulation, the order parameters of DPPC carbon chains are under careful study , because they are supposed to be greatly affected with the presence of cholesterol.

Figure 14 plots the order parameters corresponding to each hydrocarbon bond on an acyl chain. The lower curve represents the behavior of pure DPPC and it was obtained from the research done by Chiu et al. (7). The higher curve stands for the DPPC mixed with 6 cholesterol molecules.

However, there is discrepancy between our simulation data and experimental data. The portion of chain nearest to the headgroup seems to have less order of motion in our simulation result . This can be explained by referring to Figure 13 . Since

cholesterol molecules were all moving towards the bilayer center, the most affected portions of chains were also moving downwards .

Also, the space around the upper parts of the chains became larger after cholesterol molecules lowered. This provided the upper parts with more physical space for motional freedom and led to a decrease in the value of the order of parameters around those portions of the hydrocarbon chains.

Discussion on DPPC Bilayer with Cholesterol

Our exploration on the feasibility of Thermodynamic Integration for phospholipid is a new trial that has not yet been done before. The Thermodynamic method is inherently correct. An unsuccessful new trial in simulations can be attributed to many factors.

One reason could be due to the mismatch between the coupling factor and the size of cholesterol molecules. Physically, a cholesterol molecule is smaller than a DPPC molecule. When we scaled down the force field parameters of cholesterol atoms to prevent high interaction energy, the forces exerted on cholesterol molecules were much smaller than those forces among DPPC molecules and water molecules. Such a nonuniform force field caused the "ignorance" of the small cholesterol molecules by the lipids. As a result, they were less constrained and diffused rapidly to the low energy area, ie. the center of the bilayer. A solution to such a problem may be a more cautious tune on the coupling parameter so as to avoid high energy and the "ignorance" effect at the same time. Alternatively , the cholesterol molecules could be restrained in their mobility . Another reason may be the insufficient sampling rates over such a vast space. More computation power and long time scale are needed in order to improve the sampling rate.

Meanwhile, we do not exclude the possibility that there are some subtle factors that influence the way a biological system evolves . More careful investigations are needed on applying Thermodynamic Integration to phospholipid bilayers.

Results of POPC + CHOL simulation

The whole equilibration procedure for the bilayer is shown by Figure 15 and 16 which are plots of area per molecule versus time and a plot of potential energy versus time, respectively. The total time recorded is 550 picoseconds . The first 240 picoseconds is MD run at the room temperature 310K. The next 20 picosecond MD was done at 350K. Then the temperature was raised to 400K .

Condensing effect on area per molecule

In Figure 15, the area per molecule of the complex bilayer first experienced a drop from about $54.5A^2$to $54.05A^2$ per molecule. This happent after several cycles of MD run, because condensing effect caused a shrink of the area. The drop in area per molecule well matches the concept of the condensing effect derived from studying mixed monolayer of cholesterol and phospholipid (1) .

After the condensing effect, the area per molecule undergone a very slow change until we raised the temperature to 350K at the 240th ps then followed by 400K at the 260th ps. Such "heating up" steps caused an obvious effect on the system by greatly speeding up the thermal expansion procedure. Such an increasing area per molecule curve agrees with our expectation . Once the system reaches an equilibrium at temperature equal to 400K which is called local equilibrium , we need to cool the system down to the room temperature and let the system achieve its global equilibrium. However, due to the complexity of the simulated system, the time needed for the system to reach its final global equilibrium will be far more than what we had spent on the local

