Molecular Dynamics Simulation Studies of Interaction of Amphiphilic Molecules with Lipid Bilayers

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MOLECULAR DYNAMICS SIMULATION STUDIES OF INTERACTION OF AMPHIPHILIC MOLECULES WITH LIPID BILAYERS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Mechanical and Industrial Engineering

by

Jieqiong Lin
B.S., Beijing Jiaotong University, 2008
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ABSTRACT

We use molecular dynamics simulations to investigate the behavior of various amphiphilic molecules in aqueous solutions in the presence of vitamin E or lipid bilayers. Our research studies focus on two molecular systems. First, we investigate the effect of DMSO on structural properties of DMPC bilayers and calculate bilayers permeability coefficients for both water and DMSO molecules at low DMSO concentration. The simulations show that the increase of DMSO concentration in solution leads to an increase of the permeability of water through the bilayers. The permeability increase might explain the unusual ability of DMSO, even at relatively low concentrations, to allow fast relaxation of osmotic pressure imbalance present during cryopreservation protocols. The second part of our research aims at the development of a molecular-level understanding of solubilization of vitamin E by bile salts and its adsorption and positioning into cell membranes. Specifically, in a sequence of MD simulations, we investigate the aggregation behavior and interaction of cholate (CHD) and glycocholate (GCH) with oleic acid and vitamin E and adsorption, positioning, and aggregation of vitamin E molecules inside a DMPC lipid bilayer. The simulations show that at concentrations above critical micelle concentration the bile salt molecules aggregate spontaneously into small oblate micelles in just a few nanoseconds. The oblate shape is favored by bile salts unique molecular structure. The study of interaction of bile salts with oleic acid show that oleic acid molecules are solubilized spontaneously into preformed bile salt micelles. The MD study of interaction of bile salts with vitamin E show that preformed bile salt micelles are spontaneously adsorbed at the vitamin E-water interface; adsorption process that leads to important changes of interfacial energy, surface tension, and interface structure. In addition, our MD simulations demonstrate that \( \alpha \)-tocopherol incorporates spontaneously into DMPC lipid bilayers and accumulates in a relatively narrow
region, just below the membrane-water interface. This is of great significance because even if its concentration in membranes is relatively low, the spatial confinement of α-tocopherol inside the bilayer greatly enhances its concentration in this vital region, thus increasing their importance for \textit{in vivo} biological activities including oxidative stress defense.
CHAPTER 1
INTRODUCTION

1.1 Background and Motivation

Advances in computational technologies enable the use of atomistic simulations as a “computational microscope” that facilitates the gain of critical molecular-level insights into both structure and dynamics of complex systems. One of the great strengths of atomistic simulations, such as molecular dynamics (MD), is that it allows for parametric studies of the influence of various factors on systems properties and can provide critical mechanistic insights into the physical processes and interactions at length and time scales unattainable by experiments or continuum theories. In this research, using MD simulations, we investigate the structures formation and interaction of dimethyl sulfoxide (DMSO) and bile salts with α-tocopherol (vitamin E) and phospholipid bilayers. The ultimate goal is to develop molecular level mechanistic understanding of the effect of small amphiphilic molecules, such as DMSO and bile salts, on structural and permeation properties of cell membranes and on the properties of vitamin E-water interface.

Understanding the basic principles of lipid bilayer membranes, which govern and mediate various biologically relevant processes, on the cellular level is one of the great challenges in biology. To investigate the characteristics of the membranes and to obtain the intriguing physicochemical aspects of membranes systems many experiments have been performed [1-3]. Although experimental approach is still the corner stone of membrane research, it is often difficult or even impossible to obtain a thorough understanding of the phenomena taking place in lipid bilayers by experiments only. Recent development of new algorithms [4] and revolutionary advances in the computational power available to scientists has permitted computer simulations
of biological membranes to advance at a comparable pace with that of experiments [5]. Computer simulations provide unique capabilities for analyzing biomembrane properties from atomistic perspective with a degree of detail that is hard to reach by other techniques. The excellent agreement with the experiment obtained in various molecular dynamics (MD) studies [6, 7] on simple model membranes has raised the confidence in applying the atomistic simulations to even more complex systems.

The effects of non-water polar solvents on membranes are very important in many biological and medical applications. For example during freezing preservation, chemicals denoted as cryoprotective agents (CPAs) have long been utilized to minimize freezing injury [8]. Commonly used CPA’s include glycerol, dimethylsulfoxide and methanol. The specific interaction of CPAs with membranes, play a major part in cryopreservation. In a typical cryopreservation process CPAs are added to the cells being cryopreserved before the thermal insult (freezing process) and removed after the freeze-storage/thawing process. Although, the use of CPAs is quite widespread in the field of cryobiology, and large number of publications/experiments support their efficacy in reducing the freezing injury on cells and tissues, the exact cause of their cryoprotective mechanism is still subject to enquiry and debate [9]. One of the most commonly used and effective CPA is dimethylsulfoxide (DMSO), which is a solvent that has the ability to modulate cell membrane permeability and enable the cell membrane to accommodate better the osmotic and mechanical stresses during cryopreservation [10]. Hence, DMSO is the CPAs chosen for the atomistic simulation studies of this research project. Insights into how DMSO molecules modulate membrane structure and function are invaluable towards improving and regulating the cryopreservation protocols.
The first part of our research focuses on the investigation of the effect of DMSO on properties of DMPC bilayers in the absence of transient pores and on evaluation of the permeability coefficients for both water and DMSO molecules at low DMSO concentration. Given that the permeation of both water and DMSO through lipid bilayers is too slow to occur during typical MD simulation timescale, we evaluate the permeability coefficients indirectly by employing an inhomogeneous solubility-diffusion model and by computation of the free energies and diffusion coefficients profiles across the DMPC bilayer. The ultimate goal is to obtain a better understanding of the molecular mechanism and the parameters that determine the enhancement by DMSO of basal bilayer permeability in the absence of pores.

Vitamins are a class of compounds used in the food and cosmetics industries for their multiple beneficial roles in human health as biological antioxidants (vitamin E) [11], hormones (e.g. vitamins A and D), and enzyme co-factors in biochemical pathways [12, 13]. Most vitamins are not synthesized by the human body and have to be provided from outside, via food, beverages or dietary supplements. Low solubility of the compound in the mucosal fluids leads to elimination of a significant fraction of the administered dose from the alimentary canal before absorption. To ensure a high bioavailability of the vitamins it is desirable to deliver vitamins with a system which would insure constant release in the gastrointestinal (GI) tract, efficient solubilization by the GI surfactants such bile salts, and delivery and adsorption into epithelial intestinal cell membranes. Of the many vitamins that have a positive effect on humans health vitamin E stands out as one of the most important antioxidant. Halliwell & Gutteridge defined antioxidants as “any substance that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate” [14]. Antioxidants prevent cellular damage surged from chemical reactions that involve reactive
oxygen species (ROS) [15-17]. Damage caused by ROS has been related to cancer, diabetes, cardiovascular disease, inflammatory responses, degenerative diseases, aging, liver injury, cataract, and others [18-38]. A balanced food intake reach in vitamins and antioxidants has been suggested to ensure optimal tissue levels of antioxidants [39] necessary to maintain a balance between ROS formation and antioxidant defense. To optimize and improve the cellular uptake protocols of vitamin E it is imperative to develop a fundamental understanding of their solubilization by GI bile salts and adsorption into cell membranes.

Using MD simulation studies the second part of our research focuses on the development of fundamental understand of vitamin E solubilization by bile salts and their adsorption and positioning into cell membranes. The mechanistic insights revealed by our MD studies have the potential to impact directly the fundamental understanding of vitamin E solubilization and permeation through lipid membrane. In turn, this understanding can lead to the improvement and development of new optimized drugs and protocols for effective vitamin E solubilization and cellular uptake. Specifically, in a sequence of MD simulation studies, we investigate the aggregation behavior and interaction of cholate (CHD) and glycocholate (GCH), two important experimentally well-studied bile salts, with oleic acid and vitamin E. The focus is on both reveling the kinetics of the bile salts aggregation mechanism as well as on understanding the structural characteristics of the micelles formed. To further the understand of the interaction of bile salts with oily fatty phases and their role in lipid digestion we performed MD simulations of interaction and encapsulation and of oleic acid into preformed glycocholate micelles and studies of interaction of bile salts with vitamin E-water interfaces. In additions we perform extensive MD simulation focused on developing molecular level understanding of adsorption, positioning, and aggregation of vitamin E molecules inside a DMPC lipid bilayer.
1.2 Cell Membrane

Cell membranes are sheet-like assemblies of amphiphilic molecules that separate cells from the surrounding environment and are among the most important cell structures. They determine the nature of all communication through the interface and work as screening devices allowing, and sometimes even assisting, the penetration of some molecules but not others. In addition, they provide a matrix to support to membrane proteins which transport ions into and out of a cell [40, 41]. Cell membrane controls the material passage in or out of a cell and provides a stable interior environment from outside. Most small uncharged molecules are transported through membranes passively, while larger or charged molecules need active regulatory mechanisms assisted by specialized proteins [42].

The principal structural element of cell membranes is a bilayer of lipid molecules (see Figure 1.1). The origin of the lipid bilayer membrane concept is traceable to three main historic sources. Firstly, in the 17th century Hooke, after viewing the array of a cork slice under a microscope, “coined” the word cell [43]. Secondly, Newton estimated the thickness of the blackest soap film to be 3/8 x10^{-6} inches (~7 nm) [44]. Thirdly, after spreading lipids extracted from red blood cells on a Langmuir trough, Gorter and Grendel [45] concluded in 1925 that the plasma membrane of red blood cells consisted of a lipid bilayer, and likened its structure to that of a soap bubble! Thus, the works of Hooke, Newton, Gorter and Grendel led to the realization by Rudin and his co-workers in 1960 that a soap film in its final stages of thinning has a structure comprised of two fatty acid monolayers sandwiching an aqueous solution and provided the key for the formation of experimental lipid bilayer membranes [46]. Today many experimental and simulation investigations of structure and permeation properties of cell membranes circumvent
the difficulties posed by their complexities by using various simplified membrane-models such as one-component phospholipid lipid bilayers membrane or vesicles.

Figure 1.1: A molecular view of the cell membrane (Encyclopedia Britannica, Inc.).

1.3 Phospholipid Bilayers

As shown in Figure 1.1, cell membranes have as main motif a double layer of lipids and as such they are often referred to as lipid bilayers. From the large class of lipids those that contain molecular group phosphate are referred to as phospholipids (see Figure 1.2). Phospholipids are amphiphilic molecules and are composed of a hydrophilic (polar) head and two hydrophobic hydrocarbon tails. The water soluble head consists of glycerol group, a negatively charged phosphate group, and a positively charged choline group. The negative and the positive moieties confer a dipolar moment to the lipid and therefore a hydrophilic character. The two neutral fatty acid tails confer its hydrophobic characteristics. The many types of phospholipids differ from each other by either the structure of the head molecular group moieties or/and the length and
degree of saturation of their hydrocarbon tails. The degree and the location of the unsaturated bonds (i.e. carbon double bonds) play a very important role on the structural characteristics of the lipid bilayers.

Given their amphiphilic character, when placed in an aqueous solution, the phospholipids arrange themselves and aggregate into various structures such that their non-polar (hydrophobic) tails cluster together away from aqueous phase while their polar (hydrophilic) heads establish contact with the aqueous phase. There are many structures that can be formed during the aggregation process including micelles, lipid bilayers, inverted hexagonal phase, etc. The structure of the resulting aggregates can be controlled by varying lipid concentration and solvent quality. The most common structure is the lipid bilayer structure. A phospholipid bilayer is composed of two thin layers (leaflets) of lipid molecules. As shown in figure 1.3, the lipids aggregate in planar structures such that hydrophobic tails occupy the center region of the bilayer, thus avoiding contact and minimize their interaction with water molecules and the hydrophilic heads line outside bilayer region and come in direct contact with the aqueous phase. In addition to bilayer structures phospholipids can also assemble into small micelle structures when the lipids concentration is small.

1.4 Dimethyl Sulfoxide (DMSO)

DMSO ((CH₃)₂SO) is a small amphiphilic molecule consisting of a hydrophilic sulfoxide group (S=O) and two hydrophobic methyl groups. DMSO is one of the many solutes that has the ability to modulate basal permeation properties of cell membranes and therefore are found in many cell biology and medical applications. In addition, DMSO has been widely utilized to prevent cellular damage during freeze-thaw cycle in cell preservation applications. The
characteristic structural changes in membranes induced by DMSO are presumed to play a major part in the outcome of cryopreservation in cell biology.

Figure 1.2: Representation of a phospholipid molecule (http://kvhs.nbed.nb.ca/gallant/biology/phospholipid_structure.jpg)

Figure 1.3: Schematic representation of a lipid bilayer (Snapshot taken from Inex Pharmaceuticals Corporation)
Figure 1.4: The structure of DMSO molecule. Carbon atoms are represented in gray, hydrogen in white, sulfur in yellow and oxygen in red.

1.5 Bile Salts

Bile is a digestive fluid produced by liver and delivered into the small intestine from gallbladder. [47]. The major components of bile are: bile salts, phospholipids, proteins and cholesterol. Bile salts are product of the cholesterol metabolism and are the dominant species in bile. Bile plays a fundamental role digestion, especially in metabolism of fat. Specifically, the bile salts, form micelles that solubilize the sparingly soluble lipid digestion products and fat soluble molecules such as vitamin E and subsequently transfer them away from droplet surface. Table 1.1 shows a typical bile salt composition in the gallbladder [48].

<table>
<thead>
<tr>
<th>Bile salt</th>
<th>Mol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocholate</td>
<td>30.9</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>12.0</td>
</tr>
<tr>
<td>Glycochenodeoxycholate</td>
<td>29.3</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td>11.2</td>
</tr>
<tr>
<td>Glycodeoxycholate</td>
<td>9.3</td>
</tr>
<tr>
<td>Taurodeoxycholate</td>
<td>2.2</td>
</tr>
<tr>
<td>Glycolithocholate</td>
<td>0.4</td>
</tr>
<tr>
<td>Taurolithocholate</td>
<td>0.3</td>
</tr>
<tr>
<td>Glycoursodeoxycholate</td>
<td>0.4</td>
</tr>
<tr>
<td>Tauroursodeoxycholate</td>
<td>1.6</td>
</tr>
<tr>
<td>Sulfoglycolithocholate</td>
<td>1.9</td>
</tr>
<tr>
<td>Sulfotaurolothocholate</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Bile salts are amphiphilic molecules. However, unlike other typical surfactants, bile salts don’t have a well-defined hydrophilic head and a hydrophobic tail. Instead, their amphiphilic character is conferred by the asymmetric distribution of hydrophilic hydroxyl groups about bile salts planar structure with all located on one side and the hydrophobic methyl group on the other side.

1.6 \( \alpha \)-tocopherol (vitamin E)

Vitamin E is lipid-soluble antioxidant molecules that are adsorbed into cell membranes and function as a free radical scavenger to prevent lipid peroxidation. The term vitamin E is used to refer to eight molecular compounds divided up into two families, tocopherols and tocotrienols, which differ in side-chain saturation. Four of them are tocopherols and each has a fully saturated side-chain while the side-chain of each of the four tocotrienols contains three double bonds. The specific compounds in both families are named as alpha, beta, gamma and delta and vary among themselves by the number and positioning of their methyl groups. Although all eight vitamin E compounds share a number of similarities, \( \alpha \)-tocopherol has the highest biological activity being the only one that is retained by human body, and used by the \( \alpha \)-tocopherol transport protein (aTTP) which is the only known vitamin E receptor[49]. The structure of \( \alpha \)-tocopherol is shown in Figure 1.5.

![Molecular structure of \( \alpha \)-tocopherol](image)

Figure 1.5: Molecular structure of \( \alpha \)-tocopherol
1.7 References


CHAPTER 2
SIMULATION METHODOLOGY

The recent progress and developments of computer hardware and software has allowed unprecedented advances in modeling and simulation of various molecular systems. Computer simulations are able to build a bridge between the real experimental and theoretical investigations. One of the critical tasks of any computational scientist is to formulate a simplified and accurate model of the real system and then translate it in a computational model that then can be solved numerically on powerful computers. The advantages of computer simulations stem from their ability to describe the systems investigated with atomistic details and allow simulations to be performed in conditions that are often hard to achieve in experiments. Thus, scientists are able to test their models by comparing the computational results with experiments and even sometimes predict system behavior and properties before the experiment is performed.

