A Computational Study on Cardiolipin - Cytochrome C Interactions on a 5CB Liquid Crystal Droplet Surface

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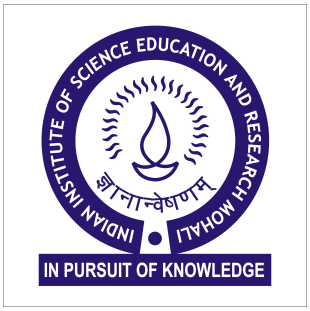
MS15159

A dissertation presented for the partial fulfilment of BS-MS Dual Degree in Science



Indian Institute of Science Education and Research, Mohali

June 2020

Dedicated to:

My Family

Certificate of Examination

This is to certify that the dissertation titled "**A Computational Study on Cardiolipin-Cytochrome C Interactions on a 5CB Liquid Crystal Droplet Surface** " submitted by **Ms. Fidha Nazreen K M** (Reg. No. MS15159) for the partial fulfilment of BS-MS dual degree programme of Indian Institute of Science Education and Research, Mohali has been examined by the thesis committee duly appointed by the institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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(Supervisor)

Dated: June 10, 2020

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Monika Sharma at the Indian Institute of Science Education and Research, Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussion. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Fidha Nazreen K M

(Candidate)

Dated: June 10, 2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Monika Sharma

(Supervisor)

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List of Figures

1.1 Cytochrome C and heme 2

1.2 Cardiolipin Structure 3

1.3 5CB Structure 3

2.1 MD Flowchart 7

3.1 5CB in water 12

3.2 Droplet Formation 13

3.3 Droplet Volume Graph 13

4.1 Lipid assembly on an LC droplet 15

4.2 Cardiolipin and 5CB in water 16

4.3 Cardiolipin structure division 17

4.4 Plots showing the penetration of tail 18

4.5 Non bonded Interactions 19

5.1 Snapshot of Cardiolipin and 5CB with Cytochrome C 22

5.2 Snapshot of Cardiolipin and 5CB with Cytochrome C 24

5.3 Helicity Plot 25

5.4 Cardiolipin-5CB Interactions in presence of Cytochrome C 26

5.5 Cardiolipin-5CB Interactions in the absence of Cytochrome C 26

5.6 Cytochrome C interactions with cardiolipin head and tail 27

5.7 Heme interactions with cardiolipin head and tail 28

5.8 Probability distribution of all the interacting residues 29

5.9 Electrostatic Profile Details 30

5.10 Electrostatic profile of Cytochrome C 30

5.11 Electrostatic profile of BSA 31

List of Tables

2.1 Simulation Details 10

Notations

5CB	4-Cyano-4'-pentylbiphenyl
C	Carbon
MC	Monte Carlo
ns	Nanosecond
O	Oxygen
P	Phosphorous
PME	Particle Mesh Ewald
ps	Picosecond

Contents

List of Figures **i**

List of Tables **iii**

Notations **v**

Abstract **x**

1 Introduction **1**

2 Methodology **5**

2.1 Molecular Dynamics 5

2.1.1 Initialisation 7

2.1.2 Heating Dynamics 8

2.1.3 Equilibration 8

2.1.4 Production 9

2.1.5 Analysis 9

2.2 Simulation Details 9

3 System 1 (5CB LC Droplet in Water) 11

3.1 Introduction 11

3.2 Methodology 11

3.3 Results 12

4 System 2 (5CB LC Droplet + Cardiolipin in Water) 15

4.1 Introduction 15

4.2 Methodology 16

4.3 Results 17

4.3.1 Cardiolipin on the Droplet Periphery 17

4.3.2 Atomic Scale Interactions 18

5 System 3 (5CB LC Droplet + Cardiolipin + Cytochrome C in Water) 21

5.1 Introduction 21

5.2 Methodology 22

5.2.1 Control 23

5.3 Results 25

5.3.1 Helicity 25

5.3.2 Cardiolipin - 5CB Interactions in the presence and absence of Cytochrome C 25

5.3.3 Cardiolipin - Cytochrome C Interactions 26

5.3.4 Heme - Cardiolipin Interactions 27

5.3.5 Nature of Interactions and Electrostatic profile 28

6 Summary and Conclusions **33**

6.1 Results 33

6.2 Conclusions 33

6.3 Future Works 34

Bibliography **35**

Abstract

Protein-lipid interactions are essential for maintaining the structural integrity of cellular membranes. Cardiolipin-Cytochrome C interactions play a vital role in the coherence of the mitochondrial membrane and apoptosis. Delineating the interaction has been a research interest for a long time, but remains elusive. This study focuses on cardiolipin-Cytochrome C interactions on a liquid crystalline droplet surface using molecular dynamics (MD) simulations, which have been used as a major tool for biophysical and biochemical studies. Significant insights into the interactions were gained through this work on the nature of interactions as well as the interacting residues.

Chapter 1

Introduction

Membranes define the outer boundary of the cell which is known as the plasma membrane and many other inner cellular compartments which are called organelles. Cellular membranes are one of the most important constituents of cell and they play a key role in cell function and fundamental life processes[Tamm 06]. They are comprised of a bilayer of lipids in an asymmetric arrangement[Corradi 18]. There are many proteins embedded as well as loosely associated with this bilayer. The protein-lipid interactions maintain the structural integrity of the membranes as well as functional integrity of proteins[Bienvenüe 94, Tamm 06].

Liquid Crystal Droplets to study Lipid - Protein Interactions

Liquid crystals (LC) are known for their low interfacial energy and sensitivity to perturbations which enable them to act as sensitive reporters of interfacial processes. They are also excellent biomimetic systems to understand biochemical interactions occurring in the cellular environment[Pani 18, Bisoyi 11]. There have been many studies utilising the property of liquid crystals to act as biosensors, as the amplification of the surface perturbations results in the change of orientation of the droplets[Gupta 98].

Understanding lipid-protein interactions at liquid crystalline interfaces have caught a lot of attention. In this regard, simulation in combination with experimental approaches can provide significant insights into these phenomena.

Cytochrome C and Cardiolipin

Cytochrome C is a small water-soluble heme protein (contains a heme prosthetic group) and it is found in between the membranes of mitochondria in loose association with the inner membrane[Manickam 17, Alvarez-Paggi 17]. It is one of the components of the electron transport chain of mitochondria and it also acts as a catalyst for several redox reactions[Yeagle 16, Souza 08]. Cytochrome C also plays a major role in apoptosis or programmed cell death[Yang 07].

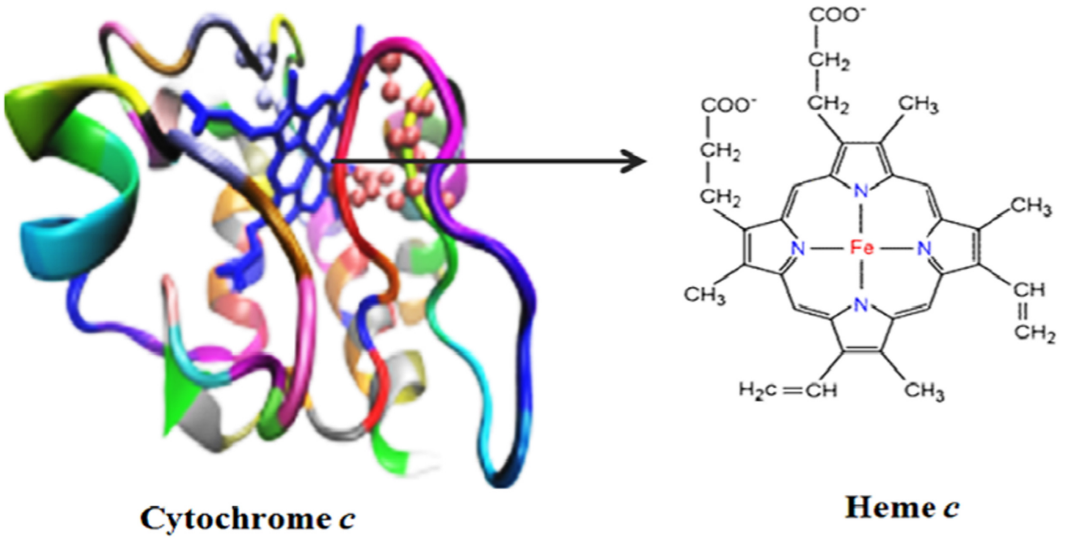

Figure 1.1: Structure of Cytochrome C and heme. Source: [Manickam 17]

The mechanism by which Cytochrome C initiate cell death occurs due to its interaction with cardiolipin. Cardiolipin is an anionic phospholipid and a major constituent of the inner mitochondrial membrane. In the presence of cardiolipin, the heme-protein interaction in Cytochrome C is weakened. This instigates peroxidase activity in Cytochrome C, which was suppressed earlier by heme. The process further leads to cardiolipin oxidation and as a result, the mitochondrial membrane loses its structural integrity, instigating cell death or apoptosis [Dykens 07, Hough 14, Belikova 06].

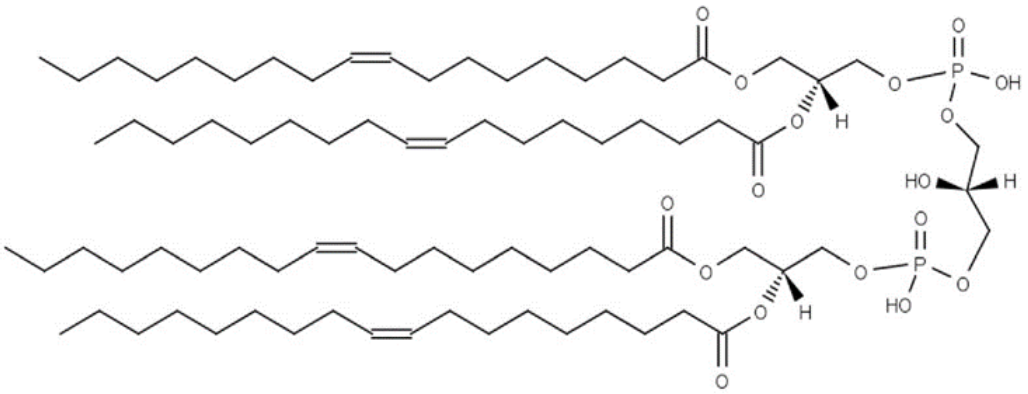

Figure 1.2: Structure of Cardiolipin. Source: https://doi.org/10.1371/journal.pone.0059267.g001

There have been a number of studies, both experimental and computational, to study cardiolipin[Dahlberg 08, Lemmin 13, Aguayo 12] and Cytochrome C individually [Wendoloski 87, Abel 10, Simonson 95]. When it comes to their interactions, there have been a considerable number experimental studies[Beales 11, Hanske 12, Sinibaldi 10]. Despite the number of studies, the clear picture of the mechanism of the interaction between cardiolipin and Cytochrome C remain obscure.

