The Effect of Cholesterol on the Stability of a DPPC-Cholesterol Bilayer

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ABSTRACT OF THESIS

Effect of Cholesterol on the stability of a

DPPC-Cholesterol Bilayer

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Cholesterol plays a very important part in regulating the properties of mixed phospholipid bilayers. In a human cell, about 50% is covered in cholesterol, and is becoming increasingly important to understand the interactions between cholesterol and the lipid in maintaining properties of the membrane, and in particular its stability more clearly. In an age of increased computer dependence, simulation models based on basic interaction parameters could prove pivotal in advancements in fields such as nano-medicine or monitoring the effect of viruses on cells. In this thesis, a mesoscopic model system of Dipalmitoylphosphatidylcholine (DPPC)-Cholesterol and water, is used to mimic a typical biological membrane. The equilibrium conformation of a membrane requires a tensionless state of the membrane. Depending on the tension, a membrane could either stretch or fold (compression). Simulations using Dissipative Particle Dynamics were used and the concentration of cholesterol was varied and the resulting tension was observed.

Acknowledgments

Over the course of the past 2 years I have met many wonderful individuals who have helped shape me into who I am today, they have provided unparalleled support and offered a lot of encouragement to me and always kept an excellent working environment, I would like to thank them for everything.

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Chapter 1

Introduction

1.1 The Cell Membrane

The term cell originated from the observations of Robert Hooke in 1665, he cut thin slices of a cork plant and observed them under a microscope and noticed that these slices were actually composed of tiny little compartments that were reminiscent of jail cells and hence the name. As time elapsed a lot more experiments were done on various structures to see if they too, like the cork plant had hidden structures; Leaves had blocks of cells with green particles, human blood was simply clusters of tiny round cells and even peoples teeth were themselves single cells[1].

That leads us to the important question, just what are cells? Cells make up all of living matter whether it is yourself or another living organism. At this level, a host of metabolic reactions occur that help keep the particular organism alive, thus the cell is also known as the fundamental unit of life. Even though cells come in a variety of shapes and small variations in size there is no relationship between the size of the organism and the cell size[2]; the larger it is, the more the number it has.

A cell can be thought of as a house, or even a factory. It is surrounded by walls and there are a host of activities that occur on the inside. There is a semi liquid that is stored inside these cell walls known as cytosol within which several organelles are located [3]. The major constituents are the nucleus, the mitochondria, the Golgi complex, endoplasmic

reticulum and of course, the cell membrane (wall). Other less common constituents that are found in specific types of cells are chloroplast, ribosomes, vacuoles and peroxisomes to names a few. Even though there are so many entities present within the cell, it is the smallest part of the organism that retains the major characteristics of the living organism[4].

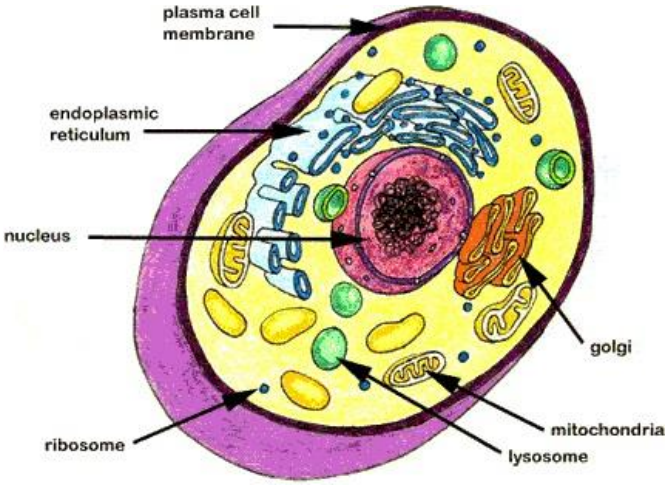


Figure 1.1. Cell membrane and components, image courtesy: www.biologycorner.com

The cell membrane is the most important organelle to any living organisms as it regulates the movement of substances into and out of the cell as well as providing structural support for the other entities within it. These membranes are composed of phospholipids organized in a regular dual layer (bilayer). As with the previous example of thinking of a cell like a house, it has selective gates that regulate the flow of different substances; this lets the cell sort of create its own personalized environment. These proteins usually span the entirety of the cell and can either be vertical (integral) or horizontal along the surface (peripheral). They can move along and even collide with each other, but cannot flip from one side of the membrane to the other, the proteins have both a hydrophobic and

hydrophilic part that facilitate with its movement along the membrane.

They can be thought of as an iceberg floating in the middle of an ocean with parts of it protruding outside the water and the rest safely “imbedded” inside.

Because they span about half of the membrane they also play a large part in the properties of the membrane as well as its weight. Although the proteins regulate movement, their presence alone is not enough to push substances in or out. With that in mind, there are 5 main methods through which this movement can occur, diffusion, osmosis, active transport, passive transport and through vesicles which undergo either endocytosis or exocytosis. The most common representation of a cell membrane is the fluid mosaic model which had been proposed by *Singer and Nicolson*[5].

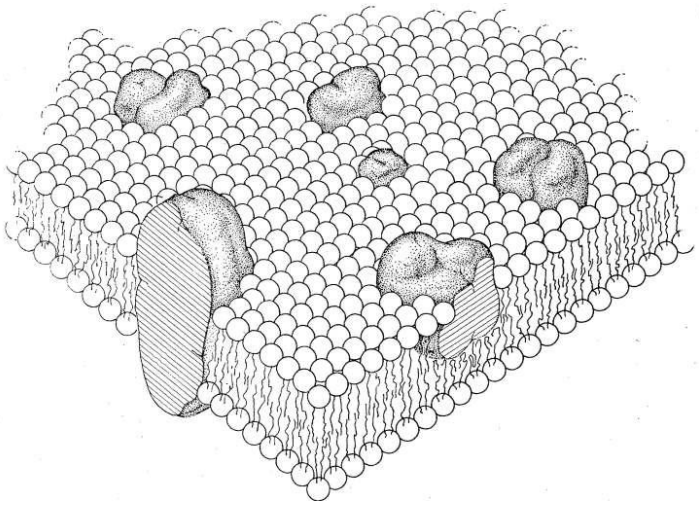


Figure 1.2 Fluid mosaic model proposed by Singer and Nicolson

1.2 Lipid Bilayer

As we have just discussed, the cell membrane is highly dependent on the lipid bilayer. It becomes increasingly important to discuss just what the lipid bilayer is all about, for that we need to start at the base and discuss lipids.

Lipids are what we refer to as amphiphilic molecules [6] which simply means that they are made up of 2 distinct parts; 1 polar-hydrophilic (water loving) head group and 1 hydrophobic (water repelling) tail group. With this unique characteristic in mind, the lipids in water tend to associate together with the hydrophobic sections coming together and being "encased" by the hydrophilic section in a process that is commonly referred to as self-assembly. Depending on the orientation and the specific structure of the lipids we can have different variations of self-assembly of which a bilayer is one of them[7]. Other formations which are possible are micelles which are heavily dependent on the size of either the head or the tail group, in the case of a larger head group they join together isolating the hydrophobic tail groups inside. The other case of the larger tail group forms something known as an inverse micelle where the hydrophobic tail beads point outwards and the hydrophilic head group are on the inside[8-9], this is highly unstable in polar solvents, however in non-polar solvents they are readily formed and act as a water store on the inside. The third and most important formation is the lipid bilayer[10]. In this case, there are generally 2 tail chains connected to a relatively large head group. These can be sort of looked at as a cylindrical molecule, this visualization becomes evident when they arrange in a regular pattern and place themselves in a parallel manner and form sheets. These sheets are functions of temperature, concentration and even pH.

The formation of a lipid bilayer is very fast and spontaneous, and is driven primarily by

hydrophobic interactions. Here the water molecules are pushed away from the tails and they get sequestered on the interior of the layer and with the aid of molecular interactions between successive tails they form a closely packed structure. The head beads also have a stabilizing factor in the form of hydrogen bonds[11].

Although there are several different types of lipids, we will only mention 3 in particular; triglycerides, steroids and phospholipids.

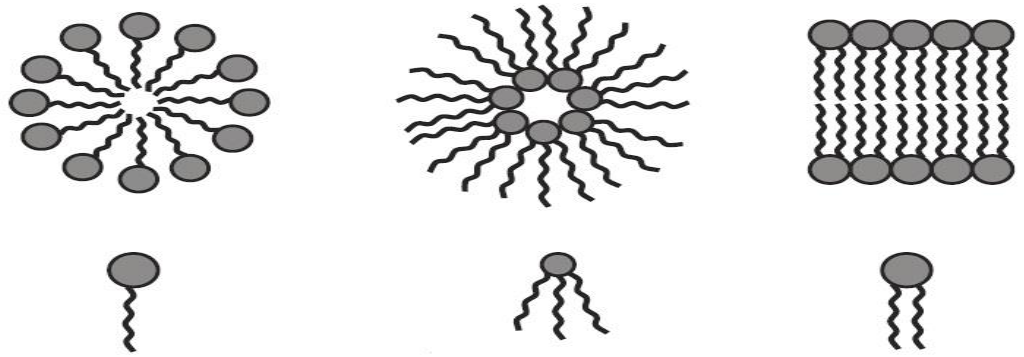


Figure 1.3 Different structures formed by lipids depending on their hydrophilic / hydrophobic balance, a micelle, inverse micelle and lipid bilayer respectively

The above image courtesy Venturoli et all [10].

Phospholipids will be the focus of our attention as they are the most abundantly found lipids in biological membranes. They composed of a glycerol molecule with a fatty acid chain (generally 2, thus is a diglyceride) and the other end is bonded to a phosphate group and a polar molecule (such as ethanolamine, serine or choline). The hydrophilic head group consists of the phosphate group and an alcohol. Different alcohols can lead to interesting variations in both structure and properties of the bilayer.

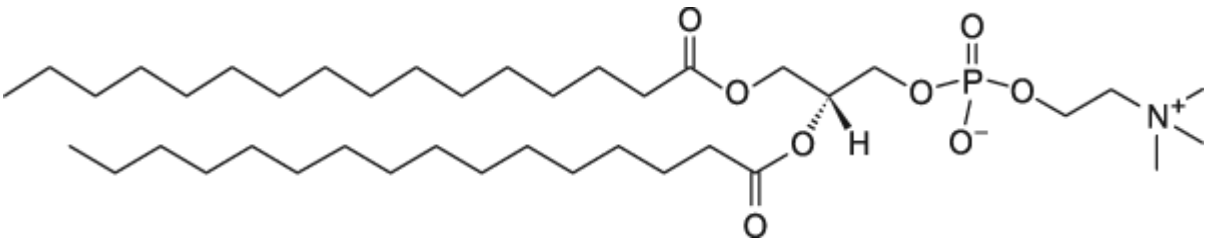


Figure 1.4 Structure of Dipalmitoylphosphatidylcholine (DPPC)

The above image is courtesy www.avantilipids.com

Another major constituent of biological membranes is sterols. Even though mammalian cells have a large number of phospholipids, they have only one major sterol [12], and that is cholesterol, which is absolutely necessary in human cells. Sterols in general vary from other lipids in the body by virtue of a steroid ring structure which is purely made up of hydrocarbons [13]. The average constitution of cholesterol in a mammalian cell is about 50%, that is, if you consider it molecule for molecule. Cholesterol molecules are a lot smaller and more rigid than their phospholipid counterparts, so if you consider the weight of the membrane which is cholesterol, it is approximately 20% [11]. Cholesterol's hydroxyl group interacts with the head groups prevalent on the membrane surface while the rest of the molecule sits nearly within the membrane, this presence also helps with lipid packing and thanks to the rigidity it provides reduced fluidity for the cell. At higher concentrations (closer to 50 %), cholesterol ensures that fatty chains do not crystallize and fuse together and become impermeable and lead to other complications [11].

