

Introduction

Lipid bilayers are fundamental components of biological membranes. They construct the structure of cellular organelles [Gutknecht and Walter, 1981] and serve as barriers to the passage of biological components into the cytoplasm. Preservation of the mobility and fluidity is a primary requisite of the cell membrane to carry out their functions, including the passive transport of chemicals needed for biological processes [Okur *et al.*, 2019]. The interactions between lipid polar head-groups and water molecules are critical for bilayer fluidity-mediated trafficking and metabolic processes [Ingólfsson *et al.*, 2014; Van Meer *et al.*, 2008; Cyran *et al.*, 2018; Chen *et al.*, 2010]. In recent years, several studies have shown that interfacial water molecules (IW) have a considerable impact on the physical properties of membranes [Debnath *et al.*, 2014; He and Maibaum, 2017; Berger *et al.*, 1997]. In this thesis, we explore the influence of structure and dynamics of the IW on the lateral organizations of membranes at various phases.

1.1 BASIC UNDERSTANDING OF CELL MEMBRANE

1.1.1 Cell

In life, cell is the most vital unit. In the year 1665, Hooke made the discovery that the tissue of a cork plant is divided into small compartments, which he termed as cells. In 1840, better observations of different tissues led to the idea that all organisms are made up of single cells or groups of cells. This hypothesis is proven after more than a century of research. One may see a eukaryotic cell as a factory where several activities take place. The cytosol, a semi-fluid, is enclosed within the cell membrane, which surrounds the cell (see figure 1.1) [Mathews and van Holde, 1990; Stryer, 1995]. The majority of the cells in higher organisms are functionally specific. Despite their various functions, they are comprised of the same lipids, proteins, nucleic acids, and polysaccharides. The cell membrane is more than just a barrier. The model of a eukaryotic cell membrane, developed by Singer and Nicolson, is known as the "Fluid Mosaic Model" [Singer and Nicolson, 1972]. The model characterises the cell membrane as a two-dimensional liquid that prevents membrane components from diffusing outward in a lateral direction (figure 1.2). The lipids organized in a bilayer provide the framework of the membrane. The bilayer serves as a solvent and a permeability barrier for the integral membrane proteins.

1.1.2 Lipids and bilayers

Lipids are the fundamental building blocks of a bilayer. Hydrophilic head groups of the lipids are preferentially associated with water whereas hydrophobic tail groups repel water. Lipid molecules are amphipathic. Due to their amphipathic nature, lipids group together in water, a process known as self-assembly. In contrast to the hydrophobic components, the hydrophilic head groups are in direct contact with the water. The lipid bilayer is one of the several macrostructures

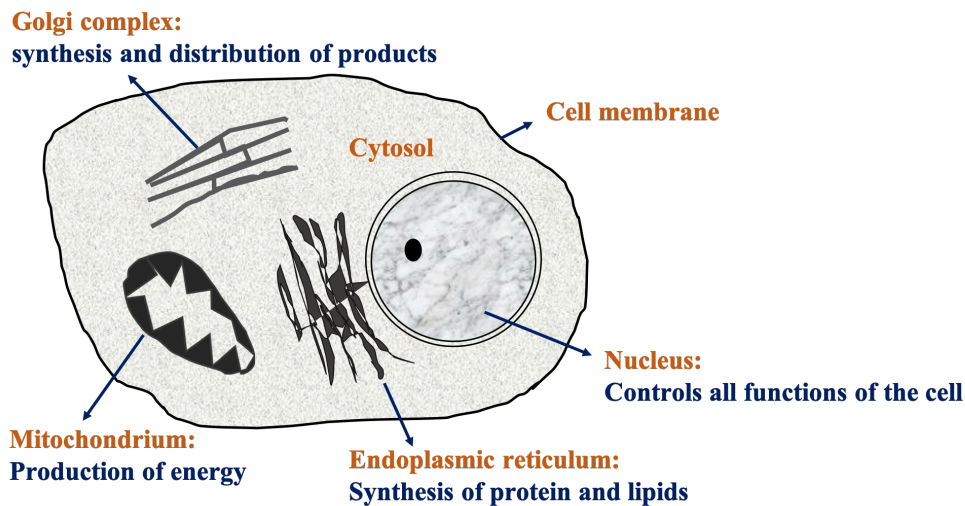


Figure 1.1 : Schematic diagram of a eukaryotic cell showing the most typical organelles.