We employed the liquid crystal mediated study of lipid-protein interactions to study the cardiolipin Cytochrome C interactions. We chose 4-Cyano-4'-pentylbiphenyl (5CB) which is the most widely used nematic liquid crystals, in this work.

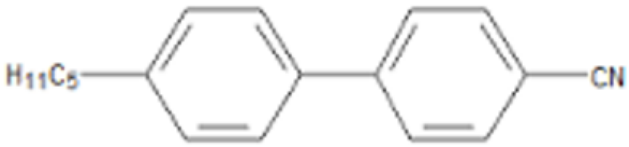

Figure 1.3: Structure of 5CB ($C_{18}H_{19}N$)

This is the first work which employs computational tools to look at cardiolipin-Cytochrome C interactions. We aim to look at the cardiolipin - Cytochrome C interactions on a 5CB liquid crystal droplet interface. This thesis primarily focuses on the molecular dynamics simulation, analysis and other computational investigations to understand the phenomena with greater clarity.

Chapter 2

Methodology

2.1 Molecular Dynamics

Classical molecular dynamics simulations are based on Newton's laws of motion. To calculate a trajectory, only the initial positions of the atoms and initial distribution of velocities are needed. The acceleration can be determined by the gradient of the potential energy function. If we know the initial conditions, the state of the system can be estimated through integration since the process is deterministic. Molecular dynamics is a very effective approach to get a deeper understanding of macromolecular structure, conformation and function.

Newton's equation of motion is given by,

$$\mathbf{F_i} = m_i \mathbf{a_i} \tag{2.1.1}$$

where $F_i$is the force acting on the particle, m_i, the mass of the particle and a_i, the acceleration of the particle. Force can also be given as the gradient of the potential energy function $\mathbf{U}$,

$$\mathbf{F_i} = -\nabla_i \mathbf{U} \tag{2.1.2}$$

Combining equations 2.1.1 and 2.1.2, acceleration can be written as the derivative of potential energy with respect to postion as,

$$\mathbf{a} = -\frac{1}{m}\frac{d\mathbf{U}}{dr} \tag{2.1.3}$$

Also,

$$\mathbf{v} = \frac{d\mathbf{r}}{dt} \tag{2.1.4}$$

$$\mathbf{a} = \frac{d\mathbf{v}}{dt} \tag{2.1.5}$$

The integration algorithms approximates the positions and velocities based on the Taylor series,

$$\mathbf{r}\left(t+\delta t\right) = \mathbf{r}\left(t\right) + \mathbf{v}\left(t\right)\delta t + \mathbf{a}\left(t\right)\delta t^2 + ... \tag{2.1.6}$$

$$\mathbf{v}(t+\delta t) = \mathbf{v}\left(t\right) + \mathbf{a}\left(t\right)\delta t + ... \tag{2.1.7}$$

The total potential energy $\mathbf{U}$ is given by sum of bonded and non bonded interactions. The bonded terms include bonds, angles, dihedral angles and improper angles, and the non bonded terms include Van der Waals interactions and electrostatic interactions. We approximate the atoms that comprise the molecules to be spheres and the bonds between them as springs. The basic stucture of a force field is given as,

$$\mathbf{U} = \sum_{bonds} K_b\left(r - r_0\right)^2 + \sum_{angles} K_\theta(\theta - \theta_0)^2 + \sum_{dihedrals} K_\phi + \sum_{impropers} K_\psi\left(\psi - \psi_0\right)^2$$

$$+ \sum_{i>j} 4\epsilon\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \sum_{i>j}\frac{q_i q_j}{4\pi\varepsilon r_{ij}}$$

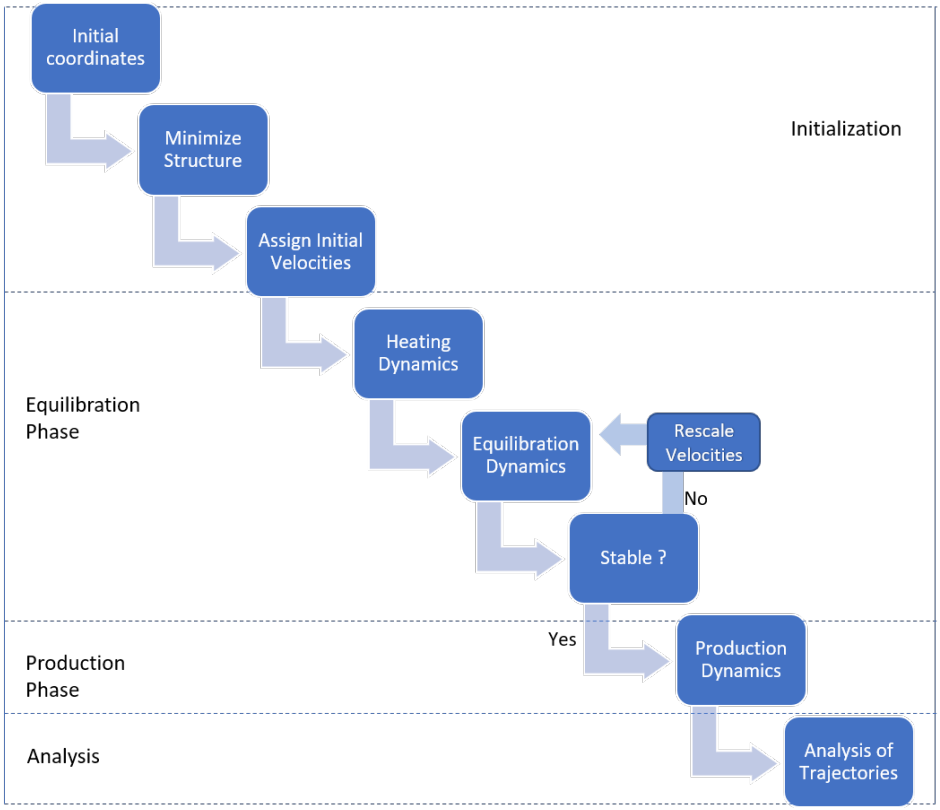

Figure 2.1: Flowchart of molecular dynamics simulation

Molecular simulations are done using two methods, molecular dynamics and Monte Carlo (MC). MD gives information about both position and momentum and also gives the temporal evolution of the system while the momentum and the time evolution cannot be determined using MC[Paquet 15]. MD was initially used by Alder and Wainwright in 1957 to study many-body system of particles[Alder 57].

The basic steps to be followed while doing an MD simulation is given in fig. 2.1 and is discussed in detail in the following subsections.

2.1.1 Initialisation

To solve any differential equation, the initial conditions are required. To estimate the positions and velocities of particles at a time t, we need the initial positions and velocities. The initial position coordinates are obtained experimentally using techniques like X-ray crystallography and NMR. Once the initial coordinates are obtained, the structure needs to be minimised relieve the strain of the conformation derived exper-

imentally as well to stabilize the structure. To do this, an algorithm like gradient descent is employed to find the local minima of the potential energy,

$$\frac{\partial \mathbf{U}}{\partial x_i} = 0, \frac{\partial^2 \mathbf{U}}{\partial^2 x_i} > 0$$

The minimised structure is then solvated (if necessary) by adding the corresponding solvent and the ions are added to neutralise the system. The initial velocities are then randomly assigned to each of these atoms from a Maxwell-Boltzmann distribution at a given temperature T,

$$P(\mathbf{v}_i) = \left(\frac{m_i}{2\pi k_B T}\right)^{1/2} \exp\left(-\frac{1}{2}\frac{m_i \mathbf{v}_i^2}{k_B T}\right) \tag{2.1.8}$$

such that the total momentum $\mathbf{P}$ cancels out,

$$\mathbf{P} = \sum_{i=1}^{N} m_i \mathbf{v}_i = 0 \tag{2.1.9}$$

2.1.2 Heating Dynamics

Now the system has to be heated to the desired temperature. The system has to be coupled with a thermostat and slowly heated to the final temperature. The simplest algorithm to achieve this is velocity rescaling. In this method, the velocities of each of the particles are multiplied by a factor λ,

$$\lambda = \sqrt{\frac{T_0}{T}}$$

where, $T_0$is the desired temperature and T is the current temperature.

2.1.3 Equilibration

The equilibration of all the molecules including solvent and ions is essential before proceeding to the dynamics of the system. To prevent the system from collapsing

various constraint algorithms that maintain the atomic distances are implemented at this. The system is relaxed for a few hundreds of picoseconds (ps) before continuing further to production.

2.1.4 Production

Upon the completion of the equilibration phase, the dynamics of the system is generated under a desired thermodynamic ensemble and the trajectory is obtained. The production phase is carried out for a few nanoseconds (ns).

2.1.5 Analysis

Once the simulation is complete, the trajectory is analysed to obtain information on the desired variable.

2.2 Simulation Details

The simulations were carried out in three parts. The first system consists of 5CB LC droplet in water. The second consists of 5CB LC droplet with cardiolipin in water. The third system consists of 5CB LC droplet, cardiolipin on the periphery of the droplet and Cytochrome C, in water. Atomic-scale molecular dynamics simulations were performed on all three systems. All simulations were carried out using GROMACS[Abraham 15]. To create the molecular topology, the CHARMM36[Huang 13] force field was used. The TIP3P[Jorgensen 83] water model was used to solvate all the systems. The initial structure of cardiolipin and Cytochrome C were retrieved from `http://www.rcsb.org/`. To define the long-range electrostatic interactions, the Particle Mesh Ewald (PME) method was used[Darden 93]. The temperature coupling was done using the velocity rescaling algorithm[Bussi 07] and the pressure was maintained using Berendsen barostat[Berendsen 84]. Further details of the simulations are mentioned in the following subsections.

System	No. of Atoms	No. of Molecules	Water	Ions
1	75320	400 5CB	26979	-
2	785609	400 5CB, 150 Cardiolipin	244703	$300 \times Na^+$
3	21596	400 5CB, 20 Cardiolipin, 6 Cytochrome C	61884	$4 \times Na^+$

Table 2.1: Simulation Details

The visualisations and the data generation was done using VMD[Humphrey 96]. MATLAB (R2019a)[MATLAB 19] was used to do the necessary calculations and data processing. All the graphs were plotted using Origin(Pro) Version 2020 (OriginLab Corporation, Northampton, MA, USA) and the probability distribution function plots were generated using MATLAB (R2019a)[MATLAB 19].