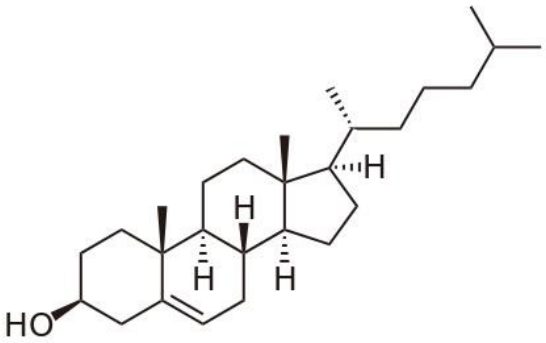


Figure 1.5 Structure of Cholesterol

1.3 Computer Simulations

Understanding the structure of a cell membrane has been an age old task with various methods used for analysis ranging from Raman spectroscopy and infra-red spectroscopy to electron microscopy and even X-rays [14]. Even though there are so many different methods in place, the overall understanding of the membrane is not completely known [15]. That is when it was thought to be beneficial to look at other alternatives to fully comprehend what is happening on the micro level. This led to the use of computer simulations in which atomistic models were created and their interactions of real compounds were mimicked so as to create a virtualization that could be used to better delve into the field.

The main methods used to do these simulations are Monte Carlo (MC), Molecular Dynamics (MD) and the most recent, Dissipative Particle Dynamics (DPD). These approaches can be broadly defined under the following headings; Deterministic or Stochastic [16].

Deterministic approaches rely on Newton's Laws of Motion to exactly pinpoint the

location of a particle at a given time. Both MD and DPD are deterministic in nature. Molecular Dynamics was first introduced by Adler and Wainwright in 1959 [17] and was designed to explain clearly and catch the smallest of interactions between particles and was done so using Newton's Equation of Motion on an atomistic scale. To elucidate this a little further, let us consider a box of fixed volume (say 20x20x20 units^3) and in this simulation box, we have 770 lipids and approximately 17000 water molecules which gives us 24000 atoms present in the system. For each of these atoms we will require intramolecular and intermolecular (Vander Waal forces, bonds, angles etc.)[14] for each time step and must be recalculated each time. These will take near astronomical times to compute, which is why MD is often restricted to a very small portion of the membrane (length scale) and the simulation is run for a very short amount of time; typically a few nanoseconds (time scale). Of course the recent innovation that led to parallel processing does eradicate this issue to a certain degree [18-19], but there are still phenomena that occur at much longer length and time scales which cannot be fully fathomed by an all atomistic approach and thus, which is why there was a need to develop newer models.

It is important to visualize what is meant by an "atom" in the previous example; it basically replaces a cluster of atoms with a bead [20].This is done under the presumption that the major aspects of the molecular structure can be conserved even though some of the atomic details are ignored. Each of these aforementioned spheres will be connected by a harmonic spring. The interactions of these particles are best described by a Leonard-Joule potential [21-23]. Dissipative Particle dynamics offers an alternative route for the same and was conceived to be more efficient than Molecular Dynamics.

Dissipative Particle Dynamics was first introduced by Hoogerbrugge and Koelman in the early 90's[24]. It has longer time and length scales which are of the order ~1000nm. DPD is sometimes known as a coarse grained version of MD[16]. The particles here interact through conservative potentials. Like Newton's laws, both momentum and mass are conserved after each collision, these collisions are considered to be a cluster of molecules that interact in a random manner (Brownian). A unique feature of this method is that between the particles there is a soft repulsive potential wherein the characteristics of both lipid and solution are described. The degree of separation is directly related to both the hydrophilicity and hydrophobicity of the lipid groups and water and their respective repulsive interaction parameters. Coarse graining further allows for larger time and length scales to be achieved and is of a few orders of magnitude better than that of MD[25].

Stochastic methods are based on probability distribution functions that are derived from a particles velocity and position. Monte Carlo falls under this category.

Monte Carlo uses stochastic methods to generate new configurations for each time step [26]. The underlying principal for running a MC simulation are, that from an initial starting configuration and position, a MC move is calculated that changes the overall configuration of the particles. This configuration can either be accepted or rejected based on an *acceptance criterion.* This makes sure they are sampled from a statistical mechanics ensemble and that the weight of the sample is also correct. For each step that occurs, a calculation of use is made and is repeated over a finite number of steps until a point of thermodynamic equilibrium is attained.

What sets MC aside from both MD and DPD is that it is not constrained by Newton's Equation of Motion, this allows for generation of multiple configurations and positions

all at once. Some of these moves may be non-trivial and can speed up the process 10 fold [27]. MC is also easily integrated with deterministic methods and together provides a very powerful simulation tool that can not only take large time and length scales, but can also drastically speed up the processing time and reduce load on processors.

On the flipside, because we do not solve Newton's equation of motion we cannot generate dynamical data from a simulation. If we consider a large system with a lot of particles it becomes increasingly difficult to simulate as there is a good probability that atoms will eventually overlap[26], especially simulations that contain an explicit solvent. The move will be rejected by the system. MC can be used in conjunction with MD or DPD to a higher efficiency [14].

1.4 Motivation

As we have described in the above sections of this chapter, it is very clear that the lipid bilayer plays a pivotal role in the successful functioning of the cell. The need to study them more in depth comes up and for a longer simulation time and length scale than previously achieved by Molecular Dynamics [27]. Dissipative Particle Dynamics is a very promising method that tackles both the length and time scale issues [25]. While there has been quite a bit of work done on pure lipid bilayers, there is a need to investigate the properties and effect that cholesterol induces whilst being an intricate part of the membrane.

The aim of this thesis is multifaceted, it provides the ideal launching pad into mixed bilayer systems that nearly perfectly mimic biological membranes and furthermore how

this model itself can be used to tackle more pressing issues surrounding drug delivery and a broader understanding of our body. Finally, I hope that this project can be used to reduce dependence on clinical trials of new drugs in the initial phases of development to help root out extreme side-effect drugs quickly and effectively.

First of all, we need to investigate what model should be used, how much coarse graining is required, what are the interaction parameters for each of these "beads" going to be, how can we differentiate them, and most importantly is our model reliable and can it be used as more than just simulation based research.

Once the characterization is completed, we will look into the properties of this mixed membrane, a few of the pressing issues that will be tackled are as follows, is our membrane system in equilibrium, is it in a tensionless state which is very important for imitating the uptake of drug particles, what exactly does cholesterol do in the bilayer; how does it affect the area per molecule of the system, and does that affect its stability when added to a single component or purely lipid bilayer system.

This thesis is broken down into the following components:

First we will delve deeper into the simulation technique which is used and how exactly it works, how we can modify these conditions to characterize a particular system and how they are stabilized.

The next step is to go into the specifics of our lipid system, what interactions are prevalent and how they were characterized. We must also run simulations that set the basis of our comparisons for the mixed membrane system.

After the lipid, we must address its counterpart, cholesterol. We will develop a pure sterol

membrane and identify under what conditions it is most likely to be stable. Although purely cholesterol bilayers are not likely to occur in nature, it provides a good starting point under which we can characterize our mixed membrane system. This also underlies the privileges of working with a computational model as opposed to purely experimental. Finally we build this model of cholesterol-lipid and tackle issues such as the effect of condensation on area per molecule, the tension state that the membrane finds itself in after equilibrating and whether or not this is similar to mammalian cells or not.

The last step is where do we go from here, what are the future applications of this work, and how will this benefit both the scientific community and potentially solve real world problems too.

Chapter 2

Computer Simulation

2.1 Introduction

There are many soft matter systems that are studied with the aid of computer simulations; a good chunk of these pertains to complex fluids. They often contain a liquid in which an object of interest is dissolved in. These could be colloids, polymers, gels, granular materials or even biological membranes [28].

The dynamics of complex systems is in general a function of its microscopic entities (structure). Now, because of this, a complex fluid cannot be fully comprehended under the use of Navier Stokes equations because of the vast differences in length and time scales [29]. That is to say, the observable phenomena are a direct cause of microscopic changes and items/objects that exist in the microscopic scale. Navier Stokes equation is heavily dependent on length and time, which poses a problem to solve using computer simulations wherein there is such a disparity between the macroscopic and microscopic scales[29] and will give rise to inaccuracies when studying the structure or effect that the microscopic entities. Both Molecular Dynamics and Monte Carlo have their shortcomings in simulating this, the former requiring overly long simulation runs to somewhat copy the macroscopic phenomena and the latter proving very difficult to calculate probable positions and structures for very large system as probability of being accepted is lower and there are dynamical issues [26],[16]. Keeping these in mind, it

becomes clear that a newer method must be devised. An intermediate length scale; the mesoscopic scale is used to simulate macroscopic phenomena while conserving the relative dynamics which are brought upon by the microscopic objects. This is achieved by coarse graining a system[20],[25]. Most of these systems lean towards symmetry or conservational laws as opposed to a mathematical backing. That is not to say they are incorrect mathematically, but conservation and symmetry are essential for observing phenomena.

Coarse graining is essentially a tool that helps connect to extreme length scales; the major variant for this is the degrees of freedom that a system has. Quite simply, the larger the number of degrees that need to be calculated, the larger the simulation time will be [30].
So it becomes important to coarse grain so that a system which has many degrees of freedom on a small scale can be successfully simulated on a larger scale with fewer degrees of freedom. Mesoscopic scales as previously mentioned are the intermediate between macroscopic and microscopic, and are very important to simulate complex fluids which are surrounded (essentially dissolved) in a solvent [31].
Despite the recent advances in computing, from developing new methods to parallel processing, there still lies a need to coarse grain subjects as it both reduces the cost of computing because of the reduced degrees of freedom and as a direct result of this, allows for much longer simulation times[32]. A coarse grained model is usually decided on multiple factors that include conserved quantities, symmetries and of course, simplicity. The created model is validated against experimental results of the pure phase [33-34].
Understanding the microscopic details are very important while making this jump.
In a nutshell, coarse graining means a reduction in degrees of freedom, a level of neglect

to atomistic details, an accelerated computation and sometimes, an over-simplification of a model.

