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Analysis of Effects of Shockwaves on Cholesterol-Containing Lipid Bilayer Models

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ANALYSIS OF EFFECTS OF SHOCKWAVES ON CHOLESTROL-CONTAINING
LIPID BILAYER MODELS

BY

RALPH KFOURY

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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OF

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UNIVERSITY OF RHODE ISLAND

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Abstract

In the following work, the effect of shock impulses ($2.5\text{--}10.0\text{ mPa} \cdot \text{s}$) on lipid bilayer membranes consisting of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and varying amounts of cholesterol (10% and 50%) has been studied using molecular dynamics. Both models were equilibrated for 7 nanoseconds in an NPT ensemble at 310 K and 1 atm. After equilibration, the 50% cholesterol model showed a larger average membrane thickness and an increased average deuterium order parameter (S_{CD}) of carbons in lipid tails when compared to the 10% model. In addition, values such as the total area per lipid and area per POPC were reduced in the 50% model when compared to the 10% cholesterol model.

After application of similar shock impulses to the 50% and 10% cholesterol models, the structural effects on each respective bilayer model were compared. The change in bilayer thickness due to shock impulse was measured for both models at all impulses tested. These values were used to calculate the percent decrease in bilayer thickness of both models. On average, the 50% cholesterol model showed a 1-2 % lesser reduction in bilayer thickness than the 10% cholesterol model at the shock impulses tested.

After application of shock impulses to both models, the temporal change of the instantaneous averaged order parameter S_{CDi} of the upper and lower monolayers of both models was calculated at shock impulses of $7.5\text{ mPa} \cdot \text{s}$ and $10.0\text{ mPa} \cdot \text{s}$. In both models, regardless of shock impulse, the S_{CDi} value of the upper monolayer decreased followed by a decrease in the S_{CDi} parameter of the lower monolayer. These effects can be linked to the collapse phase of the lipid bilayer due to shockwave. Once the

shockwave has fully traveled through the lipid bilayer, the S_{CDi} parameter of the lower monolayer begins to increase which indicates beginning of the rebound phase.

Finally, the increased fluidity of the lipid bilayer models was examined through calculation of the lateral displacement of the lipids in both models during the shockwave impulse ranges tested. The 50% model showed a slightly higher lateral displacement at the shock impulse ranges tested but not significantly higher to make any conclusions.

In addition, the efficiency of the shock impulse based on the distance of the shock water slab from the lipid bilayer was analyzed. The right boundary of the shock slab was placed 1 Å and 10 Å away from the lipid bilayer and similar shock impulses of 2.5, 5.0, 7.5 and 10 mPa * s were applied. It was shown that placement of the shock slab 10 Å away from the lipid bilayer causes a greater decrease in average bilayer thickness and slight increases in kinetic energy, temperature and pressure of the system. This could be owed to better momentum transfer among molecules achieved when the water slab is placed 10 Å away rather than 1 Å away.

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Furthermore to this, I would like thank my committee members Dr. Michael Greenfield, Dr. Philip Datseris and Dr. Chengzhi Yuan for their continued guidance and support during this process. Their patience and insight has been motivating and I am grateful for this. Along with my committee, I would like to thank my lab mates and friends Carlos Javier, Xianon Dong and Bahador Marzban for their continued presence and helpful insight throughout the course of my thesis work.

Dedication

I would like to dedicate this Master's thesis first and foremost to my mother Mary Kfoury who has been my shining light throughout any journey I have taken in my life. Without her patience, guidance, consistent love and beautiful personality, I would never have become the person that I am today.

Second, I would like to dedicate this master's thesis to Dr. Hongyan Yuan, who took a chance on me and trusted me to work in his laboratory. He has shaped me with his wisdom and continued effort to mold me into a great researcher. He challenged me to take the road less traveled, and embark in research in which I was initially unfamiliar with. His patience, intelligence and dedication to research has played a huge role on my thesis work and for that I thank him.

I would also like to dedicate this to my father Sami Kfoury who passed away in 2006 and whose memory lives on through my actions. I hope with this master's degree and my future accomplishments, I have made you and my family proud. This is only the beginning of what I intend on accomplishing so please be patient with me.

I would like to thank my brother Jimmy and my sister Joyce for being the best siblings possible and helping me in any way, shape or form throughout graduate school and in life; whether it be with provided wisdom or through action and effort. In addition to my siblings, I would like to thank those who inspired me and those who doubted me in my journey for you all played a huge role in what I have accomplished and my continuous drive and wisdom.

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Table of Contents

Abstract.....	ii
Acknowledgments.....	iv
Dedication.....	v
Table of Contents.....	vii
Nomenclature.....	ix
List of Figures.....	xi
List of Tables	xiv
Section 1 - Introduction.....	1
1.1 – Lipid Bilayer.....	1
1.2 – Phosphatidylcholines and POPC.....	6
1.3 –Lipid Bilayer Phase Behavior.....	7
1.4 – Cholesterol.....	8
Section 2 – Molecular Dynamics.....	11
2.1 – Theory Behind Molecular Dynamics.....	11
2.2 – Importance of Studying Shockwaves.....	17
Section 3 – Materials and Methods: Creation and Equilibration of Models.....	20
Section 4 – Shockwave Implentation and Setup.....	27
4.1 – Shockwave Implentation.....	27
4.2 – Shockwave Simulation Setup.....	29
Section 5 – Results and Discussion.....	34
5.1 – Equilibration Analysis.....	34

5.2 – Shockwave Simulation Analysis.....	44
Section 6 – Shock Slab Distance from Bilayer.....	64
Section 7 – Conclusions and Future Work.....	77
Appendix I – Matlab Code to Add Velocity to Water	
Molecules.....	80
Appendix II – Sample Minimization and Equilibration File for 50:50 POPC/CHL	
System.....	82
Appendix III – Sample Shockwave Simulation Configuration File for 1:1	
POPC/CHL Lipid Bilayer and 2.5 mPa * s Shock	
Impulse.....	85
Bibliography.....	87

Nomenclature

<u>Notation</u>	<u>Description</u>
mPa * s	millipascal * second
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
S_{CD}	Average Deuterium-order parameter of acyl chains
1:1 POPC/CHL	Lipid Bilayer containing 1:1 ratio of POPC to Cholesterol
9:1 POPC/CHL	Lipid Bilayer containing 9:1 ratio of POPC to Cholesterol
S_{CDi}	Instantaneous averaged order parameter
L_o	Liquid-ordered phase of lipid bilayer
L_d	Liquid-disordered phase of lipid bilayer
T_m	Melting or transition temperature from gel to liquid phase of lipid bilayer
f_i	Force acting on atom i
m_i	Mass of atom i
$\ddot{r}_i$	Second-order derivative of the position (r) of particle i
U	Potential energy
U_{LJ}	Lennard-Jones potential energy function
ε	Depth of potential well in Lennard-Jones potential function
σ	Distance where inter-particle potential (U_{LJ}) becomes zero
r_{ij}	Distance between particle i and particle j
$U_{Coulomb}$	Coulomb potential energy
r_{eq}	Equilibrium distance between two bonded atoms
θ_{ijk}	Bend angle between successive bonded atoms i, j, k

θ_{eq}	Equilibrium bend angle
ϕ_{ijkl}	Torsion angle of three successive bonds of four atoms i,j,k,l
NVE	Thermodynamic ensemble where number of particles (N), volume (V) and energy (E) are fixed
NVT	Thermodynamic ensemble where number of particles (N), volume (V) and temperature (T) are fixed
NPT	Thermodynamic ensemble where number of particles (N), pressure (P) and temperature (T) are fixed
fs	femtosecond or 10^{-15} seconds
ns	nanosecond or 10^{-9} seconds
$\hat{I}$	Specific impulse defined as the impulse per unit area

List of Figures

FIGURE	PAGE
Figure 1.1: Fluid-mosaic lipid bilayer model.....	2
Figure 1.2: Three types of structures formed by immersing phospholipids in a water solution.....	3
Figure 1.3: The structure of four different lipid molecules generally found in mammalian membranes.....	4
Figure 1.4: Structure of POPC.....	6
Figure 1.5: Structure of Cholesterol.....	8
Figure 3.1: 9:1 POPC/CHL lipid bilayer system.....	23
Figure 3.2: 1:1 POPC/CHL lipid bilayer system.....	24
Figure 4.1: Two types of water slab distances from the bilayer depicted.....	28
Figure 5.1: Average bilayer thickness of both lipid bilayer models during 7 ns equilibration compared.	35
Figure 5.2: Average area per lipid of all lipid species (POPC +CHOL) of each lipid bilayer model plotted over 7 ns equilibration time.....	36
Figure 5.3: Average area per POPC of each lipid bilayer model plotted over 7 ns equilibration time.	37
Figure 5.4: Average area per Cholesterol of each lipid bilayer model plotted over 7 ns equilibration time.....	38
Figure 5.5: Average S_{CD} order parameter for carbons in both lipids tails plotted and compared among both models.....	40
Figure 5.6: Average S_{CD} order parameter of individual carbons contained in palmitoyl lipid tails of POPC lipids in each model plotted and compared.....	41
Figure 5.7: Average S_{CD} order parameter of individual carbons contained in oleoyl lipid tails of POPC lipids in each model plotted and compared.....	42
Figure 5.8: Rise in kinetic energy of 1:1 POPC/CHL system at varying shock impulses.....	45
Figure 5.9: Rise in kinetic energy of 9:1 POPC/CHL system at varying shock impulses.	46
Figure 5.10: Rise in Pressure of 1:1 POPC/CHL system at varying shock impulses.....	47

Figure 5.11: Rise in pressure of 9:1 POPC/CHL system at varying shock impulses.....	48
Figure 5.12: Rise of temperature of 1:1 POPC/CHL system at varying shock impulses.....	49
Figure 5.13: Rise in temperature in 9:1 POPC/CHL system at varying shock impulses.....	50
Figure 5.14: Change in bilayer thickness of 1:1 POPC/CHL model at varying shock impulses.....	51
Figure 5.15: Change in bilayer thickness of 9:1 POPC/CHL model at varying shock impulses.....	52
Figure 5.16: Percent decrease in bilayer thickness at shock impulse of 2.5 mPa* s compared between lipid bilayer models.....	54
Figure 5.17: Percent decrease in bilayer thickness at shock impulse of 5 mPa * s compared between lipid bilayer models.....	54
Figure 5.18: Percent decrease in bilayer thickness at shock impulse of 7.5 mPa * s compared between lipid bilayer models.....	55
Figure 5.19: Percent decrease in bilayer thickness at shock impulse of 10 mPa * s compared between lipid bilayer models.....	56
Figure 5.20: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 1:1 POPC/CHL model due to a shock impulse of 7.5 mPa * s.....	58
Figure 5.21: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 1:1 POPC/CHL model due to a shock impulse of 10.0 mPa * s.....	58
Figure 5.22: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 9:1 POPC/CHL model due to a shock impulse of 7.5 mPa * s.....	59
Figure 5.23: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 1:1 POPC/CHL model due to a shock impulse of 10 mPa * s.....	60
Figure 5.24: The lateral displacement of lipids in the 1:1 POPC/CHL lipid bilayer at various shock impulses.....	62
Figure 5.25: The lateral displacement of lipids in the 9:1 POPC/CHL lipid bilayer at various shock impulses.....	63
Figure 6.1: Change in bilayer thickness of 1:1 POPC/CHL model at varying shock impulses and 10 Å distance of water slab from bilayer.....	65

