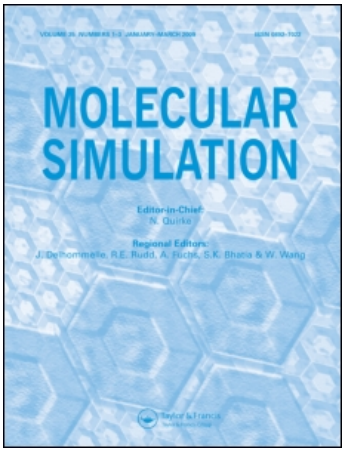

Molecular Simulation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

Lipid Membrane Structure and Dynamics Studied by All-Atom Molecular Dynamics Simulations of Hydrated Phospholipid Bilayers

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To cite this Article Stouch, Terry R.(1993) 'Lipid Membrane Structure and Dynamics Studied by All-Atom Molecular Dynamics Simulations of Hydrated Phospholipid Bilayers', Molecular Simulation, 10: 2, 335 — 362
To link to this Article: DOI: 10.1080/08927029308022172
URL: http://dx.doi.org/10.1080/08927029308022172

Molecular Simulation, 1993, Vol. 10(2-6), pp. 335-362
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LIPID MEMBRANE STRUCTURE AND DYNAMICS STUDIED BY ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS OF HYDRATED PHOSPHOLIPID BILAYERS

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(Received January 1993, accepted January 1993)

The structure and dynamics of phosphatidylcholine bilayers are examined by reviewing the results of several nanoseconds of molecular dynamics simulations on a number of bilayer and monolayer models. The lengths of these simulations, the longest single one of which was 2 nanoseconds, were sufficiently long to effectively sample many of the longer-scale motions governing the behaviour of biomembranes. These simulations reproduce many experimental observables well and provide a degree of resolution currently unavailable experimentally.

KEY WORDS: Molecular dynamics, simulations, lipids, lipid bilayers, membranes, biomembranes

INTRODUCTION

Biomembranes play a central role in a large number of life's processes. Not only do they serve as a barrier to maintain the integrity of cells, they are also the functional environment for a large number of proteins that include receptors, ion-channels, and parts of the immune system. Their amphiphathic nature and distinctive organization provide these biomembranes with the powerful and contrasting properties of low dielectric hydrophobic areas, highly charged hydrophilic regions as well as a distinctive relationship with water. An understanding of these biological constructs is central to an understanding of much of biology.

Computer simulations have proven valuable in studies of proteins and nucleic acids, two of the major classes of biomolecules [1, 2]. More recently, carbohydrates have seen some emphasis [3]. Although approached from several angles in the past, as will be discussed shortly, lipids have not benefited from the same level of theoretical and computational investigation as have the other major classes of biomolecules and are only now enjoying increasing scrutiny.

There is good reason for this lack of study. Physiologically significant phases of membranes usually are very fluid, dynamical structures that are difficult to study even experimentally, at least at the atomic level. For this reason there is little atomic-level structural data such as is available from X-ray crystallography for proteins and nucleic acids. Since many low energy conformations are available to lipids at physiological temperatures, static structures are unrealistic and dynamics plays an important part of the properties and functions of lipid assemblies. Further, the

timescales of the dynamics are long relative to the lengths of simulations practical even in the near past. Also, lipid assembles exist in a number of different phases and usually occur as mixed systems of several components, including proteins, several types of lipids, and ions. Additionally, water is a very important constituent of the structure of biomembranes – in fact lipids are defined by their amphiphilicity, their interaction with water. The character of lipid assemblies, such as membranes, is defined by poorly understood hydrophobic "forces" on one hand [4] and the perhaps only little better understood hydration of the polar headgroups on the other hand [5].

Still, the picture is not entirely bleak. As will be seen, there are many different unifying aspects of these systems that can be used to understand them as a whole. Additionally, simple homogeneous lipid systems of well-defined phase and structure have similar properties to biomembranes, a feature often exploited experimentally.

These systems present a frontier of a complexity perhaps exceeding that of proteins and nucleic acids. Accounting for their dynamical structures and accurately describing the forces holding them together will continue to provide a challenge for computer simulations for some time. However, the intense interest and debate over the structures of these assemblies have prompted many to rise to the challenge of computational study of lipid systems. These studies have ranged in detail, complexity, size, level of approximation, and in the methods that they employed. Most studies have been of monolayer or bilayer structures, although micelles have been the subject of several investigations. Simple amphiphile models have been used to probe the large-scale structure of membranes [6–10]. At the other extreme, conformational analysis has been applied to investigate specific molecules [11–13]. Lattice models have been used for some years to quantitate the properties of the hydrocarbon interior [14–18], as have models employing a rod-like representation of the lipid molecules [19, 20]. Other mathematical and statistical mechanical treatments have also proved informative [21–24]. Landmark mean-field studies have assisted in explaining experimental data [25, 26] as have Brownian dynamics simulations [27, 28] that have expanded from studies of single molecules to larger, more detailed arrays [29, 30]. Studies of larger arrays of alkane chains have expanded on the lattice field approach to understand the hydrocarbon interior [31–38] and the water/lipid interface has been the subject of many studies [39–48]. Berendsen and coworkers pioneered molecular dynamics simulations of essentially complete representations of hydrated lipid layers through a series of simulations of increasing size, length, and level of detail [49–54]. This work has been followed by others of similar detail [55–57].

Recognizing the importance of these systems to cellular function, membrane protein structure and function, and drug action, as well as the need for accurate models to explain and coordinate experimental data, we have developed a program with the goal of obtaining an accurate computational picture of lipid bilayer structure, dynamics, and function. While our initial aim was an understanding of protein-lipid interactions and drug localization and diffusion in and through the bilayer, the many questions surrounding the structure and dynamics of lipid assembles themselves have prompted us to study the lipid systems alone to provide a means of understanding experimental data on these lipid structures. Here we review our efforts in this area and present the results of molecular dynamics simulation on all-atom, fully-hydrated, liquid crystalline phospholipid bilayers. Since the natural time scales of these systems are long, several nanoseconds of simulations

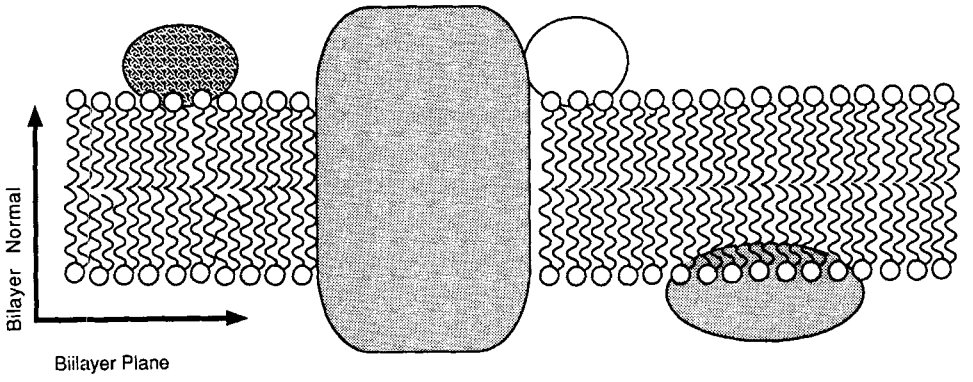


Figure 1 Schematic representation of a cross section of a biological membrane showing the bilayer structure of the the lipid molecules, an integral membrane protein, traversing the bilayer, and peripheral membrane proteins associating with one face of the bilayer and in one case with the integral membrane protein. The orientational axes will be referred to repeatedly. The polar headgroups of the lipid molecules are represented by circles and the randomly oriented hydrocarbon chains by the wavey lines.

will be discussed. The structure and dynamics of the bilayers will be detailed through examination of the results of these simulations and their comparison with those of experiment.

METHODOLOGY

The Models

Lipid molecules are characterized by their amphiphilicity; they contain both distinct polar, hydrophilic parts as well as distinct nonpolar, hydrophobic parts. In water, lipid assemblies, such as bilayers, micelles, and vesicles, form, in many cases spontaneously, with the hydrophilic parts facing the water and the hydrophobic parts aggregating in a hydrophobic core. Although there is some evidence that several different phases of lipids might play roles in biological processes, the overwhelming opinion, incorporated in the fluid-mosaic model of Singer and Nicolson [58], is that biomembranes are composed primarily of bilayers of lipid molecules in a liquid crystalline state (L_α, order in the two dimensions of the bilayer with considerable disorder within the hydrocarbon region) with proteins either loosely associated on the surface (peripheral membrane proteins) or penetrating through and residing within the membrane (integral membrane proteins) (Figure 1). For this reason, our studies have concentrated on bilayers in the liquid crystalline phase which is characterized by considerable disorder in the hydrocarbon region, complete hydration, and a large surface area of the lipid molecules within the bilayer plane. The most common of the lipids are the phospholipids, and one of the most common phospholipids are the phosphocholines. Since dimyristoylphosphatidylcholine (DMPC) (Figure 2) spontaneously forms bilayers in excess water and has been used extensively in experiments as a model membrane, it has been the focus of much of our work.