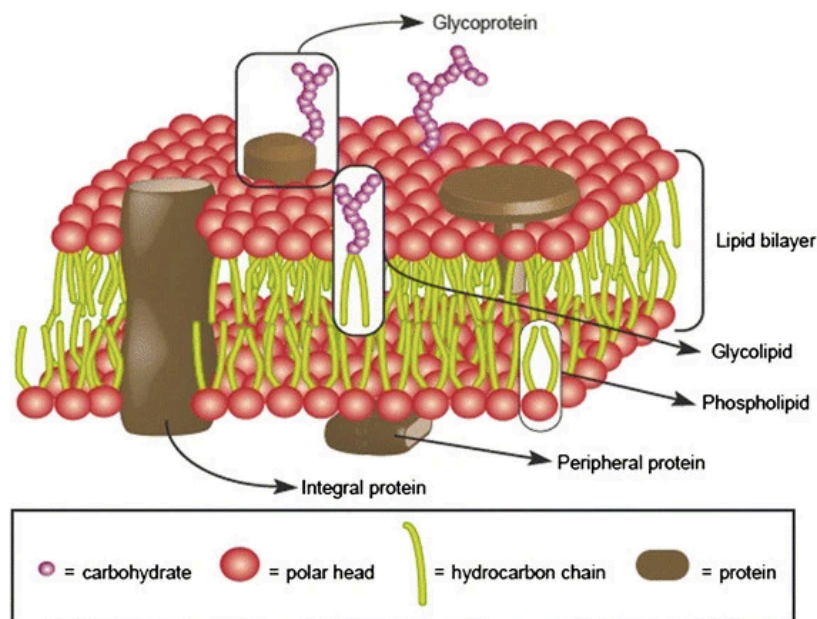


Figure 1.2 : The general representation of a cell membrane [Lombard, 2014]

that is developed based on the lipid shape and water content (figure 1.3) [Cullis and De Kruijff, 1979; Damodaran and Merz Jr, 1994; Israelachvili, 1997]. The formation of lipid bilayers is a rapid and spontaneous process that is fundamentally driven by hydrophobic interactions (i.e., the hydrophobic effect). Van der Waals forces that exist between the tails also promote tight packing. Finally, the hydrogen bonds by the head groups and electrostatic interactions stabilise the lipid bilayers [Israelachvili, 1997].

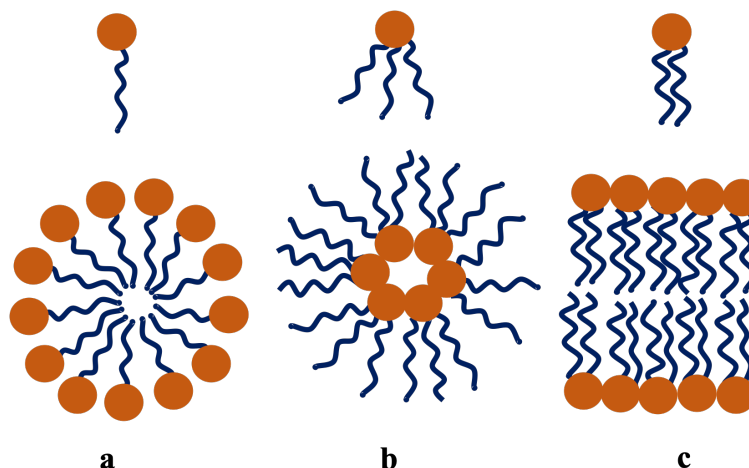


Figure 1.3 : Self assembly of lipids in water to form: (a) micelle, (b) inverse micelle, and (c) bilayer. The wavy lines: hydrophobic tails, and the filled circles: hydrophilic head groups.