Figure 6.2: Change in bilayer thickness of 9:1 POPC/CHL model at varying shock impulses with a 10 Å distance of water slab from bilayer.....	65
Figure 6.3: The evolution of bilayer thickness of the 1:1 POPC/CHL model at a 5 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).....	66
Figure 6.4: The evolution of bilayer thickness of the 1:1 POPC/CHL bilayer at a 10 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).....	67
Figure 6.5: The evolution of bilayer thickness of the 9:1 POPC/CHL bilayer at a 5 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).....	68
Figure 6.6: The evolution of bilayer thickness of the 9:1 POPC/CHL bilayer at a 10 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).....	68
Figure 6.7: Rise in kinetic energy of the 1:1 POPC/CHL system due to a 7.5 mPa shock impulse applied at different water slab distances (1 Å vs 10 Å).....	70
Figure 6.8: Rise in kinetic energy of the 1:1 POPC/CHL system due to a 10 mPa shock impulse applied at varying water slab distances (1 Å vs 10 Å).....	70
Figure 6.9: Rise in kinetic energy of the 9:1 POPC/CHL system due to a 7.5 mPa shock impulse applied at different water slab distances (1 Å vs 10 Å).....	71
Figure 6.10: Rise in kinetic energy of the 9:1 POPC/CHL system due to a 10.0 mPa shock impulse applied at different water slab distances (1 Å vs 10 Å).....	71
Figure 6.11: Rise in pressure of the 1:1 POPC/CHL system due to a 10 mPa * s shock impulse applied at different shock slab distances (1 Å vs 10 Å).....	73
Figure 6.12: Rise in pressure of the 1:1 POPC/CHL system due to a 10 mPa * s shock impulse applied at different shock slab distances (1 Å vs 10 Å).....	73
Figure 6.13: Rise in pressure of the 1:1 POPC/CHL system due to a 10 mPa * s shock impulse applied at different shock slab distances (1 Å vs 10 Å).....	74
Figure 6.14: Rise in pressure of the 1:1 POPC/CHL system due to a 10 mPa * s shock impulse applied at different shock slab distances (1 Å vs 10 Å).....	75

List of Tables

TABLE	PAGE
Table 3.1: Atom composition and number of atoms in the 9:1 POPC/CHL and 1:1 POPC/CHL lipid bilayer systems.....	21
Table 3.2 Initial Properties of the 9:1 POPC/CHL and 1:1 POPC/CHL lipid bilayer systems.	22
Table 4.1: Shockwave Simulation Setups for lipid bilayer models at varying shock impulses and water slab distances.....	31
Table 5.1: Equilibrium values of both models compared.....	43

Section 1- Introduction

1.1 – Lipid Bilayer

One of the most important components of cells, both human and bacterial, is the lipid bilayer. Serving as a barrier to the environmental world, the lipid bilayer plays a protective role for cells and helps monitor what molecules enter and leave the cell. For decades, scientists have done extensive research on lipid bilayer structure as well as ways to alter the structure to their advantage. Understanding the structural aspects of the lipid bilayer as well as how it can be manipulated is of utmost importance in many scientific areas such as drug delivery in cancer treatments as well as oligonucleotide introduction into cells as performed in gene therapy methods.

The lipid bilayer is a self-assembling, semi-permeable structure encapsulating the cytoplasm of a cell as well as membrane-bound organelles and the nucleus. It is composed of several different classes of lipid molecules including phospholipids, glycolipids, and cholesterol (Khan et al. 2013). Also present in the lipid bilayer are membrane proteins which can either serve as channel proteins such as the potassium ion channel or integral proteins, such as integrin. In addition, the lipid bilayer contains peripheral proteins which can either be attached on the outside of the membrane or on the cytosolic portion (Singer & Nicolson 1972). Overall, the lipid bilayer structure (figure 1) is composed of many different structures which all serve specific purposes both in regulation of the lipid bilayer as well as maintaining structural integrity.

Figure 1.1: Fluid-mosaic lipid bilayer model. Figure adapted from (Alberts et al. 2002)

The Amphiphilic Nature of Phospholipids

The ability of the lipid bilayer to self-assemble when exposed to water into its final structure is due to the amphiphilic nature of the lipid molecules present. The lipid bilayer is composed of phospholipids which have a hydrophilic phosphate head group and two hydrophobic fatty acid lipid tails. When exposed to water, the lipid molecules orient themselves as to maximize the interaction between water and their phosphate head groups and simultaneously minimize the exposure of the hydrophobic lipid tails to water (Alberts et al. 2002). This mechanism of assembly is also known as the hydrophobic effect. In addition, the favoring interactions between the lipid tails of each phospholipid keep water out of the hydrophobic region. This creates a sheet of lipid bilayer composed of two monolayers which is in a state of lowest free energy. In between the monolayers is the hydrophobic region which contains no water molecules.

When exposed to water, lipids can form into one of three different structures; a micelle, where a circular monolayer is formed where all lipid tails are oriented towards the center while the phosphate heads are located on the outer circumference (figure 1.2, top right), a liposome where a circular bilayer is formed containing water on the inside and outside (figure 1.2, top left) and a finally a flat bilayer sheet (figure 1.2, bottom) consisting of a planar bilayer. Experimentalists have utilized the self-assembly properties of these amphiphilic lipids to create structures such as vesicles which can be used for delivery of nanoparticles of interest.

Figure 1.2: Three types of structures formed by immersing phospholipids in a water solution. Figure adapted from (Tiwari et al. 2012)

Composition of Lipid Membranes

The mammalian lipid membrane is made up of almost 50% lipids while the remainder is proteins such as integral membrane proteins or peripheral proteins. The

specific composition of the lipid bilayer is of heavy interest because it dictates properties of the membrane such as phase transition and fluidity. The amphiphilic lipid molecules contained in the mammalian lipid bilayer vary in head and tail structure but are generally limited to four different lipid types; the first three, phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, belong to the glycerophospholipids group whose structure consists of a sn-glycero-3-phosphate core that is esterified at its C1 and C2 positions into fatty acids tails (Alberts et al. 2002). The fourth, sphingomyelin, belongs to the sphingolipid group and the structure consists of a choline molecule attached to a hydroxyl group from ceramide (Khan et al. 2013). The structures of these four phospholipids are shown in figure 1.3.

Figure 1.3: The structure of four different lipid molecules generally found in mammalian membranes. Figure adapted from (Alberts et al. 2002)

The four lipid types differ in structural composition, specifically in what type of alcohol moiety is attached to the phosphate group in each phospholipid type. More specifically a choline (phosphatidylcholine), an ethanolamine (phosphatidylethanolamine), a serine (phosphatidylserine) and a choline (sphingomyelin) represent the type of molecules linked to the phosphate groups in each of these different lipid structures. Of the four types, phosphatidylserine is the only molecule which carries a net negative charge (Quinn 2012) at physiological pH while the other three types are electrically neutral. The fatty acid tails of each lipid type can vary both in length and composition as well. Overall, these four lipids make up 50% of the mass of mammalian lipid membranes. Other phospholipids such as inositol phospholipids exist in small amounts but serve important functions in cell signaling. The remaining lipid bilayer structure is made up of cholesterol, glycolipids and membrane proteins. All these structural types interact with each other to form the semi-permeable fluid-mosaic model of the lipid bilayer shown in figure 1.1.

In addition to structural difference that exists between these phospholipid types, the specific location of each phospholipid in the lipid bilayer can vary as well. Some phospholipids exist mostly in the cytosolic monolayer of a bilayer membrane while others are predominantly in the outer monolayer of the lipid bilayer (Janmey & Kinnunen 2006). For instance, glycolipids tend to be located in the outer monolayer and are thought to play a role in formation of lipid rafts. In mammalian red blood cells, choline-containing phospholipids such as phosphatidylcholine and

sphingomyelin are primarily located in the outer monolayer while phosphatidylserines and phosphatidylethanolamines exist in the inner monolayer. Due to such a variety in lipid types, the lipid bilayer is asymmetrical and its structure and properties can vary even between lipid bilayers of the same cell types.

1.2 - Phosphatidylcholines and POPC

The most abundant phospholipid in mammalian cell membranes is phosphatidylcholine (Alberts et al. 2002). It is a constituent of human lung surfactants and serum lipoproteins and is the most frequently studied phospholipid in experimental lipid bilayer work. Therefore, the phase behavior and transition in the presence of water is of utmost interest to researchers in the field.

The phosphatidylcholine structure consists of a choline head group linked to a glycerophosphoric acid group attached to two fatty acid tails which can vary in structure and length (Alberts et al. 2002). Generally, phosphatidylcholines bear one saturated and one unsaturated fatty acid tail. An example is POPC (*1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine*), which contains a saturated (palmitoyl) and an unsaturated (oleoyl) fatty acid tail in its structure depicted in figure 1.4.

Figure 1.4: Structure of POPC. (Vigneron & Edgar181 2007)

Phosphatidylcholine lipids which contain two saturated fatty acid tails are found in nature but are less common. Regardless of structure, they are an important component in lipid bilayers and are readily studied in lipid bilayer models *in-vitro* and *in-silico*.

1.3 - Lipid Bilayer Phase Behavior

The structural components that make up the lipid bilayer heavily influence its fluidity and phase behavior. The lipid bilayer can exist in two phases, a solid two-dimensional (gel) phase and a liquid phase. In the solid phase, lipids are very immobile, unlike the liquid phase, where lipids have more freedom to move around (fluid). Another distinction is made depending how ordered the lipids are in each phase. For instance, in the liquid phase, the lipid bilayer can either be in the liquid-ordered (L_o) phase where lipids are rigid and less mobile or in liquid-disordered (L_d) phase where lipids have more freedom to move around in the lipid bilayer.

Another key characteristic of lipid membranes is the temperature at which the lipid bilayer transitions from the solid to liquid phase, also known as the melting temperature (T_m) (Berkowitz 2009). The T_m is heavily affected by the fluidity and structural components of the lipid bilayer. For instance, the structure of the fatty acid tails of lipids in the membrane can either increase or decrease the T_m . Longer fatty acid tails result in more van der WAALS attractive forces and increase the melting temperature while shorter chains decrease it (Alberts et al. 2002; Siegel et al. 1999). In addition to fatty acid tail length, the presence of double bonds in the structure creates kinks in the hydrocarbon chains and increases the mobility of lipids in the membrane. The result of this is that lipids cannot pack as tightly together then if there were no

double bonds (saturated) and therefore the T_m is decreased. In general, the higher the degree of unsaturation (more double bonds), the lower the transition temperature of the lipid bilayer. Other factors that may influence the T_m include the pH of the environment and the size of the bilayer. Overall, the combination of different lipid structures leads to mixed phase behaviors of the lipid bilayers which depend highly on which lipid types are contained in it and their orientation in the membrane.

1.4 - Cholesterol

In addition to phospholipids, another important lipid found in membranes is cholesterol, belonging to the sterol group (modified steroid). Its structure, shown in figure 1.5, consists of a hydroxyl head group linked to a steroid group attached to a hydrophobic fatty acid tail. The steroid group consists of four rings which are *trans* connected and give cholesterol a flat and rigid structure (Róg et al. 2009).