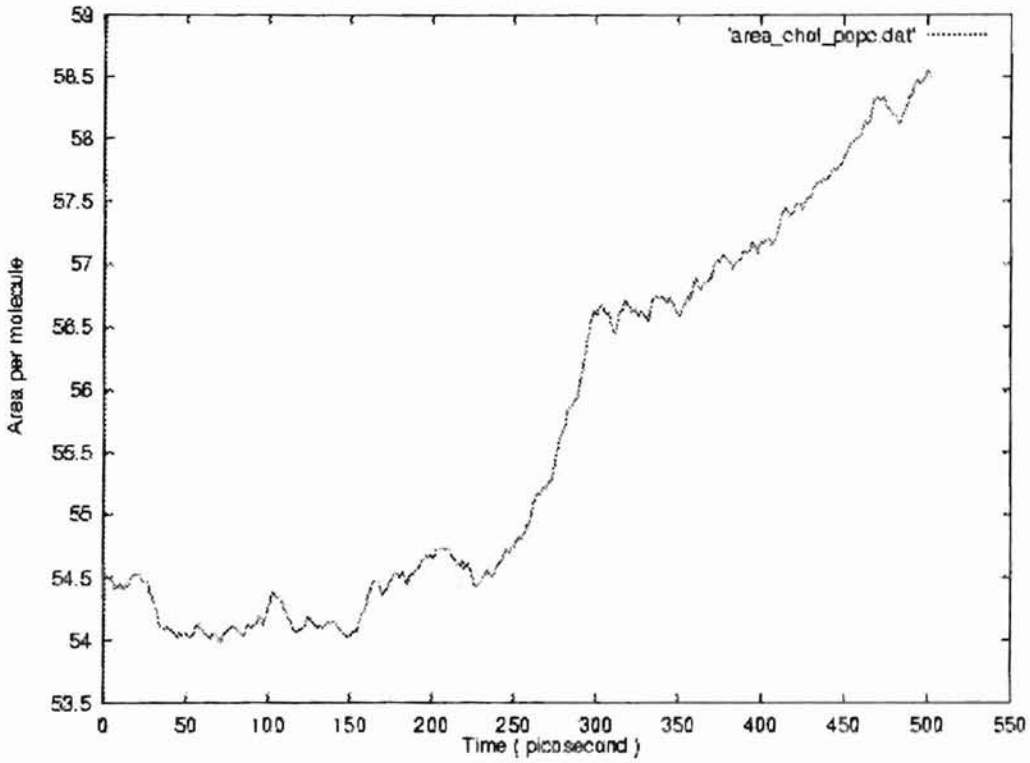

Figure 17: Area per Molecule

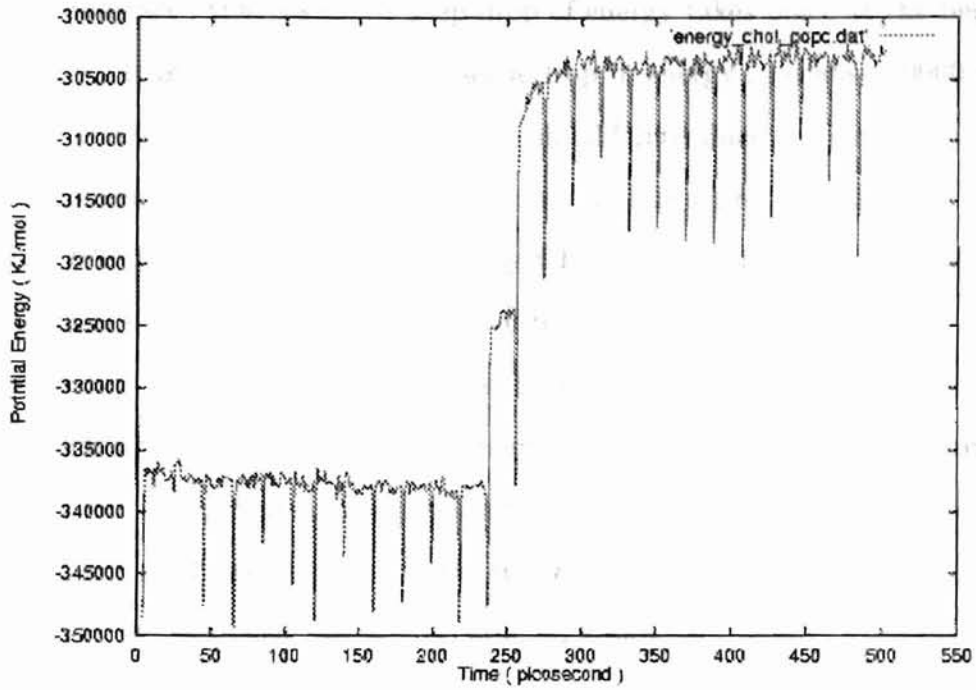

Figure 18: Potential energy versus time one the system is approaching an equilibirium.

equilibration . We will leave such a time-consuming procedure to future research . If we had enough time, we would see a drop in the area per molecule along with the decrease in temperature. Eventually, the area per molecule will reach a platea, which means the realization of equilibration state.

Potential energy

Figure 16 gives us the plot of potential energy versus time . The heating effect is clearly demonstrated in the big jumps in energy curve. They took place at 240th ps and 260th ps where the temperature was raised to 350K and 400K, respectively. The energy curve in the last 300 picoseconds seems to be very flat with slight fluctuations around some value. This again implies the approaching of local equilibrium at the temperature equal to 400K.