1.1.3 Phase transitions of lipid bilayers

The most prevalent lipids in biological membranes are phospholipids (figure 1.4) which have phosphatidylcholines (PCs) where tails can be unsaturated or saturated. The lipid head group is composed of a polar group (choline), a glycerol, and a phosphate group. Bilayers made up of phosphatidylcholines undergo phase transitions from a gel or L_β phase to a ripple or P_β phase to a fluid or L_α phase upon heating [Nagle and Tristram-Nagle, 2000; Koynova and Caffrey, 1998; Khakbaz and Klauda, 2018].

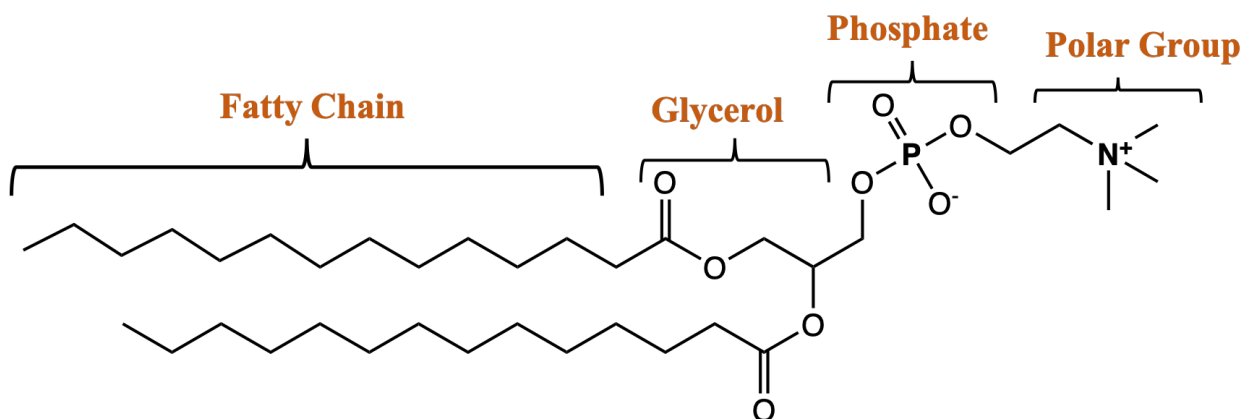


Figure 1.4 : Chemical formula of a Dimyristoylphosphatidylcholine (DMPC) lipid.

Figure 1.5 schematically depicts the phase transition of a bilayer, from a gel to a ripple to a fluid phase on heating or vice versa upon cooling. Pre-transition refers the transition from the L_β to the P_β phase that occurs when the temperature increases. The primary transition, often referred to as the chain order/disorder transition, brings the bilayer to a fluid phase or liquid crystalline (L_α) phase at the end of the process. The transition from this phase to the (L_α) phase is known as the bilayer melting because the hydrocarbon chains in this phase become disorganised. The transition temperatures are lipid composition dependent. As the tail length

increases (see table 1.1), the temperatures of the pre and main transitions rise, but they drop as the head group hydration and unsaturation of the alkyl chain increase. The degree of the tail unsaturation can have an impact on the transition temperature in addition to the chain length. The alkane chain may kink as a result of an unsaturated double bond, upsetting the periodic structure. Due to the disruption, the bilayer has more free space, which gives the neighbouring chains more flexibility. Lower transition temperatures are caused by this disturbance of packing, which decreases as the double bond strength increases. When the bilayer interacts with substances like proteins, cations, salts, or small molecules, the transition temperature shifts. Through steric interactions