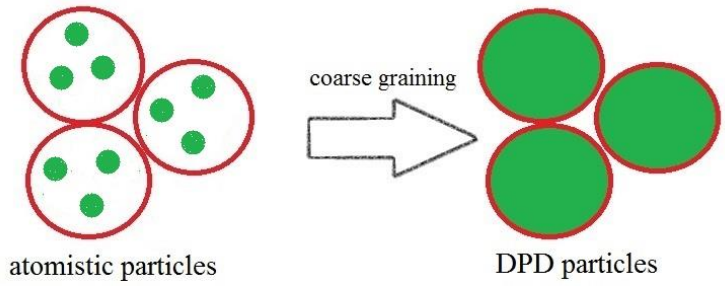


Figure 2.1 Coarse graining of 'n' particles to DPD beads

A lot of work has been done over the past few decades that cover the study of lipid bilayers using phenemological models. Biological membranes in general are not single component entities. Cholesterol is a vital part of the membrane and although there has been a bit of work done on it, there is still a vast array that is yet to be touched upon. Here we will introduce a mesoscopic model that will allow us to imitate a human cell using computer simulations. At present a majority of the simulations run using an all-atomistic approach, but they are too time consuming and thus an alternative method shall be used here. Dissipative Particle Dynamics as mentioned earlier helps accommodate for larger length and time scales.

With a human cell in mind, in the body a cell and by extension a cell membrane is not subject to any external forces and thus retains a tensionless state. Local deformities are not uncommon when interacting with foreign particles and objects such as drugs or viruses. It is very important that your system copies this and is also in equilibrium, this will be elucidated a little further in the proceeding chapters when discussing self-assembly of lipids. Lipowsky and his coworkers are largely responsible for the extreme

emphasis on simulating an area per lipid which gives us a tensionless membrane [35]. We will follow a similar methodology, wherein this area for tensionless membrane is determined iteratively.

2.2 Dissipative Particle Dynamics

DPD as mentioned earlier is a deterministic method and can accommodate for large length and time scales due to the reduced degrees of freedom as a result of Coarse Graining and is generally applicable to mesoscale systems. The pioneers in this field are Hoogerbrugge and Koelman,[24] they set the trend running by combining the best of both MD and Lattice Gas Automata (developed by Frisch et all) [36]. The method was sound, but lacked some theoretical framework required to enhance it [37]. This was duly provided by Española [38], his group provided stochastic differential equations and their equivalent Fokker-Planck equation [16] (this governs position and velocity of particles within a system) matches up nicely with Hoogerbrugge and Koelman [24]. As a result of this, it was shown that the hydrodynamic behavior of the system were consistent with those following Navier-Stokes equation. Thermodynamic equilibrium was achieved by Warren and Española when they coined a fluctuation-dissipation theorem for DPD [39].

There has been a plethora of work done utilizing DPD since its inception, since mesoscopic simulations help increase length and time scales it finds itself in many fields, some of the major work which have been done are on polymers [40-42], molecular self-assembly [43-45], colloidal suspensions [46-47] fluid mechanics [48] and even medicine [49]. *

Main Features: As we have discussed earlier, a DPD particle is formulated by taking a cluster of atoms and putting them together, by this definition, a singular DPD particle

would ideally represent the center of mass of this cluster [24],[29]. Successive particles interact via three main forces, all of which are pairwise additive.

$$\vec{F}_{i,tot} = \sum_{\substack{N \\ i \neq j}}^{1} \vec{F}_{ij}^{D} + \vec{F}_{ij}^{R} + \vec{F}_{ij}^{C}$$

Each set of particles are governed by the group of molecules it contains. Their simulations are those of soft spheres that are ruled by certain collision rules. To elaborate a little on these three forces; F^D is a dissipative force and controls the velocity differences between particles, this can be realized as a friction /drag factor on particles due to the surrounding fluid or another particle, F^R is a random force that is directed along the lines that connect the center of masses of 2 DPD spheres, it is similar to Brownian motion. They are both described as follows:

$$F_{ij}^{D} = -\gamma\omega^{D}(r_{ij})(\widehat{r_{ij}}.v_{ij})\widehat{r_{ij}}$$

$$F_{ij}^{R} = \sigma\omega^{R}(r_{ij})\zeta_{ij}.\widehat{r_{ij}}$$

Where r_{ij} and v_{ij} represent the difference in position between successive particles. $\widehat{r_{ij}}$ Is the unit vector, γ and σ are drag and noise coefficient respectively, ζ_{ij} is a random number drawn from a uniform distribution and is independent for each particle pair. These forces help maintain the hydrodynamics of the system and thus can be used for large length and time scales.

The dissipative force causes dissipation in the system, if a suitable amplitude fluctuation is added; there will be conservation of momentum. For this reason, there must be a good

balance between the weighted factors of both the random and dissipative forces. These have to be in accordance with Warren and Española' fluctuation-dissipation theorem [39].

$$[\omega^D] = [\omega^R]^2$$

$$\sigma^2 = 2\,\gamma k_B T$$

The weight function varies with r/r_c where r_c is the cut off radius that gives the extent of the interaction range.

$$\omega^R(r) = \begin{cases} 1 - \dfrac{r}{r_c} & , (r < r_c) \\ 0 & , (r \geq r_c) \end{cases}$$

Finally, F^c is a conservative force which represents the total potential stored within the particles. This force vanishes if the radius between 2 particles is greater than the cut off radius.

$$F_{ij}^C = \begin{cases} a_{ij}(1 - \dfrac{r_{ij}}{r_c}), (r_{ij} < r_c) \\ 0 \qquad\qquad , (r_{ij} \geq r_c) \end{cases}$$

Where we have a_{ij} as the repulsion strength parameter.

After these pairwise forces have been calculated, we need to attain the position and velocity of the particles; this is done by solving Newton's equations of motion. The forces are basically integrated over time to get the velocity, and similarly to attain the position, we integrate the velocities.

$$\frac{\partial v}{\partial t} = \frac{1}{m} f$$

$$\frac{\partial r}{\partial t} = v$$

Solving these equations requires a time integration scheme, there are many schemes that can be found in texts such as Euler's Scheme, Leapfrog Method, Verlet, Verlet Velocity and Predictor Calculator to name a few [50].We will adopt a variation of the Verlet Velocity Scheme which was introduced by Groot and Warren [29].

$$r_i(t + \Delta t) = r_i(t) + \Delta t . v_i(t) + \frac{1}{2} f_i(t) \Delta t^2$$

$$\tilde{v}_i(t + \Delta t) = v_i(t) + \lambda \Delta t f_i(t)$$

$$f_i(t + \Delta t) = f_i(r_i(t + \Delta t), \tilde{v}_i(t + \Delta t))$$

$$v_i(t + \Delta t) = v_i(t) + \frac{1}{2} \Delta t (f_i(t) + f_i(t + \Delta t))$$

If the value of λ is taken as 0.5, the above scheme reverts back to its original verlet velocity scheme. $\tilde{v}_i$ is the new prediction of velocity after a time step t.

Boundary conditions play an important role in determining the computational time required for a simulation to run, we will use periodic boundary conditions in this work. In periodic boundary conditions, the entire domain can be broken down into several sub divisions with each specific sub division having periodic boundary conditions on all sides which are adjoined to adjacent sub-domains. To better imagine this, we consider an office

with multiple cubicles that have people working, at a given time these employees can only move to an adjoining cubicle entering the adjacent cubicle from its opposite side. By this, the localized number of employees are changing, but as a whole is still constant (conserved).

Reflective boundary conditions are also used sometimes so that the motion of the particle can be controlled. This therefore allows very large simulations to be done.

2.3 Simulation Condition : Zero Tension

In most molecular simulations, there are a fixed number of molecules that occupy a box whose size remains fixed as well. There are exceptions sometimes where there might be replacement of solvent with particles depending on certain parameters [51]. Here we will apply periodic boundary conditions coupled with a fixed area and lipid number, and this should correspond to a large flat portion of a membrane. The lipids once in the system undergoes a process of self-assembly, in this particular case, the lipids are arranged into two separate monolayers and are static; wherein the local energies are reduced to a minimum (constant value) as the system reached equilibrium. The method by which they undergo re-arrangement depends on the interactions each particle has with its neighboring particle. This is of course assuming that there are no constraints on the system that could hinder both its energy minimization and achieving a tensionless state [52]. In earlier simulations using an all atomistic model (MD), *Feller* et all compared their results of a tensionless membrane at a particular area per lipid did not match the experimental values at the same value [53]. Their argument was that for such a small patch of the membrane, some features that are present on the macroscopic level could not be

converted or duplicated on such a scale, they resolved the issue by imposing a slight stretch to the membrane, this of course induced a positive tension on the patch, but it did match up to the experimental results. Stretching of a box will become more evident in the later chapters. After *Feller* et all completed their work, there was an increased interest in increasing the size of the simulation to see if the tensionless state could be achieved without the need of stretching the box [18], and a need to see if the membrane as simulated as a patch, could impersonate the membrane as a whole properly. This of course can be achieved by coarse graining the atomistic model and use a mesoscopic model instead. Another interesting development would be to see if coarse graining altered the results in any way, ideally, grouping atoms together of similar interaction / repulsion should not alter the overall scheme too much. There have been quite a few groups who have done work on DPD for biological membranes testing these facets of their work, notable mentions to *Lipowsky, Roland Faller, Berend Smit, Mohamed Laradji* and their respective co-workers to name a few. Recently, there has been work done on a larger membrane patch which contains in excess of 1500 lipids. The experiments were run by *Marrink and Mark* [18]; their results showed that for a stressed membrane, that if you increase the size of the system that the surface tension decreases. On the other hand, for a tensionless membrane, the area is independent of the system size and there would be no need to impose a stress to make it up to experimental values. The former corresponds well to *Feller's* conclusions.

We will use an iterative method to calculate the area per lipid that corresponds to a tensionless membrane. The tension of the membrane is directly proportional to the repulsion parameters and the size of the area occupied by each lipid. Simulations which

have been run at constant surface tension were first run by *Chiu* et all [54]. We will require sufficiently high number of calculations of velocity and position before assuming that the membrane has stabilized; so as to make tension calculations. We will be using an NVT ensemble (Number, Volume and Temperature all constant). Let us assume we have a rectangular 3-D box, with dimensions L_x, L_y and L_z , we have V = L_x. L_y. L_z and A = L_x. L_y, the number of particles depends on the size of the box, for all our simulations the box size will be 40x40x40 The density of our system is constant (ρ=3.0). For this area, and specific interaction parameters; to vary the area occupied per molecule. Once these parameters have been confirmed, the simulation is run for a sufficiently long time so as to allow it to equilibrate then tension measurements will be taken care of.

The surface tension is calculated by the following from work done by *S. Yang and R. Faller*[55]

$$\gamma(t) = \frac{L_y}{2}\left(P_y(t) + \frac{P_x(t) - P_z(t)}{2}\right)$$

Where P_i refers to the pressure along a certain direction (P_y is normal to the bilayer, while $P_{x/z}$ is along the bilayer itself) and L_y is the distance of the bilayer in the y direction.