Impressive studies have been done of biomolecules using a united atom formalism and most other lipid simulations have used this approach for the hydrocarbon

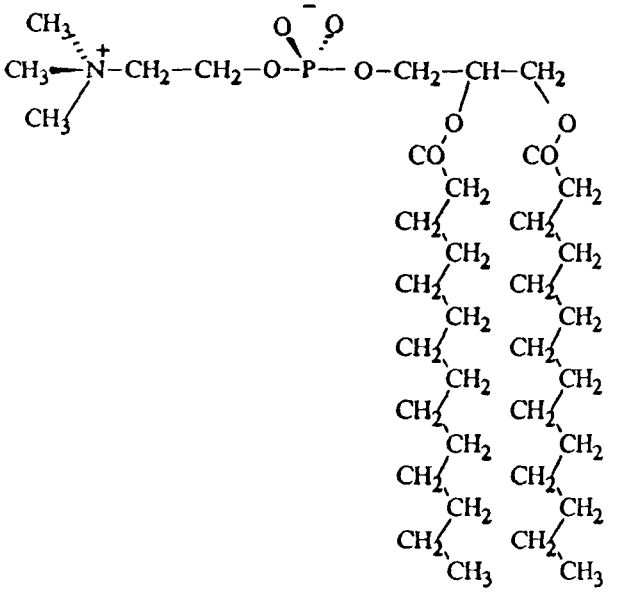


Figure 2 Structure of dimyristoylphosphatidylcholine (DMPC).

chain representation. However, we chose an all atom representation for the lipid molecules including the hydrocarbon chains. Although, this adds a considerable number of atoms over a united atom model, we feel that the explicit inclusion of hydrogens will ultimately be necessary. Since the hydrogens in the polar headgroup region of phospholipids make a considerable contribution to the electrostatics of this part of the molecule, they can not be excluded. Other theoretical studies indicate the need for explicit representations of hydrogens in hydrocarbon chains [59, 60]. Further, a primary goal of this work is the study of packing, lipid/lipid, lipid/protein, and lipid/drug. Other studies of molecular packing have indicated a need for explicit representations of hydrogens and most widely-used force fields now have such a representation. We thought it important to use this approach from the start in order to better understand the characteristics of the resulting models and force fields.

Although there is a massive amount of experimental data on lipid assemblies, there is little atomic-level data with which to build a starting model. Some lipid molecules crystallize in a bilayer arrangement [61]. Unfortunately, these structures are of little use in simulations of physiologically significant phases of lipids. Generally, the crystal structures show the hydrocarbon chains in one, the all-trans, conformation. This is due to the fact that at the low temperatures and low hydration of most of the crystals, the molecules are packed much more densely than in biomembranes where the lateral surface area is usually at least 50% larger than in the crystals. Also, this packing introduces a uniform tilt of the chains relative to the bilayer plane that is not necessarily prevalent in biomembranes.

Still, X-ray scattering, NMR, and Langmuir-trough studies provide information on the macroscopic dimensions of these systems that can be coupled with molecular

structures, speculation about conformation, and model building to provide initial bilayer models that can be refined by simulation. X-ray scattering studies provide the gross dimensions of the bilayers; the thickness and the planar area occupied by single lipid molecules has been determined for a number of lipid/water systems [62]. Further, the level of "full" hydration of bilayers (the amount of water at which bilayer properties cease to change on addition of more water and only water regions continue to swell) is known within a reasonable range [5]. Also, the temperature of phase transitions is well-established for many lipid systems.

Early work by Berendsen and coworkers found boundary effects during simulations of decane bilayers containing 16 hydrocarbon chains per monolayer that were alleviated on quadrupling the size to 64 chains per monolayer [53]. We have found also that DMPC bilayers with 36 chains per monolayer (18 molecules of DMPC), an intermediate value, also do not suffer from boundary effects. Although we have studied considerably larger systems, we found bilayers of 18 DMPC molecules per monolayer sufficient for characterizing bilayer properties. The tradeoff for size is increased sampling of dynamical properties within the same computing time.

Based on these observations, a typical system that we used consisted of a bilayer of 36 DMPC molecules (18 in each monolayer) with lateral dimensions of 34.5 Å by 34.5 Å (66 Å^2/DMPC) solvated with 481 water molecules per monolayer. Although larger systems have been studied, some containing up to 30,000 atoms, systems of this size were found sufficient for characterizing the force fields, determining optimum simulation conditions, and for investigating lipid/lipid, lipid/water, and water/water (near the lipid layers) interactions. Studies have been done using both two dimensional and three dimensional periodic boundary conditions with essentially equivalent results for the lipid molecules. In both cases, the lipid bilayer is replicated to reproduce an infinite bilayer. Three dimensional periodic boundary conditions essentially reproduce a lamellar structure. The third dimension in the two dimensional periodic boundary conditions is treated with repulsive walls to contain the waters. In both cases, the third dimension was carefully treated to provide a density consistent with the mole fraction of each of the constituents of the system [63].

The Simulations

The Verlet algorithm [64] was used with a timestep of 1.0 femtosecond which conserved energy during NEV simulations of lipid crystals, mentioned below. For the bilayer simulations, temperatures of the lipid molecules and water were separately maintained by coupling the system to a constant temperature bath [65] in almost all cases at 320 K, about 20° above the gel to liquid crystalline transition temperature. The water was a flexible SPC potential [66]. Other than the boundary conditions of constant volume, no constraints or restraints were placed on the system. Literally dozens of simulations have been conducted ranging from a minimum of 100 picoseconds to several nanoseconds in order to determine the effects of simulation conditions, equilibration and thermalization procedures, force field modifications, and models.

Potential Energy Functions

A central component for any simulation is the potential energy function that drives the dynamics. It is of particular importance in our studies since they serve not only

to evaluate the models, but also to derive equilibrium structures since no one static structure is available or realistic. Further, dynamics is as important a component of bilayer structure as is atomic bonding. Lipid simulations depend even more heavily on the force fields than do studies of, say, proteins, where the static structures determined by crystallography are of relevance. In order to establish the quality of then-available force fields and parameters for simulations of lipid systems, we conducted simulations of high-resolution crystal structures of small lipid molecules [67]. Although we found some room for improvement, particularly in the parameterization of the phosphate moiety, these tests showed that the potential energy functions for lipid molecules were on the same level of accuracy as those commonly used for proteins. Just as force fields for proteins are constantly being improved, we are currently working on a new generation of lipid force fields not only for phosphocholine and phosphoethanolamine but also for a range of other lipid molecules [68]. Further studies on crystal packing properties of a number of molecules further confirmed the reasonable quality of the Lennard-Jones parameters and atomic partial charges [69]. Also, as we will demonstrate, our simulations reproduce many bilayer properties accurately, another test, albeit long and cumbersome, of the force fields.

The electrostatic interactions were calculated as a sum of pairwise interactions between atom-centered partial point charges using Coulomb's law. In the course of our studies, the charges were determined both from Mulliken population analysis [70] and by derivation from quantum-mechanically determined electrostatic potentials [71, 72]. The great conformational flexibility of these molecules prompted us to investigate the role of conformational change in the electrostatic potential derived charges [73]. We find that charge variation and the subsequent effects on energetics and dynamics can be large; however, we also find that careful attention to and application of the charge derivation and fitting procedures can reduce these effects considerably [74]. Perhaps even more important than charge variation is the method of treating the long range component of the electrostatic force which, over long times and billions of interactions, can have a substantial influence on the properties of the system [75].

RESULTS

Stability of the Bilayers

Stability has been a problem in simulation of many biomolecules and especially so for lipid systems. Run for a sufficient length of time, many protein structures diverge considerably from their starting crystal structures. Similarly, we have been informed of a number of simulations of lipid systems that degraded, dissolved, or otherwise lost integrity after anywhere from tens to hundreds of picoseconds of simulation. Knowledge of these problems prompted us to be cautious with model-building, heating, equilibration, temperature control, and density and also was a determinant in our choice of an all-atom representation of the lipid molecules.

The result has been bilayers that we have found to be surprisingly stable yet responsive. In the course of determining optimum approaches to model building and simulational procedures and understanding and characterizing the systems, we have run literally dozens of simulations with varying models and conditions. They

have been run from hundreds of picoseconds to 2 nanoseconds and have have been subjected to treatment ranging from gentle to rough. Yet, despite considerable fluctuations, during simulations of bilayers and monolayers the lipids layers maintain their integrity. This is despite the fact that during thermalization, bilayer thickness has swelled and shrank by 4–5 Å and temperature swings in various parts of the system have been considerable. Additionally, through several methods, we have substantially altered the conformational properties of the lipid molecules. Yet, when allowed to equilibrate, the systems achieved essentially identical properties through considerable motion of the molecules, including substantial isomerization of the hydrocarbon chains and diffusion of water.