Figure 1.5: Structure of cholesterol. Three depictions of the structure of cholesterol are shown. The first two (A, B) represent the chemical structure drawn in different

representations. Finally, in C, the space filling model is shown for cholesterol. Figure adapted from (Alberts et al. 2002)

The Condensation Effect of Cholesterol

The presence of cholesterol in lipid membranes, specifically in mammalian cells heavily influences the properties of the lipid bilayer. Typically, mammalian cells can contain a range of 20-30 % mol cholesterol in their membranes and up to 50% mol in red blood cells (Róg et al. 2009) and therefore the presence of cholesterol highly affects both the structural and thermodynamic properties of the lipid bilayer. Structurally, cholesterol has been proven to have a “condensing effect” on lipid bilayers (Hung et al. 2007) (Daly et al. 2011). This condensing effect on the lipid bilayer has been shown in various experimental models (Mitchell & Litman 1998) (Oldfield et al. 1978) and in-silico models (Robinson et al. 1995) (Hung et al. 2007) (Martinez-Seara et al. 2008) . This is due to the structure of cholesterol which contains a polar hydroxyl head group which binds closely to the head groups of phospholipids around it, increasing the interaction strength between adjacent phospholipid molecules and ultimately leading to a more tightly packed lipid bilayer (Hung et al. 2007). This is the reason cells regulate the levels of cholesterol in their membranes as a way of controlling the fluidity and adjusting to different environmental conditions such as a higher or lower temperature. Overall, this condensing effect impacts structural properties of the lipid bilayer such as the thickness of the membrane, area per lipid, and the deuterium order parameter of the lipid tails (S_{CD}). Lipid bilayers with higher

levels of cholesterol have been shown to have thicker bilayers on average, a reduced total area per lipid, and an increased order parameter of carbons in acyl chains.

(Mannock et al. 2010).

In addition to structural effects, thermodynamic properties of lipid membranes are also altered by the level of cholesterol present. Specifically, the melting temperature is increased in bilayers with higher levels of cholesterol (Alberts et al. 2002). With more cholesterol, the lipid bilayer becomes more tightly packed during the gel phase and therefore requires more thermal energy to phase transition into the liquid-crystalline phase. It becomes clear that the presence of cholesterol greatly affects the properties of the lipid bilayer, one of the driving forces behind this research.

Section 2 – Molecular Dynamics

2.1 – Theory Behind Molecular Dynamics

In 1957, a group of scientists named Berni Alder and Thomas Wainwright published breakthrough research describing a numerical method to study the physical movements of atoms and molecules in an N-body simulation (Allen 2004). This research was the foundations of molecular dynamics computer simulations. Molecular dynamics simulations are commonly used to study specific phenomena at the microscopic level. Experimental work is important but is limited to studying macroscopic properties of a system in most cases. Therefore, molecular dynamics simulation can provide clues to experimentalists as to exactly what is happening to a system at the atomistic level. Many times, collaborations between experimentalist and computer simulation experts are key for unveiling the fundamentals behind important phenomena.

Foundation of Molecular Dynamics

Molecular dynamics simulation consists of numerically solving Newton's 2nd law of the classical equations of motion for a set of atoms in the system (2.1).

$$f_i = m_i \ddot{r}_i \quad (2.1)$$

To perform this task, the force acting on every atom F_i must be obtained and is derived from a potential energy function $U(r^N)$ where r^N represents the complete set of $3N$ atomic coordinates of the system studied (equation 2.2) (Allen 2004) .

$$f_i = -\frac{\partial}{\partial r_i} U \quad (2.2)$$

This is because the force can be calculated as the negative gradient of the potential energy. Before the force is obtained, the potential energy acting on every atom in the system must be calculated. To calculate the potential energy on a given atom, there are several atomic interactions which must be considered for any given system.

Non-Bonded Interactions

The first type of interactions considered is one that occurs between a pair of non-bonded atoms. The most commonly used potential to describe non-bonded interactions is the Lennard-Jones potential (U_{LJ}) shown below,

$$U_{LJ}(r) = (4\epsilon) \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right] \quad (2.3)$$

where ϵ is the depth of the potential well, σ is the distance where the inter-particle potential reaches 0 and r_{ij} is the distance between the particles (Tieleman et al. 1997). The r^{-12} term is the repulsive term and is based on the Pauli repulsion occurring between nearby atoms due to overlapping electron orbitals. Meanwhile the r^{-6} attractive term describes the attraction between two atoms due to long range forces such as van der Waals (Allen 2004). If charges are present between the set of interacting atoms, the Coulomb potential is used (equation 2.4),

$$U_{Coulomb}(r) = \frac{Q_1 Q_2}{4\pi\epsilon_0 r_{ij}} \quad (2.4)$$

where Q_1 and Q_2 are the charges of the atoms, ϵ_0 represents the permittivity of free space and r_{ij} is distance between the particles. This potential is repulsive for atoms with similar atomic charges and attractive for atoms with opposite electric charges. Traditionally, calculation of non-bonded forces between atoms in a system is the most computationally expensive part of a simulation. Generally, during simulations a cutoff distance is provided which eliminates the calculation of non-bonded forces that are separated by a certain distance threshold. This cutoff distance saves calculation and computational time during simulations.

Bonded-Interactions

The intramolecular potential between a set of bonded atoms must be calculated as well. This consists of the sum of three separate potentials which consider bond distance, bend angles and torsion angles between a set of atoms (2.5a-2.5c).

$$U_{intramolecular} = \frac{1}{2} \sum_{bonds} k_{ij}^r (r_{ij} - r_{eq})^2 \quad (2.5a)$$

$$+ \frac{1}{2} \sum_{\substack{bend \\ angles}} k_{ijk}^{\theta} (\theta_{ijk} - \theta_{eq})^2 \quad (2.5b)$$

$$+ \frac{1}{2} \sum_{\substack{torsion \\ angles}} \sum_m k_{ijkl}^{\phi, m} (1 + \cos(m\phi_{ijkl} - \gamma_m)) \quad (2.5c)$$

The first bonding potential considers the separation distance between a pair of bonded atoms where $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$. A harmonic form is utilized with a specified equilibrium distance r_{eq} . The second term considers the bend angles, Θ_{ijk} , between successive bond vectors of three atoms. Finally, the torsion angle term, Φ_{ijkl} , describes the torsion angles between three successive bonds of four atoms. Traditionally, force-field simulations packages such as CHARMM, will provide the accurate forms of equations as well as the strength parameters, k , and other constants such as r_{eq} and θ_{eq} . Generally, these force-fields are parameterized by using a combination of empirical techniques as well as quantum-mechanics calculations. The accuracy of these force-fields is tested by the ability to reproduce structural, dynamic, bulk and thermodynamic properties of small molecules whose properties have been well described experimentally.

Once the potential energy function has been calculated, the forces acting on atoms in the system can be calculated (formula 2.2). Once the forces are calculated, a numerical integration algorithm, such as the velocity-Verlet algorithm (Swope et al. 1982) can be used to calculate the positions and velocities of atoms at time $(t + \Delta t)$ given the initial coordinates and velocities of the system studied. A trajectory of the system is then produced describing the dynamic evolution of a system over an elapsed time length.

Thermodynamic Ensembles

In molecular dynamics, the choice of which statistical thermodynamic ensemble employed to study a specific model of interest depends on which properties

the researcher is interested in. Generally, there are three common thermodynamic ensembles employed during molecular dynamics simulation, the microcanonical ensemble, the canonical ensemble, and the isothermal-isobaric ensemble. In the microcanonical ensemble (NVE), the system is represented as having a fixed number of atoms (N), a constant volume (V), and a constant energy (E) (Frenkel & Smit 2002). Generally, this statistical ensemble is utilized when the energy of a system is known but no information about the internal states is available. This is an unrealistic ensemble because in experimental setups, energy is generally a varying property.

In the canonical ensemble (NVT), the system is represented as having a fixed number of atoms (N), constant volume (V), and constant temperature (T) (Frenkel & Smit 2002). This ensemble accurately represents a system that is in immediate contact with a heat bath. Finally, the isothermal-isobaric (NPT) ensemble represents a system with a fixed number of atoms (N), constant pressure (P) and constant temperature (T) (Frenkel & Smit 2002). This thermodynamic ensemble is considered the more experimentally realistic ensemble due to temperature and pressure being relatively constant during experiments. It represents an experiment being carried out in an open flask exposed to environmental temperature and pressure. Regardless, the choice of ensemble depends highly on the in-silico model to be simulated. Certain ensembles are better suited at reproducing properties for one molecule but produce errors in properties in other models placed in these ensembles.

Molecular Dynamics Time step

The vital component of any molecular dynamics simulation is the time step chosen to perform numerical integration on the system modeled. To accurately reproduce the dynamics of an all-atom system and avoid discretization errors, the time step chosen must be smaller than the fastest vibrational frequency in the system modeled. Since the vibrational frequency of hydrogen molecule is 10^{-14} s, a time step of 10^{-15} s or 1 femtosecond (fs) must be employed (Allen 2004). At such a small time step, simulating phenomena of a few microseconds becomes quite impossible without the use of supercomputers. One way to increase this time step is to constrain the bond lengths of hydrogen-containing bonds during simulation using algorithms such as SHAKE or RATTLE which allows the use of a 2 fs time step. Overall, care must be taken in choosing which phenomena to model using molecular dynamics due to the time step constraint and the time scales that can be reached.

Shockwaves and Simulation Methods

The choice of what molecular event to study using molecular dynamics is highly dependent on the time lengths which can be reached through computer simulation. In an all-atom molecular dynamics system, it is highly unlikely that a researcher will want to observe an event which occurs in the time scale of milliseconds (10^{-3} s) because simulating this event would years; depending on the number of atoms in the system and the number of CPUs (Central Processing Units) utilized to carry out the simulation. Even by coarse-graining particles in the system,

these time scales are still out of reach. Realistically, events studied should occur within the nanosecond (10^{-9}) and at most microseconds (10^{-6} s) time scale.

The high-speed phenomena that occurs during a shockwave event, is what makes this process so applicable for molecular dynamics simulation. Shockwaves occur when a wave exceeds the local speed of sound in a fluid and is characterized by a discontinuous change in pressure, temperature, and density of the medium it is occurring. The speed of sound in water is roughly 1432 m/s (Slizberg 2013) and therefore since shockwaves travel above the speed of sound, this high-speed phenomenon is easily reachable in the time scales of molecular dynamics

2.2 - Importance of Studying Shockwaves

Drug delivery and gene therapy methods heavily utilize the application of shockwaves to manipulate the permeability of the lipid bilayer contained in cells of interest. Application of shockwaves has been proven to cause temporary permeability of the lipid bilayer allowing for delivery of drugs or oligonucleotides into a patient's cells. In cancer therapy, methods such as high intensity focused ultrasound (HIFU) which utilize shockwaves are used to treat cancerous tissue and cause destruction of cancerous cells through conversion of mechanical energy into heat (Espinosa et al. 2014) . In addition, shockwave pulses are used to treat patients suffering from kidney stones in a treatment called shockwave lithotripsy (Williams et al. 1999). Although the treatment is effective, kidney tissue can be damaged due to effects of the shockwave application. Regardless of application, the study of shockwaves and the effects they can have on our cells is of great interest.