2.1 Molecular Dynamics (MD) Simulation

Nowadays, very often experimentalists investigate processes that are controlled by nanoscale characteristic phenomena and need to be resolved with atomistic resolution. This is an area in which atomistic simulation can greatly help and complement experimental investigations. Two major atomistic simulation methods have been developed. These are molecular dynamics (MD) and Monte Carlo (MC) simulation methodologies. In our investigations of interaction of various amphiphilic molecules with lipid bilayers and vitamin E we use MD simulations.

The MD simulation methodology was developed by Alder & Wainwright in the 1950’s and was applied to investigate simple gas and liquid systems modeled as simple hard core particles. Later the MD methodology was extended to systems described by more complex and realistic interactions including metals, ionic materials, polymers, and biomolecular systems. The concept
of MD simulation is relatively simply and it is based Newtonian mechanics. That is, the evolution of the many-particle system is followed in time by integrating their equation of motion. The equilibrium quantities are evaluated by time averaging of the quantities of interest. In many respects the MD simulations are very similar to the experimental investigations. The trajectories of the N atoms comprising a simulation system are obtained by integrating Newton’s classical equation of motion:

\[ \mathbf{F}_i = m_i \frac{\partial^2 \mathbf{r}_i}{\partial t^2}, \quad i = 1,2 \ldots N \]  

(2.1)

where \( \mathbf{r}_i \) are the vector characterizing the atom \( i \) position, \( m_i \) is the mass of this atom \( i \), and \( \mathbf{F}_i \) is the force acting on atom \( i \). The force, \( \mathbf{F}_i \), acting on atom \( i \) is obtained from the negative derivatives of the interaction potential function \( V(\mathbf{R}) \), where \( \mathbf{R} = (\mathbf{r}_1, \mathbf{r}_2, \ldots, \mathbf{r}_N) \) is the \( 3N \) dimensional vector representing the positions of all \( N \) atoms comprising the simulated system, and is given by:

\[ \mathbf{F}_i = - \frac{\partial V}{\partial \mathbf{r}_i} \]  

(2.2)

The interaction potential energy function, \( V(\mathbf{R}) \), is a function of the atom type, valence state, and electric charge as well as their coordinates. During the simulation the coordinates of the atoms are save for latter analysis in the so called trajectory files. The trajectory file contains important time characteristics of the systems which via averaging methodologies can be used to generate macroscopic properties [1].
2.1.1 Description of Interatomic Interactions

In general the empirical interatomic interaction potential energy function $V(\vec{R})$ is expressed as a sum over individual terms representing bonded (or internal) interactions and non-bonded contributions and can be written as:

$$
V(\vec{R}) = \frac{1}{2} \sum_{\text{bonds}} k_{ij}^b (b_{ij} - b_{eq})^2 + \frac{1}{2} \sum_{\text{angles}} k_{ijk}^\theta (\theta_{ijk} - \theta_{eq})^2
$$

$$
+ \frac{1}{2} \sum_{\text{torsions}} k_\phi (1 + \cos(n\phi - \phi_s))
$$

$$
+ \sum_{i=1}^{N} \sum_{j=i+1}^{N} \left\{ 4\epsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \right\}
$$

(2.3)

The first three terms in equation (2.3) represent the bonded interactions and the last two the non-bonded interactions. The bonded interactions consist of three parts; the bond stretching term, angles bending term and bond rotations or torsion term. The parameters, $k_{ij}^b, k_{ijk}^\theta, k_\phi$ are the force constants for the three bonding interaction terms and $b_{eq}, \theta_{eq}, \phi_s$, are the equilibrium values for the corresponding bond length, bond angle, and torsional angle respectively; $n$ represents the multiplicity and its value gives the number of minimum points in the torsional function as the bond is rotated through 360°. The non-bonded terms include the Lennard-Jones 6-12 term, which is used for the treatment of the core-core repulsion and the attractive van der Waals dispersion interaction, and the Coulomb interactions between partial point charges $q_i$ and $q_j$ of atoms $i$ and $j$ respectively. Non–bonded interactions are calculated between all atom pairs within a user-specified interatomic cutoff distance, except for covalently bonded atom pairs and
atom pairs separated by two covalent bonds. In the Lennard–Jones term the potential well depth is represented by $\epsilon_{ij}$, where $i$ and $j$ are the indices of the interacting atoms and $r_{ij}$, is the interatomic distance, and $\sigma_{ij}$, measure the interatomic separation at which the LJ term is equal to zero. Typically, $\epsilon_{ij}$ and $\sigma_{ij}$ are obtained for individual atom types and then combined using various mixing rules to yield the corresponding values for interaction atoms of different types. In the commonly used Lorentz-Berthelot mixing rules the well depth $\epsilon_{AB}$ for the interaction between the A-type atoms and B-type atoms is given as the geometric mean and the collision diameter $\sigma_{AB}$ is given as the arithmetic mean:

$$\epsilon_{AB} = \sqrt{\epsilon_{AA} \cdot \epsilon_{BB}} \quad (2.4)$$

$$\sigma_{AB} = \frac{1}{2} (\sigma_{AA} + \sigma_{BB}) \quad (2.5)$$

The empirical potential energy function given in equation (2.3) is differentiable with respect to the atomic coordinates; this gives the value and the direction of the force acting on an atom and thus it can be used in a molecular dynamics simulation. The calculation of force allows the calculation of the acceleration which tells us how the speed is changing, and from the speed variation it is possible to determine approximate positions of the atoms a very short time later. This process is called integrating equations of motion, and repeating calculation for huge number of small steps results in a trajectory with the development of positions, velocities and forces on all atoms during the simulation. A good approximation of the potential function would provide an extremely detailed description of both dynamics and equilibrium properties in the system under study. Additional information about the capabilities and limitations of the force fields used in MD simulations are given in the Appendix A.
2.1.2 Integration Algorithms

2.1.2.1 Verlet Algorithm

There are many integration algorithms that have been developed to integrate the equation of motion of atoms in a molecular dynamics approach. The most commonly used one is called Verlet algorithm and was developed by Verlet in 1967 [2]. The Verlet algorithm is based on Taylor series expression of position $r(t)$ as a function of time. For each atom the positions at $(t + \Delta t)$ and $(t - \Delta t)$ can be expressed in term of its position at time $t$ by the following two equations:

\[
\begin{align*}
  r(t + \Delta t) &= r(t) + v(t)\Delta t + \frac{1}{2} a(t)\Delta t^2 + \frac{1}{6} b(t)\Delta t^3 + O(\Delta t^4) \\
  r(t - \Delta t) &= r(t) - v(t)\Delta t + \frac{1}{2} a(t)\Delta t^2 - \frac{1}{6} b(t)\Delta t^3 + O(\Delta t^4)
\end{align*}
\]

(2.6)  
(2.7)

By adding the above two equations, we get the following equation:

\[
  r(t + \Delta t) = 2r(t) - r(t - \Delta t) + a(t)\Delta t^2 + O(\Delta t^4)
\]

(2.8)

Then by substituting ($a = F/m$) then solving for $r(t + \Delta t)$:

\[
  r(t + \Delta t) = 2r(t) - r(t - \Delta t) + \frac{F}{m}\Delta t^2 + O(\Delta t^4)
\]

(2.9)

According to the equation above, Verlet method is a fourth-order method. The position of atoms at time $t + \Delta t$ could be predicted by using the position at two previous steps.

There is also an extension to Verlet algorithm, the so called velocity Verlet algorithm. In the velocity Verlet algorithm the solution to the equation of motion is given by:

\[
  r(t + \Delta t) = r(t) + v(t)\Delta t + \frac{1}{2} a(t)\Delta t^2
\]

(2.10)
To increase the accuracy of the Verlet algorithm, the Leap-Frog algorithm has been developed. In this integration method, velocity at the midpoint between time $t$ and $t + \Delta t$ is calculated. The position $r(t + \Delta t)$ at time $t$ can be obtained by knowing $v \left( t + \frac{1}{2} \Delta t \right)$ given by

\[
v \left( t + \frac{1}{2} \Delta t \right) = v \left( t - \frac{1}{2} \Delta t \right) + a(t) \Delta tanumber{(2.12)}
\]

\[
r(t + \Delta t) = r(t) + v \left( t + \frac{1}{2} \Delta t \right) \Delta t
\]

Since the velocities in this algorithm is obtained at every half time point and the position is obtained at every integer time step, the velocities at each integer time step could written as the following equation:

\[
v(t) = \frac{1}{2} \left[ v \left( t + \frac{1}{2} \Delta t \right) + v \left( t - \frac{1}{2} \Delta t \right) \right]
\]

\[
(2.14)
\]

### 2.1.2.2 Leap-Frog Algorithm

Leap-frog is a slightly modified algorithm of the Verlet algorithm, which is using acceleration at time $t$, velocity at $t + \frac{1}{2} \Delta t$ and positions at $t$ and $t - \Delta t$ to predict the position at $t + \Delta t$. The equation in this algorithm from a Taylor expansion:

\[
r(t + \Delta t) = 2r(t) - r(t - \Delta t) + a(t) \Delta t^2
\]

\[
(2.15)
\]

The velocity was calculated first at the position $(t + \frac{1}{2} \Delta t)$, the equation is:

\[
v \left( t + \frac{1}{2} \Delta t \right) = v \left( t - \frac{1}{2} \Delta t \right) + a(t) \Delta t
\]

\[
(2.16)
\]
The velocity at time \( t \) can be also calculated as:

\[
v(t) = \frac{1}{2} (v(t + \frac{1}{2} \Delta t) + v(t - \frac{1}{2} \Delta t)) \tag{2.17}
\]

So the position at time \( (t + \Delta t) \) is:

\[
r(t + \Delta t) = r(t) + v(t + \frac{1}{2} \Delta t) \Delta t \tag{2.18}
\]

### 2.1.3 Boundary Conditions

In all MD simulations the boundaries of the simulation system have to be well defined. One approach to address this is to simply terminate the system with free surfaces (feasible in liquid and solid systems) in which case the atoms near the boundary would have less number of neighbors than atoms inside the simulation box. Unless we really want to simulate a cluster of atoms, this situation is not realistic. No matter how large is the simulated system, its number of atoms \( N \) would be negligible compared with the number of atoms contained in a macroscopic piece of matter (of the order of \( 10^{23} \)), and the ratio between the number of surface atoms and the total number of atoms would be much larger than in reality, causing surface effects to be much more important than what they should. Consider 1000 atoms arranged in a 10 *10 *10 cube. Nearly half the atoms are on the outer faces, and these will have a large effect on the measured properties. Even for \( 10^6 \) atoms, the surface atoms amount to 6% of the total, which is still nontrivial.

A solution to this problem is to use periodic boundary conditions (PBC). When using PBC, particles are enclosed in a box and the images of this box are replicated to infinity by rigid translation in all the three Cartesian directions, completely filling the space. In other words, if one of our particles is located at position \( r \) in the main simulation box, we assume that this
particle really represents an infinite set of particles located at positions \( r + la + mb + nc \), where \( l, m, n \) are integer numbers and \( a, b, c \) are box sizes along the three spatial directions. Although all these image particles move together in the same way, only one is represented and has its coordinates tracked in the computer program.

The key point is that now each particle \( i \) in the box should be thought as interacting not only with other particles \( j \) in the box, but also with their images in nearby boxes. That is, interactions can go through box boundaries. In fact, one can easily see that (a) we have virtually eliminated surface effects from our system, and (b) the position of the box boundaries has no effect (that is, a translation of the box with respect to the particles leaves the forces unchanged). In the course of the simulation, if an atom leaves the main simulation box through one facet, it re-enters the main simulation box through the opposite facet. Of course, it is important to bear in mind and to account for the imposed artifacts due to the periodicity when considering properties which are influenced by long-range correlations. Special attention must be paid to the case where the interaction potential is long ranged like for example in charged and dipolar systems.

2.1.4 Temperature and Pressure Control in MD Simulations

Prior to starting an MD simulation one is faced with the task of initializing the atomic positions and velocities for all atoms comprising the simulated system. One approach would be to start with the atoms located initially in some crystal structure or distributed randomly in within a small unit cell. The unit cell is then repeated periodically in space to fill up the desired simulation box that has the characteristic dimensions of the system to be investigated. The initial velocities are assigned according to a Boltzmann distribution at a certain temperature. This is achieved numerically by using Gaussian distributed random numbers that are multiplied by the mean square velocity which in turn is given by \( \sqrt{2k_BT/m} \) in each of the three directions.
generating the initial structure and assigning initial velocities to each both the initial temperature and the total energy of the system are predefined. The total energy of the system is given by

\[ E_{\text{tot}} = K_{\text{tot}} + U_{\text{tot}}, \]  

(2.19)

where \( K_{\text{tot}} \) is the total kinetic energy of the system and is given by

\[ K_{\text{tot}} = \sum_{i=1}^{N} \frac{1}{2} m \left( v_{x,i}^2 + v_{y,i}^2 + v_{z,i}^2 \right). \]  

(2.20)

\( U_{\text{tot}} \) is the total potential energy of the system and it is expressed as a sum of the potential energies of all atoms in the systems. Knowing the total kinetic energy at a moment in time one can use this relation to define an instantaneous temperature, \( T(t) \), at time \( t \):

\[ T(t) = \sum_{i=1}^{N} \frac{1}{k_B N} \frac{1}{m} \left( v_{x,i}^2 + v_{y,i}^2 + v_{z,i}^2 \right), \]  

(2.21)

where \( k_B \) is the Boltzmann constant, \( m \) is the mass of an atom and \( N \) is the total number of atoms in the system. In addition to temperature, the pressure also needs to calculated and often controlled during a typical MD simulation. There are several different (but equivalent) ways to calculate the average pressure in a classical N-body system. The most common methodology is based on the virial equation for the pressure. For pairwise additive interactions, one can write [3].

\[ p = nk_B T + \frac{1}{3V} \left( \sum_{i,j} \bar{f}(\bar{r}_{ij}) \cdot \bar{r}_{ij} \right), \]  

(2.22)

where \( \bar{f}(\bar{r}_{ij}) \) is the force between particles \( i \) and \( j \) at distance \( \bar{r}_{ij} \), \( n \) is particle density, \( T \) the temperature and \( V \) the volume of the system.
There are many methods that have been developed to control and maintain the temperature and pressure constant [3-5]. Commonly used methods are based on the so-called Berendsen thermostats and barostats[5]. According to Berendsen thermostat the temperature is controlled by scaling the velocities of all atoms at each time step such that \( \vec{v}_i \rightarrow \lambda \vec{v}_i \) and the scaling factor, \( \lambda \), is given by

\[
\lambda = \sqrt{1 + \frac{\Delta t}{\tau_T} \left( \frac{T_0}{T} - 1 \right)}
\]  

(2.23)

where, \( \Delta t \) is the integration time step, \( \tau_T \) is the time constant for temperature control and has to be greater than \( 100 \Delta t \), \( T_0 \) is the desired temperature and \( T \) is the current temperature. The Berendsen pressure control is implemented in a similar way by changing at each time step the volume of the simulation box and rescaling all atom positions. Accordingly, the simulation box size \( L_i \rightarrow \mu L_i \) and atoms positions \( \vec{r}_i \rightarrow \mu \vec{r}_i \), and the scaling factor, \( \mu \), is given by

\[
\mu = 1 - \frac{\kappa_T \Delta t}{3 \tau_p} (p_0 - p)
\]  

(2.24)

where, \( \Delta t \) is the integration time step, \( \kappa_T \) is the isothermal compressibility of the system, \( \tau_p \) is a time constant for pressure control, \( p_0 \) is the desired pressure and \( p \) is the current pressure. There are advantages of using these methods as they ensure steady and stable evolution of the system to equilibrium. The drawbacks are that the statistical ensemble they generate is not known.

### 2.1.5 Time and Size Limitations

Molecular dynamics evolves a finite-sized molecular configuration forward in time, in a step-by-step fashion. There are limits on the typical time scales and length scales (system size) that can be investigated and one should account for them when planning and preparing a simulation study or analyzing the simulation results. Typical MD simulations can be performed
on systems containing thousands or, perhaps, millions of atoms and for simulation times ranging from a few picoseconds to hundreds of nanoseconds. While these numbers are certainly respectable, it may happen to run into conditions where time and/or size limitations become important.

A simulation is reliable from the point of view of its duration when the simulation time is much longer than the relaxation time of the quantities we are interested in. However, different properties have different relaxation times. In particular, systems tend to become slow and sluggish in the proximity of phase transitions, and it is not uncommon to find cases where the relaxation time of a physical property is orders of magnitude larger than times achievable by simulation.