Response and equilibration times varied for different properties. Energetic properties required from 30–50 picoseconds to equilibrate and during thermalization bilayer thickness could vary by several Å over several tens of picoseconds. Torsional isomerization of the hydrocarbon chains from either all-trans or all-gauche to equilibrium values required on the order of 100–200 picoseconds. Convergence of properties from several starting configurations and thermalization and equilibration procedures provided some assurance that the systems had, in fact, come to equilibrium.

Structural and Dynamical Properties

In the following, the phosphatidylcholine bilayer is examined starting at its center, within the hydrocarbon region, and working out through the symmetric monolayers to the headgroup region, the lipid/water interface, and finally out into the solvent (Figure 3). Although small, DMPC (Figure 2) has many features important to bilayer structure. The distribution of the positions of all of its atoms is addressed, however the terminal methyl groups of the hydrocarbon chains receive special attention concurrent with investigations of the nature of the center of the bilayer, where the hydrocarbon chains of one monolayer meet those of the other. Then, the torsional isomerization, interconversion times, and overall tilt of the rest of the hydrocarbon chains are detailed. The consequences of these phenomenon are summarized by comparison to NMR and X-ray scattering data. The orientation of the carbonyl groups of the fatty acid esters is then addressed; their orientation is important in their interactions with water and in the electrostatics of the bilayer interior. It is thought that the glycerol "backbone" serves as a "pivot" for the rest of the molecule; its conformation determines the orientation of the polar headgroup. A key player in the electrostatic potential of the bilayers and in water/lipid interactions is the highly charged phosphate. It forms a strong dipole with the positive quaternary ammonium moiety two carbon atoms distant which itself is known to be a determining factor in bilayer structure [76]. The positions and motions of these groups are addressed. Finally, water/lipid interactions essentially define these amphiphathic structures and our studies of this interface are reviewed.

Bilayer Center and the Terminal Methyl Groups

Except for their attachment to the glycerol, the hydrocarbon chains are free to move. The L_α DMPC bilayer has a substantial amount of room for the chains to assume many different conformations. The lateral surface area of a DMPC molecule in this phase is about 66 $Å^2$ [62], 70% more than in its close-packed,

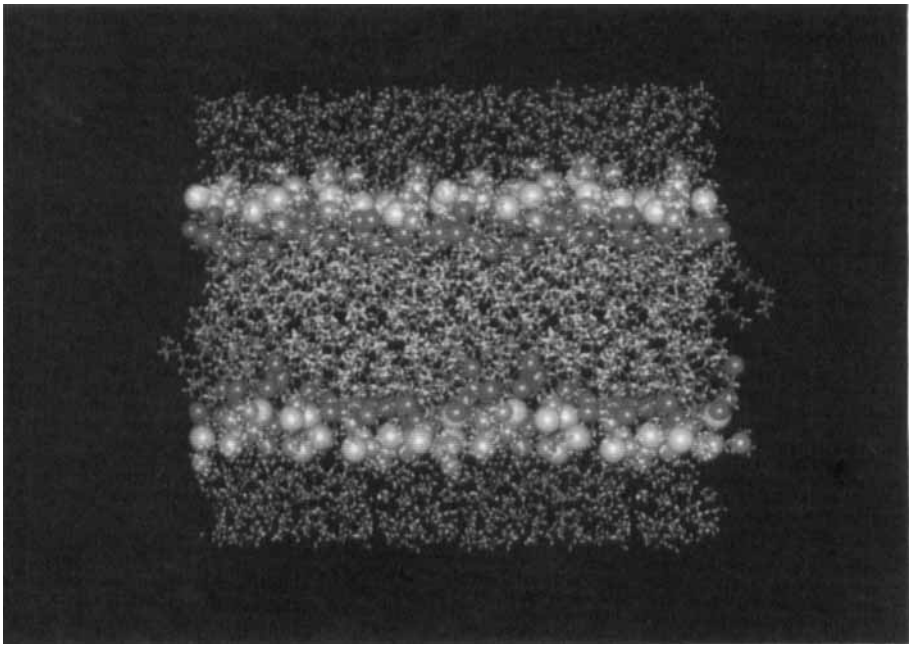


Figure 3 Structure of a simulated filly-hydrated DMPC bilayer after several hundred picoseconds of simulation. Phosphorus (magenta) nitrogen (blue) and carbonyl oxygen (red) atoms are surfaced to show the "headgroup" region. Carbon atoms, green; oxygen, red hydrogen white. (see colour plates)

all-trans crystalline phase. This more than compensates for the reduction in the apparent vertical dimension (17 Å to about 12 Å) during this phase change and results in an overall 45% increase in available volume for the chains. This apparent change in length of the molecule could arise from torsional isomerization, (which effectively reduces the length of the hydrocarbon chains from the maximum-length all-trans conformation), tilt of the chains, or interpenetration of the chains between the monolayers. Both experiment and our simulations have ruled out this last possibility. X-ray scattering shows a distinct lowering of density at the center of lipid bilayers also seen in our simulations (Figure 4) that would not be expected if the two monolayers interdigitated fully [77]. This has been over-generalized to suggest that no interpenetration occurs. However, electron density at the center is not absent, just lower than the neighboring region. Figure 5a shows the distribution of locations of the terminal methyl groups for both monolayers which indicates that they do, indeed, penetrate between monolayers. However, the frequency of this occurrence is relatively low and the peak of the distributions as well as the mean locations of the methyl groups are well within their respective monolayers.

Figure 5 further shows the broad distribution of the terminal methyl groups throughout the bilayer. They range from penetration into the opposite monolayer into the headgroup region of their own monolayer where the density of the methyl groups is found coincident with that of phosphorus, a phenomenon also observed experimentally by X-ray and neutron scattering. This range is a reflection of the

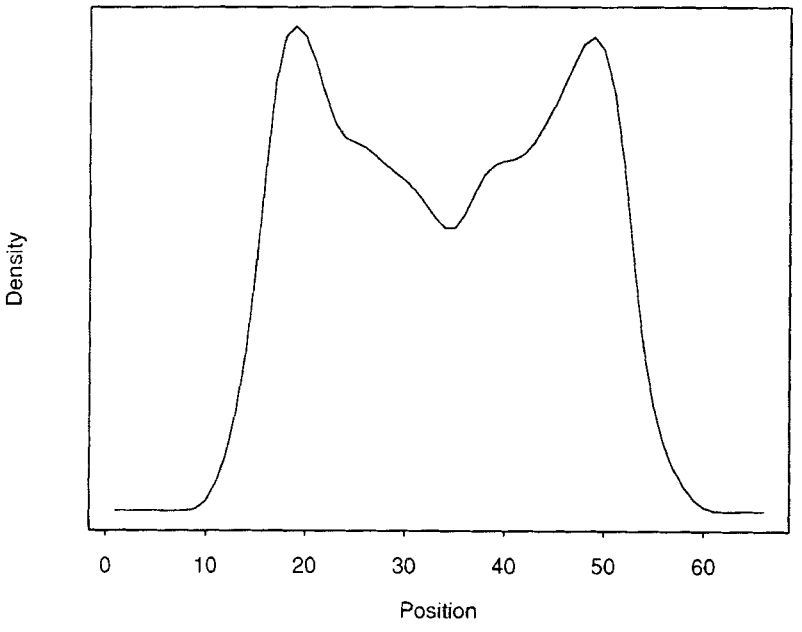


Figure 4 Electron density of a cross section of the simulated bilayer for the atoms in the lipid molecule, water molecules were excluded. Peaks represent the two phosphocholine regions.

substantial disorder found within the hydrocarbon portion of this phase of the bilayer.