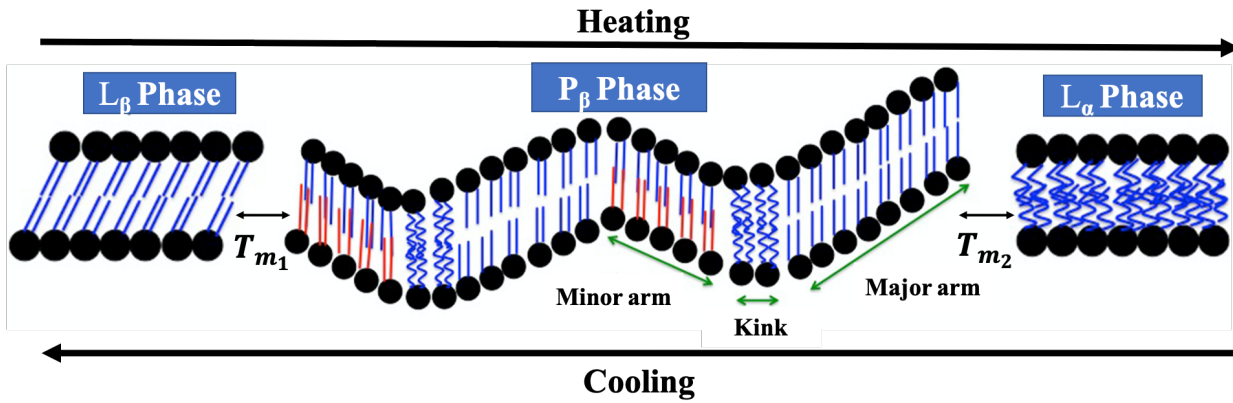


Figure 1.5 : Schematic diagrams of phase transitions of lipid bilayers due to heating or cooling: gel (L_β), ripple (P_β), and fluid (L_α) [Khakbaz and Klauda, 2018]. Pre-transition and main-transition are denoted by the symbols T_{m_1} and T_{m_2} , respectively.

amongst packed proteins, high local concentrations of membrane-associated proteins can lower the T_m of the membrane. When the hydrophobic region of a protein is exposed to water, the protein

Table 1.1 : Depending on the number of carbon atoms (n_c) in the hydrocarbon chains, different phosphatidylcholines (PCs) have different pre transition (T_{m_1}) and main transition (T_{m_2}) temperatures (in $^{\circ}\text{C}$)[Gennis, 1989].

Lipids	n_c	T_{m_1}	T_{m_2}
DMPC	14	15.3	24.0
DPPC	16	35.3	41.5
DSPC	18	51.2	54.3
DAPC	20	62.1	64.1

will become dysfunctional. Moreover, the hydration of a cell membrane is a key factor affecting how well it functions [Pal and Chattopadhyay, 2021]. The water close to the membranes controls the transit of drugs and small molecules through the cell, creation of membrane rafts, molecular recognition, and signal transduction. A growing understanding of the complex role that water molecules play in determining the composition and operation of cellular membranes is important [Pal and Chattopadhyay, 2021]. Water molecules influence the structure of membranes by forcing lipids self-assemble into membrane bilayers. Thus, understanding the behaviour (structure and dynamics) of lipid bilayers or model membranes is essential to our comprehension of how cells work.

1.2 WATER NEAR MEMBRANES

Water molecules carry out numerous biological operations of cells by forming an extensive hydrogen bond network structure [Tarek and Tobias, 2002; Chen *et al.*, 2008; Frauenfelder *et al.*, 2009; Okur *et al.*, 2019; Lawrence and Skinner, 2003; Srivastava *et al.*, 2019b; Pal *et al.*, 2003; Furse and Corcelli, 2008]. Apart from controlling membrane functions and helping protein folding and unfolding, it plays a vital role in brain physiological function by generating water channels that allow water to travel through the cell membranes of brain cells [Borgnia *et al.*, 1999]. Therefore, water is essential in forming a dynamic link between biological macro-molecules and their surroundings. Water molecules do not exist in bulk form in living organisms. Water residing near cells, proteins, DNA grooves, and so on are referred to as interfacial water (IW). Figure 1.6 shows a pictorial representation of IW near a model membrane.