A limited system size can also constitute a problem. In this case one has to compare the size of the MD cell with the correlation lengths of the spatial correlation functions of interest. Again, correlation lengths may increase or even diverge in proximity of phase transitions, and the results are no longer reliable when they become comparable with the box length.

2.2 General Theory of Small Molecules Permeation across Lipid Membranes

To investigate the permeation of small molecules through membranes an inhomogeneous solubility-diffusion model has been developed by Marrink and his group in 1994 [6]. This model enables the link of the permeation coefficients determined and used in experimental characterization of transport properties through lipid membranes to data obtained from atomistic simulation via an integral over local properties across the membrane. Next we give a brief overview of the fundamentals of this model
2.2.1 Inhomogeneous Solubility-Diffusion Model

Let’s consider the motion of an atom belonging to \( i \)th species through a lipid bilayer. Its average velocity could be written as:

\[
    u_i = -\frac{1}{\xi_i} \nabla \mu_i ,
\]

(2.25)

where, \( \mu_i \), represents thermodynamics chemical potential, and \( \xi_i \) is the frictional coefficient. The flux \( J_i \) of atoms of type \( i \) is given by:

\[
    J_i = c_i u_i = -\frac{c_i}{\xi_i} \nabla \mu_i .
\]

(2.26)

The friction coefficient \( \xi_i \) is related to the diffusion constant \( D_i \) via Einstein’s relation:

\[
    D_i = \frac{RT}{\xi_i}
\]

(2.27)

According to Fick’s 1st law of diffusion

\[
    J_i = -D_i \nabla c_i
\]

(2.28)

The linear flux relations for the case that material properties depend on one coordinate \( z \) can be written as

\[
    J_i(z) = -\frac{c_i(z)D_i(z) d\mu_i(z)}{RT} \frac{d\mu_i(z)}{dz}
\]

(2.29)

Here \( c_i(z) \) is the concentration of component \( i \), which could be replaced by equilibrium concentration \( c_i^{eq}(z) \) in the absence of an imposed gradient. The permeation resistance \( R_i^p \) could be defined as:

\[
    R_i^p = c_i^{eq} \int_{z_1}^{z_2} \frac{d\mu_i(z)}{c_i^{eq}(z)D_i(z)}
\]

(2.30)
In this equation, $c_i^*$ is the concentration in the bulk solutions on either side of the membranes. The permeation coefficient is related to the permeation resistance obtained in experimental investigations, and is defined by:

$$R_i^p = \frac{1}{P_i} \quad (2.31)$$

The ratio of local equilibrium water concentration to the bulk concentration $c_w^{eq}(z)/c_w^*$ can be computed from the simulations[6]. The local concentration is proportional to the constrained partition function $Q'$, therefore this ration could be written which is related to the potential mean force $\Delta G$ to the bulk phase:

$$\Delta G_w(z) = -RT\ln\frac{Q'(z)}{Q'(z_1)} = -RT\frac{c_w^{eq}(z)}{c_w^*} \quad (2.32)$$

Thus, according to the equation 1, the permeation resistance can also be expressed as:

$$R_w^p = \int_{z_1}^{z_2} \frac{\exp(\Delta G_w(z)/RT)}{D_w(z)} dz \quad (2.33)$$

Therefore, obtaining the potential of mean force and the local diffusion constant of water in the membrane is the key to calculate the permeation coefficient numerically.

2.2.2 Computation of Potential of Mean Force

There are currently many simulations methods that one can use to calculate the potential of mean force (PMF) or the free energy for a molecule in system as a function a reaction coordinate [1]. One of the simplest and easiest to implement, for small molecules such as DMSO, is the so called position constrain method. According to this method it is possible to determine the PMF at a given location by simply measuring the average force exerted on the molecules that is
constrained at that location. By determining the average of the constraining force at various locations along a given direction (reaction coordinate) one can find the PMF by direct integration of the average force along the reaction coordinate. Specifically, if the reaction coordinate is the position of the molecule along the z-axis, then we have:

\[
\frac{d\Delta G(z)}{dz} = -\frac{RT}{Q^*(z)} \frac{dQ^*(z)}{dz}.
\]  

(2.34)

Hence,

\[
\frac{d\Delta G(z)}{dz} = -N_{AV} \langle \frac{\partial V(r_1...r_N)}{\partial z_0} \rangle = -N_{AV} \langle F_z(z_0) \rangle.
\]  

(2.35)

Where \( \langle F_z(z_0) \rangle \) is the mean force on the constraint.

2.2.3 Computation of Local Diffusion Coefficient

Similar to the methods used for potential mean force calculation, there are various methods that one can use to compute local diffusion constant. The two of the most common methods are based on: i) the calculation of the mean-square displacement and ii) on the fluctuation-dissipation theorem.

Mean-Square displacement method for diffusion coefficient calculation is one of the easiest one to implement in MD simulations. The diffusion coefficient in z direction in this model is given by:

\[
D(z) = \lim_{t \to \infty} \langle (z(t) - z(0))^2 \rangle / 2t
\]  

(2.36)

In the second method one needs to evaluate the autocorrelation function of the random force \( \Delta F(t) \) acting on the constrained molecule which is related to \( \xi(z, t) \), the so called local time-dependent friction coefficient, by the following equation:
\[
    \xi(z, t) = \frac{\langle \Delta F(z, t) \Delta F(z, 0) \rangle}{RT}. \tag{2.37}
\]

Time integration of the above equation yields the local static friction coefficient \( \xi^S [6] \), which is related to diffusion coefficient via Einstein’s relation:

\[
    D(z) = \frac{RT}{\xi^2(z)} = \frac{(RT)^2}{\int_0^\infty \langle \Delta F(z, t) \Delta F(z, 0) \rangle \, dt}, \tag{2.38}
\]

where \( \Delta F(z, t) \) is random force and can be written as:

\[
    \Delta F(z, t) = F(z, t) - \langle F(z, t) \rangle \tag{2.39}
\]

In our research, we used the constraint force method to calculate both the PMFs and diffusivities as a function of the distance from the center of the bilayer to the center of mass for both water and DMSO molecules in the direction normal to the bilayer (z-axis).

2.3 References

CHAPTER 3
MOLECULAR DYNAMICS SIMULATION STUDY OF THE EFFECT OF DMSO ON STRUCTURAL AND PERMEATION PROPERTIES OF DMPC LIPID BILAYERS

3.1 Introduction

Lipid bilayers are the basic structures that are used by living organisms to form their cell membranes which separate the cell interior from the surrounding environment and act as barriers between the inside and outside of a cell. Understanding the transport mechanism of various molecules across membranes is of great importance for many biological processes and a key ingredient in advancing the development of many technologies such as cryopreservation of biomaterials [1, 2], drug delivery [3, 4], and gene therapy [5]. Most small uncharged molecules, such as water, oxygen, and various drugs, are transported through membranes passively, via basal pathways, at appreciable rates whereas larger or charged molecules, such as ions, sugars, and amino acids, need active regulatory mechanisms assisted by specialized proteins [6]. Important cellular processes, such as rapid attainment of osmotic balance across plasma membranes in response to rapid changes of various physical and/or chemical variables, depend crucially on the existence of a basal pathway for water to move across membranes.

There are many solutes such as methanol, trehalose, glycerol, and DMSO that have the ability to modulate basal permeation properties of cell membranes. This modulation is very important in many cell biology and medical applications. In addition, these chemicals have long been utilized to minimize freezing injury during freezing preservation. The characteristic structural changes in membranes induced by them are presumed to play a major part in the outcome of cryopreservation in cell biology.

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Besides that, is also able to induce cell fusion and cell differentiation [2] and change the properties of proteins [1]. Several experimental studies, using X-ray diffraction [7], differential scanning calorimetry [8], NMR, neutron diffraction, and infrared spectroscopy [9], have been performed to investigate the effect of DMSO on various structural and thermodynamics properties of phospholipids bilayers. Moreover, the presence of DMSO has the effect on the phase transition of bilayers [8, 10], modifies the hydration forces [34] [11], and also effect on thickness of the bilayer [12]. Despite the extensive experimental efforts, the understanding of the molecular basis for the effect of DMSO on lipid membranes is far from complete and molecular level simulations seems to provide a promising alternative.

Molecular simulations provide important mechanistic insights into the interaction of various small molecules with lipid bilayers [13, 14]. Recently, several hundred nanosecond-long all-atom [15],[16],[17],[18],[19] and coarse-grained [20] molecular dynamics (MD) simulations have been performed to investigate the effect of DMSO on various phospholipid bilayers. Sum and Pablo performed MD simulations of lipid bilayers in the presence of various DMSO concentrations[19]. Their study showed that DMSO molecules penetrate deep into the lipid bilayer and accumulate mainly in a region below the lipid head groups and act as spacers that enhance lipid-lipid separation leading to a significant increase in the area per lipid. Their simulation results also indicate that the bilayer structural changes are mainly due to the DMSO induced dehydration close to the bilayer solvent interface and to the direct interaction of DMSO with the inner bilayer region. These modes of interaction with bilayers are generic for a larger class of small amphiphilic molecules, including short chain alcohols[21],[22]. Another recent MD simulation study by Notman et al. [21], employing a coarse-grained model, found that, at higher concentration, DMSO has the ability to induce water pores in lipid bilayers, which could
be a possible mechanism for the enhancement of membrane permeability to hydrophilic and charged molecules. These findings were confirmed later by Gurtovenko et al. [15] in an *all-atom* MD simulation study of dipalmitoylphosphatidylcholine (DPPC) lipid bilayers study in which they also demonstrated that, depending on its concentration, DMSO exhibits three distinct modes of action. At low concentrations (< 7.5 mol %), DMSO induces an increase in the area per lipid. This increase is also associated with a corresponding thinning of the membrane. At larger concentrations (10 to 20 mol %), DMSO induces formation of water pores in the membrane. DMSO may even lead to disintegration of bilayer structure when DMSO concentration exceeds 20 mol %. The MD simulation study of DMPC bilayers in the presence of 11.3 mol % DMSO of Moldovan et al. [17] provides additional insight into the mechanism of pore formation in lipid bilayers. Specifically, Moldovan *et al.* rationalize the pore nucleation process in terms of a simplified free energy model that includes the entropy of pore shape and the DMSO-induced lowering of both lipid bilayer line tension and the corresponding barrier for pore creation.

Diffusion of DMSO through lipid bilayers in the presence of a concentration gradient was investigated by Leekumjorn and Sum using MD with a double-lipid-bilayer system geometry[16]. Given the inherent MD time-scale limitations they used the simulation results to estimate the required parameters that were then applied to Fick’s diffusion laws and modeled DMSO concentration profile in the system as a function of time.

In this research, we study the effect of DMSO on the properties of DMPC bilayers, in the absence of transient pores, and evaluate the permeability coefficients for both water and DMSO molecules at low DMSO concentration. Given that the permeation of both water and DMSO through lipid bilayers is too slow to occur during typical MD simulation time scale, we evaluate the permeability coefficients indirectly by employing an inhomogeneous solubility-diffusion
model and by computation of the free energies and diffusion coefficients profiles across the DMPC bilayer. The ultimate goal is to obtain a better understanding of the molecular mechanism and the parameters that determine the enhancement by DMSO of basal bilayer permeability in the absence of pores.

3.2 Simulation Model and Methodology

MD simulations were performed on two DMPC bilayer systems in which the concentration of DMSO was 0 mol % (pure water) or 3 mol % with periodic boundary conditions in all directions. Throughout the entire paper when referring to the concentration of the water-DMSO solution we used the mole percent (mol %); the quantity that is equal to the mole fraction multiplied by 100. In our case, mol % is given by the number of DMSO molecules divided by the sum of the number of DMSO and water molecules and then multiplied by 100. Both systems contain 96 lipid molecules (48 lipids in each leaflet) and 5422 water molecules (the 3 mol % system contains an additional 162 DMSO molecules). The initial configuration of the DMPC-DMSO bilayer system was generated from the pure water system by randomly replacing 162 water molecules with DMSO molecules. Additional information regarding the reparation and initial equilibration of the DMPC bilayer-pure water system can be found in our previous studies.[17, 22]

All the simulations were performed with the GROMACS 4.0 simulation package.[23] The force field parameters for lipids for both bonded and nonbonded interactions were taken from Berger et al [24]. while the partial charges were taken from Chiu et al [25]. The force field parameters of Bordat et al [26]. were used for DMSO and the simple point charge (SPC) model for water[27]. All simulations were performed at constant pressure of 1.0 bar using a Berendsen barostat and semi-isotropic coupling with time constants of 5.0 ps in the directions parallel to the
bilayer surface and 4.0 ps in the direction normal to the bilayer surface. The temperature was held constant at 323 K, which is above the phase transition temperature of DMPC bilayers in both pure water and 3 mol % DMSO systems (recall that it has been observed experimentally that DMSO has the ability to increase the phase transition temperature of DMPC bilayers from gel phase to liquid-crystalline phase[9]). At T = 323 K, both systems studied were in the liquid-crystalline phase. All non-water bond lengths were constrained by using the LINCS[28] algorithm. Water bonds and angles were constrained using the SETLLE [29] algorithm. For short-range nonbonded interactions, GROMACS uses a twin range cutoff. The inner cutoff was 0.98 nm. Forces due to van der Waals interactions in the range from 0.98 to 1.4 nm were evaluated every 10 steps. The long-range electrostatic interactions were treated using the particle mesh ewald (PME) algorithm,[30, 31] with a 0.1176 nm grid spacing, of order 4, and tolerance of 10À5. We used an integration time step of 2.0 fs. The energy minimization procedure, based on the steepest descent algorithm, was applied to the initial structures prior to the actual MD runs. The bilayers in both 0 and 3 mol % DMSO systems were prepared initially by 50 ns MD runs, out of which the last 25ns were used for evaluation of various averaged properties. The simulation system and the structure of DMPC were show in the Figure 3.1.

3.3 Result and Discussion

The main goal of this study was to investigate the effect of 3 mol % DMSO solution compared to pure water on the permeability of water and DMSO molecules in DMPC bilayers (cryopreservation experiments often employ a concentration of 2.5 mol %[2]). Evaluation of the permeability coefficients required calculation of the profiles along the perpendicular direction to the bilayer of both free energies (the so-called potentials of mean force (PMFs)) and diffusivities of water and DMSO molecules. Mass densities, charge densities, electrostatic potentials, and the
Figure 3.1: (a) Molecular structure of DMPC. (b) Cross sectional view of the DMPC membrane in the 3 mol % DMSO solution. Red spheres are used to represent the DMPC head groups (hydrophilic) and green lines refer to DMPC tail groups (hydrophobic). In DMSO, the spheres are shown as orange for sulfur atoms, blue for oxygen atoms, and green for methyl groups. The constrained DMSO molecule located at the center of the bilayer is drawn in a bigger size compared to other DMSO molecules.
orientations of water and DMSO dipole moments were calculated as a function of distance from the bilayer center, the angular and the corresponding residence time distributions of the DMPC P-N vector orientations were calculated, and the number of hydrogen bonds per water molecule was calculated for water molecules in the ester group and tail regions of the bilayers.

3.3.1 Mass Density Profile

Valuable information about the structural changes of the DMPC bilayer can be obtained by analyzing the mass distribution of various molecules or molecular groups along the direction perpendicular to the bilayer. Figures 3.2 depict the mass density profiles of lipids, water, and DMSO molecules along the normal direction to the bilayer in both 0 mol % and 3 mol % DMSO solutions. For the lipids, the mass density profile indicates the distribution of the atoms comprising the lipids along the normal direction to the bilayer. The density profiles have been shifted for clarity such that for both systems the center of the bilayers are all located at $z = 0$. Based on the lipids and water density profiles one can identify three distinct regions of the bilayer-solvent systems: i) the bulk aqueous phase, ii) the lipid-solvent interface region containing of lipid head groups, water, and DMSO, and iii) the interior of the bilayer where the tails of the two leaflets meet. The water density is high in the aqueous region, decreases steadily in the lipid-solvent interface region, and reaches very low values in the middle of the bilayer where the hydrophobic lipids acyl chains reside. There is a pronounced peak on the DMSO mass profile indicating that DMSO penetrates deep and accumulates into the lipid/water interface region and occupy positions beneath the lipid head groups in the vicinity of the ester groups. The location of the peak is determined by the amphiphilic character of DMSO in which the two methyl groups interact favorably with the hydrocarbon acyl chains while the highly polar sulfoxide group can interact favorably with lipid heads. The DMSO mass density peak is located
in the same region as the mass density peaks of the atoms delimiting the polar portion of the lipids (phosphorous and nitrogen) and the connection with the hydrophobic tails (carbonyl carbon atoms of the ester groups) as indicated in Figure 3.2(b).