Hydrocarbon Chains

Volumes can be written on the torsional isomerization of hydrocarbon chains. The temperature at the L_α phase is enough to warrant considerable chain isomerization; the energy barrier between trans and gauche conformers is 3.5–3.6 kcal/mol and the difference in energy between these two states is about 0.5–0.6 kcal/mol. Coupled with the additional volume per chain within the L_α phase relative to the close packed crystalline phase, this isomerization can occur freely. Numerous attempts to enumerate the number of possible conformations assumable by these chains usually arrive at a value of between 2–5 gauche torsions for a myristoyl chain [78]. Our studies very consistently find an average of between 2.7 and 3.3, although at any particular time individual chains have been observed with between 0 and 8 of 10 torsions in the gauche conformation. The occurrence of gauche torsions tends to be higher toward the methyl terminus than toward the ester linkage and the last torsion, that including the terminal methyl, has a much larger probability of being gauche than does any other torsion. Also, the sn-2 chain (position 2 of the glycerol) has a high probability of having gauche torsions near to the ester linkage. The reason for this is, as we will see, the glycerol tends to be oriented perpendicular to the bilayer plane. If the sn-2 chain is to "hang" perpendicular to the plane also, and parallel to the other chain, all of the torsions cannot be trans (Figure 6). Either

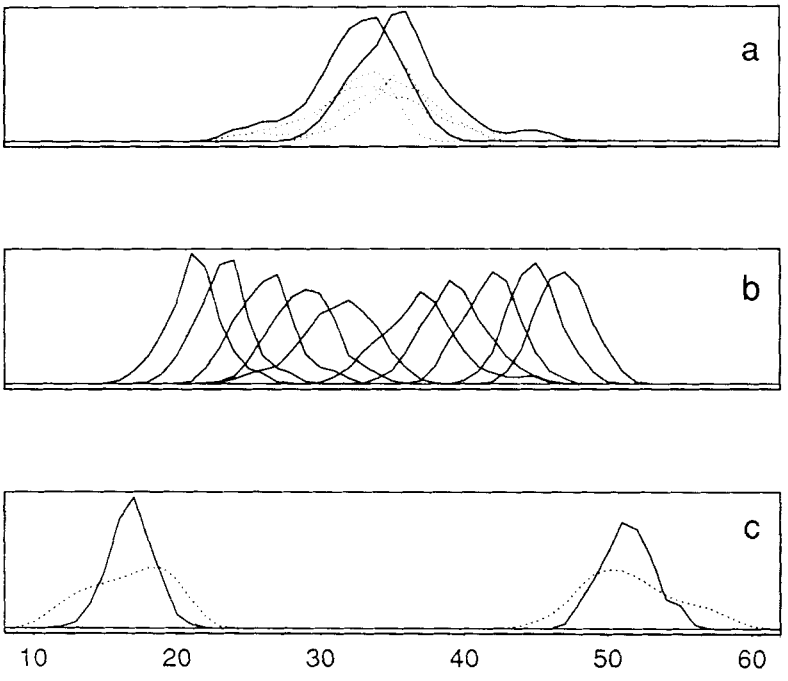


Figure 5 Distribution of atoms relative to the bilayer normal. The center of the bilayer is at approximately 34 Å a. the terminal methyl groups of the two monolayers (solid lines, averages over the two chains of the DMPC molecules; dotted lines, the distributions for the methyl groups of each chain alone showing the inequivalence of the two chains) b. every other carbon atom in the hydrocarbon chains for both monolayers. c. Phosphorus (solid) and nitrogen (dotted).

one torsion near to the ester linkage must be gauche or several in this region must combine to give the same effect. This inequivalence of the two chains is seen experimentally as well as in our simulations [79, 80].

Considerable speculation has surrounded the possibility of "kinks" in the chains [81]. A kink is a gauche^{+}-trans-gauche^{-} arrangement and serves to keep the chain perpendicular to the plane of the bilayer; the effect of the second gauche compensates for that of the first, which otherwise would have caused the chain to point parallel to the plane (as in Figure 7). Kinks maintain the cylindrical nature of the lipid molecule that is seen in the all-trans conformation. Our simulations show, on average, from .5 to 1 kink per chain, very close to experimental and theoretical estimates [78]. We see a slightly higher probability that two gauche torsions in one chain will be at the i and $i + n$ with $n = 2$ position than when n is any other value, indicating the favorable nature of this conformation. Also, when multiple torsional changes between trans and gauche occur within a chain within a short period of time, the probability is over twice as great that the changes will occur at positions i and $i + 2$ than for any other combinations. This indicates that changes in the torsions involved in a kink occur in a concerted manner. It has been speculated that such correlation might occur because it would maintain the overall cylindrical conformation of the hydrocarbon chain. Otherwise, loss of a gauche

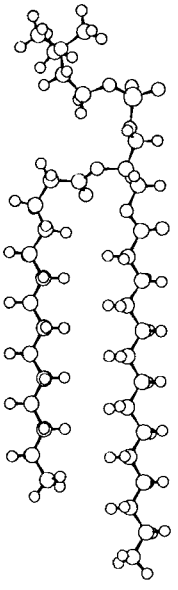


Figure 6 One conformer from the crystal structure of DMPC showing the bend in the sn-2 chain.

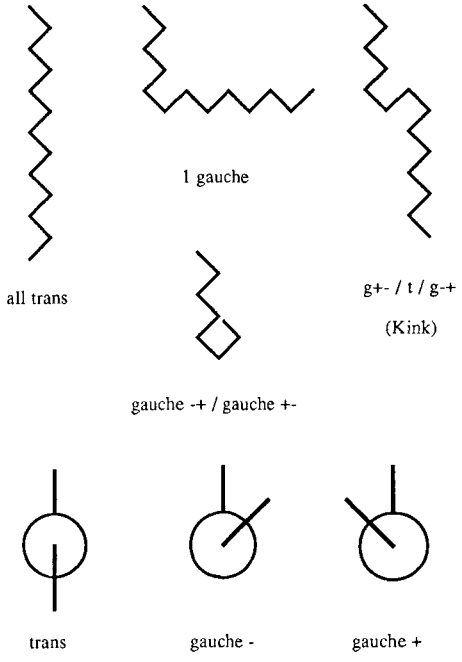
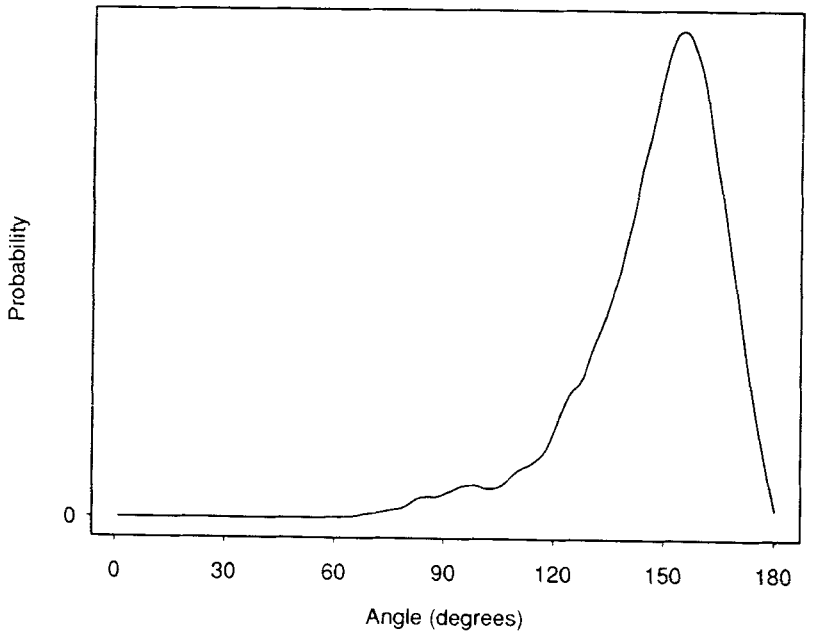


Figure 7 Features of the conformations of the hydrocarbon chains, including the effects of a gauche torsion, a "kink", and a forbidden $g^{\pm}$-$g^{\mp}$ combination.



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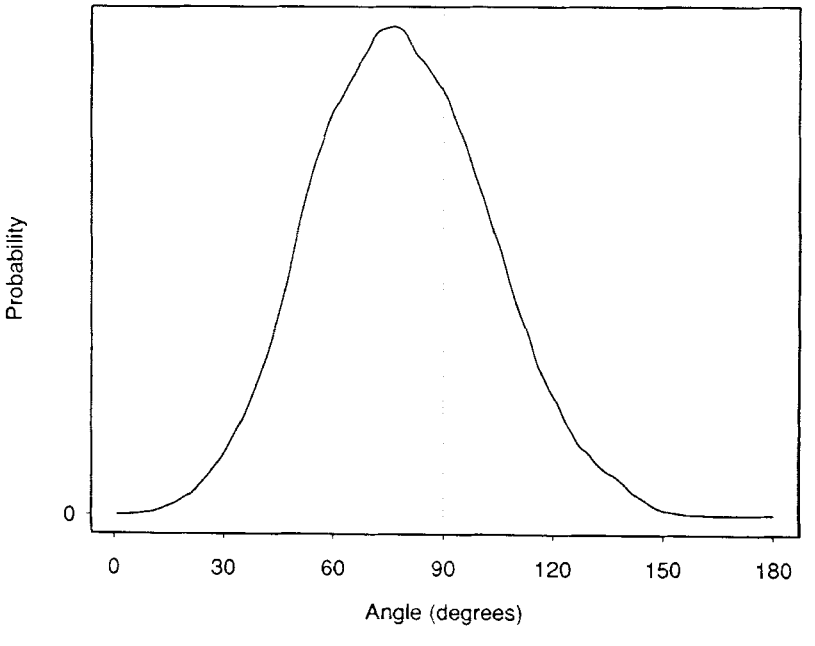