Chapter 3

System 1 (5CB LC Droplet in Water)

3.1 Introduction

Liquid crystal molecules self assemble themselves in the presence of water to form spherical droplets. The molecules orient in such a way that the polar head faces water molecules while the non-polar part remains inside the droplet. We are working with 5CB liquid crystal molecule. It has a polar C-N bond and a non-polar hydrocarbon chain. There are a large number of works involving computational simulations of 5CB liquid crystal since it is very commonly used[Tsige 99, Wang 01].

In this chapter, we aim to look at the 5CB LC droplet formation. The methodology and results are explained in detail in the following subsections.

3.2 Methodology

The initial structure of 400 5CB molecules was generated. This complex was placed in a TIP3P solvent box of dimensions 100 Å $\times$ 100 Å $\times$ 100 Å and solvated which resulted in a total of $\sim$ 786000 atoms. Minimization was done in 5000 steps and the system was equilibrated for 0.1 ns. The integration was done in steps of 2 fs and the

LINCS[Hess 97, Hess 08] constraint algorithm was employed to constrain bonds. The system was gradually heated from 0 K to 300 K. The structure was equilibrated once more under NPT conditions before proceeding to the simulations. The temperature of 300 K was maintained using velocity rescaling thermostat and pressure of 1 atm was maintained using Berendsen barostat. The structures were simulated then for 20 ns under NPT ensemble.

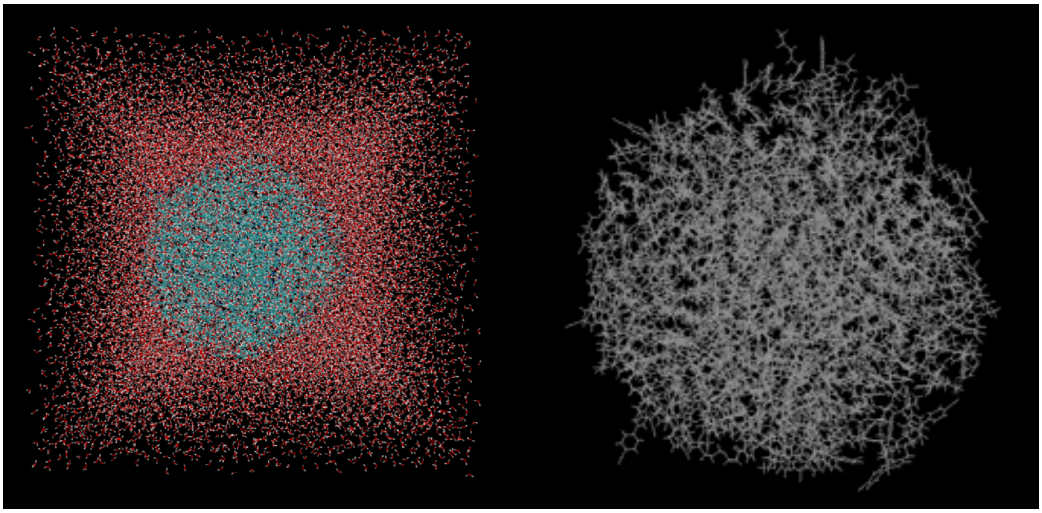

Figure 3.1: Snapshots of the 5CB in water: with and without water

To look at the droplet formation, two methods were employed. In the first method, the time evolution of the droplet was analysed from the snapshots of the 5CB LC droplet was taken at an increment of 2 ns using VMD. In the second method, the cubical volume of the 5CB LC droplet was calculated. For this, the minimum and maximum XYZ coordinates were generated for all the time frames using the Tcl script in VMD. This data was imported into MATLAB to calculate the cubical volume and then plotted.

3.3 Results

From fig. 3.2, it can be seen that the 5CB liquid crystal molecules that were spatially scattered are coming closer to form a spherical droplet. This is further illustrated in fig. 3.3, where it can be observed that the cubical volume of the space containing 5CB molecules is decreasing as the molecules aggregate to form a compact droplet.

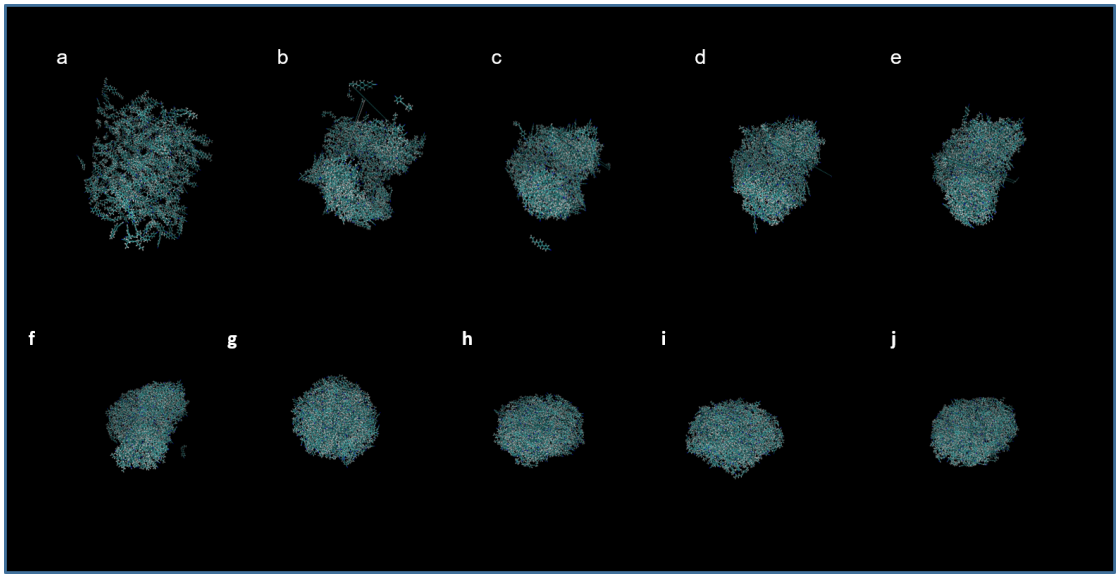

Figure 3.2: Series of snapshots showing time evolution of the 5CB LC Droplet: a-j) 5CB droplet orientation changes from initial frame to final frame in increments of 2 ns

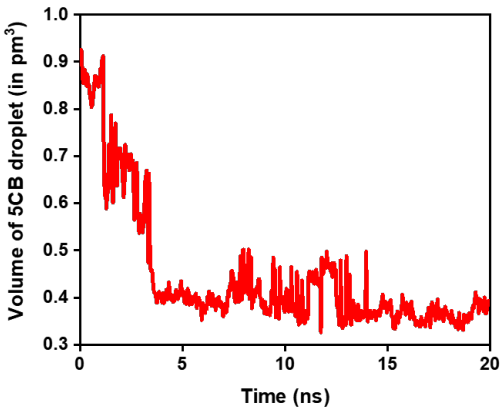

Figure 3.3: The plot showing cubical volume of the 5CB droplet against time

Hence, it can be concluded that the 5CB molecules are self-assembling themselves to form a compact droplet, in the presence of water.

Chapter 4

System 2 (5CB LC Droplet + Cardiolipin in Water)

4.1 Introduction

Amphiphiles like phospholipids assemble on liquid crystal droplets change the orientation of the droplets in a way that it is visible to the naked eye[Sidiq 15]. Penetration is found to be happening when cardiolipin molecules within the vicinity of the 5CB Droplet interact. Hydrophilic head of the cardiolipin remains outside while the hydrophobic tails are inserted into the droplet as shown in the schematic.

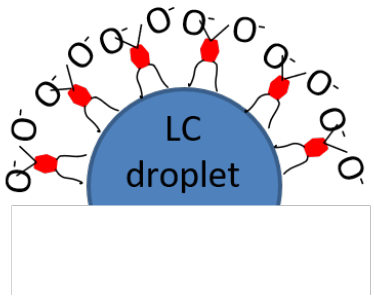

Figure 4.1: Schematic diagram that shows lipid(structure with an anionic head and hydrocarbon legs) assembly on an LC droplet (Blue)

In this chapter, we looked at the cardiolipin-5CB interactions at the droplet periphery. Furthermore, we looked at the non bonded interactions on an atomic scale between

5CB molecules and cardiolipin on the droplet periphery, since molecular dynamics simulation allows study the mechanism by which the molecules interact on a deeper level. The methodology and results are explained in detail in the following subsections.

4.2 Methodology

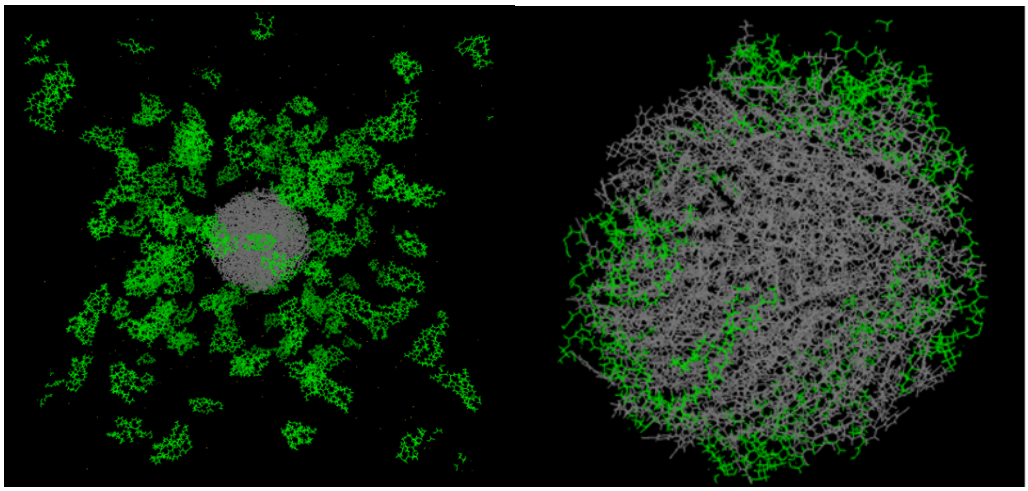

Figure 4.2: Snapshots of Cardiolipin and 5CB in water

The initial structure was generated by adding 150 cardiolipin molecules to the previous system of 400 5CB molecules. This complex was placed in a TIP3P solvent box of dimensions 200 Å × 200 Å × 200 Å and solvated which resulted in a total of ∼ 75000 atoms. After this step, 300 Na^+ ions were added to neutralise the system[Beglov 94]. Minimization was done in 5000 steps and the system was equilibrated for 0.1 ns. The integration was done in steps of 2 fs and the LINCS constraint algorithm was employed to constrain bonds[Hess 97, Hess 08]. The system was gradually heated from 0 K to 300 K. The structure was equilibrated once more under NPT conditions before proceeding to the simulations. The temperature of 300 K was maintained using velocity rescaling thermostat and pressure of 1 atm was maintained using Berendsen barostat. The structures were simulated then for 100 ns under NPT ensemble.