3.3.2 Orientation of Lipid Head Group (P-N vector)

The effect of DMSO on the orientation of the lipid head-groups was studied by evaluating the orientation distribution of the angle between the P-N vector, connecting the phosphate phosphorus atom to the choline nitrogen atom, and the outwardly directed bilayer normal. Figure 3.3(a) depicts the time evolution of the head-group orientation for two randomly-chosen lipids in the 3 mol % DMSO system. This shows that the orientation of individual head-groups may change frequently in the 0° to 150° interval and, in just a few nanoseconds, may assume multiple times very small or very large values. Consequently, the corresponding head-group orientation distribution functions are expected to be fairly wide. Figure 3.3(b) shows that, indeed, the lipid head-groups orientation distributions functions are fairly wide in both pure water and 3 mol % DMSO systems and reach their maximum values near 88° and 75° for the pure water and the 3 mol % DMSO systems respectively. These orientation angles at which the distributions reach their maximum values indicate that, in both simulated systems, the DMPC head-groups prefer orientations that are nearly parallel to the bilayer surface.

The overall tendency of the lipid head-group dipoles to adopt a more parallel orientation to the bilayer is favored by the lowering of the free energy component due to electrostatic dipole-dipole interaction associated with these bilayer configurations. As each lipid head-group can be modeled as an in plane point dipole (the plane of the bilayer leaflet) surrounded by a screening asymmetric dielectric medium (i.e., the solvent of a dielectric constant of about 78 on one side of
the leaflet and the hydrophobic lipid tails region of the bilayer interior of a dielectric constant of about 1), every one of these dipoles interacts with the field from all the other dipoles contributing

Figure 3.2: (a) The mass density of lipids, water, and DMSO along the bilayer normal in both 0 mol % and 3 mol % DMSO systems. (b) The mass densities of specific atoms such as phosphorous, nitrogen, and carbonyl carbon atoms of the ester groups along the bilayer normal in both the 0 mol % and 3 mol % DMSO systems. $z = 0$ corresponds to the center of the bilayer.
to the overall electrostatic interaction free energy component. Given that, in average, the dipoles
are tilted with respect to bilayer normal, they may be split into two components one parallel and
one perpendicular to the bilayer. The perpendicular parts become static dipoles and their
interactions will be repulsive and scale with distance as \( r^{-3} \) term that is less favorable energetically[32]. On the other hand, since the in-plane components are allowed to rotate freely around the membrane normal[32] their fluctuations will lead to attractive interactions that scale as \( r^{-6} \). The intricate combination of these electrostatic interaction terms together with those due to
the nearby dipolar moments of the polarized water and DMSO molecules will play an important
role in establishing the equilibrium the average orientation angle of lipid head-groups.
Specifically, the interaction between DMSO dipolar moment and the DMPC head-groups dipoles
may explain, in part, the shift in the P-N vector angle distribution in the presence of DMSO.
That is, the polar DMSO molecules, located mostly below the head-groups (see Figure 3.2(b)),
will tend to rotate the P-N vectors in the 3% system to point more outwardly so they can line-up
more closely with the DMSO dipole moment vectors, thus minimizing the dipole-dipole
electrostatic interaction.

3.3.3 Charge Density and Electrostatic Potential

The average presence of an electric dipole component along the normal direction in the
bilayer-solvent interfacial region gives rise to an electrostatic potential across a leaflet of the
membrane. Moreover, since the presence of DMSO has a significant effect on the P-N vector
angle distribution, it is expected that this will also affect the charge density and electrostatic
potential across the bilayer. This is because the polar head groups have relatively large partial
charges on their atoms.
Figure 3.3: (a) Time evolution of the angle between the P-N vector of the DMPC head-group and the bilayer normal for two arbitrarily chosen lipids in the 3 mol % DMSO system. (b) Time averaged distribution of the angle between the P-N vectors of the DMPC head-groups and the outwardly directed bilayer normal in both pure water and 3 mol % DMSO solutions.
Figures 3.4 shows the average charge densities across the bilayer obtained from the simulations of the 0 mol % and 3 mol % DMSO systems. Figure 3.4(a), shows the total charge density as well as the contributions from the lipids and water in the 0 mol % DMSO system. The charge distribution due to lipid head groups is mirrored and almost compensated by the opposite charge due to the water dipoles of the polarized water molecules located in the bilayer-solvent interface region. In fact, as shown in Figure 3.4(a), the comparison of the water and the total charge distributions indicate that the water molecules in the interfacial region over-compensate the lipid charge distribution. The same trend is observed in the 3 mol % DMSO system although in the presence of DMSO, as shown in Figure 3.4(b), both the lipid and water charge density profiles are not as smooth as in the pure water system. This can attributed to in plane disordering effect due to the increase spacing between the lipids heads and the presence of a larger number of water and DMSO molecules in the interfacial region of the bilayer.

The electrostatic potential, \( \psi(z) \), across the lipid bilayer can be calculated from the Poisson equation by double integrating the charge density \( \rho(z) \):

\[
\psi(z) - \psi(z_0) = -\int_{z_0}^{z'} dz' \int_{z_0}^{z''} \rho(z'') dz''
\]  
(3.1)

where \( \psi(z_0) \) represents the electrostatic potential inside the solvent region at \( z_0 \) far away from the bilayer surface. In our calculations we assume \( \psi(z_0) = 0 \) at the distance furthest from the bilayer center. Figure 3.5(a) shows the profiles of the total electrostatic potentials across the lipid bilayer in both 0 mol % and 3 mol % DMSO systems. In both systems the interior of the bilayer has a positive potential relative to the aqueous surrounding. The actual potential drop between the bilayer interior and the solvent (trans-leaflet potential) is about 575 mV and 775 mV.
Figure 3.4: Local charge density contributions from lipids, water and DMSO molecules in (a) 0 mol % DMSO system and, (b) 3 mol % DMSO systems. $z = 0$ corresponds to the center of the bilayer.
in the 0 mol % and 3 mol % DMSO respectively. Interestingly the presence of DMSO leads to an increase of the trans-leaflet potential by about 35% relative to the pure systems. These values are in very good agreement with other simulation[33] and experimental results[34]. Using the unique MD simulations capability one can gain additional insight into the origin of the trans-leaflet potential by splitting the total potential into the corresponding terms caused by different types of molecules. Figure 3.5(b) shows the contributing terms to the total electrostatic potentials due to the lipids, water, and DMSO molecules in both simulated systems. As with the charge distribution, the electrostatic potential due to water molecules mirrors and slightly overcompensates the potential component due to lipid heads. As expected, the potential component due to the DMSO molecules, although of smaller magnitude, has the same sign and shape as the component due to the water molecules. Moreover, as indicated in Figure 3.5(b) the presence of DMSO leads to an increase of the potential component due to the lipid heads. This increase can be attributed to the slight change of the average orientation of the P-N vectors towards a more out-of-plane orientation (see Figure 3.3(b)). As mentioned previously this change in orientation of the P-N vector leads to an average increase of the normal component of the lipid dipolar moments which in turn leads to a slightly larger lipid dipolar charge and larger potential change across the bilayer-solvent interface.

3.3.4 DMSO and Water Electric Dipole Orientation across Lipid Bilayers

Additional information about the DMSO and water ordering in the vicinity of the bilayer-solvent interface (hydration layer) can be obtained from studying the time averaged projections of DMSO and water electric dipole unit vectors $\hat{\mu}$ onto the bilayer normal unit vector $\hat{n}$ which can be written as:
\[ < \cos \theta(z) > = \frac{1}{|\vec{\mu}(z)|} < \vec{\mu}(z) \cdot \vec{n} > \]  

(3.2)

where \( \theta \) is the angle between the electric dipoles and normal vector to the bilayer; \( z \) is the \( z \)-component of the center of mass of the DMSO or water molecule.

Figure 3.5: (a) Total electrostatic potential across the bilayer in both 0 mol % (dashed line) and 3 mol % DMSO (solid line) systems. (b) Contributions of water, lipids, and DMSO to the total electrostatic potential in both 0 mol % (dashed lines) and 3 mol % DMSO (solid line) systems.
The mean cosine value profile is obtained by averaging, when the system is in the equilibrium regime, over dipolar orientations of all DMSO/water molecules present in the corresponding bins and over a large number of equilibrium states. Figure 3.6 shows the profiles of the averaged electric dipole projections in 0 mol % and 3 mol % systems for water and DMSO (only in 3 mol % system) molecules. Notice that a zero mean cosine average far from the bilayer center corresponds to a random orientation of the electric dipoles and that the normal unit vector, $\vec{n}$, points along the positive z direction on both sides of the bilayer. Consequently, by symmetry, the cosine average has the opposite sign in the two regions. As revealed by Figure 3.6, in or near the bilayer, both the water and DMSO molecules prefer to orient themselves such that their electric dipolar moments are oriented away from the outward bilayer normal. In fact, the maximum magnitude of $\langle \cos \theta \rangle$ corresponds to an angle that is about 180° different from the average P-N vector orientation. The P-N vector is a good approximation to the dipole moment vector of a DMPC molecule. The dipoles of the DMSO and water molecules in the bilayer point in the opposite direction of DMPC dipoles since this is the most energetically favorable orientation. For water, the ordering peak is located slightly more outward, along the bilayer normal direction, compared to the peak corresponding to the lipids (see Figures 3.2(a) and 3.6). The electric dipole ordering peaks for DMSO are wider and extend deeper into the bilayer than the corresponding peaks for water. The DMSO dipoles maintain their alignment in the opposite direction of the DMPC dipoles almost up to the bilayer center. Since only a few water and DMSO molecules penetrate deep into the lipids acyl chains region, the statistics of averaging is poor in that region resulting in noisy orientation profile curves for both molecular species. Comparing the water orientation profiles in 0 mol % and 3 mol % DMSO solutions indicate that DMSO leads to a decrease in the separation distance between the centers of the two hydration layers present on
both sides of the membrane which is directly correlated to the decrease of the bilayer thickness in the presence of DMSO.

![Graph showing the average of the cosine of the angle between dipole moment vectors of DMSO and water molecules and the outward normal to the upper leaflet (positive distance) as a function of the distance from the bilayer center in both 0 mol % and 3 mol % DMSO systems.]

**Figure 3.6**: Average of the cosine of the angle between dipole moment vectors of DMSO and water molecules and the outward normal to the upper leaflet (positive distance) as a function of the distance from the bilayer center in both 0 mol % and 3 mol % DMSO systems.

### 3.3.5 Number of Hydrogen Bonds per Water Molecule inside the Bilayer

The number of hydrogen bonds per water molecule was calculated in the regions of the bilayer containing the ester group oxygen atoms and in the bilayer tail region. A hydrogen bond was defined to exist when the donor-acceptor distance was less than or equal to 0.35 nm and the donor-hydrogen-acceptor angle was less than 30° from linear alignment. In this system, the only donor is the water oxygen atom. The acceptors considered are the water oxygen, the DMSO oxygen, and the four ester oxygen atoms. The two ester group regions were defined by averaging the upper and lower boundary positions (z direction) of the ester group oxygen atoms over all frames. The tail region was defined as the region between the two ester group regions,
except that no water molecules within the donor-acceptor cut off distance from an ester group oxygen atom were counted in this region.

Table 3.1 shows the number of hydrogen bonds per water molecule between water molecules and ester group oxygen atoms, DMSO molecules, and other water molecules; and the number density of water in the ester group and tail regions of the bilayer. One might expect that the addition of DMSO might increase the number of water hydrogen bonds with non-water molecules (ester group oxygens + DMSO). However, in the ester group region, adding DMSO reduces the number of ester group hydrogen bonds and the number of non-water hydrogen bonds actually decreases slightly with the addition of DMSO. Adding DMSO does allow more water molecules into the bilayer (increased density). With more water present, the number of water-water hydrogen bonds increases such that the total number of water hydrogen bonds also increases slightly. In the tail region, the number of water-DMSO hydrogen bonds is nearly the same as in the ester group region while the number of water-water hydrogen bonds is less than a quarter of this. This indicates that a DMSO molecule can carry a water molecule into the tail region, but clusters containing multiple water molecules are much less likely.

Table 3.1: The number of hydrogen bonds per water molecule and water number density in the regions containing the ester group oxygen atoms, and in the tail region. For comparison, the water density in bulk solvent is 32.0 and 31.0 nm$^{-3}$ in the 0 mol % and 3 mol % cases, respectively. Uncertainties are two times the standard deviation of the mean.

<table>
<thead>
<tr>
<th>System</th>
<th>Number of Water Hydrogen Bonds per Water Molecule</th>
<th>ρ$_{\text{water}}$ (nm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ester</td>
<td>DMSO</td>
</tr>
<tr>
<td>0 mol %</td>
<td>0.42 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>3 mol %</td>
<td>0.26 ± 0.01</td>
<td>0.123 ± 0.004</td>
</tr>
</tbody>
</table>
(Table 3.1 continued)

<table>
<thead>
<tr>
<th>System</th>
<th>Ester</th>
<th>DMSO</th>
<th>Non-Water</th>
<th>Water</th>
<th>Total</th>
<th>$\rho_{\text{water}}$ (nm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mol %</td>
<td>--</td>
<td>0.13 ± 0.03</td>
<td>0.13 ± 0.03</td>
<td>0.03 ± 0.01</td>
<td>0.16 ± 0.04</td>
<td>0.022 ± 0.004</td>
</tr>
</tbody>
</table>

3.3.6 Free Energy Profiles

The PMF for water and DMSO molecules in both 0 mol % and 3 mol % DMSO systems were calculated as a function of the distance from the bilayer center by numerical integration of the average constraint forces acting on the constrained molecule at different positions. Figures 3.7(a) and 3.7(b) show the PMFs calculated for water and DMSO molecules respectively in both systems. As shown in Figure 3.7(a) the PMF profile for a water molecule located far from the bilayer center is flat in both systems. As the molecule approaches the bilayer center the PMF increases steeply. In the pure water system, as the water molecule moves closer to the bilayer center the PMF exhibits an obvious peak of about 27 kJ/mol, located near the center of the bilayer, followed by a slight decrease. In contrast, in the system containing DMSO, the PMF for the water molecules increases steadily, without a clear peak, until the center of the bilayer where it reaches a maximum value of approximately 23 kJ/mol. The peak near bilayer center and minima of the PMF at the bilayer center in the pure water system are due to the low lipid density at the bilayer center (see Figure 3.2(a)). In the system containing DMSO, this density minimum is not as pronounced, so there is no peak in the PMF other than that corresponding to the center of the bilayer. As documented in Figure 7(a) the presence of 3 mol % DMSO into the DMPC
bilayer system leads to a reduction of the free energy barrier for a water molecule by about 4 kJ/mol. As shown in the following sections the decrease of the barrier in the PMF profile affects significantly the water permeability coefficient in DMPC bilayers.

As illustrated in Figure 3.7(b) the PMF profiles for a DMSO molecule moving across a DMPC lipid bilayer in both pure water system and the system containing DMSO look significantly different from the PMFs for the water molecule (see Figure 3.7(a)). Specifically, in both systems the PMFs for the DMSO molecule decrease continuously and reach a minimum value as the molecule moves from solvent into the DMPC bilayer. The minima are located approximately midway between the peaks corresponding to the lipid heads phosphate and the carbonyl group. Moreover, the profiles of the PMFs around their minimum values are fairly flat over a relatively large interval comprising the distances from bilayer center located between 1.0 nm and about 1.75 nm. Unlike the pure water system, the PMF for the DMSO molecule in the 3 mol % DMSO system shows a much smaller decrease moving into the bilayer. Both PMF profiles show a fairly steep increase when the DMSO molecule moves toward the center of the bilayer where the PMFs reach their maximum values of approximately 10 kJ/mol and 7.5 kJ/mol in the pure water system and 3 mol % DMSO system respectively. In both simulated systems the DMSO molecule experiences significantly lower PMF barriers compared to the water molecule. One can rationalize these characteristics in terms of differences in polarity of the two molecules. While water molecules are highly polar, the DMSO molecules with their slight amphiphilic character can be accommodated more easily into the hydrophobic interior of the bilayer.
Figure 3.7: The PMF profiles for (a) water molecules and (b) DMSO molecules along the normal direction to the DMPC bilayer in both 0 mol % and 3 mol % DMSO systems. Error bars are 2 times the standard deviation of the mean.
3.3.7 Diffusion Coefficients

The diffusion coefficient profiles for water and DMSO molecules in both pure water system and the system containing 3 mol % DMSO are plotted in Figures 3.8 as a function of the distance from the center of the bilayer. There are obvious similarities between the diffusion profiles for the two molecules in both systems indicating very similar diffusion mechanisms throughout the entire diffusion path spanning the bulk solution, the bilayer-solvent interface, and the interior of the bilayer. As shown in Figures 3.8(a) and 3.8(b) the water and DMSO diffusivities have the highest values in the solvent and decrease steeply and remain relatively constant as the molecules move through the bilayer-solvent interface toward the bilayer center. Interestingly, in the pure water system the diffusion coefficients for both water and DMSO increase rapidly upon moving towards the middle of the bilayer, an increase that is associated with the existence of a larger free volume in the region where the tails of the lipids comprising the two leaflets meet. One can also conclude that with the exception of a narrow region in the bilayer midsection the presence of DMSO seems to have a very limited effect on the diffusivity of both water and DMSO molecules.