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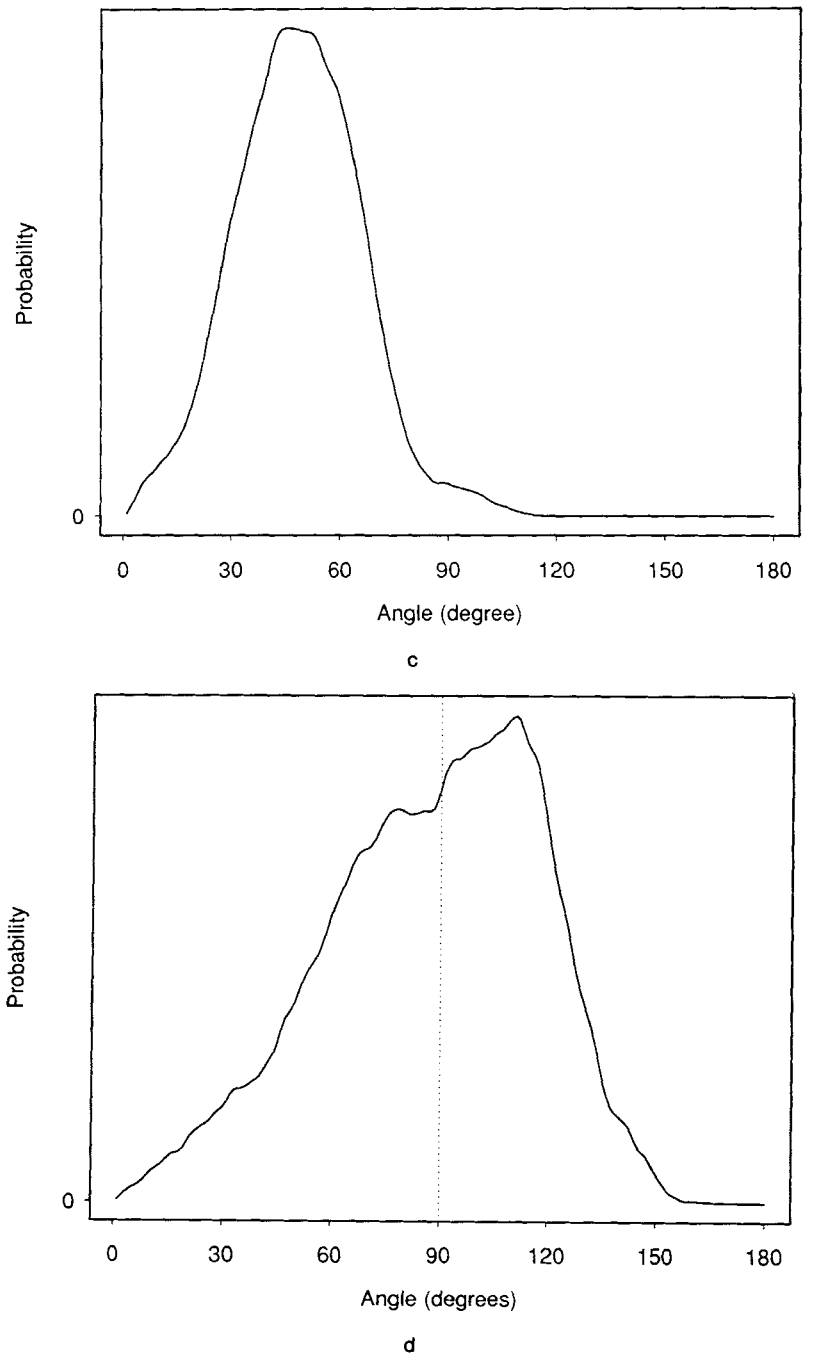


Figure 8 Distribution of the orientation of various vectors with the bilayer normal (0°, pointing from hydrocarbon to water): a. the tilt of the hydrocarbon chains b. orientation of the carbonyl (C to O) vector, c. vector of the glycerol backbone, d. the phosphorus-nitrogen vector.

conformation in one torsion would require that the previously cylindrical conformer now project at a substantial angle from the rest of the chain, possibly requiring the reorganization of neighboring chains.

Pressed by a need to simplify, early studies to enumerate the chain conformations considered a $g^{\pm}$-$g^{\mp}$ arrangement at positions i and $i + 1$ to be forbidden due to steric overlap of the hydrogens on the adjacent methylene groups (see Figure 7). The strained nature of this conformation is easy to verify using molecular models. However, our simulations utilizing flexible bonds and angles indicate that it does, in fact, occur, although infrequently (10% of the i and $i + 1$ gauche-gauche conformations). This small but significant contribution is missing in many calculations of the partition coefficient for estimating the entropy of the hydrocarbon region.

Experiment indicates that the rate of interconversion between trans and gauche is as fast as 10 picoseconds near the methyl terminus and around 100 picoseconds higher in the chain [82], although some estimates range as high as 1 nanosecond. During simulations of 500–1000 picoseconds, all hydrocarbon chain torsions interconvert at least several times and some as many as 30 times. Averaged over all of the torsions, the time between interconversions converges to 45 picoseconds, although individual values range from about 8 to 200 picoseconds. As might be expected, the range of interconversion tends to be higher toward the terminal methyl, although considerable sampling of states occurs throughout the chain.

The internal disorder of the bilayer is not caused by torsional isomerization alone. In addition, although generally oriented perpendicular to the bilayer plane, the hydrocarbon chains and the lipid molecules as a whole can tilt. Figure 8a, the distribution of tilt of the chains, shows a wide range of orientations and that tilt can be considerable. A few chains might be perpendicular to the bilayer plane, however there are some that are almost parallel to it. An average value is about 30 degrees from the perpendicular.

Torsional isomerization and chain tilt promote disorder by distributing the atoms of the chains throughout the thickness of the bilayer. This distribution was shown for the terminal methyl groups, previously. Figure 5 (b and c) shows similar plots for many of the other atoms, also. This result is very similar to those of neutron scattering of labeled lipids. Progressing down the hydrocarbon chain, from glycerol to methyl, the mean positions of the atoms in the chain progressively approach the center of the bilayer. However, their distributions are broad and overlap a great deal. Furthermore, the atoms farther down the chain have progressively greater ranges than those nearer the ester linkage, culminating in the great range of the terminal methyl groups.

At the level of the individual molecules, a large number of distinct conformations is observed. These range from essentially cylindrical to some that are quite splayed as a result of uncompensated gauche torsions and chain tilt (see Figure 9). As noted previously [29, 50], this results in a considerable amount of entangling between chains within each of the monolayers; a hydrocarbon chain of one lipid molecule can impinge on another 2–3 molecules away. This is a different picture from the view of vertical, discrete cylindrical lipid molecules packed in a layer, although the cylindrical interpretation might be a reasonable average structure over long time periods. It has interesting implications for studies of lipid/protein packing, since it implies that the lipids packed around a protein could be at some distance from the protein itself.

The order within the system can be measured by NMR and converted to an order

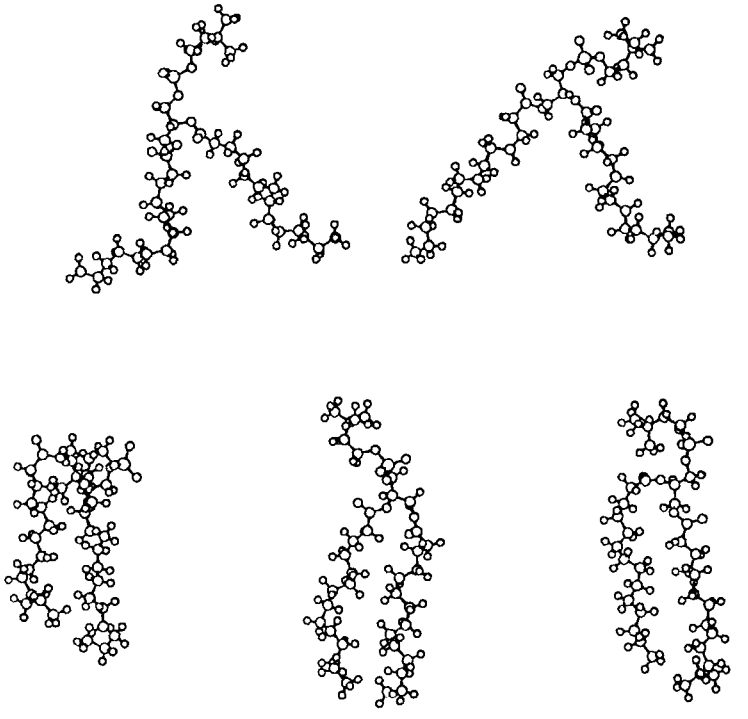


Figure 9 A small sample of conformations sampled by the DMPC molecules during the simulations. They were chosen because they demonstrate the largest (about 20 Å) and smallest distances sampled between the methyl groups, however, other conformational differences are observed, also. Note that the methyl groups can be separated by chain tilt as well as gauche torsions along the chain. Different headgroup conformers are seen, also. The choline group points into solvent in two of these (upper left and lower middle). The lower left conformer is quite compact and its choline moiety is bent down into the hydrocarbon region.

parameter for each of the methylene units [82]. The values of this number range from +1.0, which indicates that the chain is all trans and perpendicular to the plane to −.5, indicating that the chain is all-trans and parallel to the plane. The intermediate value of 0.0 indicates that the groups have an overall random orientation. Figure 10 compares the values from our simulations and NMR studies [83]. Not only are the profiles virtually identical, but also the actual values can be predicted almost quantitatively. We have found that the order parameters tend to be sensitive to simulation conditions. They have also been found to be sensitive to the experimental conditions and can change by 50% with a 20–30° change in temperature [84].