2.4 Lipid Parameterization and Construction

Repulsion Parameter (a_{ij}): As opposed to labeling individual particles as in an all atomistic simulation technique, thanks to course graining it is possible to broadly define clusters into three distinct groups based on their hydrophobicity or hydrophilicity, i.e. head groups (h), tail groups (t) and of course the solvent water (w).

The values of these repulsion parameters is such that the simulated effect of compressed DPD water is close to those of experimental value [56]. It varies for different densities; we, as mentioned earlier are using a density of 3. Some sample values which are regularly used are shown below [29].The interaction parameters a_{ij} are decided keeping in mind that the user must perfectly mimic the interactions of a solvent and an amphiphilic molecule. Like interactions are usually smaller than un-like interactions.

a_{ij}	H	t	w
h	35	80	15
t	80	15	80
w	15	80	35

Table 2.1 Model Repulsion Parameters used by Groot and Warren

Lipid Construction: As previously mentioned, lipid beads are connected by a spring potential. They can be constructed in a plethora of ways, from single tail lipids to 2-tail lipids depending on what level of coarse graining and what lipid molecule is under consideration. Other than the number of tails, the length of these chains can vary and can either be like or unlike lengths (in case of multiple tails).

The spring potential is of the form:

$$U(i, i+1) = \frac{1}{2} k_1 (|\hat{r}_{i,i+1}| - l_0)^2$$

Where l_0 is our equilibrium bond length, which in our work is taken to be 0.5. The value of $k_1 = 64$.

The chain itself, is not rigid and does not move about with the same conformation, the ends are slightly flexible with the potential to bend. To account for this movement, a bond-bending potential is added.

$$U_2(i, i+1) = \frac{1}{2} k_2(|\phi_{i,i+1}| - \phi_0)^2$$

Where k_2 and ϕ are the bending constant and angle between 2 bonds respectively (ϕ_0 is equilibrium angle).

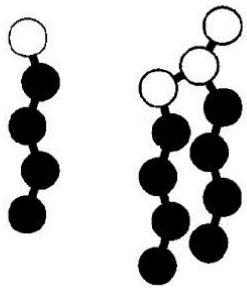


Figure 2.2 Single chain and double chain lipids as proposed by Goetz and Lipowsky [20]

Reduced Units: When working with different models, often calculations are made of physical properties to help give a better understanding of a system, key terms such as mass, density, length, time, etc. must all be calculated. It may become tedious working with an array of unites and conversions, so it is wiser to work with a system where all the quantities are reduced to a norm. This not only makes work easier to interpret, it can become applicable to all similar systems so there is no real need to remodel. As mentioned previously, this study works with in an NVT ensemble. By selecting certain predefined base units we can characterize the system and by certain manipulations derive

our dimensionless equivalent. The table below gives an analogous representation of certain units that are of use to us.

Length	Temp.	Mass	Density	Pressure	Time	Force	Energy
$L^* = \frac{L}{\lambda}$	$T^* = \frac{k_B T}{\varepsilon}$	$M^* = \frac{M}{\mu}$	$\rho^* = \lambda^3 \rho$	$P^* = \frac{\lambda^3 P}{\varepsilon}$	$t^* = t\sqrt{\frac{\varepsilon}{\mu\lambda^3}}$	$F^* = \frac{\lambda P}{\varepsilon}$	$E^* = \frac{E}{\varepsilon}$

Table 2.2 Conversion to dimensionless values where Ɛ, λ and μ are energy, length and mass respectively

Area per lipid: The membrane extends out in the x and y direction, the cross sectional area is $L_x.L_y$, The area per lipid is defined as

$$a_L = 2(\frac{L_x L_y}{N})$$

Where N is the number of lipids in the system, the number 2 is there because we have to incorporate both sides of the bilayer. This formula is important because it shows that the number of lipids is a value that primarily varies with the box size, this must be altered until a tensionless state is achieved, or for a given box size, vary the area per molecule. The bilayer thickness is calculated as the center to center distance of 2 opposite head groups.

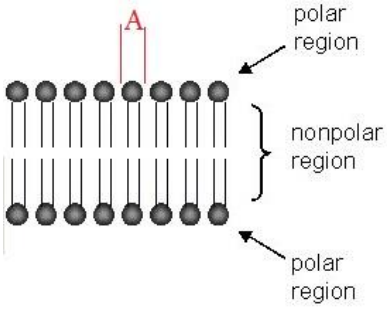


Figure 2.3 Area per lipid explained through fluid mosaic model

2.5 The Lipid Bilayer : Testing Scheme

The main aim is to get a random input of lipids to interact and assemble into a lipid membrane from which its properties and stability can be measured, the question we need to ask is how long will it take to occur? Typically, our simulations last in the region of 10^6 time steps with positional co-ordinates and other measurements taken at regular intervals of about every 2000 steps. Each time step will be around 0.02 and periodic boundary conditions will be applied. We will be utilizing "Large-scale Atomic/Molecular Massively Parallel Simulator" or LAMMPS, an initiative from Sandia National Laboratories. An ensemble of a liquid, solid or gas can be modeled with this. Although we are using this for molecular dynamics, it can be used for a variety of other works that pertain to polymers, granular structures and even metallic structures. These systems can handle simulations sizes of very high orders (billions). LAMMPS applies Newton's equations of motion to a collection of particles that either have short or long range force fields. A problem that could arise is that of particle clumping, which would lead to a localized density increase, this is taken care of by tracking the neighboring parties. These simulations can either be run on a single desktop processor or parallel processing across multiple processors. Some of the force field features of LAMMPS that we will use are as follows.

Bond style: This allows the user to set the formulae used by LAMMPS used to compute the interactions between specified atoms and keeps them in that context until the end of the simulation. Depending on the type of bonding prevalent, the bond coefficient can be altered. There are a lot of bond styles which are available namely hybrid, fene, morse and harmonic. Our simulations will use a harmonic potential.

Angle style: This dictates the angle created when 3 atoms are bonded together, in most molecular representations there are a variety of angles ranging from straight to complex shapes. The common types of angle style are none, hybrid, harmonic and cosine. Our simulations will utilize a cosine angle type.

Pair style: These are formulae that are calculated for atoms within the cut off radius distance. This because of the evolution of new positions per time step means that the interacting atoms within this distance are always changing. A few of the pair styles which are used are hybrid, colloid, Leonard-Joule and DPD. We of course, will be using DPD which covers pair-wise interactions and forces (dissipative, random and conservative).

Thermo style: Once all the bonds and angles have been sorted out for our simulation, we need to actually calculate values, this can be customized or can be read from a pre-defined set of values, they usually include any and all quantities that are required to make a calculation, for example from our surface tension calculation we need pressures along different axes, this will be included in the thermo section.

Dump: These files are used to "save a snapshot" of a cluster of atoms, it is stored every N time-steps and ends with the simulation. These in turn are used to produce images of the system which we are trying to simulate.

Chapter 3
DPPC Bilayer

3.1 Introduction

Lipids play a pivotal role in the functioning of biological membranes, they protect the components inside that are carrying out vital functions for the body[1]. Lipids are amphiphilic in nature, they have their hydrophilic head beads facing out and hydrophobic tail beads pointing inwards, away from water. In this chapter we will develop a lipid model based on DPPC, and we will initially monitor the stability of the single component bilayer. The ultimate goal of this project is to create a mixed membrane that is biologically accurate. In order to be able to monitor the tension of the membrane accurately, we need to a stable membrane of it. Our aim is to replicate that and see if we can create a stable bilayer for both lipid and cholesterol (in the next chapter) before mixing them.

3.2 Computational Details

In this segment, we will consider a two tail lipid, which corresponds to a DPPC molecule. The coarse grained model will contain a total of 9 beads, of which there will be three head beads and two tails with three beads each. A schematic representation is given below. The harmonic bond potential used here is $k_r = 64$ and equivalent bond length $r_0 = 0.5$. The bond bending potential energy shared between 3 connected beads is fixed at $K = 20$.

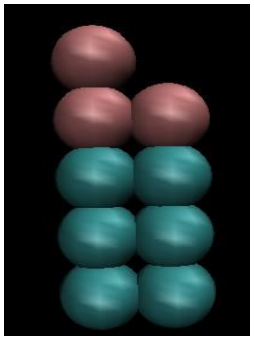


Figure 3.1 Structure of coarse grained DPPC molecule

As mentioned previously, we need to define interactions not only the beads have with each other, but also with the surrounding solvent (water). The table below gives the values of interaction parameters used in this work.

a_{ij}	h	t	w
h	25	100	15
t	100	25	100
w	15	100	25

Table 3.1 Interaction parameters of DPPC

We initially considered changing the head group interaction parameters, specifically the head-head interactions, they were varied from a_{hh} = 10 – a_{hh}= 25. These values reflect subtle changes in a system that could be observed in real life, for example, in a mammalian cell there might be certain salts present in and around the membrane that could affect these values. We will use values obtained by *Kranenburg* et all in our simulations with a slight change of a_{th}= a_{tw}=100.

Our objective finally is to see the effect cholesterol has on the stability of the bilayer and what effect the condensation caused by cholesterol due to its size and properties has on

the same [57] . This chapter will focus on being able to mimic a purely lipid bilayer of DPPC. All simulations are performed at a fixed temperature and volume. The total number of atoms present in the system will be conserved at 192000, this of course means that for different area per molecules' we will have a varying number of lipids and the solvent atom beads simply make up the remainder. The overall density will remain at $\rho=3$ [25]. The simulation run is performed by randomly putting lipids into the system and mirror it to form 2 layers that over a period of time will interact with each other and form a stable membrane. A typical simulation will last about 10^6 time steps, and data will be retrieved every 2 000 steps.

Although we expect the system to equilibrate by the stipulated time, we must ensure that there is an alternative so that the simulation need not be run again for its entirety, for this we create a restart file. Information at the n^{th} time step is stored, and a simulation after its completion can be restarted at that step (10^6 onwards) for as long as required.

There is a need to test our membranes for tension values after the bilayer has reached a state of equilibrium. This shows us that after a period of time and interactions, the membrane has reached an energy state that corresponds to its final form and minimum energy possible. If we consider a membrane with a positive tension, the membrane starts to stretch, this could lead to the formation of holes and could cause the membrane to rip or even break open altogether. On the other hand, having a membrane with a negative tension could cause the membrane to fold over and contort to different conformations. In our simulations, we noticed that for larger area per lipids, the simulations took longer to equilibrate and to maintain equal time steps and conditions, all simulations were run for approx. $1.3x10^6$ time steps (using a restart file). The lipids are inserted about the center of

the simulation box (+ direction) and a reflection on the negative direction of the axis 0-line. The initial position is important because it determines how long they will take to self-assemble, if too far apart they might not interact at all and if too close there are possibilities of overlapping lipids. The next section looks at the time evolution of a bilayer membrane. It covers from insertion to self-assembly. After the membrane is stable, we will use the tension equation discussed in the previous chapter and extract the pressures in each direction and calculate the membrane tension.