To elucidate the penetration of cardiolipin tails inside the 5CB LC droplet, two methods were employed. To get the average distances from 5CB droplet centre to different parts of the cardiolipin atoms, the carbon (C) atom on the tip of the tail of the cardiolipin, the oxygen (O) atom from the glycerol backbone and the phosphorous atom

on the head was selected as shown in fig. 4.3. The distances to each of these atoms were calculated and then averaged out. To get the number of 5CB atoms surrounding each part, the same selection of atoms was kept and the number of heavy atoms in 5CB within a cutoff distance of 5 Å was selected. For this purpose, the data was generated using Tcl script in VMD and the calculations were done using MATLAB.

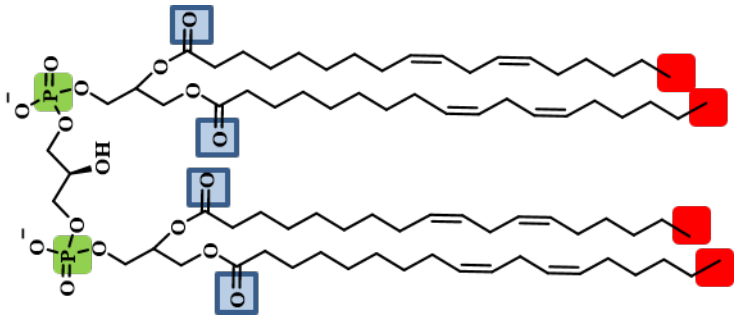

Figure 4.3: Cardiolipin structure division: Marked in red are the C atoms at the tip of the tail, marked in blue are the O atoms that constitute to the glycerol backbone and marked in green are the P atom on the head.

To look at the nonbonded interactions on an atomic scale, the interactions were grouped into interfacial and penetrating. The interfacial 5CB lie on the surface of the droplet while the penetrating 5CB lie beneath the surface, close to the cardiolipin tails that were penetrating the droplet. The interfacial and penetrating 5CB molecules surrounding the cardiolipin at the interface were selected and visualised using VMD.

4.3 Results

4.3.1 Cardiolipin on the Droplet Periphery

From fig. 4.4, it is visible that the number of atoms surrounding the tail of the cardiolipin is significantly larger compared to the head and the hydrophilic part. Also, the average distance from the centre of the 5CB LC droplet to the tail is very less compared to the head and the glycerol backbone. It can be concluded that the cardiolipin tails are penetrating the droplet while the head and the hydrophilic glycerol backbone stays outside the droplet facing the polar water molecules.

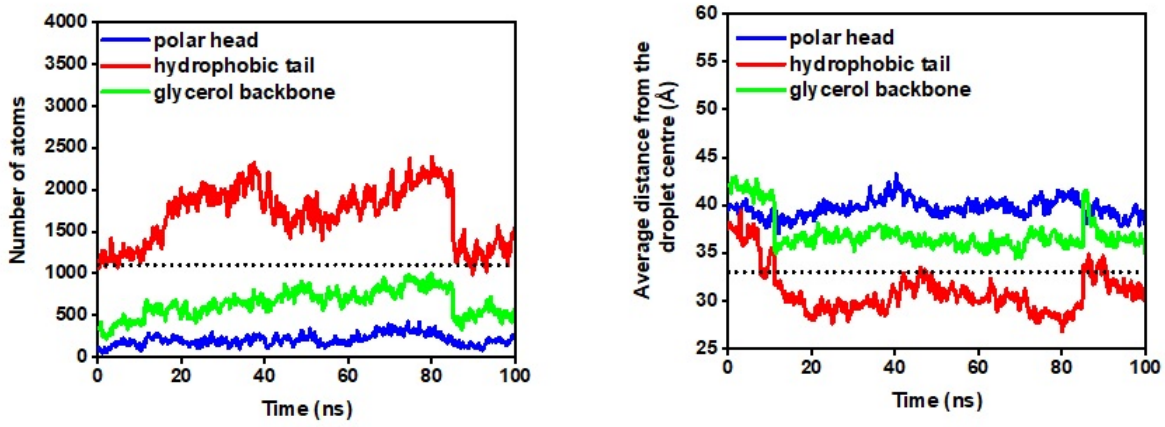

Figure 4.4: The plot of the number of 5CB atoms surrounding different parts of cardiolipin against time and the lot of the average distance from centre to cardiolipin atoms picked from different parts versus the time frames tail

4.3.2 Atomic Scale Interactions

In fig. 4.5, a, b, c corresponds to the interactions between cardiolipin and 5CB which are beneath the surface of the droplet i.e. the 5CB which surrounds the penetrating parts (tail) of the cardiolipin. From these images, it can be seen that the non-polar part of the 5CB is interacting with the cardiolipin tail, which is hydrophobic. Furthermore, it can be noted that the polar part of the 5CB always faces away from the hydrophilic head of the cardiolipin.

When the interfacial interactions are considered $(4.5 - d, e, f)$, it can be observed that some of the 5CB orients in such a way that the polar part is in close proximity of the hydrophilic head. It can also be seen that in f the 5CB in between the tails of cardiolipin is aligned in such a way that the polar part faces away from the tails. From the images, it can be concluded that the nature of interactions is hydrophobic.

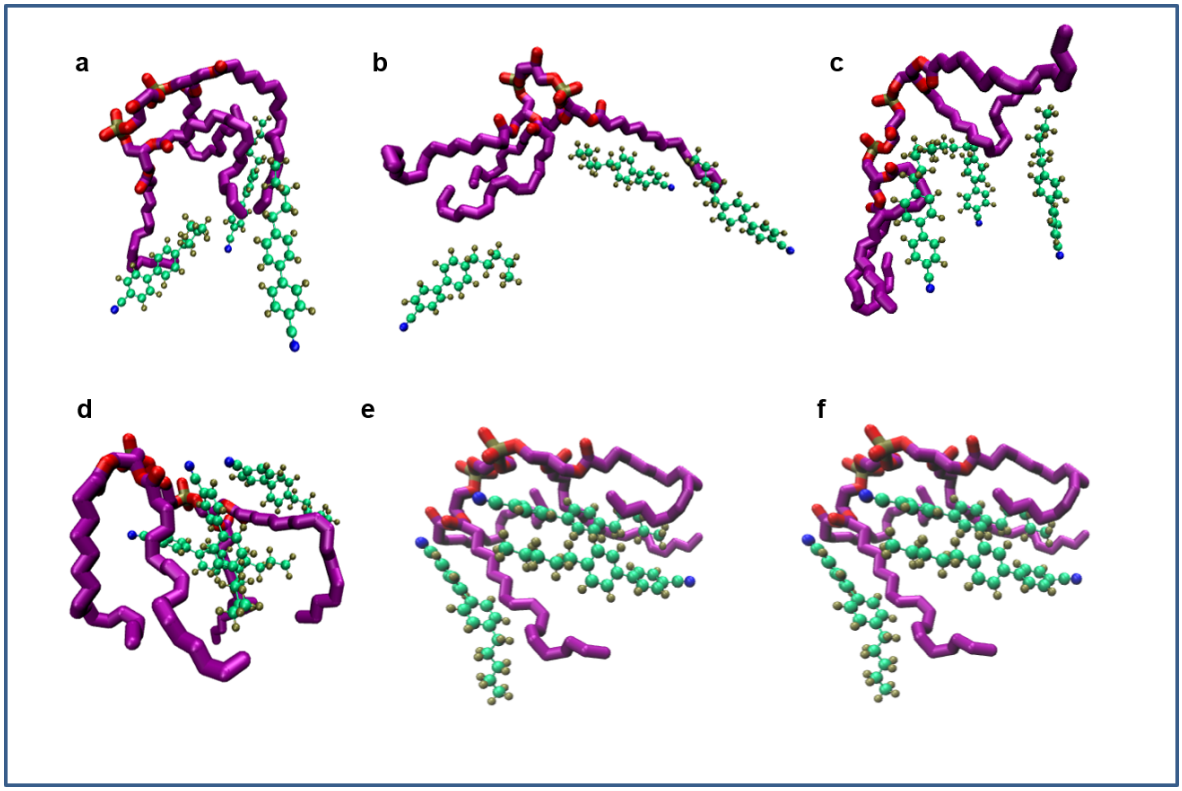

Figure 4.5: a-f) Each image shows the interaction between various interfacial cardiolipin (hydrophilic head with P atoms marked in brown, O atoms marked in red and the carbon chain in purple) and 5CB molecules (C atoms in green, H in tan and N in bright blue) a-c) Penetrating interactions d-f) Interfacial interactions

Chapter 5

System 3 (5CB LC Droplet + Cardiolipin + Cytochrome C in Water)

5.1 Introduction

Cytochrome C is known to interact with cardiolipin, as a result of which the cell death is initiated. The heme residue is known to interact with cardiolipin, weakening its association with Cytochrome C.

In this chapter, first, we tried to look at the change in helical content of Cytochrome C throughout the simulation. This step is necessary to see if the protein structure remained stable i.e. without any unfolding or denaturation. Next, the number of interactions (total and residue wise) between Cytochrome C and cardiolipin were quantified. Finally, we looked at the electrostatic profile of 5CB LC droplet, cardiolipin and one of the Cytochrome C molecule. The final step was repeated with control as well to verify the results obtained. The protein BSA was chosen as the control since it is negatively charged. The methodology and results are explained in detail in the following subsections.