Moving out of the hydrocarbon region of the bilayer, the carbonyl groups of the fatty acid ester linkages are encountered, the first of the polar atoms and the first real interface to the water region. It has been suggested that the carbonyl oxygen points toward the water layer in order to maximize hydrogen bonding [85]. The simulations show that the average position of the oxygen atoms is always about .5 Å closer to the water than is that of the carbon atoms. This is further confirmed

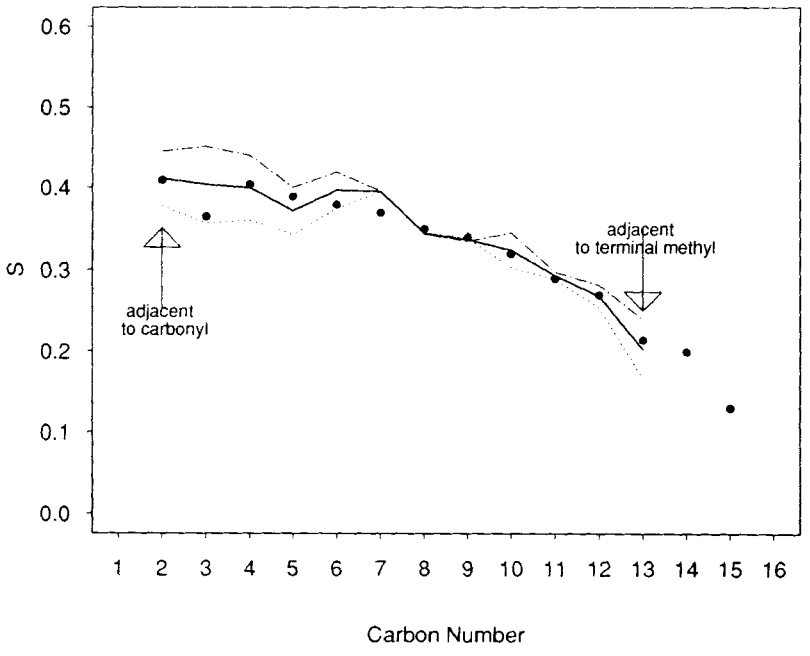


Figure 10 Comparison of calculated order parameters with experiment. The lines are from the simulation: dotted and dash-dot, the two different hydrocarbond chains, solid, average of the two chains. Dots are experimentally determined for DPPC.

by the distribution of the angle of the carbonyl with the plane of the bilayer which is fairly broad but peaks at about 30 degrees to the plane and has an average position about 10° from the plane (Figure 8b) with the oxygen pointing toward the water region. During the simulations, the carbonyl oxygen atoms hydrogen bond with (usually one, sometimes two) waters more than 50% of the time. Some speculate that this orientation of the carbonyl group, with the partial negative charge of the oxygen atom pointing out and the partial positive charge of the carbonyl carbon pointing in, could contribute to a positive potential at the center of the bilayer [85].

Glycerol

The glycerol fragment serves as the alcohol for the two acyl ester and the phosphoester linkages. It sits at the frontier to the water region and is substantially solvated. As we discussed, the glycerol is considered to be the pivot point for some of the rigid motions of the esterifying acids, the polar headgroup and the two hydrocarbon chains [86]. As previously mentioned, it is considered to orient approximately perpendicular to the plane of the bilayer [79] which is borne out by Figure 8c. The orientation of this group, as defined by the *C*1-*C*3 vector, show a broad distribution of this angle relative to the bilayer normal with a most probable value of about 60 degrees to the bilayer plane.

This portion of the molecule is conformationally mobile but its conformations are strongly influenced by repulsion between the electronegative oxygens on the

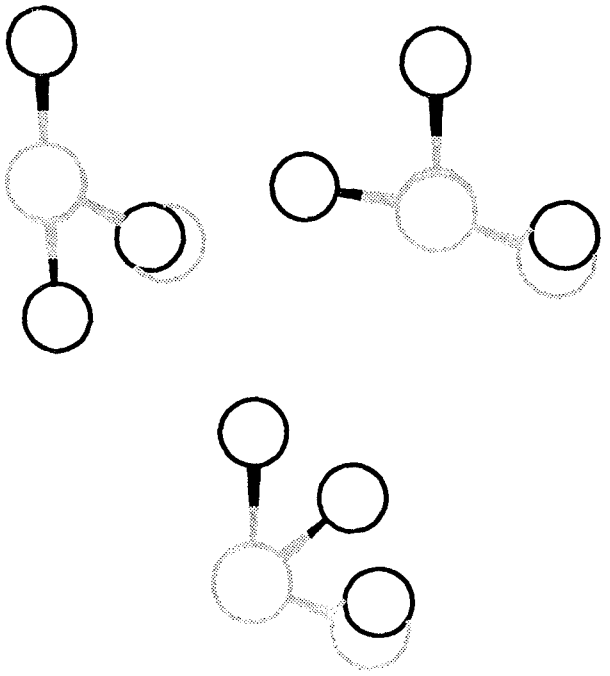


Figure 11 Conformation of the glycerol backbone showing the two favored conformations (top) and the one that is never seen (bottom) siting down the carbon-carbon bond closest to the phosphate. Oxygen atoms are represented by the darker lines, carbon atoms by the lighter lines.

neighboring carbons as well as the nearby negative phosphate group. In particular, the torsion angle about the carbon-carbon bond closest to the phosphate assumes only the g^+ and trans conformations in both crystal structures and in our simulations. This maintains the oxygen atoms on the three glycerol carbons at the maximum possible distance whereas a g^- would place the three electronegative oxygen atoms in very close proximity (Figure 11) [87]. This conformational preference is additionally reinforced by the fact that the other carbon-carbon bond also assumes only the g^+ and trans conformations, which keeps the chains oriented toward the center of the bilayer. These preferences are primarily due to intramolecular interactions and seem little affected by the environment, since they are seen in the crystal state, in *in vacuo* simulations of single lipid molecules [88], and in our bilayer simulations.

Although the two carbon-carbon torsion angles in the glycerol switch states during the simulations, their residence times are among the longest seen in these simulations and can be on the order of several hundred picoseconds.

Polar Head-group

The strong charges, electronegative atoms, and solvation of the the hydrophilic, zwitterionic, phosphocholine "headgroup" are in marked contrast with the hydro-

phobic, neutral hydrocarbon chains. The headgroup contains a strong dipole by virtue of the positively charged ammonium group about 4–5 Å from the negatively charged phosphate. Although this interacts strongly with the water, it would also be expected to interact with the dipoles presented by the other lipids, also. The consequence of this can be seen in the angle that the vector from phosphorus to nitrogen makes with the bilayer normal. Although this vector can range from almost 0 degrees (which means that the ammonium moiety is pointing out into the solvent) to values greater than 90° (which means that the ammonium is pointing into the hydrocarbon region) the average value of this angle is 90°, meaning that the dipole lies in the plane of the bilayer (Figure 8d). That the glycerol serves as the pivot point in this molecule is borne out by the distributions of the phosphorus atom and nitrogen atoms within the bilayer thickness (Figure 5c). The nitrogen has a greater range than does the phosphorus and appears to be at the end of a lever pivoting from the glycerol. Of the three possible orientations of the dipole, pointing out of the plane, into the plane, or parallel to the plane, the last option is clearly favored as shown by simulation and experiment [86]. This makes sense energetically because it allows for the charge cancellation as seen by Berendsen and coworkers [51]. There has been some discussion about the orientation of this vector, since crystal structures show up to a 30° tilt of the angle relative to what might be considered to be the bilayer "plane" in these crystals and some tilt is seen in some bilayers of low hydration. In these systems, the lipids are packed much more closely and move less than in fully-hydrated, physiologically relevant phases. They pack in such a way that the positive and negative regions of different molecules reside near each other and compensate for each other. However, for fully hydrated systems, the average position of this vector is well-established to be within the bilayer plane [89].