3.3.8 Molecular Mean First Passage Times through the Bilayers (MFPT)

In the high friction limit of the diffusive motion, the mean first passage time for a molecule to reach a distance from the bilayer center \( z_2 \) when starting from a distance \( z_1 \) can be calculated by the following equation\[35][36]:

\[
\langle \tau_{i} \rangle = \int_{z_1}^{z_2} \frac{e^{\text{PMF}_i(x)/k_BT}}{D_i(x)} dx \int_{-3.5\text{nm}}^{x} e^{-\text{PMF}_i(y)/k_BT} dy \tag{3.3}
\]

The mean first passage time curves are shown in Figures 3.9 for (a) water and (b) DMSO. The times required for a molecule located initially in the bulk solution on one side of the bilayer far from the bilayer-solvent interface (i.e., at \( z_1 = -3.5 \text{ nm} \)) to cross to the bulk solution on the other
Figure 3.8: The profiles of the diffusion coefficient, $D(z)$, for (a) water molecules and (b) DMSO molecules along the normal direction to the DMPC bilayer in both 0 mol % and 3 mol % DMSO systems. Error bars are 2 times the standard deviation of the mean.
Figure 3.9: The mean first passage time for (a) a water molecule, and (b) a DMSO molecule starting from $z_0 = -3.5$ nm away from the bilayer center to move to a new location $z$ along the bilayer normal in both 0 mol \% and 3 mol \% DMSO systems. Uncertainties (95.4\% confidence interval) were determined by sampling from the distributions for the PMF and diffusivity for each point and calculating many mean first passage times.
side \( (z_2 = 3.5 \text{ nm}) \) are shown in Table 3.2. This indicates that the mean first passage times for both water and DMSO molecules are in the microseconds range, far beyond the reach of direct MD simulation timescale. Interestingly, for both water and DMSO, the times are more than an order of magnitude smaller in the 3 mol % DMSO systems.

### 3.3.9 Permeability Coefficients

Using the free energy profiles and the diffusivities for water and DMSO and by employing an inhomogeneous solubility-diffusion model one can calculate the permeability coefficients through the DMPC bilayer. The values of the permeability coefficients are related to the local properties and can be calculated according to the following relation[13]:

\[
P_i = \frac{1}{R_i^p} = \frac{J_i}{\Delta c} ,
\]

where \( J_i \) is the flux of molecules of species \( i \), \( \Delta c \) is the concentration difference, \( P_i \) is the permeability coefficient and \( R_i^p \) is the permeation resistance given by

\[
R_i^p = \int_{z_1}^{z_2} \frac{e^{PMF(z) / k_B T}}{D(z)} \, dz .
\]

As shown in Table 2, in the pure water system (or the limiting case of infinitely diluted DMSO solution system when a single DMSO molecule is present in the solution), the permeability coefficients are 0.0119 cm/s and 4.1 cm/s for water and DMSO respectively. The value for water is within the range of experimentally measured values (e.g. \( 4.0 \times 10^{-4} \) to \( 2.4 \times 10^{-2} \) cm/s) for fluid-phase DMPC\[37\]. The permeability of both water and DMSO molecules increase in the presence of 3 mol % DMSO. While the DMSO molecules experience only a modest 36% increase of the permeation coefficient, the permeability of water molecules increases
significantly, by approximately 300%, from 0.0119 cm/s to 0.037 cm/s. This significant increase of permeation coefficient for water is mainly a direct consequence of the decrease of the water free energy barrier (PMF) located at the center of the lipid bilayer, in the presence of 3 mol % DMSO (see Figure 7(a)).

Table 3.2: Permeabilities and mean first passage times for water and DMSO molecules to diffuse (across a DMPC bilayer) between two points located, deep into the solvent, at $z_i = -3.5$ nm and $z_f = 3.5$ nm on either side of the bilayer in both 0.0 mol % and 3.0 mol % DMSO systems. The values in parentheses are error bars (-,+) for a 95.4% confidence interval.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>DMSO [mol %]</th>
<th>$P$ (cm/s)</th>
<th>$&lt;\tau&gt;$ (μs), from -3.5 to 3.5 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>0.0</td>
<td>0.0119 (0.0043, 0.0051)</td>
<td>16.2 (6.2, 8.6)</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.0</td>
<td>4.1 (1.5, 1.9)</td>
<td>1.01 (0.39, 0.64)</td>
</tr>
<tr>
<td>water</td>
<td>3.0</td>
<td>0.037 (0.011, 0.013)</td>
<td>5.77 (1.6, 2.24)</td>
</tr>
<tr>
<td>DMSO</td>
<td>3.0</td>
<td>5.58 (1.98, 1.75)</td>
<td>0.15 (0.034, 0.045)</td>
</tr>
</tbody>
</table>

3.4 Conclusions

Our MD simulation study has shown that at relatively low concentration, DMSO molecules penetrate and diffuse deep into DMPC bilayers and concentrate in the region located below the lipids polar head-groups. At 3 mol % DMSO concentration, the DMPC bilayer remains intact on a time scale of tens of nanoseconds (i.e., no stable or transient pores form in the bilayer), but the DMSO molecules have a strong effect on the area per lipid, the bilayer thickness, the orientation of the lipids heads with respect to the bilayer normal, and on trans-leaflet electrostatic potential. The calculation of the potential of mean force (PMF) and the diffusivity profiles along the direction normal to the bilayer for water and DMSO molecules indicate that while the presence of DMSO has only a small effect on diffusion coefficients of both water and DMSO, it affects significantly the corresponding trans-membrane free energy profiles. Using the free energy
profiles and diffusivities for water and DMSO and by employing an inhomogeneous solubility-diffusion model we calculated the corresponding permeability coefficients. The values of the permeability coefficients, related to an integral over the local free energy and diffusivities, enable a direct comparison between computational model and experiments. Our simulations show that in the pure water bilayer system the permeability coefficients are 0.0119 cm/s and 4.1 cm/s for water and DMSO respectively; values that are in good agreement with experimental results. Interestingly, the increase of the concentration of DMSO in the solution to 3 mol % leads to a significant increase, by about 300%, of the permeability of water through DMPC bilayer. This permeability increase might explain in part the unusual ability of DMSO, at relatively low concentrations, to reduce the osmotic pressure imbalance present during cryopreservation protocols.

3.5 References


CHAPTER 4
MOLECULAR DYNAMICS SIMULATION OF BILE SALT SOLUTIONS IN THE PRESENCE OF VITAMIN E AND OLEIC ACID

4.1 Introduction

Bile salts are among the most important biological surfactants found in vertebrates, including humans. They are the dominant components of bile which is produced by liver, temporarily stored and concentrated in gallbladder, and delivered into the small intestine. Bile plays an important role in digestion by solubilizing and dispersing nonpolar molecules such as lipids, cholesterol, fatty acids, monoglycerides, and fat-soluble vitamins. The main components of bile are bile salts (67 %, w/w), phospholipids (22%, w/w), proteins (4.5 %, w/w), cholesterol (4%, w/w), and bilirubin (0.33 %, w/w)[1]. Bile salts as the main molecular species of bile is a mixture of a large number of salt species (more than twelve) which differ from each other in the position, stereochemistry, and degree of hydroxylation and in the identity of the conjugated amino acid. Conjugation occurs primarily with glycine and taurine in a ration of approximately 3:1, with sodium (Na\(^+\)) or potassium (K\(^+\)) present as the counterions.

Structurally, all bile salt molecules have a large, relatively rigid, nearly planar, steroid moiety with a few hydroxyl groups all directed towards the same side and two methyl groups attached to the opposite side. The steroid ring is linked through a short alkyl chain to an ionic head that may contain a carboxyl group. The steroid ring is shaped like an elongated saucer with the hydroxyl groups all directed towards the concave surface (the hydrophilic side \(\alpha\)) and methyl groups towards the convex surface (the hydrophobic side \(\beta\)) (see Figures 4b, and 4c). Thus, their structure, especially their spatial distribution of the hydrophilic and hydrophobic groups, is markedly different than that of the widely used conventional surfactants that usually have a small strongly polar, or even charged, head and a long apolar hydrocarbon tail group. Therefore the
bile salt molecules have a surface that is hydrophobic and one that is hydrophilic, rather than a hydrophobic tail and a hydrophilic head as found in conventional surfactants (detergents). The special structure of bile salts affects strongly their self-assembly process and the characteristics of the resulting micelles. For example, long ago it has been shown experimentally that because of their planar polarity when the bile salt concentration is small they form smaller micelles than the classical surfactants; in the region of 2-10 molecules per micelle [2, 3]. One can rationalize this result by observing that it is difficult to assemble a large aggregate and maintain contact between water and all of the hydrophilic faces. However, unlike other amphiphilic character, the mean aggregation number of bile salts increases with the bile salt concentration, due mostly to the formation of the so-called secondary micelles [4]. Also as result of their peculiar planar amphiphilic character, the bile salts seem to not exhibit a well-defined critical micellar concentration (cmc) with micelle formation occurring as a continuum association process [5] and it makes it more challenging to predict self-assembly solubilization process when interacting with other amphiphiles such as fatty acids produced by lipid digestion or with fat-soluble vitamins and drugs.

Because of their biological importance bile salts aggregation and interaction with other molecules has been the subject of many experimental, theoretical, and computational studies. The observation of bile salts spontaneous aggregation behavior in aqueous solutions was reported as early as 1942 [6, 7]. Many studies have focused on developing a mechanistic understanding of bile salts self-assembly process in both pure and mixed systems containing lipids and cholesterol [8-14]. Experimental investigations have been carried out by using modern investigation techniques such as X-ray scattering [15, 16], light scattering [8, 17], and nuclear magnetic resonance (NMR) measurements [18, 19]. These studies lead to the conclusion
that the bile salt aggregation occurs in two stages, the so called “primary-secondary model”[20], Accordingly in the primary stage model the aggregates (micelles) are relatively small characterized by a small aggregation number and the driving force being the hydrophobic interaction. At larger bile salt concentration a secondary stage is present in which larger micelles form by clustering of smaller ones; process believed to be controlled by the intermolecular hydrogen bond interactions.

Molecular modeling approaches such as molecular dynamics and Monte Carlo simulations simulations have been used for studying the structure and self-assembly of micellar structures [21-24]. The biggest challenge for MD simulations has been the limitations in both system size and the simulation time. Despite the great advances the MD simulation can provide to this area so far there have been a relative small number of MD simulation studies focusing on developing molecular level understanding of bile salts systems [21-24].

In this study we present results of our MD simulations carried out to investigate the aggregation behavior of two important, well studied experimentally bile salts, cholate (CHD) and glycocholate (GCH) in aqueous solution. Glycocholate is known as one of the most abundant component of the seven bile salt molecules comprising the human bile; in fact GCH accounts for about 30.9 mol% of the bile salts mixture. Our focus is on both reveling the kinetics of the aggregation mechanism as well as on understanding the structural characteristics of the micelles formed. To better understand the interaction of the bile salts with fatty acids and their role in lipid digestion we performed MD simulations to investigate the interaction and encapsulation and of oleic acid into preformed glycocholate micelles. In addition, we present the results of our MD simulations studies aimed at the development of a molecular level understanding of the
effect of bile salts on surface tension and interfacial energy of the $\alpha$-tocopherol – water interface [$\alpha$-tocopherol is one of the main components of vitamin-E].

4.2 Simulation Systems and Methodology

4.2.1 Initial Structures and Simulations Setup

MD simulations of the sodium cholate (CHD) and sodium glycocholate (GCH) self-assembly were performed at the concentration of 100mM, which is the typical bile salts concentration in the small intestine after they are released from gallbladder. Figure 4.1 shows the chemical and spatial 3-dimensional structures of two types of bile salts considered the glycocholate and cholate molecules. In addition, Figure 4.2 shows the chemical structures of $\alpha$-tocopherol (vitamin E) and oleic acid, the molecular species whose interaction with bile salts was also investigated in this study. In the first part of the study our focus was on understanding the aggregation behavior of the two bile salts glycocholate and cholate in aqueous solutions. Two system sizes, one containing 31 bile salt molecules and the other containing 186 sodium glycocholates, were considered. Figure 4.3 depicts the initial structure of the simulation system containing 31 cholates.

Self-assembly of both cholate and glycholate were investigated in the 31 bile salt molecules systems. The initial structure was created by randomly distributing the 31 bile salt molecules in a cubic simulation box of 8 nm size and by solvating the resulting structure with 16,000 water molecules. In addition to the neutralizing Na$^+$ ions, a background salt concentration of 0.15M was added to the system. To gain additional insight into the statistical effects on self-assembly process we also carried out self-aggregation study of GCH system that was 6 times larger. This system was constructed in a similar way and contained 186 GCH molecules, 96,000 waters and 462 Na$^+$ and 276 Cl$^-$ ions to account for charge neutrality and add a 0.15M
background salt to the aqueous solution. The bile salts concentration in both CHD and GCH systems (both large and small systems) was 100 mM, value that is well above the critical micelle concentration (cmc). Critical micelle concentration is the minimum concentration of bile salts at which micelles begin to form. At equilibrium, if the system is large enough and the surfactant concentration is above cmc the number of bile salts in the free monomer state (e.g., those that are not part of any larger aggregates) is constant.

To better understand the mechanism of oil solubilization in bile salts we also investigated the adsorption and transfer of oleic acid molecules from aqueous solution into three preformed glycocholate micelles. Figure 4.4 shows the initial structure of this simulation system that contains three GCH micelles. The preformed micelles were taken from the previous simulations of 31 GCH molecules and contain 12, 11, and 6 GCH molecules respectively (in addition the system contained two GCH molecules that were not incorporated in any of the three well-formed micelles). This simulation system was obtained from the last configuration of the previous, 31 GCH molecules system, by removing the water molecules and ions and by adding ten oleic acid molecules that were initially randomly distributed throughout the simulation cell. After the addition of the oleic acid molecules the system was rehydrated and neutralizing and background salt ions were added to the solution.

To better understand the solubilization of vitamin E by bile salts we performed multiple MD studies of two other systems. In caring on these studies our goals was to better our understanding of adsorption and dissolution of preformed bile salt micelles at the vitamin E - water interface as well as the understanding of the change of the interfacial properties, (e.g. interfacial energy, surface tension and structure of the interface) of vitamin E-water interface in the presence of bile salts. The initial structures used in these studies are represented in Figures 4.5 and 4.6. Both
Figure 4.1: (a) and (c) Chemical and (b) and (d) 3-dimensional structures of glycococholate (GCH) and cholate (CHD) ions respectively used in the MD studies. In panels (b) and (d) the carbon, oxygen, nitrogen and hydrogen are colored in cyan, red, blue, and white, respectively.

Simulation systems contain a slab of vitamin E immersed in aqueous solutions that contain the Na\(^+\) and Cl\(^-\) ions as well as the GCH and CHD ions respectively. Under the 3-dimensional periodic boundary conditions setup this simulation system contains two water-vitamin E interfaces. In the study of adsorption and dissolution of GCH micelles at the vitamin E-water interface (Figure 4.5) the vitamin E slab was 3 nm thick and contained 287 vitamin E molecules. In the initial structure the preformed micelles were all placed at a distance at least 2 nm away from any of the two vitamin E – water interfaces. In the second system simulations, shown in Figure 4.6, aimed at understanding the effect of bile salt molecules adsorbed at the vitamin E-water interface on surface tension, interfacial energy, as well as on the structure of the interface, the vitamin E slab contained 382 molecules and was 4 nm thick. We performed a total eleven
simulations in which the number of CHD molecules located at each of the two interfacial regions was incrementally increased from 1 to 11. The simulations were initially carried on at constant number of particles, constant pressure $p=1$ atm, and constant temperature $T=300k$, for 20ns to allow the system to equilibrate and the switched to constant volume simulation for 100 ns. The last 40 ns along each trajectory were used for calculation of the averaged properties.