5.2 Methodology

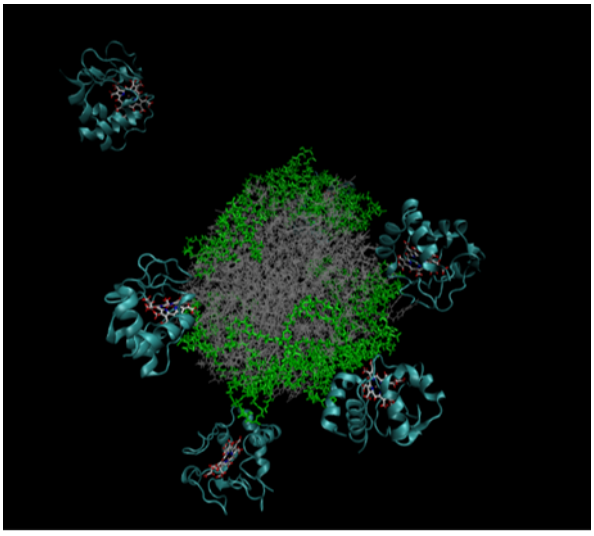

Figure 5.1: Snapshot of Cardiolipin and 5CB with Cytochrome C

The initial structure was generated by adding 6 Cytochrome C molecules to 20 cardiolipin molecules on the periphery of 400 5CB molecules (since we are only interested in CL molecules at the droplet periphery). This complex was placed in a TIP3P solvent box of dimensions 130 Å × 130 Å × 130 Å and solvated which resulted in a total of ∼ 22000 atoms. After this step, 4 Na^+ ions were added to neutralise the system[Beglov 94]. Minimization was done in 5000 steps and the system was equilibrated for 0.1 ns. The integration was done in steps of 2 fs and the LINCS constraint algorithm was employed to constrain bonds[Hess 97, Hess 08]. The system was gradually heated from 0 K to 300 K. The structure was equilibrated once more under NPT conditions before proceeding to the simulations. The temperature of 300 K was maintained using velocity rescaling thermostat and pressure of 1 atm was maintained using Berendsen barostat. The structures were simulated then for 200 ns under NPT ensemble.

First, to look at the helical content of Cytochrome C, a tcl code was written to obtain the percentage content of α-helix in Cytochrome C across time frames from VMD. This data was plotted against time.

To quantify the number of interactions between Cytochrome C and cardiolipin, the cardiolipin was divided into two parts, head and tail ad the list of heavy atoms were

generated. The interactions were defined in such a way that if an atom from a cardiolipin residue comes in 10 Å range of at least 50% of the head or tail heavy atoms, then it is said to interact with the respective part. Each interaction corresponds to 1 and if there is no interaction, it corresponds to 0. Such a binary list was generated and in the end and the interactions were summed up. After this, the residue wise interaction with cardiolipin head and tail were calculated. The total number of interactions as well as a separate plot of heme- cardiolipin interactions was generated. The probability distribution of the total number of interactions, heme interactions and residue wise interactions were also generated. The calculation and plotting of the probability distribution function were done using MATLAB.

To look at the electrostatic profile, the entire system of molecules was grouped based on its electrostatic nature. Cytochrome C was divided into three categories, negatively charged (includes negatively charged and polar negative), positively charged (includes positively charged and polar positive) and uncharged (includes polar neutral and non-polar) residues. 5CB molecules were grouped into the polar N atoms and non-polar hydrocarbon chains. The cardiolipin molecules were classified into polar P and O atoms and the nonpolar hydrocarbon chains. VMD allows you to classify atoms and residues by default or manually. Here, this was done manually. Surf calculations in VMD use the molecular surface solver[Varshney 94] to build the surface and display it after colouring, based on the desired grouping of molecules. Surf calculations were employed here in combination with the above-mentioned classification of atoms and residues to generate the electrostatic profile, showing different patches based on their charge and polarity. The time evolution of this system was then analysed through the snapshots generated using VMD.

5.2.1 Control

For the third system, we also did a control simulation. Since Cytochrome C is a positively charged molecule, it was essential to look at the behaviour of the cardiolipin and 5CB LC droplet in the presence of another protein which has different properties. For this purpose, we chose BSA, which is a neutrally charged protein, and simulated the system with similar conditions as that of the third system in a rectangular box of

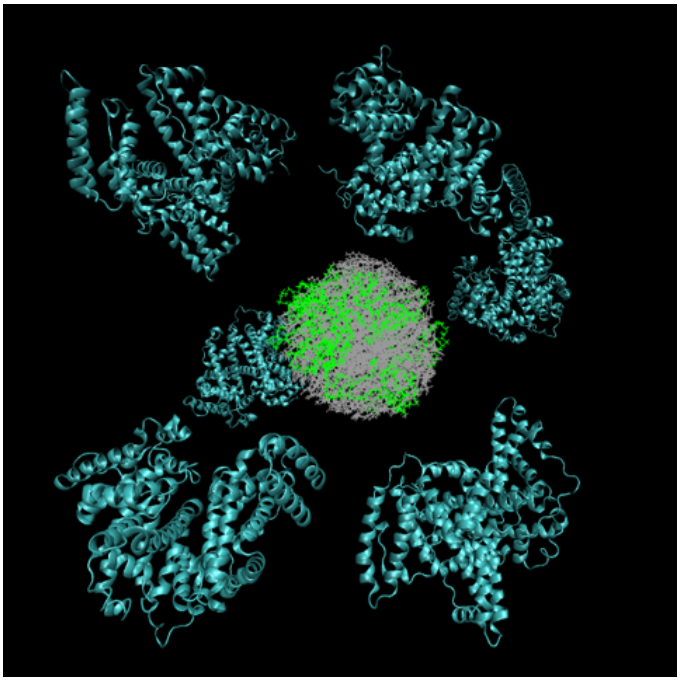

Figure 5.2: Snapshot of Cardiolipin and 5CB with Cytochrome C

dimension 210 Å × 210 Å × 210 Å. The initial structure was generated by adding 6 BSA molecules to 20 cardiolipin molecules on the periphery of 400 5CB molecules. To define the long-range electrostatic interactions, the Particle Mesh Ewald method was used. Minimization was done in 5000 steps and the system was equilibrated for 0.1 ns. The integration was done in steps of 2 fs and the LINCS[Hess 97, Hess 08] constraint algorithm was employed to constrain bonds. The system was gradually heated from 0 K to 300 K. The structure was equilibrated once more under NPT conditions before proceeding to the simulations. The temperature of 300 K was maintained using velocity rescaling thermostat and pressure of 1 atm was maintained using Berendsen barostat. The structures were simulated then for 200 ns under NPT ensemble.

Here, we looked at the dynamics of the system and the electrostatic profile time evolution. For this, the final analysis for the third system was repeated here as well.

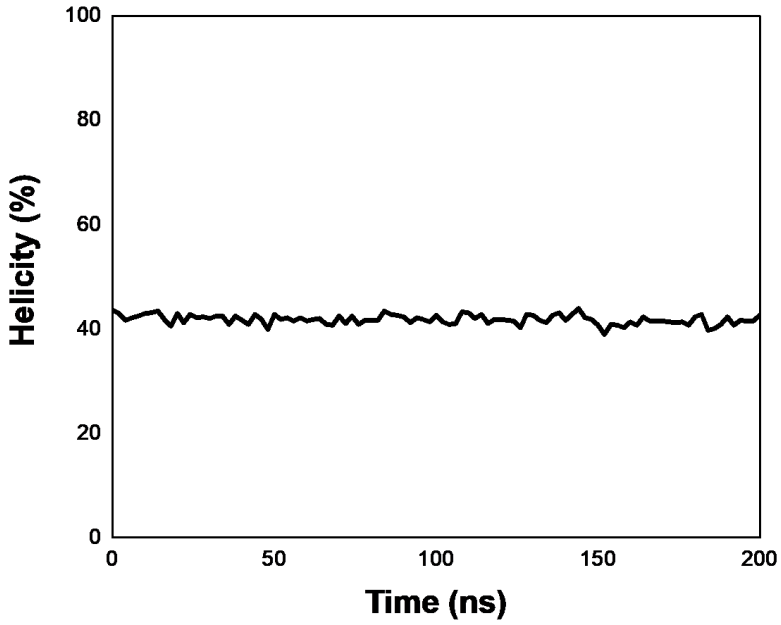

Figure 5.3: Helicity Plot

5.3 Results

5.3.1 Helicity

The three-dimensional structure of Cytochrome C has five α-helices into which the protein backbone is folded. Fig. 5.3 shows the change in percentages of α-helices in the whole structure or the helical content of Cytochrome C with time. It can be seen that the helical content of the protein is not undergoing significant changes. This means that the secondary structure of the protein remains intact throughout the simulation.

5.3.2 Cardiolipin - 5CB Interactions in the presence and absence of Cytochrome C

Comparing Fig 5.4 and 5.5 we can find that there is no significant difference in the average distance to head and tail from the droplet centre as well as the average number of 5CB atoms surrounding each part. Only a slight change is visible. We

cannot comment anything about how the cardiolipin-5CB interactions are changing when Cytochrome C is introduced.

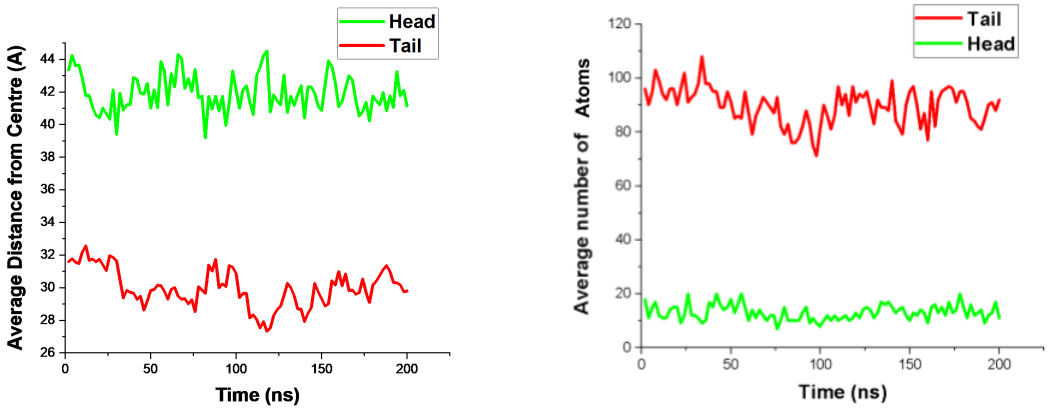

Figure 5.4: Plots showing the average number of 5CB atoms surrounding different parts of cardiolipin against time and the average distance from centre to cardiolipin atoms picked from different parts versus the time frames in the presence of Cytochrome C