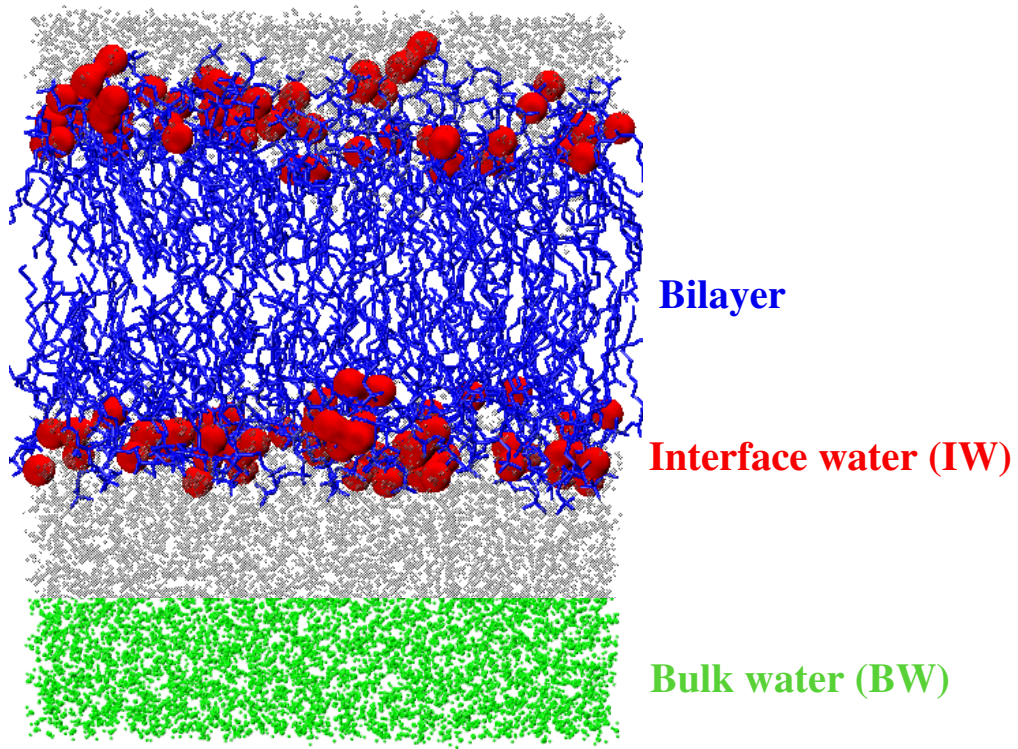


Figure 1.6 : Two water regions: Interface water (IW) (Red: QuickSurf), and Bulk water (Green: VDW) near a simulated hydrated DMPC lipid bilayer (Blue: licorice).

Properties of the IW are different than the bulk [Das *et al.*, 2013; Pal and Chattopadhyay, 2021]. According to molecular dynamics simulations, IW near lipid head-groups strongly correlate to phospholipid bilayers. The existence of hydrogen bonding and chemical confinement of interfacial water near lipid heads causes interfacial fluids to slow down in their movements [Srivastava and Debnath, 2018]. The study of the interactions of the lipid bilayer with macromolecules in the presence of water is useful in a variety of applications, including drug delivery. The dynamic coupling between bilayers is mediated by hydrogen bonding between lipid-water-lipid [Pronk *et al.*, 2015]. The first step in determining how the IW influences various biological, chemical, and physical processes is to understand the correlation of water and membrane dynamics and their influence to the membrane lateral organizations. The macroscopic topology of membranes changes as hydration levels increase [Koster *et al.*, 1994; Stachura *et al.*, 2019]. Membranes can

change their phases in response to hydration levels. Since membrane organization is related to their biological functions, establishing the correlation between the structure and dynamics of the IW and membrane will enable us to connect the activity of biomolecules with the structure and dynamics of the IW. This will help in developing and creating novel medications for boosting specific chemical processes [Jung and Marcus, 2007]. As the membrane organization is a direct consequence of their phases, a summary on the literature review on the membrane phases and the IW dynamics has been presented below.