Figure 4.2: Chemical structures of (a) $\alpha$-tocopherol (vitamin E) and (b) oleic acid.
Figure 4.3: Initial structure of the MD simulation system containing 31 cholates randomly
distributed in aqueous solution. The coloring scheme is as follows: water is represented in tan,
Cl\(^-\) ions in green, Na\(^+\) ions in orange. Cholate (CHD) ions are represented in licorice and have C
atoms in cyan, O in red and H in white.

Figure 4.4 Initial structure of the MD simulation system containing three glycocholate micelles
taken from a previous self-assembly simulation, ten oleic acid molecules, Na\(^+\) and Cl\(^-\) ions, and
water. The coloring scheme is as follows: water is represented in tan, Cl\(^-\) ions in green, Na\(^+\) ions in orange. Glycocholate (GCH) ions are represented in licorice and have C atoms in cyan, O in red, N in blue, and H in white.
Figure 4.5 Initial structure of the MD simulation system containing three glycocholate micelles taken from a previous self-assembly simulation interacting with vitamin E-water interfaces. Periodic boundary conditions in all three dimensions are considered. For better illustration of the two vitamin E-water interfaces part of the periodic images along the z-direction (vertical direction) are also represented. The coloring scheme is as follows: water is represented in tan, Cl$^-$ ions in green, Na$^+$ ions in orange. GCH ions are represented in licorice and have C atoms in cyan, O in red, N in blue, and H in white. Vitamin E molecules in the slab are represented with lines with C atoms colored in cyan and O in red.
Figure 4.6 Initial structure of the MD simulation system containing a 4nm thick slab of vitamin E in aqueous solution in the presence of two CHD molecules adsorbed at the vitamin E-water interfaces. Periodic boundary conditions in all three dimensions are considered. The coloring scheme is as follows: water is represented in tan, Na\(^+\) ion in orange. CHD ions are represented in licorice and have C atoms in cyan, O in red, and H in white. Vitamin E molecules in the slab are represented with lines with C atoms colored in cyan and O in red.

### 4.2.2 Methodology

All MD simulations were performed with GROMACS 4.0 simulation package.[25]. The force field parameters for bile salts were obtained by combining separately the parameters obtained from the existing topologies for the 4 ring planar head part of the cholesterol with the parameters for the tail part obtained by parameter fitting done on the ATB website [http://compbio.biosci.uq.edu.au/atb/]. The simple point charge (SPC) model was used for water [26]. All bile salt self-assembly simulations were performed at constant pressure of 1.0 bar using a Berendsen barostat and isotropic coupling. The temperature was held constant at 300 K. All
non-water bond lengths were constrained by using the LINCS[27] algorithm. Water bonds and angles were constrained using the SETLLE [28] algorithm. For short-range non-bonded interactions, GROMACS uses a twin range cutoff. The inner cutoff was 0.98 nm. Forces due to van der Waals interactions in the range from 0.98 to 1.4 nm were evaluated every 10 steps. The long-range electrostatic interactions were treated using the particle mesh ewald (PME) algorithm,[29, 30] with a 0.1176 nm grid spacing, of order 4. We used an integration time step of 2.0 fs. The energy minimization procedure, based on the steepest descent algorithm, was applied to the initial structures prior to the actual MD runs.

4.3 Results and Discussion

4.3.1 Self-Aggregation of Bile Salts

The process of spontaneous aggregation of bile salts into micelles in both small (31 CHD or 31 GCH molecules) and large simulation systems (186 GCH) are illustrated in Figures 4.7, 4.8 and 4.9. In all three systems the molecules were placed randomly in the simulation box in the initial state. Because the bile salt molecules have hydrophobic faces that interact unfavorably with the aqueous environment they very quickly start to associate into smaller micellar aggregates (~5 ns) containing two or three bile salts molecules and continue to grow to larger aggregates forming micelle with stable size. The larger micelles although they are more stable in size they are still quite dynamic structures as individual bile salt molecules can detach or attaché from/to the existing structures. As documented in Figures 4.7 (b) and 4.8 (b), the simulations show that after about 50 ns in the 31 CHD molecules system and 60 ns in the 31 GCH molecules system the aggregate structures reach steady stable states characterized by the presence of two and three well-formed micelles in the CHD and GCH simulation systems respectively. In our system a bile salt molecule was defined to be part of a micelle when its center of mass is located
to a distance less than 0.51 nm away from the center of mass of at least one molecule belonging to the micelle. Based on this criterion, the clustering and the corresponding aggregation numbers were monitored throughout the self-assembly process in both small and larger bile salts systems. In addition to determine the overall shape of the micelles we also evaluated the principal radius of gyration for each micelle, radii of gyration that were averaged over the last 20 ns of the trajectories. The bile salt aggregation numbers and the radii of gyration are presented in the Table 4.1. Compared to the experiment results, the aggregation numbers in the small systems simulations are relatively small which is probably due to the limitations of the MD simulations imposed by both small systems size and short simulation times. The aggregation number varies between 6 for one of the GCH micelles and 18 for the largest of two CHD micelles.

Table 4.1: The aggregation number and principal radii of gyration of the micelles obtained in the self-assembly process in both 31 glycocholate and 31 cholate systems

<table>
<thead>
<tr>
<th>Bile salt type</th>
<th>Micelle size</th>
<th>R_x (nm)</th>
<th>R_y (nm)</th>
<th>R_z (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocholate(GCH)</td>
<td>12</td>
<td>0.74</td>
<td>0.98</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.73</td>
<td>0.93</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.59</td>
<td>0.80</td>
<td>0.85</td>
</tr>
<tr>
<td>Cholate (CHD)</td>
<td>18</td>
<td>0.82</td>
<td>1.13</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.71</td>
<td>1.02</td>
<td>1.08</td>
</tr>
</tbody>
</table>

As documented in Table 4.1 the principal radii of gyration of both GCH and GHD micelles indicate that the overall shape of the micelles is oblate rather than spherical with R_x, the smaller radius, ranging between 0.59 to 0.82 nm and the other two radii R_y, and R_z, approximately of the same value, ranging between 0.80 and 1.13 nm and 0.85 and 1.20nm respectively. The oblate shape of the micelles is consistent with specific packing of the bile salt molecules, controlled by the planar distribution of the hydrophobic and hydrophilic moieties of bile salt molecules [see Figures 4.1 (b) and 4.1 (d)]. That is the molecules pack such that the hydrophobic methyl groups of the molecules point towards the inside the micelle while hydrophilic hydroxyl groups point
Figure 4.7: Snapshots depicting the self-assembly process of 31cholate (CHD) molecules. (a) In the initial structure the surfactants was uniformly distributed in the aqueous solution containing both neutralizing and background NaCl ions. (b) Two well-formed micelles are observed in the final structure. The coloring scheme is as follows: Cl\(^-\) ions in green, Na\(^+\) ions in orange, CHD ions are represented in licorice and have C atoms in cyan, O in red, and H in white. Water is omitted for clarity.
Figure 4.8: Snapshots depicting the self-assembly process of 31 glycocholate (GCH) molecules. (a) In the initial structure the surfactants was uniformly distributed in the aqueous solution containing both neutralizing and background NaCl ions. (b) Three well-formed micelles are observed in the final structure; two GCH are not part of the three micelles formed. The coloring scheme is as follows: Cl⁻ ions in green, Na⁺ ions in orange, GCH ions are represented in licorice and have C atoms in cyan, O in red, N in blue, and H in white. Water is omitted for clarity.
Figure 4.9: Snapshots depicting the self-assembly process of 186 glycocholate (GCH) molecules. (a) In the initial structure the surfactants was uniformly distributed in the aqueous solution containing both neutralizing and background NaCl ions. (b) Both large (containing more than 15 GCH) and smaller well-formed micelles are observed in the final structure. The coloring scheme is as follows: Cl\(^-\) ions in green, Na\(^+\) ions in orange, GCH ions are represented in licorice and have C atoms in cyan, O in red, N in blue, and H in white. Water is omitted for clarity.
toward the aqueous solution such that to maximize their interacting area with polar and charged groups.

Another parameter useful in characterizing the dynamics and the stability of aggregates formed during self-assembly process is the solvent accessible surface area (SASA). SASA is defined as the surface area of all bile salt molecules in the system that can have access to the water molecules at a given time. Figure 4.10 shows the 60 ns time evolution of the SASA for the 31 GCH molecules system during the self-assembly process. As expected the surface area accessible to water is large at the beginning when the GCH molecules are distributed randomly in the aqueous solution. As the bile salt molecules aggregate the SASA decreases. The rate of decrease of the SASA can be viewed as an indicative of the rate of aggregation. As the 3 micelles structure remains stable during the last 20 ns of the simulation so does the SASA which fluctuate around a pretty stable average value.

Figure 4.10: Time evolution of the solvent accessible surface area (SASA) of GCH molecules during self-assembly in the 186 GCH molecules system.
The aggregation process in the large 186 GCH molecules system, of the same 100mM bile salt concentration, shows the formation of much larger structures. As depicted in Figure 4.9 (b) in about 100 ns the system coarsens to just 8 large micelle clusters. As documented in Figure 4.11 the number of clusters in the system is about 150 at the beginning of the simulation and decreases down, and remains relatively stable (see inset in Figure 4.10), to just 8 cluster after about 100 ns. The small number of clusters present after 100 ns shows the presence of some large ones. The 186 GCH system shows the presence of large clusters that contain up to 30 bile salt molecules, aggregation number that is much larger than the typical micelle sizes that aggregate under the driving force of hydrophobic interactions. These larger clusters are the result of the secondary aggregation process in which smaller micelles are attracted toward each other and form stable clusters stabilized by formation of hydrogen bonds between bile salt molecules that are part of neighboring micelles.

Figure 4.11: The number of bile salt clusters in the 186 glycocholate molecules system after 100 ns simulation time. The inset details the time evolution of the number of clusters during the last 25 ns of the 100 ns trajectory.
4.3.2 Adsorption of Oleic Acid into Preformed Glycocholate Micelles

To further the understanding of the role of bile salts in digestion of lipid-based formulations we investigated the interaction and adsorption of oleic acid molecules in three preformed GCH micelles. As described previously in section 4.2.1 the initial state consists of three preformed GCH micelles and 10 oleic acid molecules that were initially distributed randomly in the aqueous solution far from any of the three micelles. The three GCH preformed micelles have aggregation numbers of 12, 11, and 6. Figure 4.12 shows two snapshots of the simulation system depicting the initial structure of the simulation system and the structure after 10 ns. As seen from Figure 14 (b) in within 10 ns all 10 oleic acid molecules are incorporated in the three GCH micelles. Detailed atomistic analysis reveals that oleic acid molecule penetrate with hydrocarbon tails first deep inside the hydrophobic region of the micelles. The 10 oleic acid molecules are incorporated into the three micelles as follows: the 12 GCH molecules micelle incorporates 5 oleic acid molecules, the 11 GCH molecules micelle incorporates 3 oleic acids and the 3 GCH molecules micelle incorporates 2 oleic acids. As evident from Table 4.2 the incorporation of oleic acid molecules leads to swelling of the micellar structures and results in both micelle size increase and shape change. Specifically, the micelles shape change from oblate to more spherical.

Table 4.2: The characteristics of the three preformed GCH micelles upon adsorption of 10 oleic acid molecules. The average values were evaluated by averaging over the last 8 ns of the trajectory.

<table>
<thead>
<tr>
<th>Bile salt type</th>
<th>Micelle size</th>
<th>Number of oleic acid</th>
<th>R_x (nm)</th>
<th>R_y (nm)</th>
<th>R_z (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocholate (GCH)</td>
<td>12</td>
<td>5</td>
<td>0.98</td>
<td>0.99</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3</td>
<td>0.90</td>
<td>0.95</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>0.74</td>
<td>0.77</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Figure 4.12 (a) Initial and (b) the final snapshots delineating the rapid adsorption and entrapping (about 10 ns) of oleic acid molecules in three preassembled GCH micelles (taken from a previous self-assembly simulation). The coloring scheme is as follows: Glycocholate (GCH) ions are represented in licorice and have C atoms in cyan, O in red, N in blue, and H in white. Oleic acid molecules are represented in VDW and have the polar heads in red and the hydrocarbon tails in gold.
4.3.3 Interaction of Preformed GCH Micelles with Bulk Vitamin E

To develop a fundamental understanding of how bile salts interact with vitamin E we performed an MD simulation study of interaction of preformed GCH micelles with bulk vitamin E. Specifically, our goal was to understand how preformed bile salt micelles interact with the water-vitamin E interface. In our MD simulation the simulation system consists of a 3nm thick slab of vitamin E immersed in an aqueous solution containing three preformed GCH micelles that were obtained from the previous simulation focused on GCH molecules self-assembly. Under the 3d periodic boundary conditions the simulation system contains two planar vitamin E-water interfaces (see Fig 4.5 for details of the initial simulation structure). In the initial state the three micelles were placed at least 2nm away from the water - vitamin E interfaces. Starting from this initial state we performed NVT MD simulation for 160 ns total simulation time.

Figures 4.13 depict time evolution of the simulation system over 160 ns. On can see that the three micelles are attracted and move, as intact units, towards one of the two vitamin E-water interfaces. Once a micelle makes contact with the interface it loses its integrity and the constituent GCH molecules are adsorbed in the interface and spread uniformly across its entire area. In our simulation the large micelle containing 12 GCH molecules was adsorbed into one vitamin E-water interface and the other two micelles containing 11 and 6 GCH molecules respectively were adsorbed into the other vitamin E – water interface.

Figure 4.13: Snapshots depicting the time evolution of adsorption of three preformed GCH micelles into two vitamin E – water interfaces. The two interfaces are created by immersion of a slab of vitamin E into an aqueous solution. Simulation structure at: (a) initial time, (b) 50 ns, (c) 100 ns and (d) 160 ns. Periodic boundary conditions in all three dimensions are considered. The coloring scheme is as follows: water is represented in tan, Cl⁻ ions in green, Na⁺ ion in orange, GCH ions are represented in licorice and have C atoms in cyan, O in red, N in blue, and H in white. Vitamin E molecules in the slab are represented with lines with C atoms colored in cyan and O in red.
time = 0 ns
(a)

(b) time = 40 ns

(c) time = 100 ns

(d) time = 160 ns
To gain additional insight into the distribution of the GCH molecules across the vitamin E-water interface and gain information about the width of the interface we investigated the mass distribution of various molecular species along the normal direction to the interface (the z direction). Figure 4.14 depicts the mass density function of glycocholate, vitamin E, and water along the normal direction. The distributions represent averages obtained over the last 40ns of the 160ns trajectory. It should be mentioned that the three micelles adsorb and stred across the interface in less than 120 ns; that is during the last 40 ns of the 160 ns trajectory the all GCH molecules are located in the interface region. The density profiles demonstrate clearly that the vitamin E- water interface remains relatively sharp and that indeed all GCH molecules are located in one of the two interfaces. The differences in height and width of the GCH profiles (shown in Figure 4.14 (b)) reflect the fact that the numbers of GCH molecules in the two interfaces are not the same. Moreover as illustrated in Figure 4.14 (b) upon adsorption into the interface the GCH molecules reorient themself such that methyl groups point towards the vitamin E phase and the hydroxyl group point towards the aqueous phase.

One can gain additional insight into the kinetic of adsorption of GCH micelles into the Vitamin E – water interfaces by monitoring the time evolution of the radii of gyration. Figures 4.15 show the time evolution of the three radii of gyration for the three micelles as they are adsorbed into the vitamin E- water interfaces. The initial aggregation numbers of the three micelles are 12, 11, and 6 respectively. The rapid and steady increase in time of the radii of gyration is an indication of the rapid change in shape of the micelle which in our case coincides with its flattening due to interaction and eventually adsorption into one of the two vitamin E – water interfaces. As shown in figure 4.15 the 12 GCH molecule micelle and the 6 GCH molecules micelle start interacting with the interfaces pretty early and are adsorbed in the
interfaces in less than 30 ns. The 11 GCH molecule micelle on the other hand remains stable for about 70 ns. However once it makes contact with the interface it gets adsorbed and spreads across the interface in less than 30 ns.