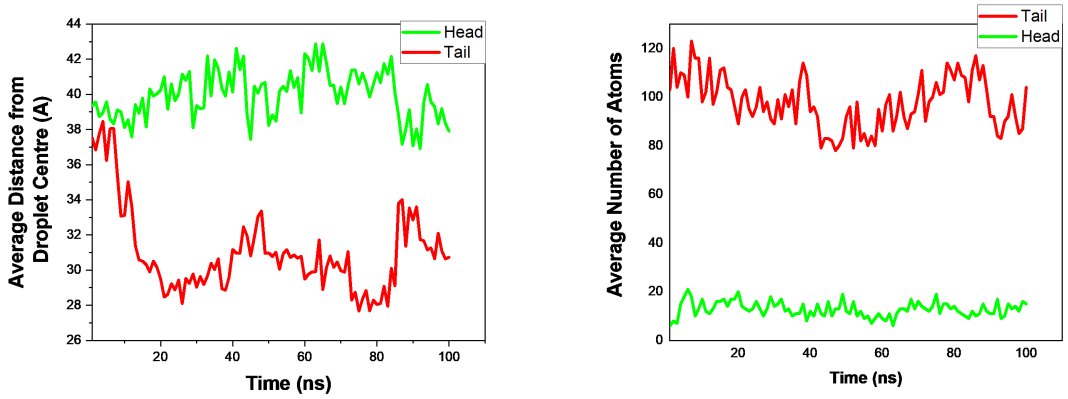

Figure 5.5: Plots showing the average number of 5CB atoms surrounding different parts of cardiolipin against time and the average distance from centre to cardiolipin atoms picked from different parts versus the time frames in the absence of Cytochrome C

5.3.3 Cardiolipin - Cytochrome C Interactions

It can be seen from fig. 5.6 that the part of cardiolipin that is interacting with Cytochrome C is the head. This conclusion can be drawn from both the time evolution plots as well as probability distribution function plots. The time evolution plot shows a contrast between the number of interactions with the head and tail per time frame.

Looking at the probability distributions, it can be observed that the mean number of interactions is 10 for tail while it is 200 for the head.

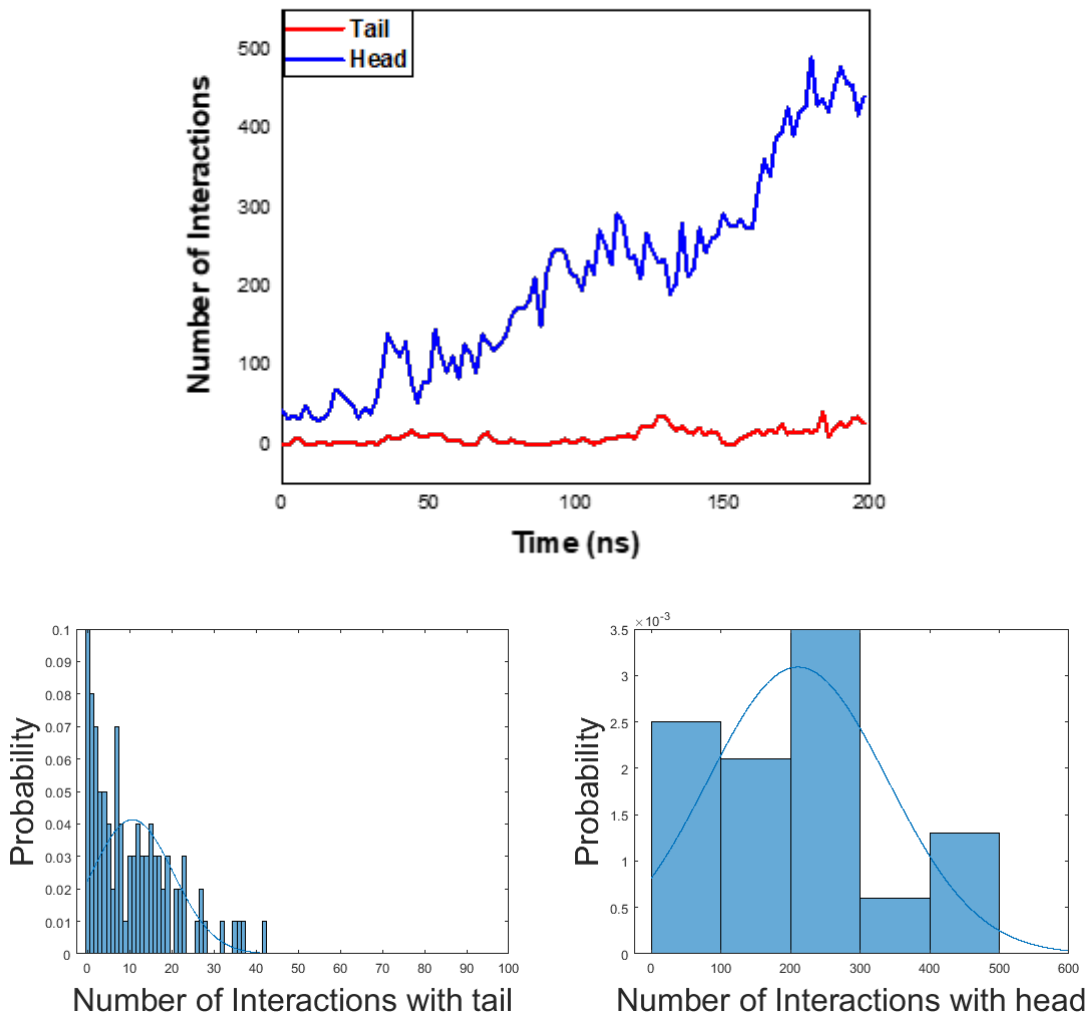

Figure 5.6: Plots showing the time evolution as well as probability distributions of Cytochrome C interactions with cardiolipin head and tail.

5.3.4 Heme - Cardiolipin Interactions

Heme is showing a significant number of interactions with cardiolipin head, despite the number of residues present per Cytochrome C molecule i.e. one residue per Cytochrome C. Fig. 5.7 shows the time evolution and probability distribution function of heme interactions with cardiolipin head and tail. The probability distributions show that the mean number of interactions is 0 for tail while it is 15 for the head. The

observations perfectly match with our hypothesis that the positively charged heme residue interacts with the polar negative head of the cardiolipin.

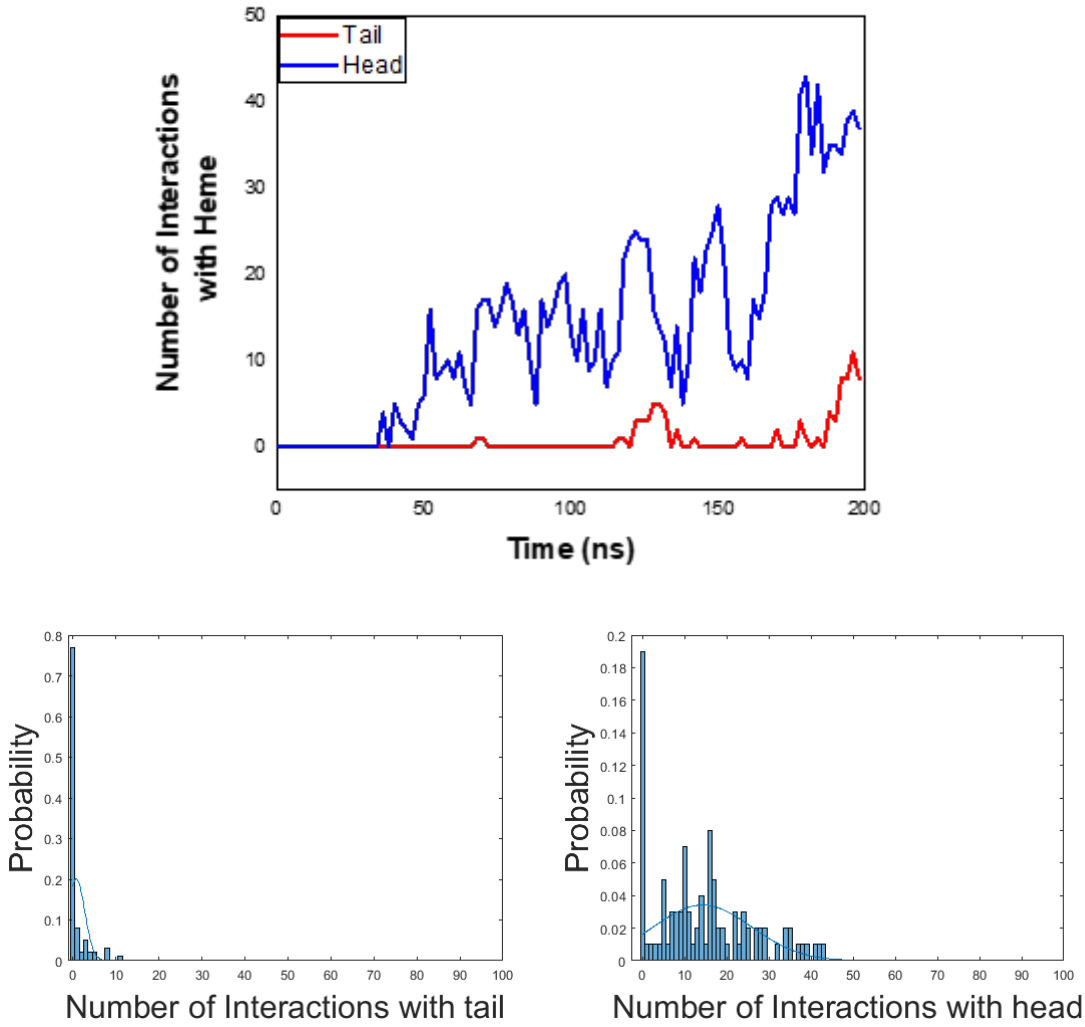

Figure 5.7: Plots showing the time evolution and the probability distributions of interactions between heme residue of Cytochrome C and cardiolipin - both head and tail.

5.3.5 Nature of Interactions and Electrostatic profile

Apart from heme, the interacting residues are lysine, isoleucine, threonine and glutamic acid. The probability distribution of the interaction with these residues is given in fig. 5.8 It can be observed that cardiolipin shows the most number of interactions with lysine, which is a basic (positively charged) amino acid. From this, we can conclude that the nature of interactions is electrostatic.