Previous work done by K. Smith, D. Jasnow and A. Balazs [58] (single lipid architecture) as well as G. Illya, R. Lipowsky and J. Shillcock [59] (for multiple lipid architectures) attained the following distributions,

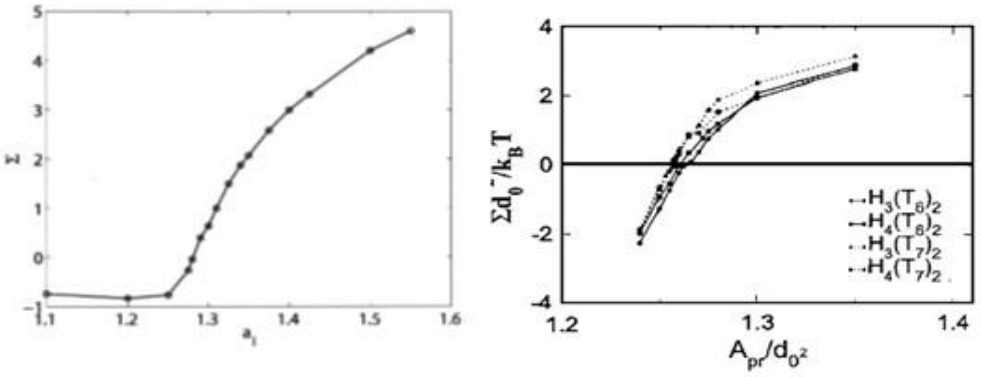


Figure 3.2 Results obtained by Smith et al and Illya et al respectively

The tension is calculated for each time step that information is collected, so for a typical simulation that runs 10^6 times and information is *dumped* every 2^3 steps we will have 500 positional data points. Each of these will have its own tension value, it is evident that the

value of tension is evolved over the time steps, and it is important to include all of these values as opposed to just taking the final tension value, these tension values vary as the membrane conformation varies. From all of these values, our objective is to find the lowest possible average of tension. This is achieved by *box averaging* [60]. In this technique, the tension values over 5 data points are averaged and re-averaged until a singular value remains. This helps reduces the local membrane free energy to a minimum. This technique is useful because it shows a marked reduction in error compared to single averaged tension value results while not changing the total number of data points collected over the simulation. A similar approach has been used in the recent past [60],[61].

3.3 Results and Discussion

Our simulations will run over an area per lipid range of 1.12 – 1.34, the table below shows the variation of tension with the area per lipid. In work presented here, the error bars have been left out because its value was $\geq 5\%$

Area per lipid	overall average Tension	numbered average Tension
1.12	-0.321	-0.300
1.13	-0.285	-0.279
1.14	-0.21	-0.215
1.15	-0.19	-0.190
1.16	-0.113	-0.113
1.17	-0.0897	-0.0925
1.18	-0.0313	-0.0546
1.19	0.0101	0.00961
1.2	0.1004	0.1000
1.21	0.265	0.244
1.22	0.372	0.368
1.23	0.566	0.563
1.24	0.843	0.824
1.25	1.052	1.038
1.26	1.278	1.264
1.27	1.432	1.400
1.28	1.51	1.517
1.29	1.634	1.626
1.3	1.745	1.732
1.31	1.844	1.833
1.32	1.9103	1.901
1.33	1.963	1.957
1.34	2.000	1.990

Table 3.2 Tension values for each area per lipid.

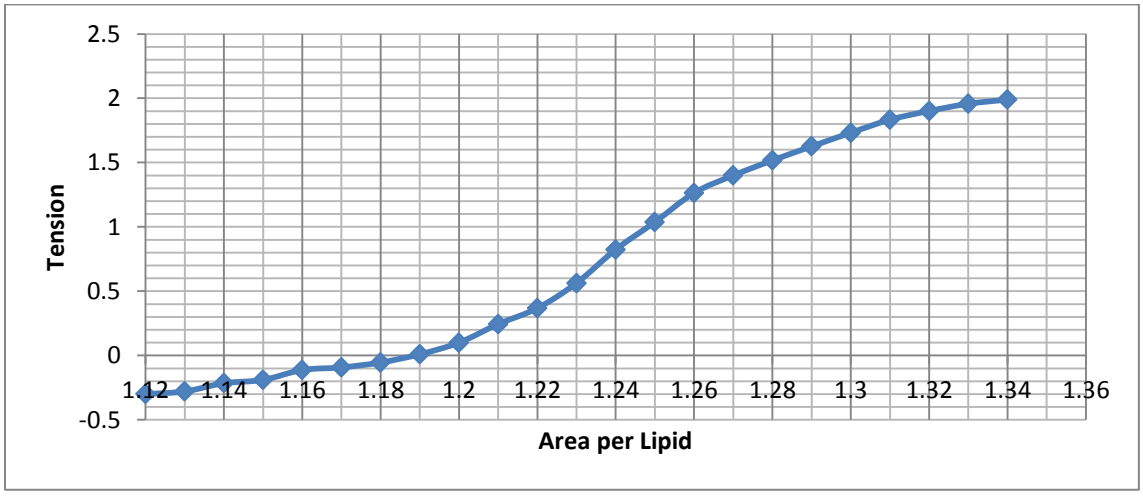


Figure 3.3 Graph of variation on Tension versus Area Per Lipid.

The first thing we had to check was whether or not a stable membrane was forming. Initially the lipids are placed a short distance from each other. As time passes, due to their respective interaction parameters the lipids start organizing themselves and mixing (per monolayer). While they re-orient themselves, the 2 layers come within contact and start forming a bilayer. It is important to note that once this bilayer has formed that lipids are not fixed to a certain position but can move about. This elucidates the need to continuously monitor the bond, angle and pair energies, while it is seemingly stable, it is not at its lowest energy conformation. This is measured by plotting the respective energies (bond, angle and pair energies) against time. They usually fluctuate with high amplitude initially and eventually wane off to a certain value and attain equilibrium. Once we can quantitatively confirm that our membrane has reached equilibrium we can start our tension calculations. We will utilize the box averaging method stated above and compare it with the overall singular average. As the area per molecule is varied, we have a changing number of lipids in our system (overall number of atoms is always constant), this means that for a fixed volume, as we increase the area per molecule, there is likely to be fewer lipids and as a direct consequence, its stability will vary. From our results we can see that an increase in this area causes the membrane tension to become more positive. That means that the membrane is transitioning from closely packed to a more loose conformation, this if increased too much will cause the membrane to stretch and eventually form holes in the membrane or break up altogether. As previously mentioned, we require a tensionless membrane which will neither bend nor stretch. By interpolating the graph of tension versus area per lipid we attained a tensionless state to be at approximately A_0=1.185.

The below diagrams reached equilibrium a little faster with fewer fluctuations because the simulation was reset every few thousand steps initially, meaning that the initial large fluctuations could be avoided and leave very minor fluctuations and eventually reach stability. The comparison between velocity reset and original energy values are shown, as we can see, they eventually equilibrate at the same time, just the initial spikes which are observed are churned out and those values because they are in the formation stage affect tension results which is undesirable.

The following information is from calculations at an area per lipid of 1.20 (overleaf).

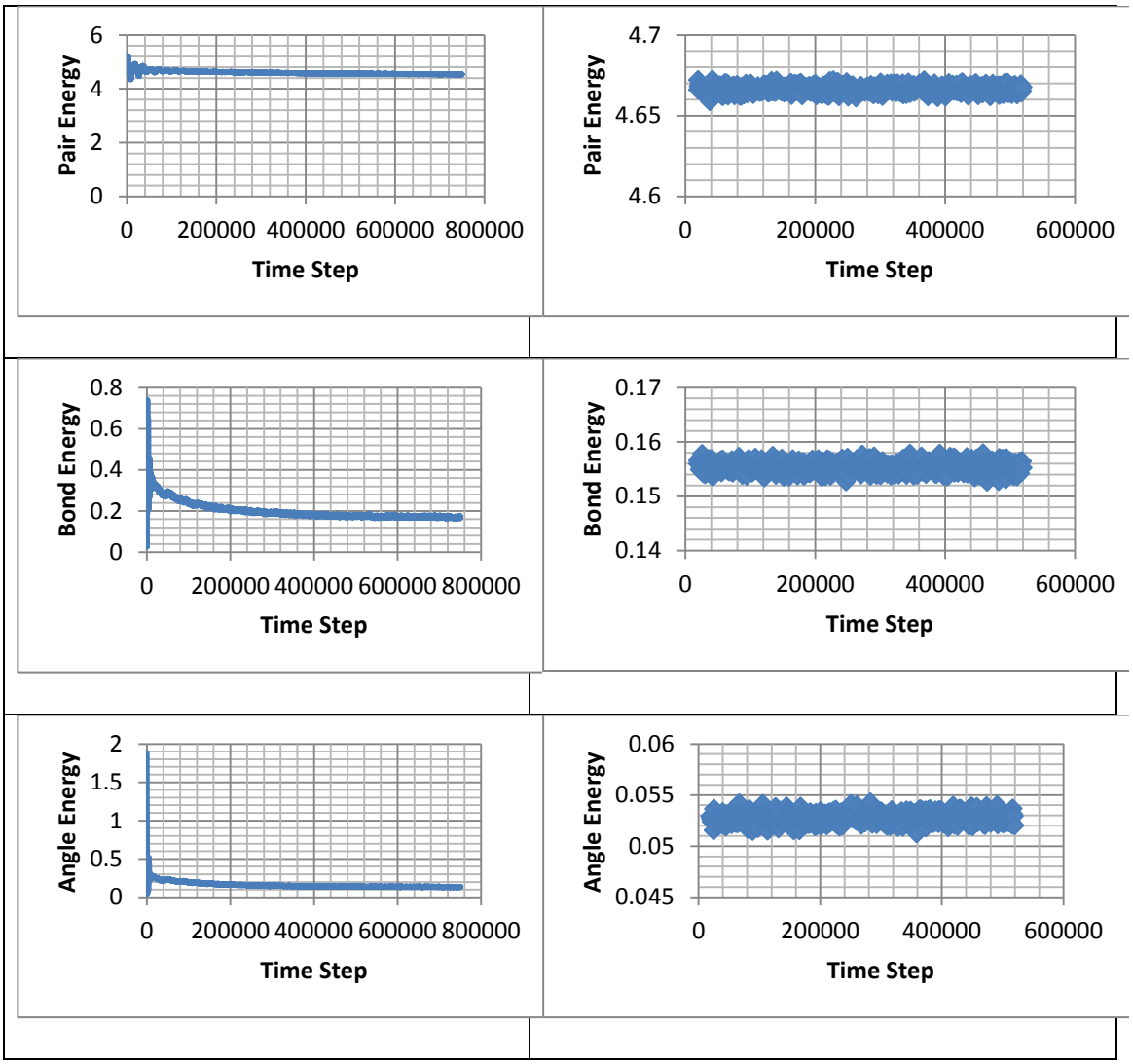


Figure 3.4 Energy evolution over time for regular simulation versus velocity reset values.