The variability of this vector has some important consequences for the electrostatics and solvation of the bilayer. As the *P-N* vector swings out of the plane, it presents its positive ammonium group as a diffuse positive sphere beyond the strong negative potential of the phosphate group. In that position, as we will see, the ammonium is completely solvated. In contrast, as the vector swings the other way, the ammonium group invades the hydrophobic hydrocarbon chain region and presents a positive charge to this low dieletric region which already has a positive potential due to the orientation of the carbonyl groups. With the ammonium in this position, the water neighboring the bilayer now sees a potential effectively more negative due to the negative potential of the phosphate. For the ammonium to move in this way requires the loss of some water of hydration. This is shown by a plot of the water coordination number (Figure 12) that shows a bimodal distribution due to those ammonium groups that project into the water and become fully hydrated and those that are in the plane of the bilayer or project into the hydrocarbon region and lose some of the hydration. The penalty for this loss may not be as great as one might think. Although the ammonium carries a positive charge, the charge is diffuse and is spread out over the nitrogen, three methyl groups, and a methylene. Additionally, this quaternary amine presents no groups for hydrogen bonding. The enthalpy of the choline/water interaction is certainly considerably less than that of the phosphate/water interaction which commands a much more ordered water environment (Figure 13a,b), and preliminary calculations indicate that the choline/water interaction energy is close to that of water for itself [90]. Although an ion, the energetic implications of desolvation might not be severe and might be more than compensated for by favorable entropy of desolvation.

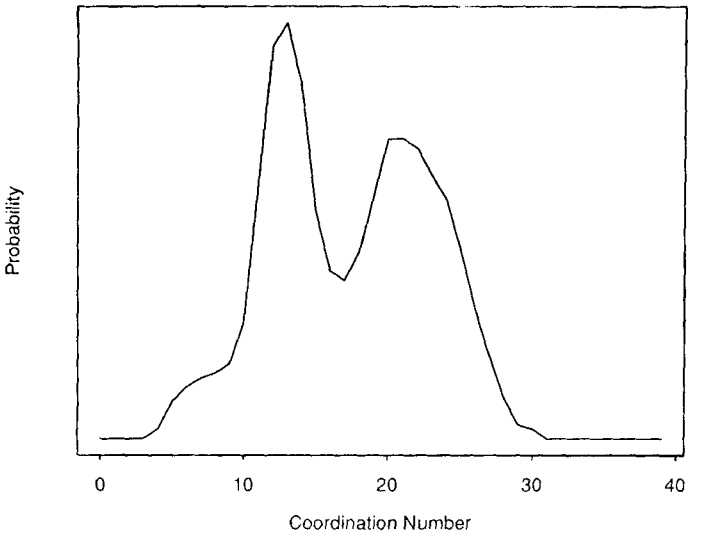
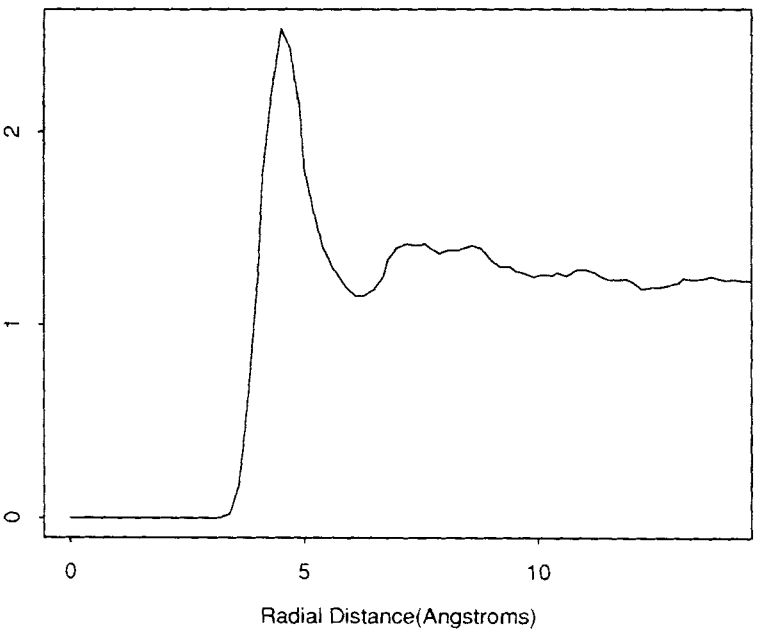


Figure 12 Coordination number of water oxygen atoms about the choline ammonium group. The bimodal distribution is due to two populations of the ammonium group, pointing out of the bilayer and completely solvated, or into the bilayer and only partially solvated.

The dynamics of the headgroup is quite varied. A number of different conformations are available to this group, although some torsions are restricted. From our simulations, we see that the restricted torsions can be explained based on steric factors, although explanations based on electrostatics have been proposed. Most of the torsions along the "backbone" of this group switch between low energy rotamers fairly freely during the simulations. Residence times for a particular rotamer vary from tens to many hundreds of picoseconds; however, a typical value is several hundred picoseconds. NMR studies of the phosphate group suggest that the torsions about the phosphorus-oxygen bonds vary on the order of 400–700 picoseconds [91], in good accord with our results which also show long residence times for these particular torsions. The motion of the headgroup as a whole, determined by the autocorrelation times of the P-N vectors, is very long in most cases (greater than 500 picoseconds), although there are exceptions. This suggests that at least some of the more rapidly varying torsion angles within the headgroup change in concert to create crankshaft motions that maintains the overall orientation of this group.

The dynamics of the lipid molecules as a whole can be probed by their lateral diffusion within the bilayer plane. Fluorescence recovery after photobleaching studies provide values for DMPC at 45°C of about 10 to 12 $\times$ 10^{-8} cm^2/sec [92] in good agreement with those of 9.5 $\times$ 10^{-8} cm^2/sec determined from several hundred picoseconds of simulation at 320 K. There is some indication that these values might, over long time periods, be sensitive to conditions of the simulation.



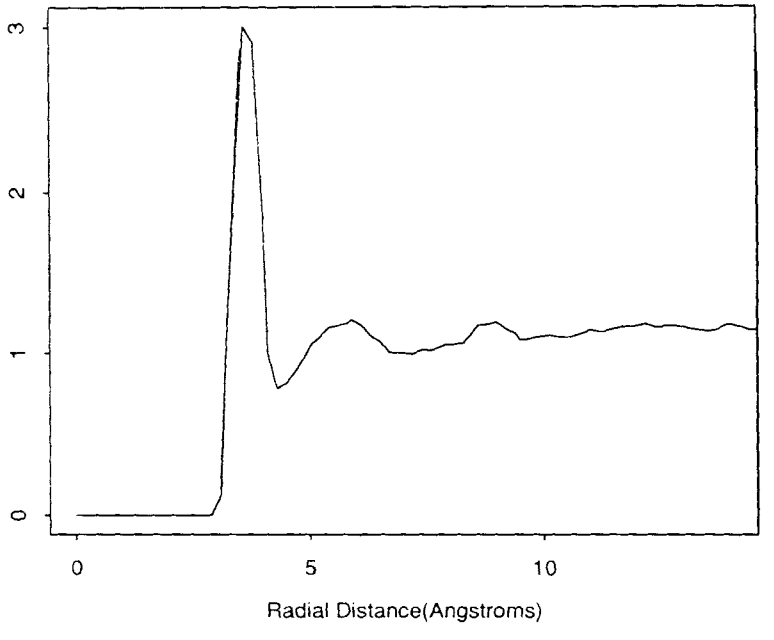


Figure 13 Radial distribution functions of water about a. the ammonium group and b. the phosphate.

Water/Lipid Interface

We have examined the lipid component of the bilayer, from hydrocarbon chains to glycerol to phosphate and ammonium. Briefly we have examined the intimate water-lipid contacts. Another important component of lipid assemblies is the solvating water, a region of considerable interest and experimental study [5, 93–98]. This region is tricky to study, both experimentally and by simulation. It is governed by strong electrostatic forces, hydrogen bonding, water structure, and the dynamics of both water and lipids. We have studied "lamellar" water between lipid bilayers, but have also studied monolayers at the "air"/water interface using large bodies of water in order to free our observations of the effects of one lipid layer from the perturbing effects of the other. The presence of a second monolayer masks some of the effects of the lipids on the water structure. Through exhaustive simulations of this region, we are confident in saying that results are strongly dependent on the conditions of the simulation and that long range forces on the order of greater than 20 Å play a role in the properties of this region [75]. The unique orientation of these systems expose the effects of the long-range forces clearly, however, it is likely that these same perturbations will occur in simulations of other solvated biomolecules. Recent observations by Schreiber and Steinhauser of the effects of long range forces on the dynamics of solvated peptides also show the effects of omission of forces beyond distances even as large as 14 Å [99].