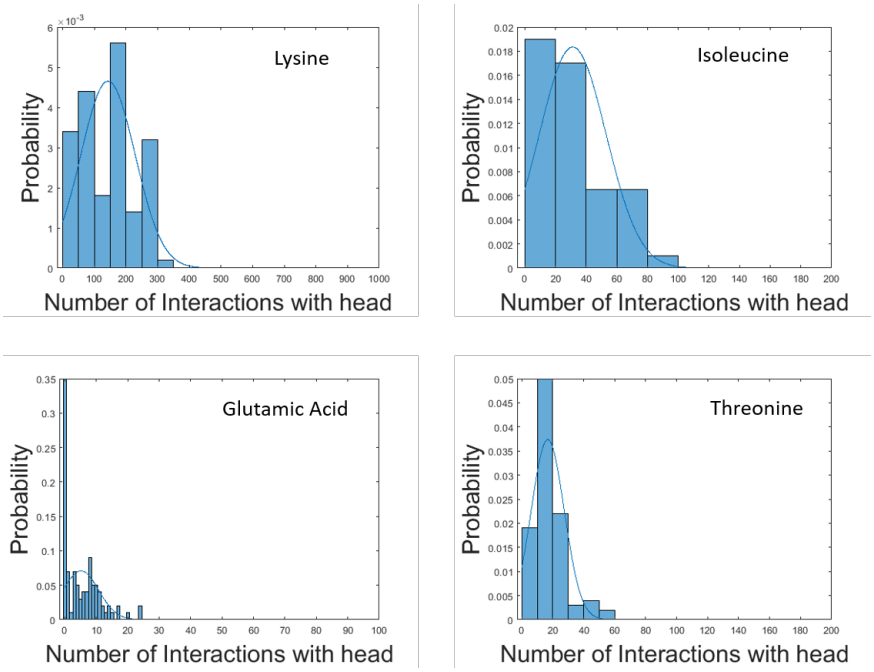

Figure 5.8: The probability distribution of the interactions of all the interacting residues with cardiolipin head

Fig. 5.9 shows the different regions of the electrostatic profile of Cytochrome C as well as the control, BSA along with the 5CB droplet coated with cardiolipin in detail. Figures 5.10 and 5.11 shows the time evolution of both the systems.

From fig. 5.10, it can be seen that the positively charged Cytochrome C is coming closer to the cardiolipin coated 5CB droplet, whose nature is mostly non-polar with a few polar negative parts.

Meanwhile, from fig. 5.11, we can see that the negatively charged BSA is staying more or less in the same position and is not coming closer to the cardiolipin coated 5CB droplet.

The comparison between the electrostatic profiles of Cytochrome C and BSA electrostatic profiles, further illustrates that the nature of interactions in this system is electrostatic.

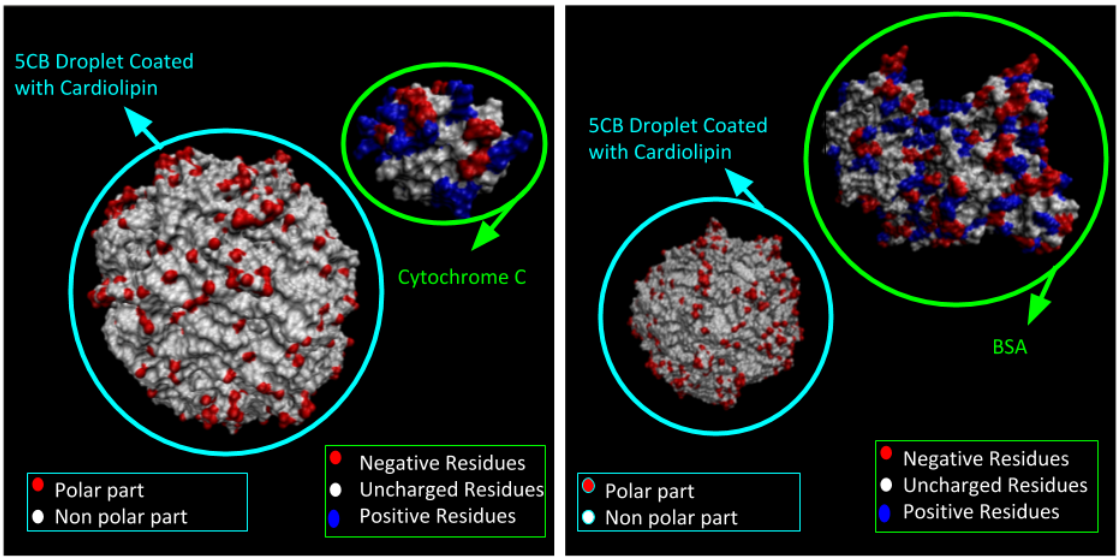

Figure 5.9: Inside the cyan oval is the 5CB droplet with cardiolipin on its surface: Polar part corresponds to P and O atoms in cardiolipin mostly (because cardiolipin covers most of the 5CB LC surface) and a very few N atoms in 5CB. Non-polar part corresponds to hydrocarbon chains in cardiolipin (mostly) and 5CB. Inside the green oval are the proteins Cytochrome C and BSA in the corresponding images: Blue patches correspond to positively charged and polar positive residues, red corresponds to negatively charged and polar negative residues and white corresponds to uncharged residues

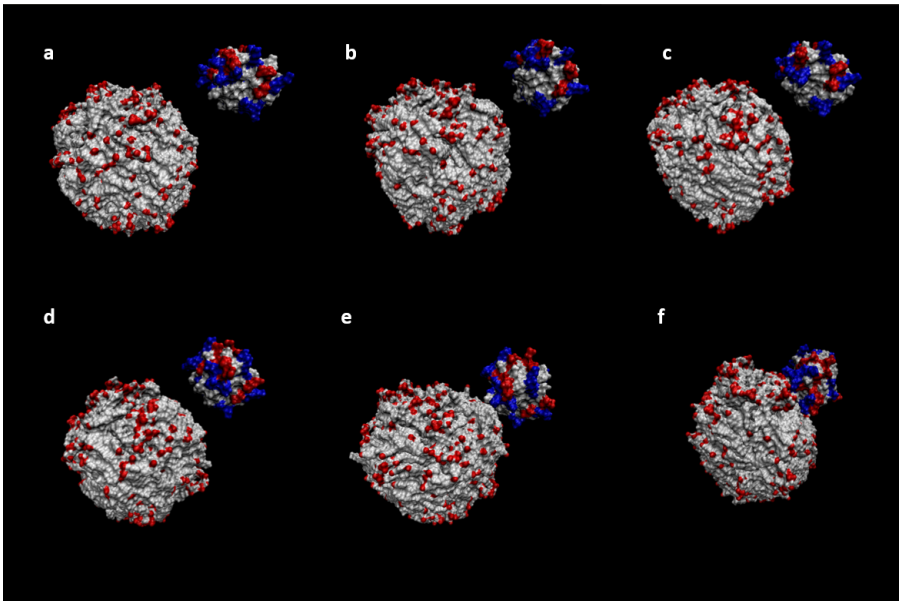

Figure 5.10: a-f)The time evolution of Cytochrome C with respect to the 5CB LC droplet coated with cardiolipin (the LC droplet along with cardiolipin on the left and Cytochrome C molecule on the right).

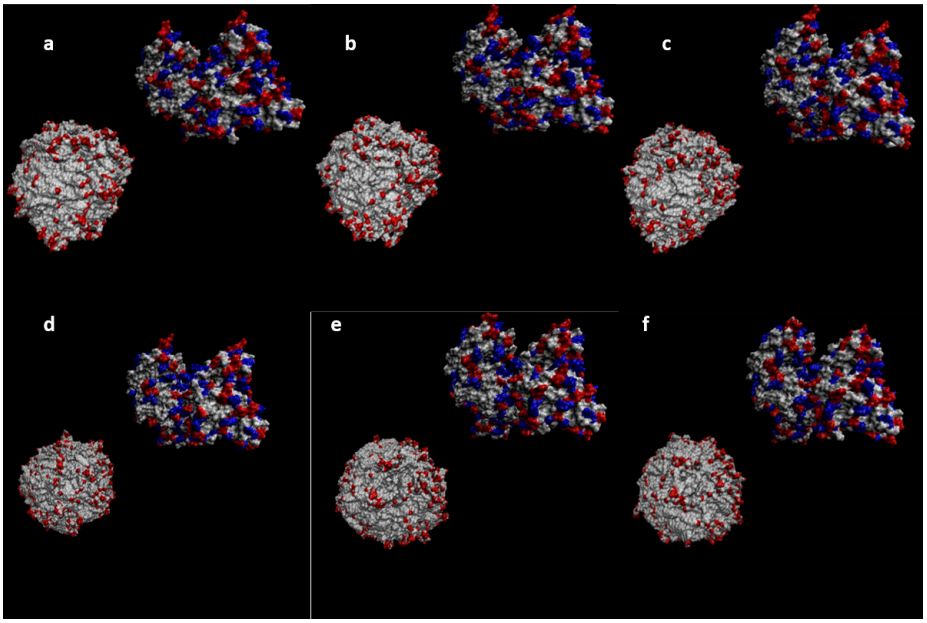

Figure 5.11: a-f)The time evolution of Cytochrome C with respect to the 5CB LC droplet coated with cardiolipin (the LC droplet along with cardiolipin on the left and Cytochrome C molecule on the right).

Chapter 6

Summary and Conclusions

6.1 Results

- In the first system, the liquid crystal droplets self assemble to form a compact droplet. Hydrophobic interactions are the main interactions happening in this system.

- In the second system, the cardiolipin inserts its tail into the 5CB droplets. The nature of interactions in this system is hydrophobic as well.

- In the third system, the anionic cardiolipin head interacts with the positively charged residues as well as the polar residues of Cytochrome C. Furthermore, the heme residue shows considerably strong interactions with cardiolipin head. The analyses done in this system reveals that the nature of interactions between cardiolipin and Cytochrome C is electrostatic.

6.2 Conclusions

- The computational studies can offer deeper insights into the biophysical and biochemical phenomena and paint a more precise picture, to the level of atomic-scale interactions.

- 5CB liquid crystals can be employed as a medium to study cardiolipin and cytochrome c interactions. Similarly, other liquid crystals can also be used for this purpose as they are good biomimetic systems.
- The same method which was used to model cardiolipin - Cytochrome C interactions can be employed to study other protein-lipid interactions as well.
- The cardiolipin - 5CB system may behave similarly in the presence of any other positively charged (basic) protein since the nature of interactions is electrostatic.

6.3 Future Works

- If the cardiolipin-5CB system behaves the same way it did with Cytochrome C in the presence of other positive proteins, we might be able to develop the system as a sensor for positive proteins. To do this, we need to look at the behaviour of the system in the presence of a good number of other proteins as well.
- Since the cardiolipin - Cytochrome C interactions were successfully modelled on a 5CB liquid crystal droplet, we wish to extend the study to other lipid-protein systems and look at all the systems that can be studied in this manner.