Finally, the images below are to illustrate the physical differences in the membrane when under different sets of tension, we can clearly see the folding and bending on the negative tension as well as the gaps formed with a highly positive tension. All images were taken at the same time-step.

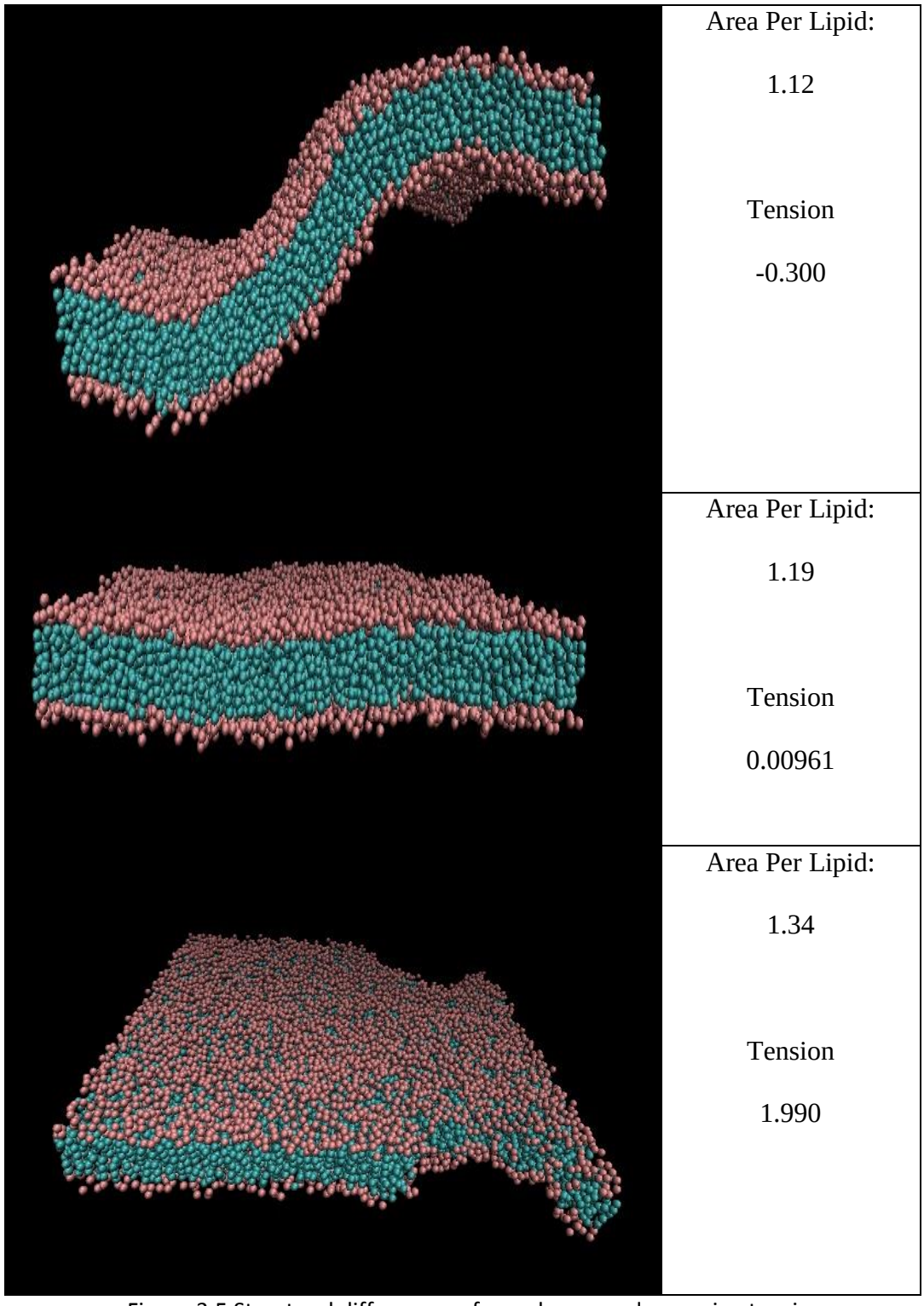


Figure 3.5 Structural differences of membrane under varying tension

Chapter 4

Cholesterol Bilayer

4.1 Introduction

In the previous chapter we discussed the stability of a purely lipid bilayer. In order to mimic a biological membrane, we need to add cholesterol as it is an intricate part of the cell and maintains several features of the cell including its stability and fluidity [62]. These are amphiphilic molecules as well with a ring structure and a loose tail [12]. Cholesterol bilayers in nature don't really exist, but in order to make our calculation of molecule sizes when dealing with a mixed membrane we use this computational model to help us attain a value that has no tension on it. We will design the cholesterol in the same manner as the lipid, and perform several calculations to check for its tensionless area per molecule value.

4.2 Computational Details

Here we will discuss the construction of the sterol molecule that we will use. This molecule corresponds to cholesterol and is visualized as a cyclic compound with a loose tail and connected to two head beads (slightly more hydrophobic cholesterol variation). This molecule will also have a total of 9 DPD-beads; same as that of the DPPC molecule. A schematic is given below. Along the same lines as the previous chapter, interaction parameters, angles and coefficients are also used. For simplicity, these have kept the same; harmonic bond potential $k_r = 64$ and equivalent bond length $r_0 = 0.5$. The bond bending potential energy shared between 3 connected beads is fixed at $K = 20$.

a_{ij}	h	t	w
h	25	100	25
t	100	25	100
w	25	100	25

Table 4.1 Interaction parameters of cholesterol and water.

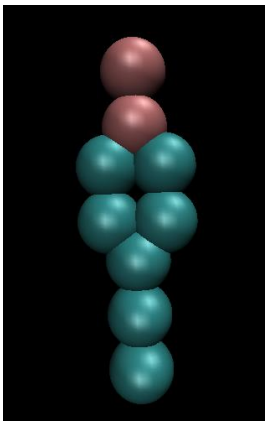


Figure 4.1 Structure of coarse grained Cholesterol molecule

The interactions between all 3 beads are given above

The only difference here is our head-water interactions which is slightly less hydrophilic than the lipid. While this difference is not predominant, it is used to illustrate the fact that the lipids and cholesterol are never perfectly aligned in the bilayer.

The cholesterol molecule when compared to the lipid is a lot smaller [57], this means that the range of area per molecules must be a lot smaller to maintain accuracy. This value is measured iteratively with sizes varying from just smaller than the lipid. For this set of experiments we will use sizes varying from A_0=1.00 to A_0 = 1.15.

This gives rise to a very tantalizing prospect once we consider the mixed membrane, with such a small area per molecule, there would be a large number cholesterol relative to lipid and subsequently, think about whether or not we can implement both tensionless states, and whether or not these states will correspond to a mixed membranes lowest energy.

It is essential that all the simulations are run under the exact same conditions; once again we will be using a box of 40x40x40 and fixed temperature. The total number of atoms is constant at 192,000 but will have a lot more of the cholesterol molecules owing to its small size. Simulations will be run for 10^6 time steps, with positional information collected over regular intervals.

Because it is highly unlikely that a cholesterol bilayer will ever be formed in nature, this particular set of tests show the beauty of a computational technique. The area per lipid which corresponds to a tensionless state of a mixed membrane could be thought of as the combined effect of the individual tensionless states. By simply assigning interaction parameters, we can "negotiate" a membrane with our system for cholesterol in the same manner as the lipid, and extract its tensionless state from a range of values.

4.3 Results and Discussion

Once again, the area per molecule that corresponds to the lowest energy balance for cholesterol molecule must be found. The same procedure described in chapter 3 for collecting and calculation of information from these runs will be followed. Because the area per lipid is very small compared to DPPC, there; for a system with constant number of atoms will include a lot more cholesterol atoms, and much fewer of the solvent

molecules. The packing of cholesterol for various area per lipids is key to describing the tension values attained. Similarly to before, the cholesterol molecules are placed within a short interacting region of each other, initially the orientation is that of a translation about the line x=0. The bilayer is formed almost instantly but there is still a lot of re-alignment and “fidgeting” which leads to the membrane carrying that unstable energy which must be dissipated before being termed stable. Once that has happened, tension values can be calculated. The distribution of tension across the different area per lipids is given below.

Area Per Molecule	overall average Tension	numbered average Tension
1	-0.356	-0.349
1.01	-0.323	-0.316
1.02	-0.361	-0.356
1.03	-0.288	-0.281
1.04	-0.121	-0.113
1.05	0.151	0.148
1.06	0.467	0.463
1.07	0.552	0.531
1.08	0.998	0.993
1.09	1.24	1.21
1.1	1.51	1.492
1.11	1.538	1.535
1.12	1.594	1.584
1.13	1.562	1.551
1.14	1.745	1.729
1.15	1.876	1.833