Electrostatics of the Membrane/Water Interface

It is interesting to speculate about the approach, interaction, and diffusion of small molecules, peptides, and proteins to, with, and through, lipid membranes. Although continuum approaches, such as Gouy-Chapman, apparently provide an accurate macroscopic view of many of the properties of such interactions, the importance of individual hydrogen bonds and electrostatic interactions should not be ignored. For example, as noted, the polar head group can present its ammonium group either out into the solvent or else partially embedded in the hydrocarbon region. The phosphate group, however, being on what might be considered the immobile end of a lever, moves much less. As the positive ammonium varies in position, a molecule approaching the membrane surface from the aqueous region would "see" very different views. Figure 14, the electrostatic potential of the lipid surface as calculated by the Poisson-Boltzman method [100], illustrates this. The concentrated negative charge of the phosphate group, as well as the partial negative charges on the glycerol group, impart a strong, far-reaching negative field to the bilayer, punctuated by the more diffuse positive spheres of the ammonium group. These positive spheres can move above and below the broader negative field. In addition, they can move parallel to the plane. Coupled with the number of possible orientations and motions can provide a large number of different motifs to an approaching molecule, such as a protein or peptide hormone. It is interesting to speculate whether different proteins might recognize different motifs. Additionally, although ours is a homogeneous system, most biomembranes are heterogeneous, which adds additional complexity and additional possibilities for specific interactions.

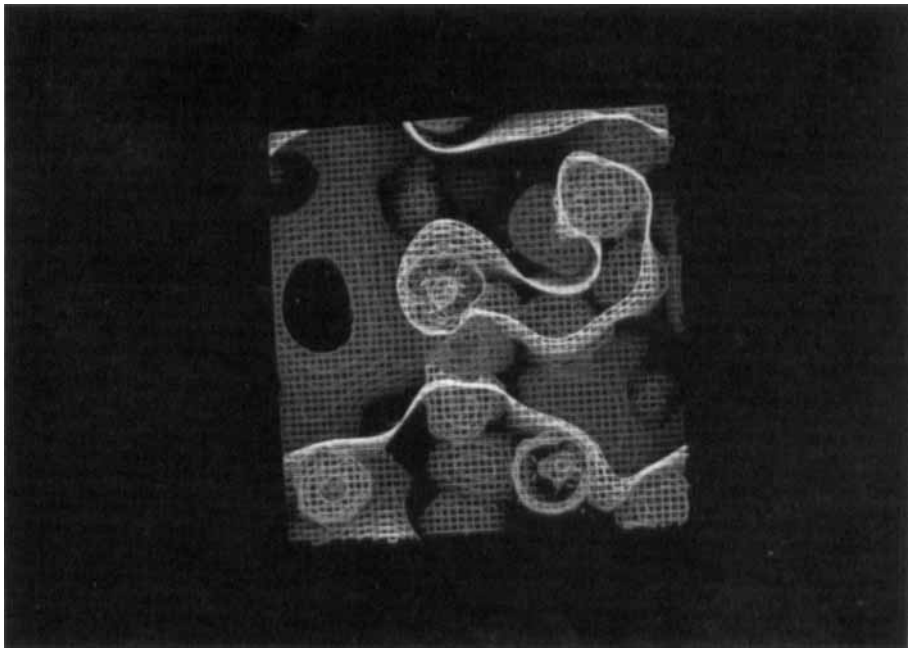


Figure 14 Poisson-Boltzmann plot of the electrostatics of the bilayer surface looking down onto the surface. Blue represents 1 kT contours, red, −1 kT, green 0.0 kT. The blue balls are the positively charged choline ammonium groups. (see colour plates)

CONCLUSIONS

We hope that this monograph provides a detailed insight into the atomic level structure and dynamics of phospholipid bilayers. It provides yet more evidence that computer simulations and models provide not only a means of explaining and coordinating experimental data, but also a way to better understand the details of that data.

The simulation of lipid bilayers of realistic size and for realistic times, even using all-atom models such as we describe here, are now approachable. The study of the natural time scales of these biological structures require long simulations and their necessary large size adds to the computational demand. However, the increasing availability of computing resources makes these simulations ever more feasible.

It is rewarding to find that relatively simple potential energy functions and classical molecular dynamics simulations can reproduce so many structural and dynamic properties so well. The results of the studies presented here, as well as that of the rapidly increasing number of related studies, provide some confidence that simulation of lipid bilayers can provide physically realistic results. Although the bilayers themselves require more study, the success of the simulations to-date opens up a wide range of possibilities for computational study of important biochemical processes. These include the effects of the membrane environment on peptide

hormone conformation, structural effects of lipids on integral membrane proteins, and transport of small molecules across lipid bilayers. The nature of protein/lipid packing is of some interest in understanding the structure and function of integral membrane proteins such as receptors and ion-channels. An understanding of the perturbation in the bilayer structure caused by the presence of proteins will no doubt aid in understanding local phase changes, membrane fusion, and communication and interaction of proteins within the membranes. Preliminary studies of protein/lipid interactions in other laboratories have been exciting [38, 52, 101] and we have expanded our studies to include protein/lipid interactions (Figure 15). Simulations are also in progress to study the diffusion of small molecules and their effects on lipid structure and dynamics, which are important to an understanding of drug transport and anesthesia. Hopefully, carefully planned simulations will help to circumvent the difficulties posed by the long time scales of these phenomena. Additionally, the effects of unsaturation within the hydrocarbon chains, as well as study of lipids other than the phosphocholines, such as the phospatidylethanolamines, are underway.

However, our studies, and others like them, represent only the first of what must necessarily be a long series of steps in the computational study of biomembranes. These structures are complex and heterogeneous, consisting of many kinds of lipids, normally a substantial amount of protein (both peripheral and integral, some perhaps having a structural function), carbohydrates, ions, and, of course, water. Additionally, although the L_α phase of the bilayer appears to be the one most relevant phase and structure for biomembranes, experimental evidence supports speculation that other phases in addition to the L_α and structures other than the bilayer might play important roles in biochemical processes such as membrane fusion. Clearly there is much to do and the field of simulation of lipid systems is rich with a complexity and diversity that could easily rival that of proteins.

Acknowledgements

Howard Alper played a pivotal role in the studies of the water/lipid interface as well as studies of phosphatidylethanolamine and unsaturation. Donna Bassolino also played a key role in the water/lipid studies and as well as in the studies of small molecule diffusion. Malcolm Davis generated the Poisson-Boltzmann plots of the electrostatic potential. My gratitude goes to Jiri Novotny for his support in this work and to all four of the above for a careful review of this manuscript. Thanks to Richard Shaginaw, John Stringer, and Richard Gopstein of the Bristol-Myers Squibb High Performance Computing Center who play key roles in our work by orchestrating our Cray Y-MP and Silicon Graphics computing network. Portions of this work were initiated while the author was resident in the Laboratory for the Structure of Matter of the Naval Research Laboratory and he would like to thank K. Ward and J. Karle for their support. He would also like to thank A.T. Hagler and P.A. Kollman for valuable discussions and encouragement.

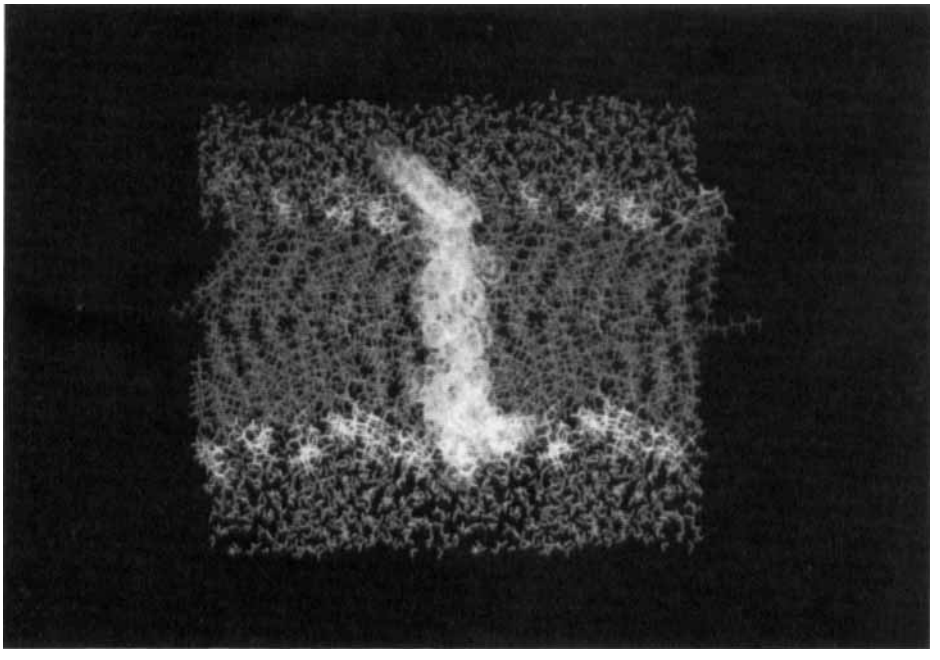


Figure 15 Membrane spanning alpha helix of glycophorin, an abundant erythrocyte protein, in a DMPC bilayer. (see colour plates)

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