Bibliography

[Abel 10] Stéphane Abel, Marcel Waks & Massimo Marchi. *Molecular dynamics simulations of cytochrome c unfolding in AOT reverse micelles: The first steps.* The European Physical Journal E, vol. 32, no. 4, pages 399–409, 2010.

[Abraham 15] Mark James Abraham, Teemu Murtola, Roland Schulz, Szilárd Páll, Jeremy C Smith, Berk Hess & Erik Lindahl. *GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers.* SoftwareX, vol. 1, pages 19–25, 2015.

[Aguayo 12] Daniel Aguayo, Fernando D González-Nilo & Christophe Chipot. *Insight into the properties of cardiolipin containing bilayers from molecular dynamics simulations, using a hybrid all-atom/united-atom force field.* Journal of chemical theory and computation, vol. 8, no. 5, pages 1765–1773, 2012.

[Alder 57] Berni Julian Alder & Thomas Everett Wainwright. *Phase transition for a hard sphere system.* The Journal of chemical physics, vol. 27, no. 5, pages 1208–1209, 1957.

[Alvarez-Paggi 17] Damian Alvarez-Paggi, Luciana Hannibal, María A Castro, Santiago Oviedo-Rouco, Veronica Demicheli, Veronica Tortora, Florencia Tomasina, Rafael Radi & Daniel H Murgida. *Multifunctional cytochrome c: learning new tricks from an old dog.* Chemical reviews, vol. 117, no. 21, pages 13382–13460, 2017.

[Beales 11] Paul A Beales, Chris L Bergstrom, Nienke Geerts, John T Groves & T Kyle Vanderlick. *Single Vesicle Observations of the Cardiolipin- Cytochrome c Interaction: Induction of Membrane Morphology Changes.* Langmuir, vol. 27, no. 10, pages 6107–6115, 2011.

[Beglov 94] Dmitrii Beglov & Benoit Roux. *Finite representation of an infinite bulk system: solvent boundary potential for computer simulations.* The Journal of chemical physics, vol. 100, no. 12, pages 9050–9063, 1994.

[Belikova 06] Natalia A Belikova, Yury A Vladimirov, Anatoly N Osipov, Alexandr A Kapralov, Vladimir A Tyurin, Maksim V Potapovich, Liana V Basova, Jim Peterson, Igor V Kurnikov & Valerian E Kagan. *Peroxidase activity and structural transitions of cytochrome c bound to cardiolipin-containing membranes.* Biochemistry, vol. 45, no. 15, pages 4998–5009, 2006.

[Berendsen 84] Herman JC Berendsen, JPM van Postma, Wilfred F van Gunsteren, ARHJ DiNola & Jan R Haak. *Molecular dynamics with coupling to an external bath.* The Journal of chemical physics, vol. 81, no. 8, pages 3684–3690, 1984.

[Bienvenüe 94] Alain Bienvenüe & Josette Sainte Marie. *Modulation of protein function by lipids.* In Current topics in membranes, volume 40, pages 319–354. Elsevier, 1994.

[Bisoyi 11] Hari Krishna Bisoyi & Sandeep Kumar. *Liquid-crystal nanoscience: an emerging avenue of soft self-assembly.* Chemical Society Reviews, vol. 40, no. 1, pages 306–319, 2011.

[Bussi 07] Giovanni Bussi, Davide Donadio & Michele Parrinello. *Canonical sampling through velocity rescaling.* The Journal of chemical physics, vol. 126, no. 1, page 014101, 2007.

[Corradi 18] Valentina Corradi, Eduardo Mendez-Villuendas, Helgi I Ingolfsson, Ruo-Xu Gu, Iwona Siuda, Manuel N Melo, Anastassiia Mous-

satova, Lucien J DeGagne, Besian I Sejdiu, Gurpreet Singh*et al.* *Lipid–protein interactions are unique fingerprints for membrane proteins.* ACS central science, vol. 4, no. 6, pages 709–717, 2018.

[Dahlberg 08] Martin Dahlberg & Arnold Maliniak. *Molecular dynamics simulations of cardiolipin bilayers.* The Journal of Physical Chemistry B, vol. 112, no. 37, pages 11655–11663, 2008.

[Darden 93] Tom Darden, Darrin York & Lee Pedersen. *Particle mesh Ewald: An N log (N) method for Ewald sums in large systems.* The Journal of chemical physics, vol. 98, no. 12, pages 10089–10092, 1993.

[Dykens 07] JA Dykens. *Comprehensive Medicinal Chemistry II.* 2007.

[Gupta 98] Vinay K Gupta, Justin J Skaife, Timothy B Dubrovsky & Nicholas L Abbott. *Optical amplification of ligand-receptor binding using liquid crystals.* Science, vol. 279, no. 5359, pages 2077–2080, 1998.

[Hanske 12] Jonas Hanske, Jason R Toffey, Anna M Morenz, Amber J Bonilla, Katherine H Schiavoni & Ekaterina V Pletneva. *Conformational properties of cardiolipin-bound cytochrome c.* Proceedings of the National Academy of Sciences, vol. 109, no. 1, pages 125–130, 2012.

[Hess 97] Berk Hess, Henk Bekker, Herman JC Berendsen & Johannes GEM Fraaije. *LINCS: a linear constraint solver for molecular simulations.* Journal of computational chemistry, vol. 18, no. 12, pages 1463–1472, 1997.

[Hess 08] Berk Hess. *P-LINCS: A parallel linear constraint solver for molecular simulation.* Journal of chemical theory and computation, vol. 4, no. 1, pages 116–122, 2008.

[Hough 14] Michael A Hough, Gary Silkstone, JAR Worrall & Michael T Wilson. *NO Binding to the Proapoptotic Cytochrome c–Cardiolipin*

Complex. In Vitamins & Hormones, volume 96, pages 193–209. Elsevier, 2014.

[Huang 13] Jing Huang & Alexander D MacKerell Jr. *CHARMM36 all-atom additive protein force field: Validation based on comparison to NMR data*. Journal of computational chemistry, vol. 34, no. 25, pages 2135–2145, 2013.

[Humphrey 96] William Humphrey, Andrew Dalke & Klaus Schulten. *VMD – Visual Molecular Dynamics*. Journal of Molecular Graphics, vol. 14, pages 33–38, 1996.

[Jorgensen 83] William L Jorgensen, Jayaraman Chandrasekhar, Jeffry D Madura, Roger W Impey & Michael L Klein. *Comparison of simple potential functions for simulating liquid water*. The Journal of chemical physics, vol. 79, no. 2, pages 926–935, 1983.

[Lemmin 13] Thomas Lemmin, Christophe Bovigny, Diane Lancon & Matteo Dal Peraro. *Cardiolipin models for molecular simulations of bacterial and mitochondrial membranes*. Journal of chemical theory and computation, vol. 9, no. 1, pages 670–678, 2013.

[Manickam 17] Pandiaraj Manickam, Ajeet Kaushik, Chandran Karunakaran & Shekhar Bhansali. *Recent advances in cytochrome c biosensing technologies*. Biosensors and Bioelectronics, vol. 87, pages 654–668, 2017.

[MATLAB 19] MATLAB. version 7.10.0 (r2019a). The MathWorks Inc., Natick, Massachusetts, 2019.

[Pani 18] Ipsita Pani, Diksha Sharma & Santanu Kumar Pal. *Liquid Crystals as Sensitive Reporters of Lipid-Protein Interactions*. General Chemistry, vol. 4, no. 2, 2018.

[Paquet 15] Eric Paquet & Herna L Viktor. *Molecular dynamics, monte carlo simulations, and langevin dynamics: a computational review*. BioMed research international, vol. 2015, 2015.

[Sidiq 15] Sumyra Sidiq, Indu Verma & Santanu Kumar Pal. *pH-driven ordering transitions in liquid crystal induced by conformational changes of cardiolipin.* Langmuir, vol. 31, no. 16, pages 4741–4751, 2015.

[Simonson 95] Thomas Simonson & David Perahia. *Microscopic dielectric properties of cytochrome c from molecular dynamics simulations in aqueous solution.* Journal of the American Chemical Society, vol. 117, no. 30, pages 7987–8000, 1995.

[Sinibaldi 10] Federica Sinibaldi, Barry D Howes, Maria Cristina Piro, Fabio Polticelli, Cecilia Bombelli, Tommaso Ferri, Massimo Coletta, Giulietta Smulevich & Roberto Santucci. *Extended cardiolipin anchorage to cytochrome c: a model for protein–mitochondrial membrane binding.* JBIC Journal of Biological Inorganic Chemistry, vol. 15, no. 5, pages 689–700, 2010.

[Souza 08] Jose M Souza, Laura Castro, Adriana María Cassina, Carlos Batthyany & Rafael Radi. *Nitrocytochrome c: synthesis, purification, and functional studies.* In Methods in enzymology, volume 441, pages 197–215. Elsevier, 2008.

[Tamm 06] Lukas K Tamm. Protein-lipid interactions: from membrane domains to cellular networks. John Wiley & Sons, 2006.

[Tsige 99] Mesfin Tsige, Milind P Mahajan, C Rosenblatt & PL Taylor. *Nematic order in nanoscopic liquid crystal droplets.* Physical Review E, vol. 60, no. 1, page 638, 1999.

[Varshney 94] Amitabh Varshney, Frederick P Brooks & William V Wright. *Computing smooth molecular surfaces.* IEEE Computer Graphics and Applications, vol. 14, no. 5, pages 19–25, 1994.

[Wang 01] Zhiqiang Wang, James A Lupo, Soumya Patnaik & Ruth Pachter. *Large scale molecular dynamics simulations of a 4-n-pentyl-4-cyanobiphenyl (5cb) liquid crystalline model in the bulk and as a*

droplet. Computational and Theoretical Polymer Science, vol. 11, no. 5, pages 375–387, 2001.

[Wendoloski 87] JJ Wendoloski, James B Matthew, PC Weber & FR Salemme. *Molecular dynamics of a cytochrome c-cytochrome b5 electron transfer complex*. Science, vol. 238, no. 4828, pages 794–797, 1987.

[Yang 07] Fang Yang, Bing-Rui Zhou, Peng Zhang, Yan-Feng Zhao, Jie Chen & Yi Liang. *Binding of ferulic acid to cytochrome c enhances stability of the protein at physiological pH and inhibits cytochrome c-induced apoptosis*. Chemico-biological interactions, vol. 170, no. 3, pages 231–243, 2007.

[Yeagle 16] Philip L Yeagle. The membranes of cells. Academic Press, 2016.