It is also clearly seen that a sudden deep drop of energy takes place at the beginning of each cycle . It is a surprise for us to see a drop in energy , because 10000 CBMC steps done at the end of the previous cycle should introduce higher energy due to possible bad contacts between water molecules and headgroups. The simulations on pure POPC and DPPC bilayers done by Chiu and Scott, et al. both showed a "jump" in energy at each discontinuity caused by CBMC . Such a discrepancy between pure phospholipid bilayer and mixed bilayer is really an interesting phenomenon. We attribute such a sudden drop in potential energy to the effect of energy minimization , in which the simulation was done without a heat reservoir so that it caused a decrease in potential energy. Once such a decrease of energy in EM overpasses the increase of energy after CBMC, we shall see a drop in energy plot.

Order parameter profiles

Another important structural data is the order parameters. Figure 17 and 18 demonstrate the order parameter profiles for the longer and shorter hydracarbon chains , respectively.

In Figure 17, there are three order parameter versus carbon number curves. The Lowest curve is the parameter profile for the long chains of pure POPC bilayer. It was obtained from the simulation done by Chiu, Scott, et al (26). In comparison, we plotted the other two curves from the data in our simulation. The middle one corresponds to the POPC long chains within the cutoff radius of one of the 6 cholesterol molecules. The top one represents the POPC long chains out of the cutoff range ($0.5A$) to the cholesterol. As we can see, there are large increases in order parameters for the chains in both positions. This well matches the decrease in the area per molecule.

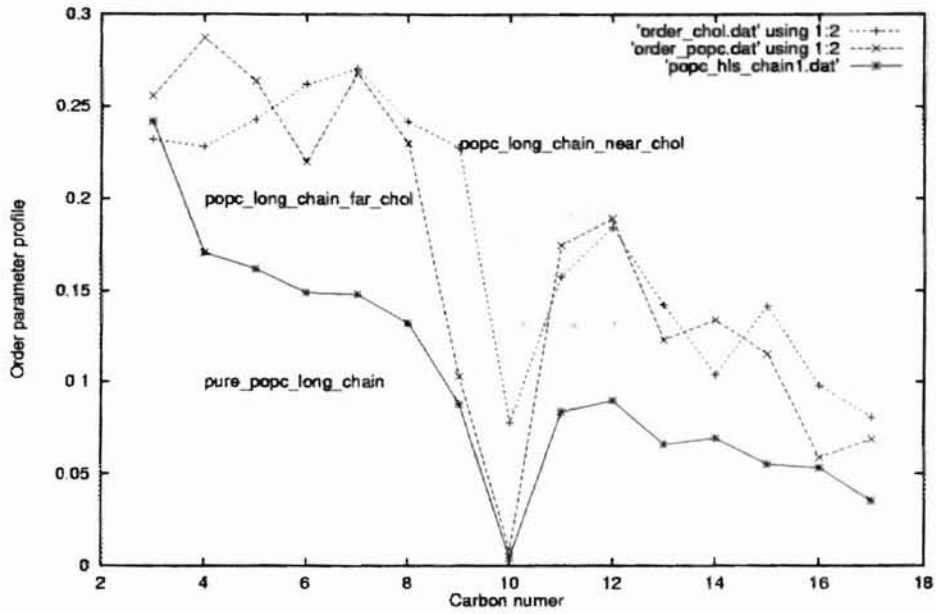

Figure 19: The comparison between the long chains for pure POPC, POPC near CHOL and POPC far away from CHOL.

The H_2 NMR studies of deuterium-labeled phopholipids and ESR studies of spin-labeled lipids (1) conclude that, with the presence of cholesterol in lipid bilayer, an increase in motional order of the phospholipid chains can be seen around the upper and middle portions of the chains, while less change in order parameter happens at the chain tails . The apparent effect on the upper and middle part of the POPC chains can be attributed to the vicinity of the rigid steroid rings , which hinder the motions of chains around them. In contrast, the chain tails near the center of the bilayer are less affected by cholesterol molecules and experience more motional freedom .

Figure 18 shows the order parameter profiles for the shorter chains of POPC. Same as Figure 4, we have curves of the pure POPC , POPC near cholesterol and POPC far from cholesterol shown here. Similarly, we can see larger affects on the motional order of the upper and middle parts of chains whereas less affect on the tails. This is consistant with the situation of long chains. Meanwhile, we see larger increase in