At the macroscopic level, the effect of shockwaves on the lipid bilayer has been studied intensively. Microscopically, much work remains to be done to understand the effects on the lipid bilayer structure at the atomic level. This knowledge can provide the medical field with ways to make improvements to currently implemented methods which utilize shockwaves.

Past research has probed the effects of shockwaves on lipid bilayer structural properties *in-silico* through molecular dynamics research. Specifically, the Koshiyama group (Koshiyama et al. 2006a) modeled the effects of shockwaves on DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine)-containing lipid bilayer models. They noted changes in the bilayer structure due to shockwaves such as the rebound and collapse of the lipid bilayer and increased water permeation. Properties such as the accumulated lateral displacement and the temporal evolution of the instantaneous averaged deuterium order parameter of lipid tails were measured. Two years later (Koshiyama et al. 2008), this same group tested the effects of shock impulses impacting at various incident angles to DPPC lipid bilayer models. In addition, several groups tested effects of shockwaves on coarse-grained lipid bilayers using different coarse grained models as well as different force fields; the first using the DPDE force field (Ganzenmüller et al. 2011) and the second group using the MARTINI force field (Santo & Berkowitz 2014). Although this research was important, the all-atom *in silico* lipid bilayer models tested were very simplistic, only containing one phospholipid type and did not contain cholesterol, a vital component in mammalian cell membranes. While in contrast, the coarse-grained models lacked both atomic detail and cholesterol in their membranes modeled. Regardless, the addition of

cholesterol to lipid membranes alters structural properties such as the average membrane thickness, the area per lipid and the deuterium order parameter of acyl chains. The models previously tested, have different structural properties since they lack cholesterol. In addition, cholesterol-free lipid mammalian membranes hardly exist in nature and therefore, this research will provide a more realistic analysis of the effects of the lipid bilayer due to shockwave than prior research has.

The first goal of this research is to apply shock impulses of varying magnitude (2.5 - 10 mPa * s) on two different in-silico lipid bilayer models. The first model contains a 9 to 1 ratio of POPC to cholesterol (10% cholesterol). This model will be referred to as the 9:1 POPC/CHL model. The second model consists of a 1 to 1 ratio of POPC to cholesterol (50% cholesterol). This model will be referred to as the 1:1 POPC/CHL model throughout this thesis. Structural effects such as the change in bilayer thickness, the temporal change in instantaneous average deuterium order parameter S_{CDi} of lipid tails, and the lateral displacement of lipids will be quantified at the range of shock impulses tested for both models.

The second goal of this thesis research is to analyze the effect of the distance of the water slab from the lipid bilayer on the model systems. This will be tested by placing the shock slab 1 and 10 Å away from the lipid bilayer models studied and measuring the effect on the change in bilayer thickness, kinetic energy and pressure on the systems studied. This will elucidate which mechanism is more effective in this setup for producing the strongest shock impulse.

Section 3 – Materials and Methods: Creation and Equilibration of Models

Setup of Initial Lipid Bilayer Model Structures Containing POPC and Cholesterol

The membrane models tested in these simulations were created using the online software, CHARMM-GUI (Lee et al. 2016). This program allows the user to select which lipid species as well as the number of molecules of each species to include in the membrane models created. For the models tested in this research, POPC and cholesterol were chosen as the two lipid species and two models containing 10% cholesterol and 50% cholesterol were created. The 10% cholesterol (9:1 POPC/CHL) membrane contained 216 POPC and 24 cholesterol molecules while the 50% cholesterol (1:1 POPC/CHL) membrane consisted of 120 POPC and 120 cholesterol molecules. These molecules were evenly divided among the monolayers thereby representing a symmetric bilayer as opposed to an asymmetric one which contains an unequal number of lipids in each monolayer. Therefore, for the 9:1 POPC/CHL model, each monolayer contained 108 POPC molecules and 12 cholesterol molecules while the 1:1 POPC/CHL model contained 60 POPC and 60 cholesterol molecules in each monolayer.

Solvation and Ionization

Once the initial bilayer models were created, they were solvated explicitly using the TIP3P (Jorgensen et al. 1983) model for water molecules. A total of 23 nanometers (nm) of solvent was added in the z dimension. More specifically, 20 nm of solvent was added to the right of the bilayer model (z+) and 3 nm to the left (z-). The excess solvation in the z+ side of the lipid bilayer is needed in order to allow

shockwave propagation to be modeled accurately, as previously noted by the Koshiyama group (Koshiyama et al. 2006a). Solvent was not added in the x and y dimension due to the lack of water molecules in the hydrophobic region of the bilayer. Once solvated, sodium and chlorine ions were added to both systems until a concentration of .15M was reached. The solvation and ionization was performed using the program VMD (Visual Molecular Dynamics) (Humphrey et al. 1996). Table 3.1 below lists the total number of atoms in each system as well as the number of atoms of each molecule type while table 3.2 lists some key initial properties of each system respectively.

Molecule Type	10 % Cholesterol Model	50% Cholesterol Model
	Atom Count	Atom Count
Total	241968	208535
POPC	28944	16080
Cholesterol	1776	8880
Sodium	198	160
Chloride	198	160
Water	210852	183255

Table 3.1: Atom composition and number of atoms in the 9:1POPC/CHL and 1:1 POPC/CHL lipid bilayer systems.

Lipid Bilayer Model	Total Number of Lipids	Lipid Bilayer Composition	Bilayer Size (nm)	System Size (nm)	Total Number of Atoms
9:1	240	216 POPC 32 CHOL	9.88 x 9.61 x 4.65	9.88 x 9.61 x 27.66	241,968
1:1	240	120 POPC 120 CHOL	8.79 x 8.87 x 4.66	8.79 x 8.87 x 27.67	208,215

Table 3.2 Initial Properties of the 9:1 POPC/CHL and 1:1 POPC/CHL lipid bilayer systems.

In addition, snapshots of the final systems for both models are shown (figure 3.1a, 3.2a) as well as top view snapshots of each model (figure 3.1b, 3.2b) where POPC and cholesterol molecules are colored differently in order show the distribution of cholesterol in the models created. Snapshots were rendered using VMD (Humphrey et al. 1996)

a)

b)

Figure 3.1: 9:1 POPC/CHL lipid bilayer system. Figure 3.1a is a snapshot of the 9:1 model containing water (red), POPC (green) and cholesterol (blue). Figure 3.1b is a top-view representation of the 9:1 model showing the distribution of POPC (green) and cholesterol (blue) in the upper monolayer.

a)

b)

Figure 3.2: 1:1 POPC/CHL lipid bilayer system. Figure 4.3a is a snapshot of the 1:1 model containing water (red), POPC (green) and cholesterol (blue). Figure 3.2b is a

top-view representation of the 1:1 model showing the distribution of POPC (green) and cholesterol (blue) in the upper monolayer.

Equilibration of Lipid Bilayer Models

An initial 10,000 steps of minimization was carried out for both models using the conjugative gradient method (Fletcher & Reeves 1964) in NAMD (Malterer et al. 2005). Following minimization, equilibration of models was carried out in an NPT ensemble at 310 K and 1 atm using a time step of 2 fs. To use this timestep, all hydrogen-containing bonds were constrained using the SHAKE algorithm described here (Ryckaert et al. 1977). Constant temperature was maintained using Langevin dynamics with a damping coefficient of 1/ps. Constant pressure was controlled using a Nosé-Hoover Langevin piston algorithm (Feller et al. 1995) with a Langevin piston period of 50 fs and a Langevin piston decay of 25 fs. The three orthogonal dimensions of the periodic cell were allowed to fluctuate independently. The ratio of the unit cell in the x-y plane was kept constant while allowing fluctuations along all axes. This option is required for the previous one. Periodic boundary conditions were employed in all three dimensions. All atoms exiting the boundaries were wrapped accordingly. Long range electrostatic were treated using the Particle Mesh Ewald method (Essmann et al. 1995) which has been shown to be a good method in accurately calculating long-range forces in membrane simulations (Vermeer et al. 2007). The CHARMM 36 force field for lipids was used and is described here (Venable et al. 2010). An example equilibration configuration file for the 1:1 POPC/CHL model can be found in the appendix (APPENDIX II) provided. This configuration file is similar to the 9:1 model, varying only in file names.

The equilibration runs were set to 20 ns but after 7 ns of equilibration, values such as average membrane thickness, average area per lipid, temperature and pressure had converged for each system and therefore the equilibration runs were terminated. The final restart velocities and coordinates of these equilibrations were used in the following shockwave simulations

Section 4 – Shockwave Implementation and Setup

4.1 – Shockwave Implementation

The previously established methods of Koshiyama et al. (Koshiyama et al. 2006a) were used to model the shockwaves in the following simulations. The impulse in this work is defined as the impulse per unit area ($\hat{I}$), generally called the specific impulse. The specific impulse can be defined as the change of momentum(M) over the area (A) where the pressure is exerted as shown in the formula below,

$$\hat{I} = \frac{M(t+) - M(0)}{A} \quad (4.1)$$

Based on previous experiments by Kodama et al. (Kodama et al. 2000) that applied varying shock impulses to leukemia cells in water, it was shown that at the initiation of a shockwave, the shockwave front had not reached water and cells ahead of it and therefore the momentum of water molecules at shock initiation was 0. After a small distance traveled at time (t_+), the momentum of water molecules was shown to be the impulse applied times the area ($I \times A$). From this finding, the shockwaves in the simulations were modeled as a change of momentum of water molecules in a slab of volume $L_z * A$ directly adjacent to the lipid bilayer; where L_z represents the thickness of the water slab and A represents the lateral area of the water slab in the x and y plane. The water slab will be termed “**shock slab**” from this point on in the thesis. To implement this change in momentum, a formula was used to calculate an average velocity to add to the z-component of the thermal velocity of water atoms in the shock slab adjacent to the bilayer. This velocity is added only to the z component of the

thermal velocity of water atoms because this shockwave is impacting the lipid bilayer with an incident angle of 0° normal to the bilayer plane (xy). The average velocity added to water molecules in the shock slab is calculated using the formula below,

$$V_{added} = \frac{\hat{I} * A}{m * N_w} \quad (4.2)$$

where $\hat{I}$ is the specific impulse, A is the area of the simulation box, m is the mass of one water molecule (2.99×10^{-26} kg) and N_w is the number of water molecules in a shock slab of thickness $5 \text{ \AA}$. The far right boundary of the slab was placed $1 \text{ \AA}$ away from the lipid bilayer in the first set of simulations and $10 \text{ \AA}$ away from the lipid bilayer in the second set of simulations as shown in figure 4.1 below.

Figure 4.1: Two types of shock slab distances from the bilayer depicted. In figure 4.1, the 50:50 POPC/CHL lipid bilayer is depicted with POPC molecules (red) and cholesterol (green). The blue slab represents the shock slab whose right boundary is

placed 1 Å away from the lipid bilayer while the white slab is placed 10 Å away.

Other atoms are removed for visualization purposes.