Figure 4.14: (a) The mass density profiles along the normal direction to the vitamin E – water interfaces for: water, vitamin E, GCH, and methyl and hydroxyl groups of GCH molecules.(b) Mass distribution of GCH and its hydrophobic (methyl) and hydrophilic (hydroxyl) groups across vitamin E – water interfaces.
Figure 4.15 Time evolution of the three principal radii of gyration for three micelles during their interaction and adsorption into vitamin E-water interfaces.
4.3.4 The Effect of Bile Salts on Vitamin E – Water Interfacial Properties

It is known that bile slats absorb at the water-oily phases interfaces and lead to significant changes of interfacial properties including interfacial energy, surface tension, and interface structural characteristics [1, 5]. Moreover, the modifications of the interfacial properties depend on the nature and the concentration of bile salt molecules adsorbed at the interface. In this study we used MD simulations to investigate the changes induced by CHD molecules on the interfacial properties of vitamin E – water interface. Specifically we investigated the effect of interfacial bile salt concentration on interfacial energy, surface tension and structure.

The geometry of the simulation system is presented in Figure 4.16 and consists of a 4 nm thick slab of vitamin E immersed in an aqueous solution together with a pre-determined number of vitamin E molecules that are symmetrically and uniformly distributed across the two vitamin E – water interfaces. In addition for each CHD molecule a charge compensating Na\(^+\) ion was added to the system. We performed a total of twelve simulations in which the number of CHD molecules located at each of the two interfacial regions was incrementally increased from 1 to 11 (that is, the total number CHD molecules was varied from 2 to 22). All simulations were initially carried out at constant number of particles, constant pressure, P=1atm, and constant temperature, T=300K, for 20ns to allow the system to equilibrate and then switched to constant volume, constant temperature simulations simulation for 100 ns. The last 40ns of each simulation were used to calculation of the change of the interfacial energy, surface tension, and structural properties of the interface at various prescribed interfacial CHD concentration.
Figure 4.16 The MD simulation system containing a 4nm thick slab of vitamin E in aqueous solution in the presence of 6 CHD molecules adsorbed at each vitamin E- water interface. Periodic boundary conditions in all three dimensions are considered. The coloring scheme is as follows: water is represented in tan, Na\textsuperscript{+} ion in orange. CHD ions are represented in licorice and have C atoms in cyan, O in red, and H in white. Vitamin E molecules in the slab are represented with lines with C atoms colored in cyan and O in red.
To quantify the effect of the adsorption of CHD molecules at the vitamin E – water interface we calculated the change of the interfacial energy, \( \Delta E_{\text{interf}} \), due to adsorption of bile salt molecules defined as follows:

\[
\Delta E_{\text{interf}} = \frac{E_{\text{total}} - (nE_{\text{bile, single}} + E_{\text{vitaminE-water}})}{A_{\text{interf}}}
\] (4.1)

where, \( E_{\text{total}} \), \( E_{\text{bile, single}} \) and \( E_{\text{vitaminE-water}} \) denote the energies of the whole system, the total energy of a single bile salt (CHD) molecule in vacuum at the same temperature (calculated from a separate MD simulation), and the total energy of a system with no bile salt molecule adsorbed at the interfaces. The variable \( n \) is the actual number of CHD molecules adsorbed into the two vitamin E – water interfaces present in our simulation system. Since the area of each vitamin E-water interface was maintained fixed (8.5nm \( \times \) 8.5nm) in all simulations, the subsequent addition of pairs of CHD molecules to the interfaces amounts to an incremental increase of the CHD concentration in the interface. To put the interfacial concentrations considered in this study into perspective we should mention the following. It is generally accepted that bile salt concentration at a vitamin E-water interface (or any water-oily phase interface for that matter) is a function of the concentration in the bulk phase when this is below the cmc (critical micelle concentration) but it saturates when the bulk concentration is above the cmc. Accordingly, in our study we assume that the interface bile salt concentrations considered correspond to bulk concentration below the cmc. This is reasonable as in our simulation no bile salts molecules were observed to leave the interface and move into the bulk region.

Figure 4.17 shows the change in the interfacial energy of the vitamin E-water interface with the increase of the CHD ions in the interface. The simulation results show that in within the
range of interfacial concentrations considered, which appears to be well below the saturation value, the decrease in the interfacial energy with the increase of CHD ions concentration is linear. Specifically, in the concentration range addition of a single CHD molecule decreases the interfacial energy by about 0.75 mJ/m². This linear decrease is consistent with what one would expect at small bile salts interfacial concentrations in which the interaction between individual bile salts can be neglected. Given the magnitude of interfacial energy decrease per CHD molecule on can infer that the bile salts are quite effective in lowering the vitamin E-water interfacial energy.

One can gain additional insights into the interfacial effect of bile salt molecules by calculating the vitamin E-water interfacial tension. We calculated the interfacial tension, $\gamma$, in our bile salt-laden vitamin E–water interface by using the mechanical definition [31]

$$\gamma(t) = \frac{L_z}{n} \left( \frac{P_{zz}(t)}{2} - \frac{P_{xx}(t) + P_{yy}(t)}{2} \right)$$

(4.2)

where $z$-axis is chosen along the normal to the interfaces (see Figure 4.16), $P_{zz}(t)$ is the instantaneous normal component with respect to the interface of the pressure tensor and $P_{xx}(t)$ and $P_{yy}(t)$ are the instantaneous tangential pressure components; $L_z$ is the height of the simulation box along the $z$-axis and $n$ is the number of interfaces parallel to the $xy$-plane (in our case $n=2$). The averaged surface tension $\gamma$ was evaluated by time averaging the $\gamma(t)$ over the last 40 ns of the trajectory.
Figure 4.17 Variation of the change of interfacial energy of the vitamin E-water interface with the concentration of CHD molecules adsorbed in the interface.

Figure 4.18 shows the profile of the surface tension as a function of the concentration of CHD molecules in the vitamin E–water interface. The interfacial concentration of CHD molecules is defined as the number of molecules across an interface divided by the interface area. Overall the simulation results indicate that in within the error bars the adsorption of CHD ions at the vitamin E–water interface causes just a small decrease of the effective interfacial tension. This result is not surprising after all because although the presence of CHD leads to a significant decrease of the interfacial energy, at the atomic scale the structure of the water-vitamin E interface remains largely unchanged. However, the macroscopic scale the adsorption of CHD molecules in the interface can and will lead to an effective reduction of the surface tension. The
effective reduction of the surface tension at the macroscopic scale arises because the adsorbed CHD molecules generate a two-dimensional pressure $\Pi$ that opposes the tendency of surface tension to make interfaces contract and minimize their area. The macroscopic effective surface tension $\gamma$ of the CHD–laden vitamin E–water interface will be given by $\gamma = \gamma_0 - \Pi$, where $\gamma_0$ is the surface tension of the CHD free vitamin E–water interface. Ultimately, the surface pressure $\Pi$ is determined by the entropy and effective CHD–CHD interionic repulsion.

![Figure 4.18 Variation of the effective surface tension of the vitamin E–water interface with the concentration of CHD molecules adsorbed in the interface.](image-url)

Figure 4.18 Variation of the effective surface tension of the vitamin E–water interface with the concentration of CHD molecules adsorbed in the interface.
Similar to the case of adsorption of preformed GCH micelles into the vitamin E – water interface the presence of CHD molecules at interface will lead to important structural changes across the interface. Specifically, Figure 4.19 depicts the mass density function of cholate ions, vitamin E, and water along the normal direction. The distributions represent averages obtained over the last 40ns of the 120ns trajectory. The density profiles demonstrate clearly that the vitamin E- water interface remains also relatively sharp in the presence of CHD ions. Moreover as illustrated in Figure 4.19 (b) once placed at the interface the CHD molecules undergo relaxation reorientation motion such that their methyl groups point towards the vitamin E phase while the hydroxyl groups point towards the aqueous phase.

4.4 Summary and Conclusions

In summary, we performed sets of molecular dynamics simulations aimed at revealing the kinetics and structural characteristics of the self-assembly process of two well-studied bile salt molecules the cholate (CHD) and glycocholate (GCH). Our studies show that at concentration above critical micelle concentration (~100mM) in both systems the bile salt molecules aggregate spontaneously into small micelles in just a few nanoseconds. The average micellar aggregation numbers are 9.6 and 15.5 for GCH− and CHD− systems respectively and the shape of micelles is oblate instead of spherical; oblate shape favored by bile salts unique molecular structure. Our study of interaction of bile salts with oleic acid show that the oleic acid molecules are solubilized spontaneously into preformed bile salt micelles. The MD study of interaction of bile salts vitamin E show that preformed bile salt micelles are spontaneously adsorbed at the vitamin E – water interface, adsorption process that leads to important changes of interfacial energy, surface tension and interface structure.
Figure 4.19: (a) The mass density profiles along the normal direction to the vitamin E – water interfaces for: water, vitamin E, CDH, as well as methyl and hydroxyl groups of CHD molecules. (b) Mass distribution of CHD molecules and their hydrophobic (methyl) and hydrophilic (hydroxyl) groups across vitamin E – water interfaces.
4.5 References


CHAPTER 5
MOLECULAR DYNAMICS SIMULATION STUDY OF ADSORPTION AND POSITIONING OF VITAMIN E INTO DMPC LIPID BILAYERS: IMPLICATIONS FOR THEIR ANTIOXIDANT ROLE

5.1 Introduction

Vitamin E is a lipid-soluble antioxidant molecule that is usually located in cell membranes and function as a free radical scavenger to prevent lipid peroxidation. Understanding the positioning of vitamin E in lipid bilayers can provide valuable insight into the mechanism of lipid-peroxidation inhibition at the molecular level. Historically vitamin E was firstly defined as factor X which is a necessary nutrient for reproduction of rats;[1] then this fat-soluble factor was proved to be a vitamin and named as vitamin E by the same group in 1925.[2] There are eight different forms of vitamin E which include tocopherols and tocotrienols derivatives. They are named as alpha, beta, gamma and delta for both types, determined by the various position and number of their methyl groups. Since α-tocopherol is the primary form of vitamin E and has the highest biological activity, a number of researches of investigating the interaction with lipid membrane have been reported.[3-5] The structure of α-tocopherol is shown in Figure 5.1(a).

Oxidation is an unavoidable reaction in human body, and usually leads to the production of free radicals. These oxidation products are able to cause chain-reactions which in turn may lead to cell damage or death. The long-term accumulated cell damage may result in some serious diseases, such as cancer and heart disease. A number of studies have proved that α-tocopherol is an excellent fat-soluble and reaction-chain-breaking antioxidant which is able to prevent cellular level damage.[6-8] In the past numerous experimental methods such as X-ray diffraction and NMR have been used to investigate the interaction between vitamin E and cell membranes[9-14]. These studies indicate that the presence of α-tocopherol decreases the transition temperature in lipid bilayers from gel to liquid crystal phase and reduce the enthalpy of transition from gel to
liquid phase[14] A recent study shows the antioxidant activity of α-tocopherol takes place exclusively at the membrane’s surface.[15] Their result shows α-tocopherol stayed at the lipid-water interface all the time and its tail interacting with lipid acyl chains. In recent years, more and more computational simulations have been performed to calculate the antioxidant behavior of α-tocopherol in membranes. A series of molecular dynamics simulations have been used to investigate the structural and kinetic properties of α-tocopherol interaction with various type of lipid at different temperature. They pointed out that the α-tocopherol head does not stay perfectly vertical in membranes at different temperatures without flip-flop; the lateral diffusion coefficient was also calculated in various type of membrane, which is in good agreement with experiment result.[16].

In this study we report results of our MD simulation studies focused on developing molecular level understanding of adsorption, positioning and aggregation of α-tocopherol molecules into a DMPC lipid bilayer.

5.2 Simulation Methodology

MD simulations were used to investigate the interaction of α-tocopherol (vitamin E) with DMPC lipid bilayers (see Figure 5.1 (b) for the structure of DMPC molecule). We focused our attention on three important molecular processes pertaining to the fundamental understanding of interaction of vitamin E with DMPC bilayers. Specifically, we investigated i) the adsorption and incorporation of an α-tocopherol molecule in a DMPC bilayer, ii) the mechanism of α-tocopherol flip-flop between bilayer leaflets, and iii) the interaction and aggregation of 2 α-tocopherols in a DMPC bilayer. The interaction of α-tocopherol with DMPC bilayers was investigated by evaluating the free energy profile (PMF) of an α-tocopherol molecule moving across the bilayer. The PMF calculations were performed with umbrella sampling methodology. Two PMFs for
$\alpha$-tocopherol entering the DMPC bilayer were obtained using umbrella sampling (47 windows). These simulations systems consists of 256 DMPC molecules (128 lipids in each leaflet), 2 $\alpha$-tocopherols and 16660 water molecules. The two $\alpha$-tocopherols were spaced 4.6 nm apart and the simulations were run until the two PMF curves converged (about 20 to 40 ns / window).

All MD simulations were performed with the GROMACS 4.0 simulation package.[17]. The simulations were performed at constant pressure (1 bar) using semi-isotropic pressure coupling and constant temperature (T-323K) using Berendsen barostat [18]. The force field parameters for lipids for both bonded and non-bonded interactions were taken from Berger et al [19]. The force field parameters for $\alpha$-tocopherol were generated using Automated Topology Builder (ATB) [http://compbio.biosci.uq.edu.au/ATB]. The simple point charge (SPC) model was used for water[20]. All non-water bond lengths were constrained by using the LINCS[21] algorithm. Water bonds and angles were constrained using the SETLLE [22] algorithm. For short-range nonbonded interactions, GROMACS uses a twin range cutoff. The inner cutoff was 1.02 nm. Forces due to van der Waals interactions are 1.4 nm were evaluated every 10 steps. The long-range electrostatic interactions were treated using the particle mesh ewald (PME) algorithm.[23, 24]. We used a time step of 0.2 fs. The energy minimization procedure, based on the steepest descent algorithm, was applied to the initial structures prior to the actual MD runs.
Figure 5.1: The structure of (a) α-tocopherol and (b) DMPC molecules.
5.3 Result and Discussion

5.3.1 Free Energy (PMF) of α-tocopherol in a DMPC Lipid Bilayer

The free energy, or the so-called potential of mean force (PMF), for an α-tocopherol inside a DMPC lipid bilayer as function of the distance from the center of the bilayer was evaluated using umbrella sampling approach. Figure 5.2 shows the profile of the PMF when the α-tocopherol molecules moves from the center of the bilayer all the way into the aqueous environment. The PMF is constant when the α-tocopherol is outside lipid bilayer and starts starts to drop steeply as the α-tocopherol enters the bilayer and it reaches a relatively narrow minimum when the α-tocopherol center of mass is located about 0.9 nm from the bilayer center. As the α-tocopherol molecule moves toward the bilayer center the PMF change course and starts to increase reaching a small peak right at the bilayer center. As shown Figure 5.2 and using the symmetry of the bilayer system one can identify two energy barriers. One of about 25kT representing the energy barrier for an α-tocopherol molecules to move from the stable minima into the aqueous environment outside the DMPC bilayer and the other one of about 4 kT for the α-tocopherol to move across the center of the bilayer into the opposite bilayer leaflet. Given the magnitude of the two energy barriers one can infer that α-tocopherol has a very strong affinity for the interior of lipid bilayers; α-tocopherol spontaneously leaving the DMPC membrane interior is an extremely rare occurrence. Because the PMF barrier at the center of the bilayer is just a few times larger (~4kT) than thermal energy one would expect that the α-tocopherol molecule would flip-flop from one bilayer leaflet to the other quite frequently.
5.3.2 Positioning of α-tocopherol inside DMPC Bilayer

To gain additional information regarding the location of α-tocopherol when adsorbed into DMPC bilayers we evaluated the profiles along bilayer normal direction of the positions of various molecular groups belonging to both α-tocopherol and lipid molecules. These include the hydroxyl oxygen and tail end carbon in α-tocopherol and choline nitrogen, phosphate and ester oxygen atoms of lipids. Figure 5.3 shows the distribution of these molecular groups along the normal direction to the bilayer. It is evident that the tail of the α-tocopherol (blue solid line) is located at the center of bilayer and the average position of the hydroxyl group of α-tocopherol (red solid line) is located close to the ester oxygen group of the lipids heads, a little lower than the phosphate oxygen group. The positioning of the α-tocopherol head is determined by its hydrophilic property which is favored energetically by the interactin with the polar ester oxygen
groups of lipids. Likewise the hydrophobic tail of the α-tocopherol is located close to the center of the bilayer which is highly hydrophobic.