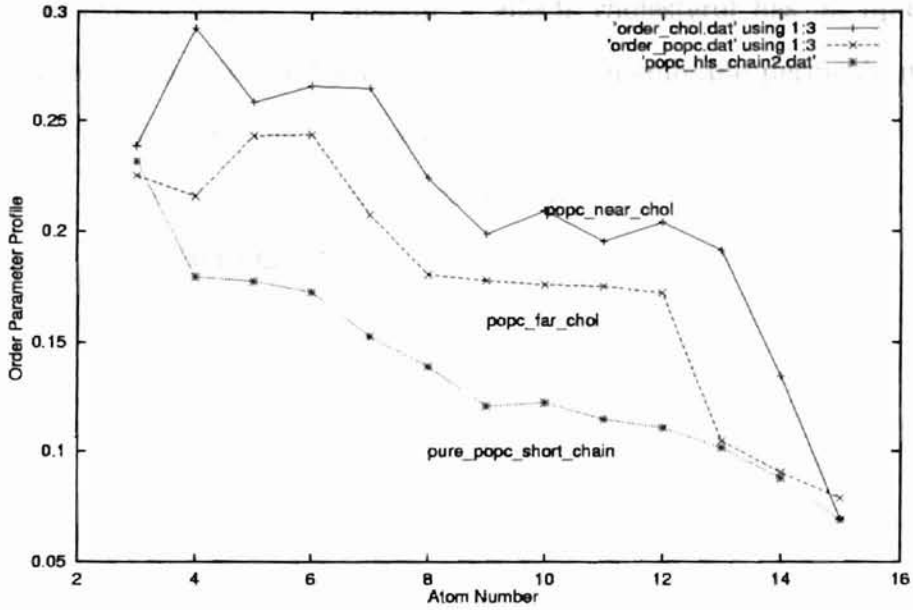

Figure 20: The comaprison between short chains.

the motional order of POPC chains near cholesterol. This is a good match with our theoretical analysis and expectation.

Discussion on POPC with Cholesterol

The simulation clearly demonstrates the increased order parameters on both chains of POPC molecules when mixed with cholesterol molecules. More interesting thing is the larger effect due to cholesterol than we thought. Since few data are available on such a mixed system, we need to do more thorough investigation on the complex bilayers.

The equilibrium obtained in this simulation is just a local one . The structural data in analysis are characteristic of local also. Although they sufficiently prove the validity of our simulation method and results, we still don't have the complete picture of the whole equilibration procedure of a realistic bilayer. Such a goal requires much longer time with the current computation power.

Experimental results suggest us that more effects cholesterol has on lipid bilayers should be investigated. These include the effect on membrane permeability, lateral diffusion, phase transitions, etc. Simulations studies on some topics can involve huge number of physical factors and parameters so that they demand more powerful computing facility and better algorithms.

References

[1] Philip L. Yeagle, "The Membranes of Cells." San Diego, CA: Academic Press, Inc. 1993

[2] H.L.Scott, Eric Jacobsson, and Shankar Subraniam, "Simulations of Lipid Membranes With Atomic Resolution." Computers In Physics, VOL.12, No.4, Jul/Aug 1998

[3] S.J.Singer, G.Nicholson, " The Fluid Mosaic Model for Biophysical Membranes", Science 175, 720 (1972)

[4] E.Gorter and F.Grendel, "On Bimolecular layers of lipids on the chromocytes of the blood," J. Exp.Med. 41 439-443. 1925.

[5] J.D.Robertson. "The molecular structure and contact relationship of cell membranes." Biophysical chemistry. VOL.10, 1960

[6] D.E.Green and J.Purdu, " Membranes as expressions of repeating units," Proc. Natural Acad. Sci. U.S.A VOL.55, 1966.

[7] S.W.Chiu, M.M.Clark, Eric Jakobosson, Shankar Subramaniam, H.Larry Scott, " Application of a Combined Monte Carlo and Molecular Dynamics Method to the Simulation of a Dipalmitoyl Phosphatidylcholine Lipid Bilayer", 1998.

[8] D.P.Tieleman, S.J.Marrink and H.J.C.Berendsen. "A computer perspective of membranes: molecular dynamics studies of lipid bilayer systems", Biochimica et Biophysica Acta 1331, 235-270, 1997

[9] Merz and Roux. "Biological membranes: A molecular perspective from computation and experiment". Birkharser, Boston. 1996

[10] D.P.Tieleman. "Theoretical studies of membrane models: Molecular dynamics of water, lipids and membrane proteins". Universal Press, Veenendaal,Netherlands, 1998.

[11] A.J.Kox, J.Michels and F.W.Wiegel. "Simulation of a lipid monolayer using molecular dynamics." Nature VOL.287, 1657-1666, 1980.

[12] P.van der Ploeg, H.J.Berendsen. "Molecular dynamics simulation of a bilayer membrane". Chem. Phys. VOL.76 3271-3276. 1982

[13] B.Jonsson, O.Edholm, O.Telieman. "Molecular dynamics of a sodium octanote micelle in aqueous solution." J. Chem. Phys. VOL.85 22559-2271. 1986

[14] E.Egbert and H.J.C. Berendsen. " Molecular dynamics simulation of a semetic liquid crystal with atomic detail". J.Chem.Phys. VOL.89, 1988.