Finally, to add the average velocity calculated, a MATLAB code was written to calculate the average velocity added to each water molecule in the slab by implementing the formula above. This MATLAB code calculates the added average velocity in Å/ps (NAMD units) based on the shock impulse range input by user. Once this velocity is calculated, it is added to the z-component of the thermal velocity of only water molecules in the slab of designated coordinates. For more information, please refer to the MATLAB code included in the appendix section (APPENDIX I) of this thesis.

4.2 – Shockwave Simulation Setup

Shock impulses of 2.5, 5, 7.5, 10 mPa * s were applied to each model. The shockwave simulations were carried out in an NVE (constant number of atoms, N, volume, V and total energy, E) ensemble in order to analyze non-equilibrium dynamics. An example configuration file for the shock simulations is provided in the appendix (APPENDIX III).

To determine the time length of each shockwave simulation at varying shock impulse, the approach by the Koshiyama group (Koshiyama et al. 2006) was used for a system simulated using periodic boundary conditions. The distance from the right boundary of the shock slab to the opposite boundary of the simulation box was divided by the average velocity added to determine length of simulation.

For instance, if the length from shock initiation to the opposite boundary of the simulation box is 200 Å and the average velocity added is 200 Å/ps, then the shockwave simulation will be carried out for a total of 1 picosecond. Table 4.1 list the added average velocities to water atoms in each respective slab, as well as length of simulation time and time step employed for both models at both shock slab distances from lipid bilayer tested. For higher velocities added, smaller time steps were employed to prevent disruption of simulation.

a)

1:1 POPC/CHL Model							
<i>Shock Slab 1 Å distance</i>				<i>Shock Slab 10 Å distance</i>			
Impulse (mPa *s)	Vel. Added (Å/ps)	Timestep (fs)	Time (fs)	Impulse (mPa* s)	Vel. Added (Å/ps)	Timestep (fs)	Time (fs)
2.5	50.786	0.5	7000	2.5	50.786	0.5	7100
5.0	101.572	0.5	3500	5.0	101.72	0.5	3600
7.5	152.358	0.5	2350	7.5	152.58	0.5	2400
10.0	203.144	0.1	1750	10.0	203.44	.1	1800

b)

9:1 POPC/CHL Model							
Shock Slab 1 Å distance				Shock Slab 10 Å distance			
Impulse (mPa *s)	Vel. Added (Å/ps)	Timestep (fs)	Time (fs)	Impulse (mPa* s)	Vel. Added (Å/ps)	Timestep (fs)	Time (fs)
2.5	51.047	0.5	6500	2.5	51.068	0.5	6550
5.0	102.09	0.5	3200	5.0	102.13	0.5	3300
7.5	153.14	0.5	2150	7.5	153.20	0.5	2200
10.0	204.19	0.1	1600	10.0	204.27	0.1	1700

Table 4.1: Shockwave Simulation Setups for lipid bilayer models at varying shock impulses and shock slab distances. The shockwave simulation setup details for the 1:1 POPC/CHL model (4.1a) and the 9:1 POPC/CHL model (table 4.1b) are listed for both shock slab distances.

Analysis of Equilibration and Shockwave Production Runs

Analysis of equilibration of the models and shockwave simulations was carried out using the software MEMBPLUGIN (Guixà-González et al. 2014). Membrane properties such as the average membrane thickness, area per lipid, and carbon-deuterium order parameter of lipid tails were calculated with this software. The

following descriptions below describe how each relevant bilayer property is calculated by this software.

Average Membrane Thickness

The *Membrane Thickness* tool contained in this software calculates the average membrane thickness over a trajectory by measuring the distance between the two density peaks of user-selected atoms (phosphorous) belonging to head group of POPC as well as the location of the middle point between them defined as the first and second central moment corresponding to the mass density profile of the phosphorous atoms.

% Decrease in Average Membrane Thickness

In addition, the percent decrease in the average membrane thickness was calculated for both models using the formula below

$$\% \text{ Decrease} = \left(\frac{Th_t (\text{\AA}) - Th_{t+1} (\text{\AA})}{Th_t (\text{\AA})} \right) * 100 \quad (4.3)$$

Where Th_t is the average bilayer thickness at time t and Th_{t+1} is the average bilayer thickness at time $t+1$.

Deuterium Order parameter S_{CD} of Lipid Tails

The *Deuterium Order Parameter S_{CD}* tool measures the deuterium order parameter of acyl chains in phospholipids. This parameter is typically derived through NMR experiments (Vermeer et al. 2007) and represents the orientational mobility of the carbon-hydrogen methylene bonds along the lipid tails of the phospholipid model

(Heller et al. 1993). The formula below is used to measure the S_{CD} parameter of lipid tails in this software,

$$S_{CD} = (-3/2(\cos^2\Theta_{CD})-1) \quad (4.4)$$

where Θ_{CD} is the angle between a CD- (Carbon-deuterium) bond in experiment or a CH- (carbon-hydrogen) bond in simulations and the membrane normal (z-axis). The averaging is done among all lipids in the sample for length of sample time. Higher S_{CD} values represent a less fluid lipid bilayer with lipid tails that are more ordered (straight).

Area per Lipid

The *Area per Lipid* tool calculates the total average area per lipids for all lipid species as well as the average area per individual lipid species such as POPC or cholesterol using a user-defined selection of atoms. The x and y coordinates of the selected species are projected onto a plane which is delimited by the simulation box. This plane is then subsequently divided into polygons and the area of each polygon is calculated.

Lateral Displacement

The two-dimensional (x and y plane) lateral square displacement LSD is calculated using formula 4.5,

$$LSD = \langle \frac{1}{N_L} [\sum_{i=1}^{N_L} [r_i(t) - r_i(0)]^2] \rangle \quad (4.5)$$

where N_L is the number of lipids, and $r_i(t)$ the position of lipid I at time t.

Section 5- Results and Discussion

5.1 – Equilibration Analysis

For any good in-silico bilayer model, equilibrium properties must be measured during equilibration to monitor how accurately the properties of these models, such as the area per lipid, match experimental values of similar models. Therefore, the model systems were equilibrated for a total of 7 ns and these equilibration trajectories used to calculate properties such as the average membrane thickness, average area per lipid, and average deuterium order parameter S_{CD} for individual carbons in all lipid tails (palmitoyl + oleoyl) combined or for each lipid tail separately. In addition, the deuterium order parameter was calculated separately for carbons of lipid tails only in either the upper or lower monolayer.

Equilibration Analysis: Average Membrane Thickness

The average bilayer thickness calculated over the 7 ns equilibration trajectory is plotted and compared in figure 5.1 for both models. The final value of average membrane thickness of the 1:1 POPC/CHL model was $45.38 \pm 0.52 \text{ \AA}$ while the 9:1 POPC/CHL model was $38.80 \pm 0.43 \text{ \AA}$.

Figure 5.1: Average bilayer thickness of both lipid bilayer models during 7 ns equilibration compared.

Overall, the 50% cholesterol model showed an increase in average bilayer thickness. Through experiments, it has been shown that the addition of higher levels of cholesterol will result in a thicker lipid bilayer than in models with a lower or no amount of cholesterol. Therefore, it is expected that the model 1:1 model with 50% cholesterol, should have a thicker membrane than the 9:1 model containing only 10% cholesterol.

Equilibration Analysis: Area per Lipid

During equilibration, the average area per lipid for both models was calculated for all lipid species (POPC +CHOL), as well per each individual lipid (POPC or CHL) specie. In figure 5.2, the average area per lipid for all lipid species in the system is computed and compared among the two models.

Figure 5.2: Average area per lipid of all lipid species (POPC +CHOL) of each lipid bilayer model plotted over 7 ns equilibration time.

Owing to cholesterol's condensation effect, the system with 1:1 POPC/CHOL ratio shows the lowest total area per lipid for all lipid species in the system.

In addition to the total area per lipid, the area per POPC molecule was calculated throughout the course of equilibration as well. In figure 5.3, the average area per POPC for each model is compared.

Figure 5.3: Average area per POPC of each lipid bilayer model plotted over 7 ns equilibration time.

The addition of cholesterol lowers the average area per POPC as well. Cholesterol causes lipids in the membrane to pack closer to each other and therefore this result is expected.

Finally, in figure 2c, the average area per cholesterol of each system is plotted.

Figure 5.4: Average area per Cholesterol of each lipid bilayer model plotted over 7 ns equilibration time.

On average the area per cholesterol is higher in the 50% cholesterol model when compared to the 10% cholesterol model.

Overall, the addition of cholesterol to the lipid bilayer causes the POPC lipids to pack tighter (condensation effect) thereby decreasing the area of the lipid bilayer. This causes a decrease in the the total area per lipid and area per POPC when compared to the 10% model with a lower range of cholesterol. In POPC only models (1:0 POPC/CHL), the calculated average area per lipid has been determined experimentally as $68.3 \pm 1.5 \text{ Å}^2$ (Venable et al. 2010) . This value has also been validated in computational models of POPC-only membranes (Kučerka et al. 2006). In contrast,

the average area per POPC of the 1:1 POPC/CHL model is $57.09 \pm 1.444 \text{ \AA}^2$ while the average area per cholesterol molecule is $30.398 \pm 1.605 \text{ \AA}^2$. Meanwhile, in the 9:1 POPC/CHL model, the final average area per POPC is $65.1129 \pm 1.455 \text{ \AA}^2$ and average area per cholesterol is $27.29 \pm 2.909 \text{ \AA}^2$. The area per lipid is reduced slightly in the 9:1 POPC/CHL system and greatly in the 1:1 POPC/CHL system when compared to a POPC model without cholesterol. This is expected due to the well-known condensing effect of cholesterol (Hung et al. 2007) (Daly et al. 2011) and has been replicated in computer models (Smondyrev & Berkowitz 1999) (Zhang 2009) (Forrest & Sansom 2000) as well.

Equilibration Analysis: S_{CD} (carbon-deuterium order parameter) of Lipid Tails

The deuterium order parameter (S_{CD}) of lipid tails in the lipid bilayer is an important parameter when it comes to analysis of lipid bilayer models. The deuterium order parameter is a measurement of how “ordered” lipid tails of phospholipids in the lipid bilayer are (Tieleman et al. 1997). It is expected that the addition of cholesterol will lead to a higher average deuterium order parameter of the carbons in the lipid tails of the POPC lipid species in the system due to cholesterol’s condensation effect. This condensation effect leads to a tighter packed lipid bilayer and lipid tails which are more ordered “straight” as demonstrated both experimentally and in-silico (Róg et al. 2009). In figure 5.5, average order parameter S_{CD} through equilibration time is calculated for all individual carbons in all lipid tails (oleoyl + palmitoyl).

Figure 5.5: Average S_{CD} order parameter for carbons in both lipid tails plotted and compared among both models.

As expected, the order parameter of carbons in acyl chains of POPC on average is increased more significantly in the model in the 50% cholesterol model than in the model only containing 10% cholesterol. Specifically, the order parameter of carbons closer to the lipid head group (lower-numbered carbons) show a much more dramatic increase in order parameter than ones at the end of the lipid tails (carbons no. 16,17,18). Along with this, a useful property to calculate is the mean of the individual deuterium-order parameters calculated for every individual carbon. This is calculated using formula 5.1 below,

$$Mean S_{CD} = \frac{\sum_{i=1}^{n_c} S_{CDi}}{n_c} \quad (5.1)$$

Where n_c is the total number of carbons being summed, in this case 17, and S_{CD} is the long-time average value of the S_{CD} parameter computed for each individual carbon over 7 ns of equilibration time contained in both lipid tails.