1.2.1 Membrane phase behavior

The dynamics and the structure of the membrane and the IW are measured using a variety of experimental approaches mainly like Vibrational Sum Frequency Generation (VSFG), Infrared spectroscopy (IR), X-ray Diffraction analysis (XRD), Magnetic Resonance (NMR) spectroscopy, Quasi Elastic Neutron Scattering (QENS) spectroscopy, Differential Scanning Calorimetry (DSC), Scanning Tunneling Microscopy (STM), Overhauser Dynamic Nuclear Polarisation (ODNP), Atomic Force Microscopy (AFM) and so on. ^1H NMR spectroscopy is used to analyse membrane phase transitions across a wide range of temperatures, which can aid in the development of membrane-protective cryoprotectants and the research of freezing-tolerant species [Wolfe and Bryant, 1999; Mandal and van der Wel, 2016]. The phase behavior of the lipid membrane is studied using high sensitivity DSC [Mabrey and Sturtevant, 1976]. STM is first used to determine the structure of the DMPC bilayer in the P_β phase. Different phases of the lipid bilayer and their transitions are investigated using different experimental techniques [Tristram-Nagle *et al.*, 2002; Akabori and Nagle, 2015; Findlay and Barton, 1978; Jørgensen *et al.*, 1993]. Membrane related protein topology, structure, and immersion depth, are probed using the IW using ODNP-enhanced NMR relaxometry investigations [Cheng *et al.*, 2013]. The structure and dynamics of lipid bilayers, as well as the causes of water orientational relaxation rates, are studied using QENS and NMR spectroscopic methods. These spectroscopic investigations with great resolution can look for hydration water near membranes and membrane proteins.

1.2.2 Slow relaxation of water near membranes

Water near membranes affect the relaxation rates of lipids [Molugu *et al.*, 2018]. AFM investigations using frequency modulation reveal the presence of two hydration layers near supported dipalmitoylphosphatidylcholine bilayers [Fukuma *et al.*, 2007]. The influence of hydration numbers on DMPC bilayers above and below the primary phase transition temperature is measured using neutron scattering studies [Trapp *et al.*, 2010]. The location of hydration water near the polar head-group, as well as at the carbonyl and glycerol moieties of the lipid membrane, is revealed by ^{13}C NMR [Volke *et al.*, 1994; Volke and Pampel, 1995]. Columnar order in liquid crystalline phases of stacked lipid bilayers has been seen due to a link between two-dimensional intralayer domain development and interlayer smectic ordering aided by solvents [Tayebi *et al.*, 2012]. During dehydration, an increasing proportion of well-structured, low density network water around the POPC bilayer is discovered using IR spectroscopy [Binder, 2007]. As the hydration of water diminishes, a slowing in orientational relaxation of water near artificial multibilayers of the phospholipid dilauroyl phosphatidylcholine DLPC has been detected using ultrafast polarisation selective vibrational spectroscopy [Zhao *et al.*, 2008]. Using QENS, multilamellar DMPC bilayers are investigated with very low to moderate hydration levels across the gel to liquid phase transition of the bilayers [Swenson *et al.*, 2008]. At a very low temperature, 260 K, mainly rotational

water dynamics is observed which changes to a translational motion at 290 K, slightly below the gel-to-liquid transition. Experiments with VSFG spectroscopy are carried out to gain molecular level information on water present at membrane interfaces [Mondal *et al.*, 2012]. The high sensitivity of VSFG, vibrational spectra of water at the lipid interface may be obtained, providing information on the structure of the IW. However, NMR signals are naturally modest in intensity due to the low magnetic moment. As a result, NMR cannot identify substances in micromolar quantities. The majority of NMR equipment is motion sensitive, resulting in signal distortions during quantitative measurements [Spiess, 2017; Chatham and Blackband, 2001; Emwas, 2015]. Cell-NMR is a strong tool for analysing the dynamics of cell membranes at atomic levels in theory. However, it has a few drawbacks like the cell life span within the NMR tube is insufficient for obtaining high-quality relaxation data, and protein interactions contaminate relaxation time scales, resulting in erroneous findings [Li and Liu, 2013]. In order to quantify any frequency dependent time-correlation trait, it is necessary to consider these effects while analysing the structure and dynamics of water [Schmidt *et al.*, 2005]. As a result, accurately probing water dynamics becomes challenging [Yamada *et al.*, 2017]. Molecular dynamics (MD) simulations can overcome some of the shortcomings of the experimental techniques which are generally versatile, otherwise.