[15] W.F.van Gunsteren, W.F.P.Kruger, S.R.Billeter, AE.Mark, A.A.Eising, W.R.P.Scott, P.H.Huneberger and I.G.Tironi.. "Biomolecular Simulation: The GROMOS96 Manual and User Guide", Biomos b.v., Zurich, Groningen, 1996

[16] W.L. Jorgensen and J.Tirado-Rives. " The OPLS potential function for proteins. Energy minimizations for crystal peptides and crambin". J.AM.Chem.Soc. VOL 110 1988

[17] B.R.Brook, R.E.Bruccoleri, B.D.Olsafson, D.J.States,S.Swaminathan and M.Karplus. "CHARMM: A program for macromolecular energy, minimization and dynamics calculations." J.Chem.Soc. VOL106. 1984

[18] Daan Frenkel and Berend Smit, " Simulation of phase coexistence for complex molecules", Computer in Physics, VOL, NO.3 May/June 1997

[19] S.W.Schiu, M.Clark, V.Balaji, S.Subramaniam, H.L.Scott and E.Jakobsson. "Incorporation of surface tension into Molecular Dynamics Simulation of a Interface: A Fluid Phase Lipid Bilayer Membrane". Biophysical Journal, VOL69, 1995

[20] Daan Frenkel, Berend Smit. "Understanding Molecular Simulation: From Algorithms to Applications." Chestnut Hill, MD:Academic Press., 1996

[21] Xaver Daura, Alan E. Mark, Wilfered F. Van Gunsteren, " Parameterization of Aliphatic CHn United Atoms of GROMOS96 Force Field", Jounal of Comput. Chemistry,Vol.19, 535-547, 1997.

[22] R.R. Gabdoulline, G.Vanderkooi, C.Zheng. "Comparison of the structure of Dimyristoyl phosphatidylcholine in the presence an d absence of cholesterol by molecular dynamics simulation". J.Phys. Chem. VOL.100, 1996.

[23] J.F.Nagle, R. Zhang, S.Tristram-Nagle, W.Sun, H.Petrache and R.M.Suter. " X-Ray Structure Determination of Fully Hydrated L phase Dipalmitoyl phosphatidylcholine Bilayer". Bio. Phys. J. VOL.70, 1419-1431.1996

[24] S.Tristram-Nagle, H.Petrache and J.Nagle. "Structure and Interactions of Fully Hydrated Dioeoylphosphatidycholine Bilayer". Bio.Phys. J. VOL.75, 917-925, 1998

[25] Scott E. Feller, Daxu Yin, Richard W. Pastor, Alexander D. Mackerell, Jr. 1997 " Molecular Dynamics Simulation of Unsaturated Lipid Bilayers at Low Hydration: Parameterization and Comparison with Diffraction Studies", Biophy. Journal, Vol.73, 2269

[26] S.W.Chiu, M.M.Clark, Eric Jakobosson, Shankar Subramaniam, H.Larry Scott, "Combined Monte Carlo and Molecular Dynamics Method to the Simulation of Dioleyl and Palmitoyl-oleyl Phosphatidylcholine Lipid Bilayers" ,1999

[27] R.Gabdoulline, G.Vanderkooi, and C.Zheng "Comparison of the Structure of Dimyristoylphopsphatidylcholine in the Presence and Absence of Cholesterol By Molecular Dynamic Simulation", J.hysi. Chem, Vol 100, 1996

[28] D.Frenkel and B. Smit, "Understanding Molecular Simulation: From Algorithms to Applications" . San Diego, CA, USA: Academic Press. 1996

[29] T. J. Mcintosh, " The effect of cholesterol on the structure of phosphatidylcholine bilayers," Biochem. Biophys. Acta 1978

VITA

Xueli Hou

Candidate for the Degree of

Master of Science

Thesis: COMBINED MONTE CARLO AND MOLECULAR DYNAMICS SIMULATIONS OF CHOLESTEROL IN PHOSPHOLIPID BILAYERS

Major Field: Physics

Biographical :

Education: Received Bachelor of Science degree in Wireless Communication from Northern Jiaotong University , Beijing, P. R. China in July 1997. Completed the requirement for the Master of Science degree with a major in Physics at Oklahoma State University in July, 1999.

Professional Experience: Teaching Assistant at the Department of Physics, Oklahoma State University, 1997–1999; Research Assistant at Department of Physics, Oklahoma State University, 1998–1999; Member of American Physical Society, 1997–1998.