The mean S_{CD} order parameter for the 9:1 POPC/CHOL is .153 and is close to the average equilibrium value of .16 calculated experimentally in POPC-only models (Magarkar et al. 2014). The mean S_{CD} order parameter for the 1:1 POPC/CHOL model is .208. Additionally, the S_{CD} order parameter is calculated separately for carbons contained in each individual fatty acid tail; palmitoyl (figure 5.6) or oleoyl (figure 5.7) and compared among models.

Figure 5.6: Average S_{CD} order parameter of individual carbons contained in palmitoyl lipid tails of POPC lipids in each model plotted and compared.

Figure 5.7: Average S_{CD} order parameter of individual carbons contained in the oleoyl lipid tails of POPC lipids in each model plotted and compared

The palmitoyl S_{CD} parameter shows a similar trend as the average order parameter of both tails combined (figure 5.5) where lower-numbered carbons closer to the head group of the phospholipid show an increase in average order parameter than higher-numbered carbons further away from the head group. The increase in order is also observed in the average S_{CD} parameter for the oleoyl lipid tails when comparing the 1:1 model. Carbons 9-10 are the location of the double bond and therefore motion is restricted and the average order parameter of these carbons is similar in both models.

In addition, the S_{CD} parameter for carbons in lipid tails in the upper monolayer and the lower monolayer was calculated separately during equilibration and are shown

in figures 5.8 and 5.9. These individual S_{CD} order parameter values for lipids in the upper monolayer and lower monolayer will be important when analyzing the subsequent shockwave simulations and the collapse and rebound of the lipid bilayer that occurs. Some relevant equilibrium properties are shown in table 5.1

Model	Averaged S_{CD} Order Parameter	Final System Temp (K)	Final Bilayer Thickness (nm)	System Size (nm) (x,y,z)
9:1 POPC/CHL	Bilayer	310.012	3.984	10.478 x 10.505 x 33.29
	.153			
	Upper Monolayer			
	.156			
	Lower Monolayer			
	.161			
1:1 POPC/CHL	Bilayer	310.259	4.479	8.89 x 8.362 x 39.25
	.208			
	Upper Monolayer			
	.201			
	Lower Monolayer			
	.194			

Table 5.1: Equilibrium values of both models compared.

5.2 - Shockwave Simulation Analysis

The implementation of a shockwave impulse using the current formulated method of Koshiyama et al. (Koshiyama et al. 2006a) leads to drastic changes on the lipid bilayer systems tested. Addition of a calculated average velocity to water molecules in the water slab leads to rises in the kinetic energy, temperature and pressure of each system. Therefore, to validate that the shockwave impulse is being added correctly, these variables must be measured and should show an initial increase due to the added velocity followed by a slow decrease until an equilibrium value is reached for the system tested. This is only the case in an NVE ensemble because temperature and pressure are not controlled in contrast with an NPT ensemble where pressure and temperature are kept constant by thermostats and barostats and therefore the added kinetic energy is dissipated through the thermostat while the added pressure is dissipated through the barostat. The following set of analysis is carried out for both the 1:1 POPC/CHL and 9:1 POPC/CHL models at a range of shock impulses (2.5, 5.0, 7.5, 10 mPa * s). In addition, the utmost right boundary of the water slab was placed 1 Å away from the lipid bilayer in each respective system.

Rise in Kinetic Energy

The addition of an average velocity to water molecules in the chosen water slab adjacent to the bilayer induces a rise in the kinetic energy of the system. With higher applied shockwave impulses, a higher rise in kinetic energy should be observed. The rise in kinetic energy due to shockwave impulses applied to each system is shown separately. Figure 5.8 plots the kinetic energy for the range of

shockwave impulses tested on the 1:1 POPC/CHL model. Figure 5.9 represents the rise in kinetic energy for the range of impulses tested on the 9:1 POPC/CHL model. These plots are simply a validation that the added velocity due to shockwave impulses is being applied correctly.

Figure 5.8: Rise in kinetic energy of 1:1 POPC/CHL system at varying shock impulses.

Figure 5.9: Rise in kinetic energy of 9:1 POPC/CHL system at varying shock impulses.

Rise in Pressure Due to Shockwave

Similar to kinetic energy, the application of an added velocity to water atoms in the system induces a rise in pressure as well. Figures 5.10 and 5.11 plot the respective rise in pressures induced by shockwave impulses for both models respectively. Once again, this is simply another validation that the added velocity is being applied correctly to both systems. With increase in shockwave impulse, an increase in the initial rise in pressure is seen as well when compared to lower impulses.

Figure 5.10: Rise in Pressure of 1:1 POPC/CHL system at varying shock impulses.

Figure 5.11: Rise in pressure of 9:1 POPC/CHL system at varying shock impulses.

Rise in Temperature Due to Shockwave

Finally, in addition to an increase in kinetic energy and pressure, the temperature in the system is increased rapidly as well due to shockwave impulses. A rise in kinetic energy leads to a rise in the temperature of the systems studied. Figures 5.12 and 5.13 plot the rise of temperature induced by the range of shockwave impulse added to each respective system.

Figure 5.12: Rise of temperature of 1:1 POPC/CHL system at varying shock impulses.

Figure 5.13: Rise in temperature in 9:1 POPC/CHL system at varying shock impulses.

As the plots show, the temperature increase is proportional to the strength of the shockwave impulse added. After all these validations are made, the analysis on the shockwave simulations and the structural effects can now be made.

Shockwave Simulation Analysis: Change in Bilayer Thickness

As the Koshiyama group discussed in their shockwave simulations performed on their DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) models, one of the major structural effects due to shockwaves is the collapse and subsequent rebound of the lipid bilayer. This effect can be analyzed by measuring the change in the average

bilayer thickness throughout the course of the shockwave simulations. In figure 5.14 and 5.15 respectively, the change in average bilayer thickness induced at varying shockwave impulses is plotted for the 1:1 POPC/CHL and the 9:1 POPC/CHL model. An initial collapse phase, where the average membrane thickness decreased, is followed by a rebound phase where the membrane rebounds to its original thickness value. These two distinct phases are observed for both models at all shock impulses applied.

Figure 5.14: Change in bilayer thickness of 1:1 POPC/CHL model at varying shock impulses.

Figure 5.15: Change in bilayer thickness of 9:1 POPC/CHL model at varying shock impulses.

In more detail, after the initial collapse phase, the shockwave has propagated through the lipid bilayer and therefore the rebound phase begins and occurs at a range of 500-1000 fs for the respective models.

In addition, the higher the shock impulse applied, the greater the reduction in membrane thickness. Also at higher shockwave impulses, the rebound phase begins earlier because the shockwave has interacted and passed through the lipid bilayer faster than at lower shockwave impulses as expected. Finally, another observation is that the membrane thickness returns close to its normal value faster at higher shock

impulses (7.5, 10 mPa * s) then at the lower range of impulses tested (2.5, 5.0 mPa * s).

In addition to analyzing the evolution of bilayer thickness of both models due to shockwaves separately, a comparison of the bilayer thickness changes due to shockwave impulses was carried out as well. To allow for this comparison, the percent decrease in bilayer thickness due to shockwave at similar shock impulses was calculated using equation 4.3

Since the starting membrane thickness in both models is different, calculating percent decrease in bilayer thickness by incorporating the starting membrane thickness values allows for a comparison between models to be made. Once the percent decrease in bilayer thickness was calculated for both systems at the range of shockwave impulses tested, plots were constructed comparing the percent decrease in bilayer thickness of the respective bilayer models at the range of shockwave impulses tested (figures 5.16, 5.17, 5.18 and 5.19) .

Figure 5.16: Percent decrease in bilayer thickness at shock impulse of 2.5 mPa* s compared between lipid bilayer models.

Figure 5.17: Percent decrease in bilayer thickness at shockwave impulse of 5 mPa * s compared between lipid bilayer models.

Figure 5.18: Percent decrease in bilayer thickness at shock impulse of 7.5 mPa * s compared between lipid bilayer models.

Figure 5.19: Percent decrease in bilayer thickness at shock impulse of 10 mPa * s compared between lipid bilayer models.

When comparing the percent decrease in bilayer thickness at varying shock impulses between models, there is a small difference observed. In general, the 1:1 POPC/CHL model shows a 1-2% less decrease in bilayer thickness corresponding to approximately a 1 Å less reduction in average bilayer thickness when compared to the 9:1 POPC/CHL model. This small difference is not enough to draw any conclusions about the different models and the effect increased cholesterol concentration has on mitigating this effect.

Instantaneous Average Deuterium Order Parameter of Upper and Lower Lipid Monolayers During Shockwave

One of the primary effects of shockwaves is the collapse and rebound of the lipid bilayer caused by a change in the membrane thickness. Membrane thickness is known to be highly reliant on the length of acyl chains contained in the bilayer. Related to acyl chain length is the deuterium order parameter (S_{CD}) of lipid tails. The chain length of lipids decreases if the disorder (S_{CD}) of the chain bend angles increases. Therefore this is a good quantity to measure during shockwave impact of the lipid bilayer models. Although the S_{CD} parameter is generally computed as a long-time average, in the case of these shockwave simulations, this should be bypassed. Instead, the averaged instantaneous deuterium order parameter S_{CDi} (equation 5.1) is computed separately at various time intervals for the upper and lower monolayers of the bilayer studied. The temporal evolution of the average S_{CDi} for each separate monolayer is presented for the 1:1 POPC/CHL model at shock impulses 7.5 mPa * s (figure 5.20) and 10.0 mPa * s (figure 5.21). and for the 9:1 POPC/CHL model during shock impulses of 7.5 mPa * s (figure 5.22) and 10 mPa * s (figure 5.23)

Figure 5.20: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 1:1 POPC/CHL model due to a shock impulse of 7.5 mPa * s.

Figure 5.21: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 1:1 POPC/CHL model due to a shock impulse of 10.0 mPa * s.

Figure 5.22: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 9:1 POPC/CHL model due to a shock impulse of 7.5 mPa * s.

Figure 5.23: Temporal Evolution of the instantaneous averaged deuterium order parameter of the upper and lower monolayers of the 1:1 POPC/CHL model due to a shock impulse of 10 mPa * s.

As can be seen in figures 5.20-5.23, the instantaneous averaged deuterium order parameter S_{CD_i} of the upper monolayer begins to decrease first as the shock interacts with this monolayer. This is followed by a slight lag period of roughly 250 femtoseconds in which is followed immediately by a decrease in the order parameter of the lower monolayers. Both values of upper and lower monolayer approach a minimum then begin to increase up to their original values. Once the S_{CD_i} of the lower

monolayer order parameter values begin to rise, this is the beginning of the rebound phase (roughly 500 femtoseconds) of the membrane mentioned earlier in analysis of change in membrane thickness due to shock impulse. This rebound time matches with the change in bilayer thickness plots (figures 5.14 and 5.15) which show a rebound of the membrane thickness at the same time values for these specific impulses (7.5,10 mPa *s) as to when the S_{CDi} parameter of the lower monolayers begin to decrease.