Table 4.2 Variation of Tension with Area Per Molecule

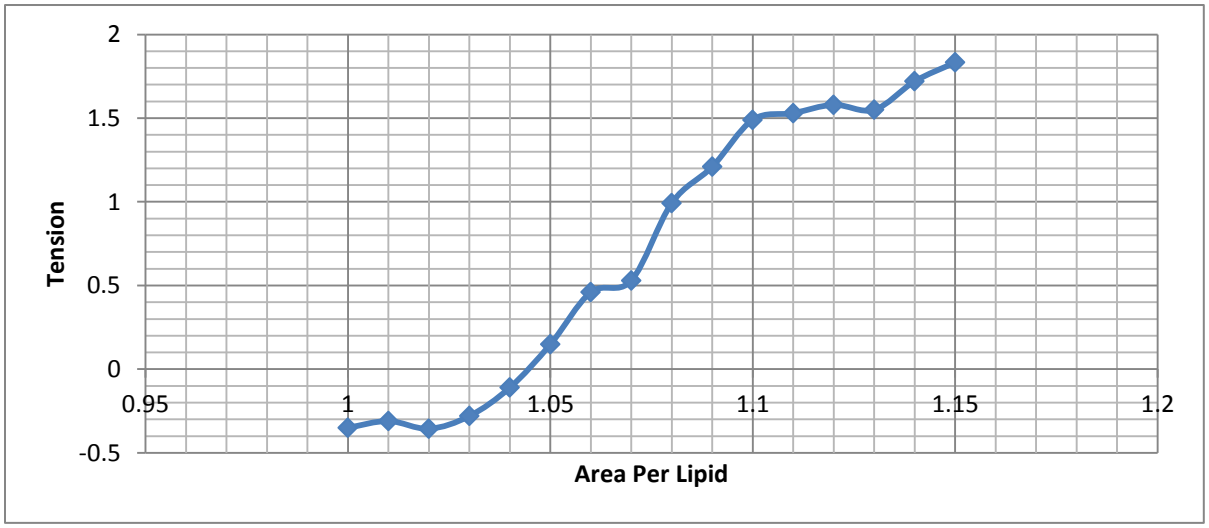


Figure 4.2 Variation of Tension with Area Per Molecule

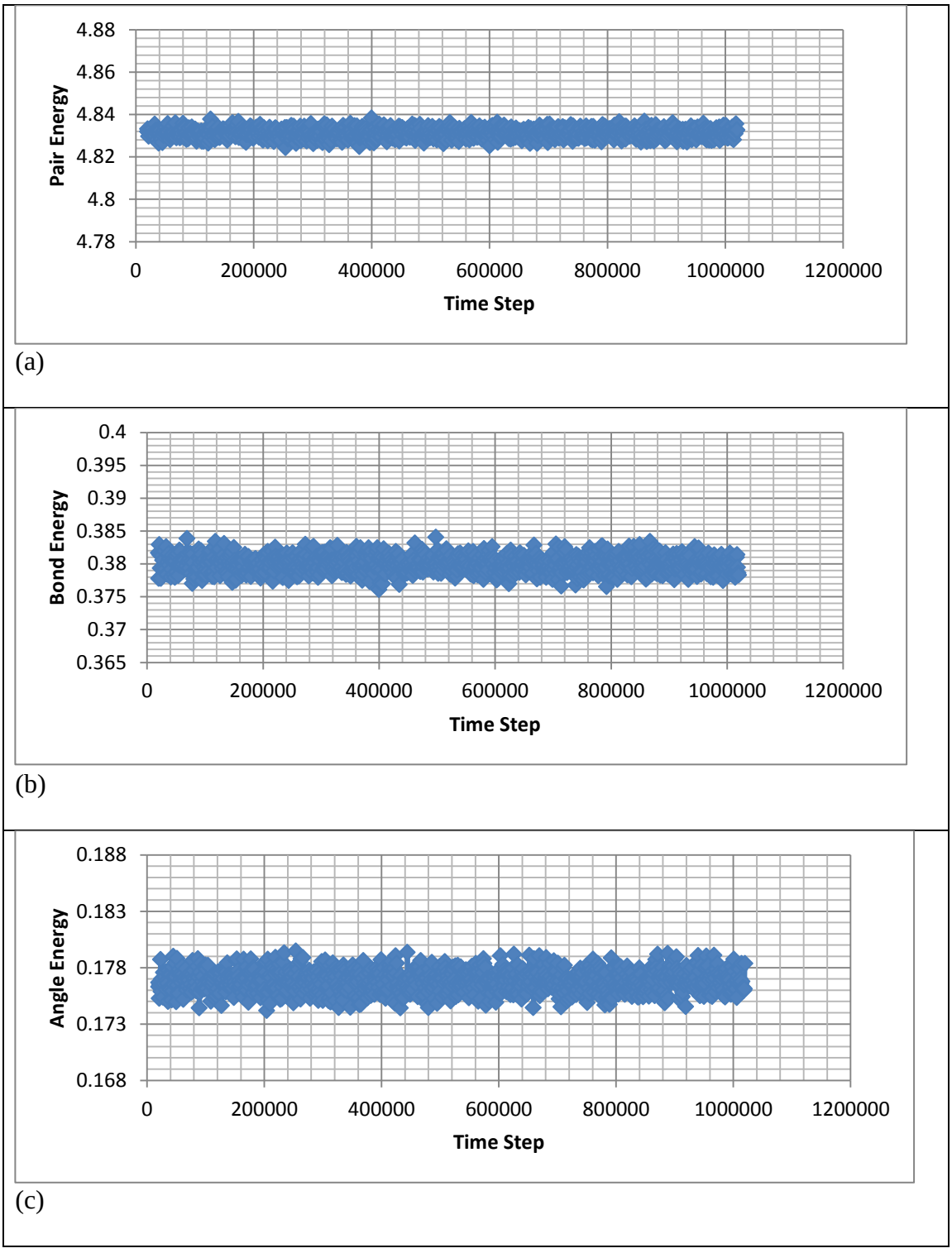
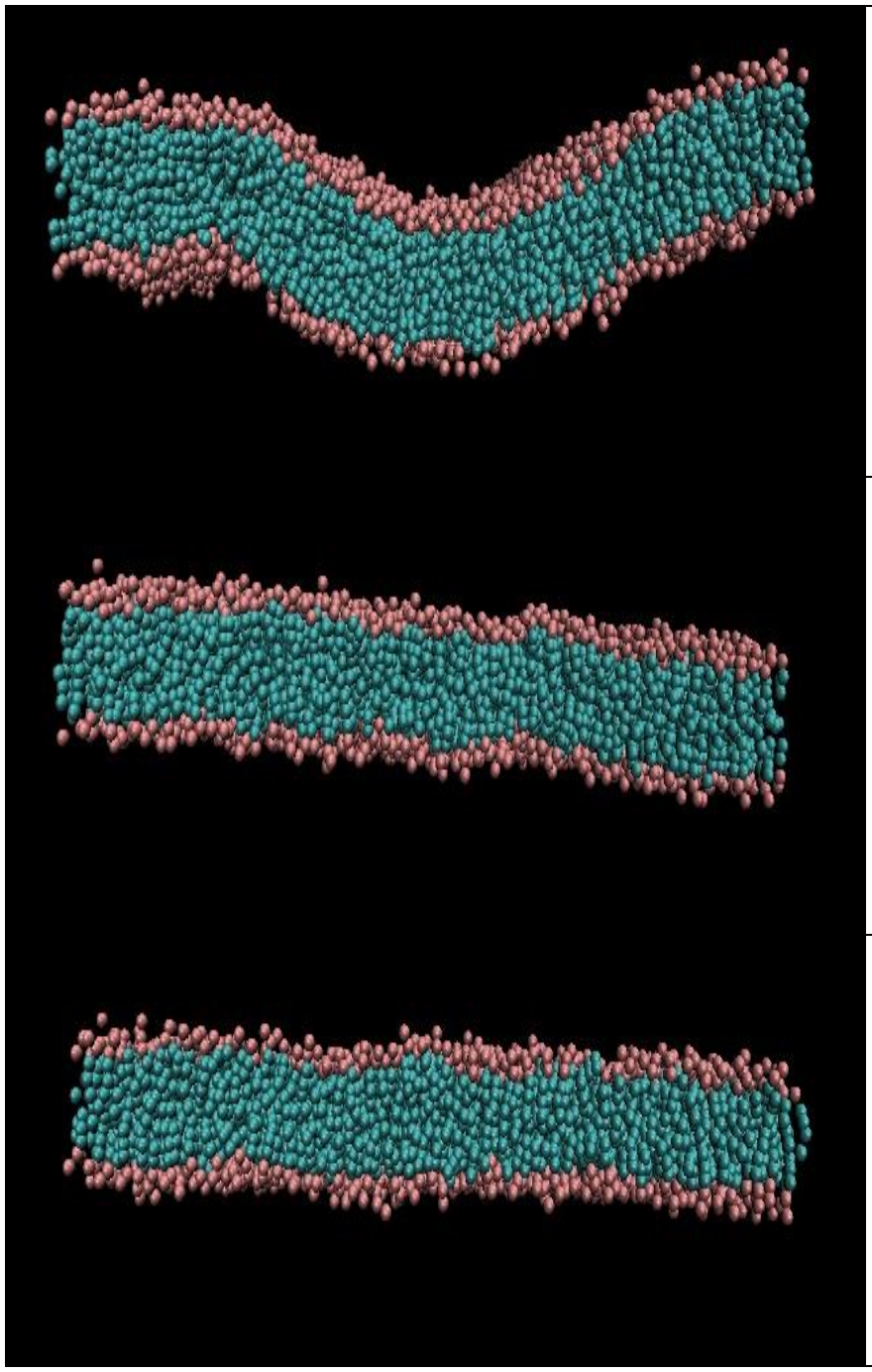


Figure 4.3 Variation with time of (a) Pair Energy, (b) Bond Energy and (c) Angle Energy

From our results we can see that the results do match up well with results obtained by our DPPC bilayer. The point to note is that while for an area per molecule for 1.185 is the

zero tension measurement for DPPC, the equivalent value for cholesterol is interpolated to be approximately 1.045, this is drastically lower than DPPC, which means that there will be a discrepancy when mixing the 2 because for the same area occupied for both tensionless states the number of molecules will vary. This will be discussed in the next chapter. Upon comparing all their respective energies, we can see that they both take about the same time to equilibrate but cholesterol in general has a higher energy for all pairs, bonds and angles, this is due to the architectural complexity of cholesterol over that of DPPC.

The images below illustrate the variation of structure of the membrane for extremity values of area per lipid, like the previous chapter, the negative tension is marked by a folding of the membrane. For the strongly positive tension we expect slight gaps and holes starting to form in the lipid bilayer, this does not necessarily need to happen for the tension to be highly positive. Because cholesterol is so small, it can attain a strong positive tension yet still appear to be stable from its images(comparison of images (b) and (c)). This could possibly be due to the fluid tail which might plug any gaps that might be formed. This will generally result in a lowering of the membrane thickness as well.



	Area Per Lipid: 1.01 Tension: -0.349
	Area Per Lipid: 1.045 Tension: 0.0062
	Area Per Lipid: 1.15 Tension: 1.833

Figure 4.4 Conformations of bilayer under different tension values

Chapter 5

DPPC-Cholesterol Bilayer

5.1 Introduction

In the previous chapters we have discussed the properties of single component bilayers. The human cell has a mixture of lipids and cholesterol, with cholesterol usually having a concentration of about 50% in the membrane. Cholesterol plays an important role in regulating the fluidity in a membrane as well as several other key functions [62]. But just what does cholesterol do to the lipid bilayer? F. de Meyer and B. Smit illustrated the effect of this by discussing the condensing effect caused by cholesterol on the area per molecule of the bilayer and how that affected its phase structure [57]. The condensing effect is essentially the non-ideal behavior shown when cholesterol and lipids are mixed ideally, with the area per molecule recorded being strikingly different from ideal value [57]. We will be taking a slightly different route by using that explanation of condensing into how it affects the stability and tension exerted on the cell trying to keep its formation. We initially construct our mesoscopic model and vary the concentration of cholesterol for a fixed temperature and volume.