Figure 5.3: Position profile of choline nitrogen, phosphate oxygens and ester oxygens of the lipids and hydroxyl oxygen and tail end carbon of α-tocopherol along the bilayer normal.

### 5.3.3 The Orientation of α-tocopherol inside Lipid Bilayer

The interaction of α-tocopherol with the DMPC bilayer has also strong effect on its orientation while in the adsorbed state. This characteristic was studied by evaluating the orientation distribution of the angle between two arbitrarily chose vectors along the α-tocopherol molecule and the bilayer normal. Figure 5.4 illustrates the definition of the two vectors $\vec{h}$ and $\vec{l}$ defined such that they characterize α-tocopherol head and tail the directions. In addition to characterize α-tocopherol orientation with respect to DMPC bilayer we define two angles $\theta_h$ and $\theta_t$, Equations 5.1 and 5.2, that the two vectors make with the normal to the bilayer. Figures 5.5
depicts the probability distribution of the angle between head and tail vectors and bilayer normal direction. In these figures, y-axis represents the equilibrium distance of head from bilayer center, sum of each row at a certain distance is equal to 1. Figure 5.5 (a) illustrates that the angle between the head vector of α-tocopherol and bilayer normal decreases when the α-tocopherol is close to the bilayer center, in other words, the head of α-tocopherol is in a more vertical position when the α-tocopherol goes into bilayer. Figure 5.5(b) illustrates the probability distribution of angle between tail vector of α-tocopherol and bilayer normal direction.

\[
\theta_h = \cos^{-1}\left(\frac{\vec{h} \cdot \vec{n}}{|\vec{h}|}\right)
\]

(5.1)

\[
\theta_t = \cos^{-1}\left(\frac{\vec{t} \cdot \vec{n}}{|\vec{t}|}\right)
\]

(5.2)

Figure 5.4: The head and tail vectors characterizing the orientation of α-tocopherol molecules.
Figure 5.5: The probability distribution of $\alpha$-tocopherol for (a) head vector and (b) tail vector with respect to bilayer normal.
5.3.4 The α-tocopherol Head and Tail Order Parameter

Another commonly measured quantity is the order parameter which characterizes the orientation of hydroxyl oxygen group and the carbon tails. In this study, the order parameter $S_h$ and $S_t$ have been defined in terms of angle $\theta_h$, $\theta_t$, defined as:

$$S_h = \frac{\langle 3\cos^2\theta_h - 1 \rangle}{2} \tag{5.3}$$

$$S_t = \frac{\langle 3\cos^2\theta_t - 1 \rangle}{2} \tag{5.4}$$

where $\langle \ldots \rangle$, refers to time averaging over specified trajectory. The order parameter value is in the range from -0.5 (perpendicular to the bilayer normal) to 1 (aligned with bilayer normal), a value 0 means no orientational order. In Figure 5.6, black solid line represents the order parameter of the α-tocopherol head hydroxyl group while the red solid line refers to the tail vector order parameter. Both head and tail of α-tocopherol are parallel to the bilayer when the α-tocopherol is away from the lipid bilayer, the position of α-tocopherol start to become vertical when it enters the lipid bilayer. The tail end-to-end vector become perpendicular to the bilayer normal near the center of the bilayer while the head vector remains parallel to the bilayer normal. Then both the head and tail are perpendicular to the bilayer normal when α-tocopherol in the center of the bilayer, and ready to start flip-flop.

To further investigate the orientation of α-tocopherol inside the lipid bilayer we also evaluated the angle between its head and tail. This angle was defined as: $\langle \theta_h \rangle - \langle \theta_t \rangle$, where brackets refers to the time averaging. In Figure 5.7, positive values indicate that the tail is more aligned with the bilayer normal while negative value refers to the fact the head is more aligned with bilayer normal direction. Moreover we can see that the tail is more aligned to the bilayer
normal when the α-tocopherol is outside the membrane. Then the tail become perpendicular to the bilayer normal, as the $\langle \theta_h \rangle - \langle \theta_t \rangle$ value drops below zero.

Figure 5.6: The order parameters of head and tail vectors of α-tocopherol with respect to bilayer normal as a function of the distance of α-tocopherol head group from bilayer center.

Figure 5.7: The average angle between α-tocopherol head and tail vectors.
5.3.5 Lipid Tail Order Parameters

Additional information regarding the effect of the presence of \( \alpha \)-tocopherol inside lipid bilayer on the structure of the lipids is obtained by analyzing the ordering in the lipid carbon tails. The commonly used parameter to measure this property, the alignment of lipid tails, is called deuterium order parameter. Deuterium order parameter can be obtained from both experiments and simulations. In this work, the deuterium order parameter, \( S_{cd} \), is related to the angle between C-H bonds in the lipid tail and bilayer normal:

\[
S_{cd} = \frac{3\cos^2\theta_{CH} - 1}{2}
\]  

(5.5)

where

\[
\theta_{CH} = \cos^{-1}\left(\frac{\text{CH} \cdot \vec{n}}{|\text{CH}|}\right)
\]

(5.6)

Again in Equation 5.5 and 5.6 the brackets \(<...>\) represent time averaging over the simulation time. \( \theta_{CH} \) is the angle between the CH bond vector and the bilayer normal. Since we use the united atoms in the simulation, we need to reconstruct the CH bond by using the position of the carbon group in the tail. The plot of order parameter versus the number of carbon is shown in Figure 5.8. Two head equilibrium distance of 2.3nm from bilayer center (red solid line) and in the center of bilayer (black solid line) have been chosen to investigate the order parameter of the lipid tail. The simulation result shows a trend of decrease of ordering when the \( \alpha \)-tocopherol is in the center of bilayer. There is a pronounced peak in the middle of the carbon tail. Figure 5.8 collected the simulation result of normalized \( <S_{cd}> \) over all carbons as a function of the distance of the head of \( \alpha \)-tocopherol from bilayer center.
Figure 5.8: Deuterium order parameter for DMPC lipid tail.

Figure 5.9: Averaged deuterium order parameter over all carbons.
5.3.6 α–Tocopherol Flip-Flop and Aggregation

To investigate the flip-flop mechanism of α-tocopherol, we performed separate MD simulations in which two α-tocopherol molecules were inserted inside the DMPC bilayer in different leaflets, some distance apart and released. The simulations show that the two tocopherols are attracted and move towards each other and eventually aggregate in one leaflet in less than 70ns. As Figure 5.10 shows, in the initial state the two α-tocopherols are located in different leaflets about 2 nm apart. As the time progresses they move towards each other, one tocopherol flip-flops into the other tocopherol and spontaneously aggregate. Detailed analysis shows that the aggregate is held together by the formation of hydrogen bonds. The number of hydrogen bonds between the two α-tocopherol molecules is also showed in the Figure 5.10.

![Figure 5.10: Time evolution of the distance between the heads of two α-tocopherol molecules that aggregate in a DMPC bilayer and the number of hydrogen bonds between the α-tocopherol molecules during aggregation.](image)
5.3.7 Formation of Hydrogen Bonds during Aggregation of two $\alpha$-Tocopherols in a DMPC Bilayer

Table 5.1 shows the number of hydrogen bonds per $\alpha$-tocopherol molecule between hydroxyl group in the head of $\alpha$-tocopherol and the ester oxygen atoms, phosphate oxygen atoms and water. The number of hydrogen bonds with the phosphate oxygens and with the ester oxygens of the DMPC was approximately equal for both $\alpha$-tocopherol molecules, indicating that the $\alpha$-tocopherol hydroxyl oxygen resides halfway between them. No $\alpha$-tocopherol – water hydrogen bonds were observed in a 6 nanosecond trajectory.

In our simulations a hydrogen bond was defined to exist when the donor-acceptor distance was less than or equal to 0.35 nm and the donor-hydrogen-acceptor angle was less than 30° from linear alignment. In these simulations, the only donor we considered is the hydroxyl group in $\alpha$-tocopherol, and the accepters considered are oxygen atoms in ester and phosphate groups in DMPC lipids.

Table 5.1: The number of hydrogen bonds between of $\alpha$-tocopherol while adsorbed inside the DMPC bilayer and various molecular groups present in the simulation system.

<table>
<thead>
<tr>
<th></th>
<th>Ester</th>
<th>Phosphate</th>
<th>Water</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-tocopherol 1</td>
<td>0.1152</td>
<td>0.1107</td>
<td>0</td>
<td>0.2259</td>
</tr>
<tr>
<td>$\alpha$-tocopherol 2</td>
<td>0.0997</td>
<td>0.0997</td>
<td>0</td>
<td>0.1993</td>
</tr>
</tbody>
</table>

5.4 Conclusions

Using MD simulations we studied the adsorption, positioning and orientation of $\alpha$-tocopherol into DMPC lipid bilayers. The PMF calculations show that $\alpha$-tocopherol has a very strong affinity for the interior of lipid bilayers. Specifically, the free energy required for moving an $\alpha$-tocopherol molecule from inside a lipid bilayer into the aqueous environment is about 25
times the thermal energy, value which indicates that α-tocopherol spontaneously leaving a membrane is an extremely rare occurrence.

The MD simulations demonstrate that α-tocopherol is incorporated in DMPC lipid bilayers and is concentrated in a relatively narrow region, just below the membrane/water interface. This is of great significance because even if their concentration in membranes is relatively low, the spatial confinement of α-tocopherol inside membranes greatly enhances their concentration in this vital region, thus increasing their importance for in vivo biological activities including oxidative stress defense.

Our studies also show that when two α-tocopherol molecules are located in close proximity inside a lipid bilayer they aggregate spontaneously. Upon aggregation they hydrogen bond with each other. This strong interaction indicates that it is unlikely that α-tocopherol is evenly distributed inside cell membranes.

5.5 References


APPENDIX A
CHARACTERISTICS OF THE FORCE FIELDS USED IN MD SIMULATIONS

As described in Section 2.1.1 the typical empirical interatomic interaction potential energy function $V(\vec{R})$ that describes the interactions in a molecular system can be represented as a sum over individual terms representing bonded and non-bonded interactions (see Equation (2.3)).

The three energy terms of the bonded interaction, $V_{\text{bonded}}$, are illustrated schematically Figure A1 and is given by:

$$V_{\text{bonded}} = V_{\text{bond-stretch}} + V_{\text{angle-bend}} + V_{\text{torsion}} \quad (A1)$$

Figure A1. Representation of (a) bond stretching, (b) angle bending, and (c) torsional components of intramolecular bonded energy term.

The first term in (A1) is a harmonic potential representing the interaction between atomic pairs (Fig where atoms are separated by one covalent bond, i.e., 1,2-pairs. This is the approximation to the potential of atoms $i$ and $j$ joined by a covalent bond as a function of displacement from the ideal bond length.

$$V_{\text{bond-stretch}} = \sum_{1,2 \text{ pairs}} \frac{k_{ij}^b}{2} (b_{ij} - b_{eq})^2 \quad , \quad (A2)$$
where, $k^b_{ij}$ is a force constant that describes the stiffness of the actual bond and $b_{eq}$ is the equilibrium length of the bond. Both equilibrium length of the bond and force constant are specific for each pair of bounded atoms, i.e. depend on the chemical type of the atom constituents. The harmonic representation of the bond stretching term was chosen for its simplicity and ease of implementation in the MD codes. A true bond stretching potential is not harmonic and it is better represented by a Morse potential that has the form:

$$V_{Morse} = \sum_{1,2\text{ pairs}} D_e\{1 - \exp[-a(b_{ij} - b_{eq})]\}^2 ,$$

(A2)

where, $D_e$ is the depth of the potential energy minimum, $a$ is a parameter characterizing the bond stiffness and $b_{eq}$ is the reference equilibrium bond length. The Morse potential is not usually used in MD simulations because it is not particularly suited to efficient computations and because it requires three parameters to be specified for each bond. An what is more important it is extremely rare in MD simulations for bonds to deviate significantly from their equilibrium values. Values of force constant are often evaluated from experimental data such as infrared stretching frequencies or from quantum mechanical calculations. Values of bond length can be inferred from high resolution crystal structures or microwave spectroscopy data.

The second term in (A1) is associated with change of bond angle, $\theta_{ijk}$, from the equilibrium value, $\theta_{eq}$, for the atoms $i$, $j$, $k$ (where $i$, $j$, $k$ are bonded together with $i$ bonded with $j$ and $j$ bonded with $k$) and can be described using a Hooke’s law or harmonic potential function,

$$V_{\text{angle-bend}} = \sum_{\text{angles}} \frac{k^\theta_{ijk}}{2} (\theta_{ijk} - \theta_{eq})^2$$

(A3)

The contribution of each angle is characterized by a force constant, $k^\theta_{ijk}$, and a reference value $\theta_{eq}$. The values of $k^\theta_{ijk}$ and $\theta_{eq}$ depend on the chemical type of the atoms forming the bond.
angle. Rather less energy is required to distort an angle away from its equilibrium than to stretch or compress a bond, and the force constants are proportionally smaller.

The third term represents the torsion angle potential function which models the presence of steric barriers between atoms separated by 3 covalent bonds (1,4 pairs). The motion associated with this term is a rotation, described by a dihedral angle and coefficient of symmetry \( n=1,2,3 \), around the middle bond. This potential is assumed to be periodic and is often expressed as a cosine function.

\[
V_{\text{torsion}} = \sum_{1,4 \text{ pairs}} \frac{k_\phi}{2} (1 + \cos(n\phi - \phi_s)) \tag{A4}
\]

with \( k_\phi \) being the force constant that describes the strength required to distort the dihedral angle formed between four bonded atoms. The existence of barriers to rotation about chemical bonds is of great importance to understanding the structural properties of molecules and conformational analysis. Quantum mechanical calculations suggest that the barrier to rotation about a chemical bond can be considered to arise from antibonding interactions between atoms on opposite ends of the bonds connected to the bond about which the torsion occurs; in general the antibonding interactions are minimized when the conformations are eclipsed. Many force fields are used for modeling flexible molecules where the major changes in conformation are due to rotations about bonds. It is therefore essential that the force field properly represents the energy profiles of such changes.

The potential term representing the contribution of non-bonded interactions in the total potential function (Eq 2.3) has two components, the Van der Waals interaction energy and the electrostatic interaction energy. The non-bonded interactions are computed on the basis of a neighbor list (a list of non-bonded atoms within a certain radius), in which exclusions are already
removed. In the potential energy function, these interactions are accounted by the electrostatic and Van der Waals interactions.

\[ V_{\text{non-bonded}} = V_{\text{van der Waals}} + V_{\text{electrostatic}} \]  
(A5)

The van der Waals interaction between two atoms arises from a balance between repulsive and attractive forces. The repulsive force arises at short distances where the electron-electron interaction is strong. The attractive force, also referred to as the dispersion force, arises from fluctuations in the charge distribution in the electron clouds. The van der Waals interaction is most often modeled using the Lennard-Jones 6-12 potential which expresses the interaction energy using the atom-type dependent constants \( \varepsilon_{ij} \) and \( \sigma_{ij} \). Values of \( \varepsilon_{ij} \) and \( \sigma_{ij} \) may be determined by a variety of methods, like non-bonding distances in crystals and gas-phase scattering measurements.

\[ V_{\text{van der Waals}} = \sum_{\text{nonbonded pairs}} 4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] \]  
(A6)

The electrostatic interaction between a pair of atoms is described by Coulomb term,

\[ V_{\text{electrostatic}} = \sum_{\text{nonbonded pairs}} \frac{q_i q_j}{4\pi \varepsilon_o \varepsilon_r r_{ij}} \]  
(A7)

where \( q_i \) and \( q_j \) are the partial charges on atoms i and j. The permittivity of free space is designated by \( \varepsilon_o \) and \( \varepsilon_r \) is the relative permittivity. The distance between the atoms is denoted by \( r_{ij} \).

Over the past decades a variety of force fields for biomolecular simulations have been developed. Typical examples of force fields include AMBER[1-3], CHARMM[4-6], ECEPP[7], ENCAD[8, 9], GROMOS[10-12] and OPLS[13, 14]. These biomolecular force fields have a similar form of the interactions function (like the ones considered in this Appendix), yet they
differ considerably in their parameterization philosophy and parameter values. Because the latter can be obtained in a variety of ways, by fitting to a range of molecular properties of small molecules against different sets of quantum mechanical and experimental data, different parameter set may yield widely different results when applied to large complex biomolecular systems. With all the variety of available force fields it is often the responsibility of the end users of molecular simulation tools to choose the best force field suited for their particular application.

References


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