Overall, by calculating the temporal change of the instantaneous averaged order parameter S_{CDi} , the time at which the collapse and rebound phase of the lipid membrane occur can be identified. In addition, it can be said that the decrease and increase of the membrane thickness due to shock impulse was due to an initial shortening of the chain lengths (greater disorder) followed by an increase in chain lengths (decreased disorder) observed during the rebound phase.

Lateral Diffusion of Lipids During Shockwave

Generally, lipids undergo two types of movements in the lipid bilayer. The first type, tranverse diffusion, involved lipids flip-flopping from monolayer to monolayer and is a generally slow process. The second type of movement, the lateral diffusion, consists of lipids moving laterally (side to side) in the lipid monolayer they reside in. In contrast to transverse diffusion, the lateral diffusion of lipids occurs rapidly and can be measured in such short simulations. To measure the lateral fluidity, the 2-dimensional (xy plane) lateral mean square displacement of lipids (LSD) can be calculated (equation 4.5) and is presented for both the 1:1 POPC/CHL model (figure

5.24) and the 9:1 POPC/CHL (figure 5.25) models at the range of shockwave impulses tested.

Figure 5.24: The lateral displacement of lipids in the 1:1 POPC/CHL lipid bilayer at various shock impulses.

Figure 5.25: The lateral displacement of lipids in the 9:1 POPC/CHL lipid bilayer at various shock impulses.

As can be seen in the figures above, the lateral fluidity of lipids during shockwave impulse increases rapidly and is proportional to the strength of the impulse applied. On average, the 1:1 model shows a slightly higher lateral fluidity of lipids during shockwave impact at all shockwave impulses tested when compared to the 9:1 model.

Generally, the diffusion coefficient is a good measurement of the fluidity of the lipid bilayer. This value is usually derived from the lateral displacement values obtained over a long-time average. Since these shockwave simulations are in the picosecond time length, the calculation of the diffusion coefficient in these simulations would be irrelevant (Almedia & Vaz 1983).

Section 6 – Shock Slab Distance from Bilayer

The results presented above for both models consisted of applying an added velocity to water molecules in a water slab of thickness 5 Å placed adjacent to the lipid bilayer where the far right end of the slab was placed approximately 1 Å away. One variable not tested by the Koshiyama group and other researchers during in-silico shockwave simulations on lipid bilayers is whether the distance placed between the lipid bilayer and the shockwave water slab is significant. In reality, the implementation of shockwaves using the methods presented in this research rely heavily on the efficiency of momentum transfer between water molecules, specifically water molecules in the water slab and in close proximity to it. Therefore, a similar set of simulations was carried out where the water slab was placed 10 Å away from the bilayer. The velocities applied at both distances for each respective model are very similar. The only variable is the distance of the water slab from the lipid bilayer.

As with the systems tested above where water slabs were placed 1 Å away from the lipid bilayer, the evolution of bilayer thickness at varying shockwave impulses is plotted for the 1:1 POPC/CHL (figure 5.26) model and the 9:1 POPC/CHL (figure 5.27) model where the water slab was placed 10 Å from the lipid bilayer.

Figure 6.1: Change in bilayer thickness of 1:1 POPC/CHL model at varying shock impulses and 10 Å distance of water slab from bilayer.

Figure 6.2: Change in bilayer thickness of 9:1 POPC/CHL model at varying shock impulses with a 10 Å distance of water slab from bilayer.

As can be observed by comparing evolution of bilayer thickness at different water slab distances (figure 5.14 vs figure 5.26) for the 1:1 model and for the 9:1 model (figure 5.15 vs figure 5.26), there is a reduction of bilayer thickness observed in the models where the water slab is placed 10 Å away from the lipid bilayer as opposed to 1 Å. This increased effect on the membrane thickness size can be observed in both models.

To take a closer look, the change in bilayer thickness of the 1:1 POPC/CHL model at 5 mPa * s and 10 mPa * s at both distances of water slab is plotted (figures 5.28, 5.29)

Figure 6.3: The evolution of bilayer thickness of the 1:1 model at a 5 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).

Figure 6.4: The evolution of bilayer thickness of the 1:1 POPC/CHL bilayer at a 10 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).

The same analysis for the different water slab distances tested on the 9:1 POPC/CHL system were done and are depicted in figures 5.30 (5 mPa * s) and 5.31 (10 mPa * s).

Figure 6.5: The evolution of bilayer thickness of the 9:1 POPC/CHL bilayer at a 5 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10Å).

Figure 6.6: The evolution of bilayer thickness of the 9:1 POPC/CHL bilayer at a 10 mPa * s shock impulse compared for different water slab distances tested (1 Å vs 10 Å).

There are two trends observed in both models when comparing the two different water slab distances tested. First, unexpectedly, there is a greater reduction in average membrane thickness in the systems where the water slab was placed 10 Å away from the lipid bilayer. Expectedly, the maximum decrease in bilayer thickness occurs later in time than the systems where the shock slab is placed 1 Å away. This is expected because a shock placed further away from the bilayer will take longer to reach it.

In an effort to clarify why this increased bilayer thickness reduction is seen in the 10 Å distance simulations, the rise and dissipation of kinetic energy is compared between both systems. The rise in kinetic energy is compared for the 1:1 model at 7.5 and 10 mPa * s impulses and different slab distances (figures 6.7, 6.8). The same comparison was carried out for the 9:1 POPC/CHL model at different water slab distances (figures 6.9, 6.10).

Figure 6.7: Rise in kinetic energy of the 1:1 POPC/CHL system due to a 7.5 mPa shock impulse applied at different water slab distances (1 Å vs 10 Å)

Figure 6.8: Rise in kinetic energy of the 1:1 POPC/CHL system due to a 10 mPa shock impulse applied at varying water slab distances (1 Å vs 10 Å).

Figure 6.9: Rise in kinetic energy of the 9:1 POPC/CHL system due to a 7.5 mPa shock impulse applied at different water slab distances (1 Å vs 10 Å).

Figure 6.10: Rise in kinetic energy of the 9:1 POPC/CHL system due to a 10.0 mPa shock impulse applied at different water slab distances (1 Å vs 10 Å).

As the kinetic energy plots comparing distance of water slab show, at a 10 Å distance, the kinetic energy of the system is slightly higher than at a 1 Å distance in both models at both shock impulses compared. This is representative of an increased momentum transfer efficiency that occurs when there is a greater distance between the lipid bilayer and the water slab that contains water molecules with the added average velocities.

Rise in Pressure Due to Shockwave Compared

As noted earlier, the implementation of a shockwave using the methods described, leads to a rise in the pressure of the system. Therefore, a comparison of pressures in both the 1:1 POPC/CHL and 9:1 POPC/CHL models at different shock slab distances is carried out. Figures 5.36-5.39 compare the rise in pressure due to shockwave in both models at 1 Å and 10 Å shock slab distance at shock impulses of 7.5 and 10 mPa * s.

Figure 6.11: Rise in pressure of the 1:1 POPC/CHL system due to a 7.5 mPa shock impulse applied at different shock slab distances (1 Å vs 10 Å).

Figure 6.12: Rise in pressure of the 1:1 POPC/CHL system due to a $10 \text{ mPa} \cdot \text{s}$ shock impulse applied at different shock slab distances ($1 \text{ \AA}$ vs $10 \text{ \AA}$).

Figure 6.13: Rise in pressure of the 9:1 POPC/CHL system due to a $7.5 \text{ mPa} \cdot \text{s}$ shock impulse applied at different shock slab distances ($1 \text{ \AA}$ vs $10 \text{ \AA}$).

Figure 6.14: Rise in pressure of the 9:1 POPC/CHL system due to a 10 mPa * s shock impulse applied at different shock slab distances (1 Å vs 10 Å).

In both models, at the 10 Å distance of shock slab from the lipid bilayer, the system pressure is higher throughout simulations at both shock impulses plotted, as can be seen in figures 6.

Therefore, the placement of the shock slab has an impact on the momentum transfer efficiency of water molecules in the slab as well as in between the slab and the lipid bilayer. It can be seen in the results above that, applying similar shock impulses, but increasing the distance between the shock slab and the lipid bilayer has implications on system properties such as the kinetic energy and pressure as well as structural properties of the lipid bilayer such as the change in bilayer thickness.

Regardless, the goal of application of shockwaves to cells and more specifically the lipid bilayer relies heavily on alteration of the lipid membrane structure. By conducting this comparison of the distance of the shock slab relative to the lipid bilayer, this gives more insight to researchers on how distance of shock from target, in this case the lipid bilayer, effects the strength of the shock impulse applied.

Section 7 – Conclusions and Future Work

The goal of this research was to first analyze the effects of shockwaves of varying impulses on the structural effects on in-silico lipid bilayer models containing varying amounts of cholesterol. By providing this analysis, more realistic results can be provided in comparison to prior membrane models studied which contained no cholesterol. In the first part of this thesis, an equilibrium analysis was done on both models post-equilibration to show exactly just how different the structural properties of models with different amounts of cholesterol can be. Although this analysis has been performed previously on membrane models containing POPC + cholesterol, this was merely a verification of the two models. As shown previously, the model with 50% cholesterol displayed a higher average membrane thickness than the 10% cholesterol model. In addition, the 50% cholesterol model showed a reduced average area per lipid, and average area per POPC while showing an increased average area per cholesterol. Finally, the averaged deuterium order parameter of individual carbons in lipid tails was higher for all carbons in the 50% model, specifically carbons closer to the head group of the lipid. This is expected since POPC lipids in membranes with a higher range of cholesterol added demonstrate a more ordered structure compared to membranes with less or no cholesterol added.

Upon applying a range of shock impulses (2.5 -10 mPa * s) to the respective bilayer models, the average bilayer thickness change was measured for both models. In addition, the percent decrease in bilayer thickness was calculated to allow a comparison among both models tested. On average, the 1:1 POPC/CHL model showed a lower percent decrease (1-2 %) at all shock impulses than the 9:1

POPC/CHL model. This was not enough of a difference to make any conclusions of how the level of cholesterol in a membrane might mitigate the impact of shock. In addition to bilayer thickness change, the temporal change in the instantaneous averaged deuterium order parameter S_{CDi} due to shock impulse was calculated and plotted for the upper and lower monolayers separately. By doing this, it was shown that monitoring these values during these simulations can be indicative of when the collapse and rebound phase start and terminate. It was also shown that these values go hand in hand with the change in membrane thickness values and that the change in membrane thickness due to shockwave can be linked to the increase and decrease of the instantaneous order parameter of the upper and lower monolayers. Finally, the lateral displacement of both models at varying impulses was measured in order to give a representation of the fluidity of the lipids in these models during various shock impulses.

In addition to the effects of shockwaves, the distance of shock slab placement relative to the lipid bilayer was compared for a 10 Å distance and for a 1 Å distance. On average, at the same shock impulses, the 10 Å water slab system demonstrated greater membrane thickness decrease, a slight increase in kinetic energy of the systems tested, and a slight pressure increase. This can be attributed to the increased momentum transfer efficiency that occurs between water molecules in the slab and near it at the 10 Å distance when compared to the 1 Å. At the 1

In future work, the effect of shock impulses on cancerous-like lipid membranes will be probed. Cancerous cell membranes have a different lipid bilayer composition than mammalian cells. Ideally, by doing this type of research, this is directly applicable to

available cancer cell drug therapy methods that rely on permeabilizing the cancerous cell membrane in order to introduce therapeutic drugs into it.