5.2 Computational Details

Now that the tensionless states of lipid and cholesterol have been established, we will now turn our focus to the main part of our project, which is to see the effect of cholesterol on the stability of a multi-component bilayer membrane. Although both lipid and cholesterol are comprised of the same number of beads, their area per lipids are different

with cholesterol being a much smaller molecule than DPPC. The first issue that we must address is that for 2 different values of A_0 the number of lipids/cholesterol will not be equal. With this in mind, it would be incorrect to use an average value of the two. Instead a slight variation will be made wherein we will calculate their numbers individually. This number will vary with the concentration of cholesterol used. We will vary our concentration range from 10% - 50%. For each of these concentrations, the effect of cholesterol will be monitored to see whether or not a stable membrane is formed, or if the condensation effect induced by having cholesterol in our system alters the value at which the membrane is tensionless.

$$N_{Total} = N_{chol} + N_{Lipid}$$

$$N_{chol} = \frac{L_x . L_y}{A_C}$$

$$N_{Lipid} = \frac{L_x . L_y}{A_L}$$

This calculates the overall number of lipids and cholesterol, but this currently is independent of concentration, the actual number which is put into the system is governed by monitoring the number of lipids, after a certain number has been attained, the system will stop putting this lipid and start putting in cholesterol. As per previous chapters, all the interaction parameters are exactly alike. The only extra interactions are those with the two lipid types.

a_{ij}	h_{lip}	h_{chol}	t_{lip}	t_{chol}	w
h_{lip}	25	25	100	25	15
h_{chol}	25	25	100	100	25
t_{lip}	100	100	25	25	100
t_{chol}	100	100	25	25	100
W	15	25	100	100	25

Table 5.1 Interaction parameters of mixed membrane

Our simulation conditions (NVT) are identical to our other runs. When molecules are put into the system, they are organized in a regular manner, we set a certain value(depending on concentration) for the lipid number and cholesterol number. The lipid and cholesterol are both assigned different bead designations to better differentiate them.

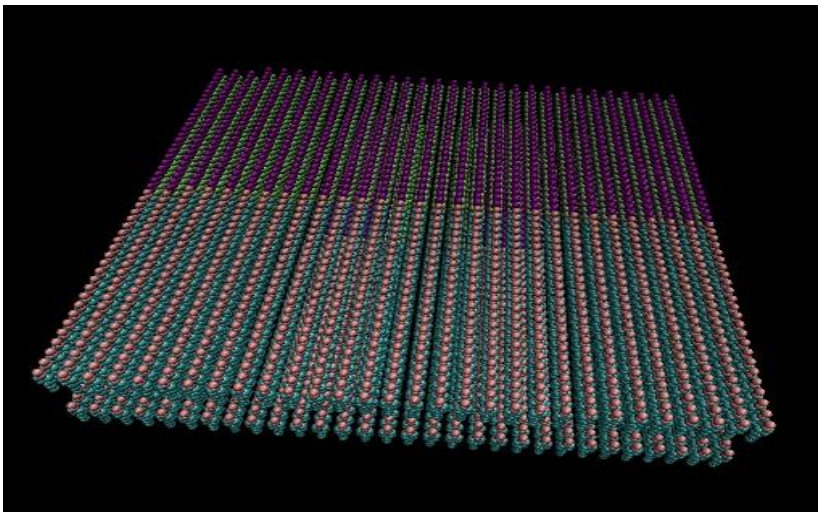


Figure 5.1 Top view illustrating lipids and 40% cholesterol

5.3 Results and Discussion

The addition of cholesterol to a lipid bilayer is an intricate part of being able to successfully mimic mammalian cells. This work provides the key tool into understanding how this cholesterol affects the stability and by extension, the tension attained by a membrane under certain conditions and varying concentration of it. While a typical biological membrane has typically 50% cholesterol, it is important to work with a range so as to fully develop an understanding of the effects. We will be working with concentrations from 10% - 50% cholesterol for which tension measurements will be made.

% cholesterol	10	20	30	40	50
tension	-0.132	-0.112	-1.50	-1.513	-1.766

Table 5.2 Variation of tension with concentration of cholesterol

From the results we can see that all the tension values are highly negative and might fold irreversibly rendering the cell useless, this is rather surprising considering we have used the tensionless state area per lipid to calculate the number of lipids and cholesterol molecules. This is explained by the fact that the cholesterol molecule is much smaller than that of the lipid, so it may cause slight folding of the formed membrane. The excessive bending is also attributed to the fact that as they start to bend, the head-head groups of different lipids might come into close enough contact for more repulsion and eventually cause the cell to break.

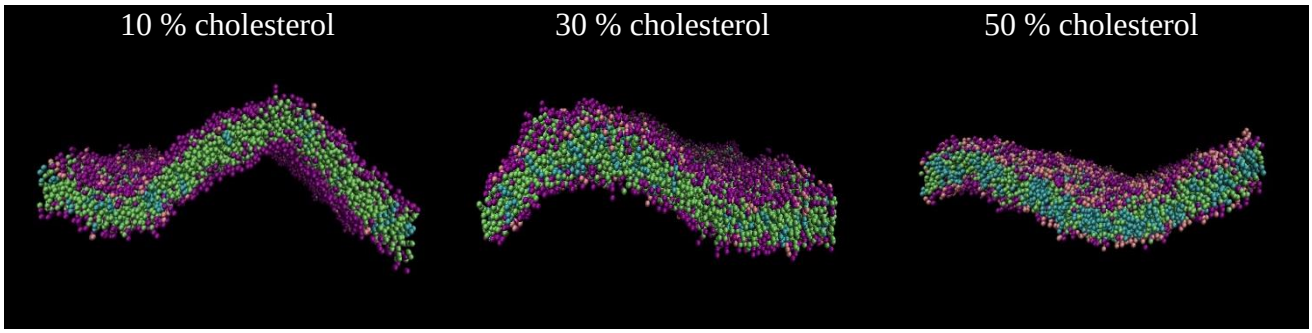


Figure 5.2 Visualization of membrane tension for different concentrations of cholesterol

Interestingly, even though the tension results show an increase as concentration increases, the lower concentration shows more bending, it shows the effect of having a large imbalance in the components, where the odd molecules cause a pile up locally and therefore cause deformation. This of course reduces as the system mixes over time.

Since we have already established the zero tension values, we will attempt to stretch [63] the box size and increase the volume. This function will keep all external conditions constant but stretch the box along the y direction (there will be corresponding changes in the vertical direction to keep volume constant). This will allow more space for the membrane to spread, and as a direct result, make the tension more positive. Typically boxes are stretched by a very little so as to maintain the overall integrity of the simulation. We will adjust the box sizes and monitor the variation of tension with stretching of 1% - 4%. These simulations will be run for a 50% cholesterol bilayer.

% stretching	1	2	3	4
tension	0.0289	0.0378	0.0379	0.0331

Table 5.3 Effect of stretching on mixed membrane tension

From the above table we can see that stretching the membrane by even as little as 1% each run causes our membrane to eventually reach a more or less stable conformation. These results make it clear that condensation effect of cholesterol has a part to play in the

stability of the membrane even though both number of cholesterol and lipid are governed by separate equations based on their individual area per molecules.

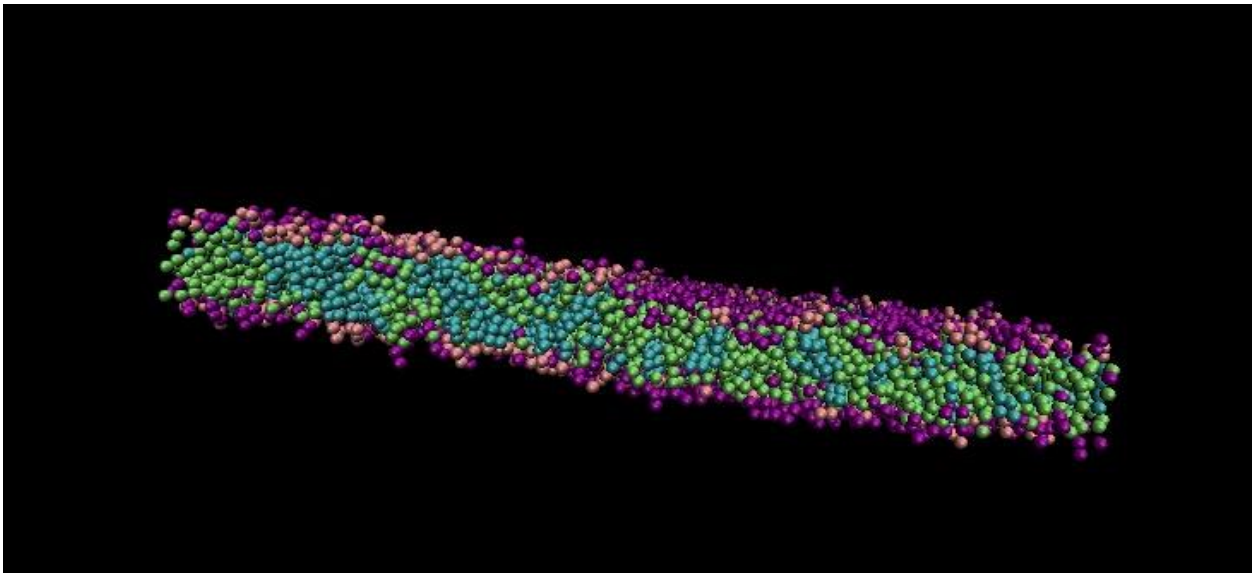


Figure 5.3 Stability of stretched mixed membrane

Chapter 6

Summary and Future Scope

6.1 Summary

The effect of cholesterol on the stability of a DPPC-cholesterol bilayer was studied. Initially, a purely lipid based model was used to evaluate the area per molecule corresponding to a tensionless state after it had reached its minimum free energy. Subsequently, the same was repeated for a purely cholesterol bilayer. The area per lipid affects how many molecules are present in a system. This number is much higher for cholesterol than DPPC because it has a much smaller area per molecule. The two components were mixed and the lipid-cholesterol bilayer was made. The concentration of cholesterol was varied and the tension became more negative. This was a result of the condensation effect caused by cholesterol. The simulation box was finally stretched and a tensionless mixed membrane was achieved.

6.2 Future Scope

Understanding the interactions of mixed lipid bilayers which are close to human cells are of the upmost importance and there are many branches that extend from this project that could be our next steps. The most obvious perhaps is its applications in drug discovery. While formulating a drug, this model can be used to inspect the potential pros and cons of this drugs which are modeled based on their interaction parameters. They can be monitored for their effect on the membrane and evaluate how effectively the cell cooperates with certain drugs.

A slight step from that would be to evaluate how these particular drugs enter the system. The method of uptake can be closely monitored and the effect of cholesterol on the uptake of drugs can be monitored.

There has been much talk about nano-medicine, the mere fact that you can administer so much more drug on a smaller scale makes an invaluable addition to any research work. But just how do these particles interact with the cell, using this model we can evaluate not only 1 particle interacting with the cell, but thanks to DPD's large length and time scale, we can now effectively simulate several particles at once and evaluate their cooperative effect on the cellular uptake.

Testing of new drugs is both expensive and time consuming. With the help of a well-designed model, there can be less dependence on clinical trials. By inspecting potential drugs on a simulation, we can eradicate a few of the options by observing their effects directly, this both reduces the number of trials that need to be done, and also allows for a more safe testing scheme.

There are also several variations that can be studied to help improve the simulation quality and increase its reliability.

Phase segregation can be effectively studied for a mixed membrane and perhaps the cause of heterogeneities present in the same system. The system can also be used to test the effect of different sized and shaped nanoparticles have on the uptake into a cell to help develop more effective drugs.

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