APPENDIX

Appendix I – Matlab Code to Add Velocity to Water Molecules

```
function calculateaveragevelocitytoaddtowatermoleculesinshockslab
%%THIS MATLAB CODE IS USED TO CALCULATE AND ADD AN AVERAGE VELOCITY
TO THE
%%THERMAL VELOCITY OF WATER MOLECULES OF CHOSEN COORDINATES IN WATER
BOX
%%RALPH KFOURY
%%UNIVERSITY OF RI,CHEMICAL ENGINEERING DEPARTMENT
%% Namd units    length (angstroms) mass (dalton) pressure (bar)
%% System Size {-45.29999923706055 -42.43899917602539 -
112.5739974975586} {43.66400146484375 41.183998107910156
279.9989929199219}
%% Bilayer Size {-45.29999923706055 -42.43899917602539 -
68.39299774169922} {43.66400146484375 41.183998107910156 -
2.7690000534057617}
%% Water Shock Slab Dimensions {-38.13199996948242 -
36.547000885009766 -74.3949966430664} {36.42100143432617
35.71900177001953 -69.4010009765625}
%% Water Atoms in 5 ang shock slab    = 2662
%% Water molecules in 5 ang shock slab = 887

for I=2.5:2.5:10

    Ip = I/1000                                %Convert impulse from mPa to Pa
    Xi= (38.13199996948242+36.42100143432617)/10    %total length of
    water slab in x dimension (Angstrom)
    Yi= (36.547000885009766+35.71900177001953)/10    %total length of
    water slab in y dimensions (Angstrom)
    X= Xi/(10^9)                                %convert length of
    water slab in x direction (meters)
    Y= Yi/(10^9)                                %convert length of
    water slab in y direction (meters)
    A= (X*Y)                                    %calculate Area of
    water slab (m^2)
    Mk= 2.99*(10^-26)                            %Mass of Water
    Molecule in kilograms (kg)
    LipidBilayerminZ=-68.39299774169922            %Minimum Z coodinate
    of Lipid bilayer
    Gap=1                                         %Gap between bilayer
    and shock slab placement
    Nw= 887                                     %Number of water
    molecules in Pulse slab
    z = 5 ;                                     %Thickness of water
    slab (Angstroms)
    z_right= LipidBilayerminZ-Gap                %Z max of shock slab
    z_left = LipidBilayerminZ-Gap-z              %Z min of shock slab
    AvgVsi = (Ip*A)/(Mk*Nw)                      %avg velocity added
    (pascal*second*meters)/kilograms (SI units) = m/s
    v_z_shock= AvgVsi* (10^-2)                  %avg velocity of
    shock in NAMD units (Ang/ps)
```



```

fi_coor =
'step10Final_equilibration50POPC50CHLLipidBilayer.restart.coor';
%Restart Coordinate File of Lipid Bilayer Equil
fi_vel =
'step10Final_equilibration50POPC50CHLLipidBilayer.restart.vel';
%Restart Velocity File of Lipid Bilayer Equil

fid_coor = fopen(fi_coor, 'r');

fid_vel = fopen(fi_vel, 'r');

fid_V_added=fopen(['LipidBilayerVelocityAdded' num2str(I) '.txt'],
'w+');
fprintf(fid_V_added,'Atom   Vstart       V Final           ZCoord\n');

fid_new=fopen(['LipidBilayer50_50CHLShockwave' num2str(I) 'Impulse'
num2str(v_z_shock) 'Vel' num2str(z)
'AngstromPulseLength.restart.vel'], 'w+');
chartemp=fgetl(fid_coor);

xyz = textscan(fid_coor,'%s %d %s %10c %f %f %f %f %f %s %s ') ;

chartemp=fgetl(fid_vel);

vel = textscan(fid_vel,' %s %d %s %9c %f %f %f %f %f %s %s ')

a = xyz{2};
z = xyz{7};           % z coordinate colum of coordinate file
v = vel{7};           % z vel component of velocity file
w = xyz{9} ;          % beta column of coord file set to 1 for water
atoms to ensure vel only added to water atoms not ions

for i=1:length(z)      ;
    if z(i)> z_left & z(i)< z_right & w(i)>0;
        fprintf(fid_V_added,'%1d %2.4f', a(i),v(i));
        v(i) = v(i)+v_z_shock ;
        fprintf(fid_V_added,'           %2.4f           %2.4f\n',v(i),z(i));
    end
end

vel{7} = v ;

vel{4}= cellstr(vel{4}) ;

nrows = length(v);
formatSpec = '%4s %6d %4s %9s %11.3f %7.3f %7.3f %4.2f %2.2f %9s
%1s\n';
fprintf(fid_new,'REMARK RESTART VELOCITIES WRITTEN BY NAMD AT
TIMESTEP 10000\n')
for row = 1:nrows

fprintf(fid_new,formatSpec,vel{1}{row,1},vel{2}{row,1},vel{3}{row,1},

```

```

vel{4}{row,1},vel{5}(row,1),vel{6}(row,1),vel{7}(row,1),vel{8}(row,1)
,vel{9}(row,1),vel{10}{row,1},vel{11}{row,1});
end
fprintf(fid_new,'END');

end
fclose(fid_coor);
fclose(fid_vel);
fclose(fid_new);

```

Appendix II – Sample Minimization and Equilibration File for 50:50 POPC/CHL System

```

structure          50POPC50CHLLipidBilayer_Solvated_Ionized.psf
# Structure file of lipid bilayer

coordinates        50POPC50CHLLipidBilayer_Solvated_Ionized.pdb
# Coordinate file of lipid bilayer

set temp           310.0;
# Desired temperature of system

temperature        $temp;

outputName         LipidBilayer5050POPCCHLMinandEquilibration;
#Output name of files produced

firsttimestep      0;    # First time step of simulation

restartfreq        500;  # restart file every 500 steps = 1ps

dcdfreq           500;  # dcd file every 500 steps = 1ps
dcdUnitCell       yes;

xstFreq           500;  # xst file every 500 steps = 1ps

outputEnergies     50;  # Number timesteps between each energy
output of NAMD

outputTiming       500;  # Number Timesteps between each timing
output shows

# Force-Field Parameters
paraTypeCharmm     on;  #USING CHARMM PARAMATER FILE (YES)
parameters         toppar/par_all36_lipid.prm
parameters         toppar/toppar_all36_lipid_cholesterol.str
# These are specified by CHARMM
exclude           scaled1-4

```

```

1-4scaling factor 1.0
1-4scaling          1.0
switching           on      #Use switching function (yes)
cutoff              12.0; #Cutoff distance for nonbonded
switchdist          10.0; #Switch function distance
pairlistdist        16.0; #Neighbor list distance
stepspercycle       20;
pairlistsPerCycle   2

# Integrator Parameters
timestep            2.0;          # time step used fs/step

rigidBonds           all;    #H bonds constrained to fixed length

nonbondedFreq        1; #nonbonded forces calculated every step

fullElectFrequency   1; #PME calculated every step

#Periodic boundary conditions box

cellBasisVector1     99.0 0.0  0.0;  # vector to the next image
cellBasisVector2     0.0 96.0 0.0;
cellBasisVector3     0.0  0.0 277  ;

cellOrigin            -1.0271649360656738 -0.5152723789215088
83.73059844970703

wrapWater            on;          # wrap water to central cell
wrapAll              on;          # wrap other molecules too

# PME (for full-system periodic electrostatics)
PME                  yes;
PMEInterpOrder       6;  # interpolation order (spline order 6 in
charmm)

PMEGridSizeX         99;          # should be close to the cell size

PMEGridSizeY         96;          # corresponds to the charmm input
fftx/y/z

PMEGridSizeZ         277;

# Constant Pressure Control (variable volume)

```

```

useGroupPressure      yes;          # use a hydrogen-group
based pseudo-molecular viral to calculate pressure and
has less fluctuation, is needed for rigid bonds
(rigidBonds/SHAKE)

useFlexibleCell        yes;          # yes for anisotropic system
like membrane

useConstantRatio       yes;          # keeps the ratio of the unit
cell in the x-y plane constant A=B

langevinPiston          on;          # Nose-Hoover Langevin piston
pressure control

langevinPistonTarget    1.01325;    # target pressure in bar 1atm =
1.01325bar

langevinPistonPeriod    50.0;        # oscillation period in
fs. correspond to pgamma T=50fs=0.05ps
                                     # f=1/T=20.0 (pgamma)
langevinPistonDecay     25.0;        # oscillation decay
time. smaller value corresponds to larger random
                                     # forces and increased
coupling to the Langevin temp bath.
                                     # Equal or smaller than
piston period
langevinPistonTemp      $temp;        # coupled to heat bath

# Constant Temperature Control
langevin                on;          # langevin dynamics
langevinDamping          1.0;        # damping coefficient of
1/ps (keep low)
langevinTemp             $temp;      # random noise at this
level
langevinHydrogen         off;        # don't couple bath to
hydrogens

minimize 10,000;        # minimize system 10,000
steps

run      10000000;      #Run equilibration (20ns)

```

APPENDIX III – SAMPLE SHOCKWAVE SIMULATION CONFIGURATION FILE

```

#####
## JOB DESCRIPTION                                     ##
#####
SHOCKWAVE SIMULATION FILE 1:1 POPC/CHL Lipid Bilayer

#####
## ADJUSTABLE PARAMETERS                               ##
#####

structure          50POPC50CHLLipidBilayer_Solvated_Ionized.psf

outputName
LipidBilayer50_50CHLShockwave2dot5mpa_PS_5AngPulseLength_1nm

coordinates
step10Final_equilibration50POPC50CHLLipidBilayer.restart.coor

velocities          LipidBilayer50_
50CHLShockwave2.5Impulse50.786Vel5AngstromPulseLength.restart.v
el

extendedSystem
step10Final_equilibration50POPC50CHLLipidBilayer.restart.xsc

#####
## SIMULATION PARAMETERS                               ##
#####

# Input
paraTypeCharmm      on

parameters           toppar/par_all36_lipid.prm
parameters           toppar/toppar_all36_lipid_cholesterol.str

# Force-Field Parameters
exclude              scaled1-4
1-4scaling           1.0
cutoff               12.
switching            on
switchdist           10.0
pairlistdist         16.0
timestep             0.5;                # fs/step
rigidBonds           all;                # Bound constraint all
bonds involving H are fixed in length
nonbondedFreq        1;                  # nonbonded forces every
step
fullElectFrequency   1;                  # PME every step

```

```

wrapWater          on
wrapAll            yes

#PME (for full-system periodic electrostatics)
if {1} {
PME                yes
PMEGridSpacing     1.0
}

binaryoutput off
binaryrestart no
restartfreq        200      ;# 200steps=100fs
dcdfreq           200
xstFreq           200
outputEnergies    200
outputPressure    200

#####
## EXECUTION SCRIPT                                ##
#####

run 14000

```

Section 8 – Bibliography

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