Understanding many fundamental biochemical phenomena requires atomic-level explanation of the membrane water interface [Berger *et al.*, 1997]. The atomic level trajectory is routinely obtained using MD simulations. Figure 1.7 provides the flow chart of the steps generally followed in a typical MD simulation. MD simulations have been widely utilised to deduce how cell membranes behave in various biological processes [Patra *et al.*, 2003]. To understand the function of cell membrane, different phases of bilayers that are studied using MD simulations [Tu *et al.*, 1996; de Meyer *et al.*, 2010; Högberg and Lyubartsev, 2006]. Depending on the amount of resolution and the time scale required to comprehend the phase transitions, coarse-grained (CG) and all-atom (AA) molecular simulations will be employed to study the distinct phases of lipid bilayers [Lee and Larson, 2006; Stouch, 1993]. The amount of hydration has a significant impact on membrane structural features such as bilayer thickness, order parameter, area per lipid, order parameter, diffusion, reorientation, and so on. Membranes tend to approach a more ordered form at low hydration levels or low temperatures, when their acyl chains stiffen and the alignment of the lipid head-group goes parallel to the membranes' plane. The degree of hydration is the most important need for membranes to operate properly, followed by the influence of temperature. Although major activities of membranes, such as hosting trans-membrane proteins, exchanging biomolecules for transport, and medication administration, are important at fully hydrated states, a wide range of species may exist in anhydrobiosis for lengthy periods of time [Tan *et al.*, 2015]. To understand the orientation of water molecules at the bilayer surface when the hydration number changes, the reorientational auto-correlation function (RACF) is computed. From high to low water content, the water relaxation time slows down [Högberg and Lyubartsev, 2006; Calero *et al.*, 2016]. IW near the phospholipid bilayer is tightly connected to the polar groups of the lipid bilayer via hydrogen bonds (HBs) [Calero and Franzese, 2019]. The suitable force field should be chosen in order to capture phase transitions across an appropriate temperature range. In AA-MD [Aoun *et al.*, 2016; Pluhackova *et al.*, 2016; Ogata and Nakamura, 2015], the AMBER and CHARMM lipid force fields have been utilised to investigate lipid phase transitions, while the Martini force field is often employed in CG-MD [Marrink *et al.*, 2007]. The transition phases of the DPPC and DPPE bilayers are investigated using AA-MD [Leekumjorn and Sum, 2007]. Surprisingly, a drop in hydration levels has a significant impact on lipid dynamics, but only a little impact on structural features [Högberg and Lyubartsev, 2006; Zhao *et al.*, 2008]. Temperature, ionic strength, pH, salt content, and other environmental conditions have an impact on membrane characteristics. Among these factors, effect of temperature [Veatch and Keller, 2005; Blicher *et al.*, 2009], pressure [Chen *et al.*, 2011], etc. have been investigated exhaustively over the past few decades. The transport properties of small molecules (steroids, glycerols, drugs) [Orbach and Finkelstein, 1980; Majumdar *et al.*, 2004], etc.

are strongly affected by local orientational structure of water near membranes [Yang and Hinner, 2015]. The reorientation of water at membrane surfaces differs from that of the bulk water. IW in the vicinity of membrane head-group areas has a preference for lipid heads. All the discussed literature clearly show that the dynamics of the IW near membranes slows down compared to the BW. The slow relaxation originates from dynamical heterogeneity [Srivastava *et al.*, 2019a].

1.2.3 Confined fluid as an alternative for supercooled liquid

In the fields of biology, chemistry, geology, and others where system features are heavily dependent on water concentration and its structural and dynamical characteristics, the study of confined water has taken on a remarkable amount of significance. The fundamental understanding on the structure and dynamics of the IW or confined water is of tremendous importance to understand supercooled water in no man's land as confined water refuses to freeze in a crystalline state, as demonstrated by supercooled water. Thus water confinement has been widely used for exploiting features of bulk water at supercooled temperatures. It has been suggested that water contained in small volumes, such as protein-binding pockets, tiny membrane pores, membrane protein channels, etc., might help in acquiring a deeper understanding of the behavior of bulk water in deep supercooled areas [Rasaiah *et al.*, 2008; Li *et al.*, 2013]. This is due to the fact that enclosed water can outperform the crystallisation process [Capponi *et al.*, 2011; Cervený *et al.*, 2016]. Supercooled bulk water and water contained in silicon substrate have been compared using experimental techniques including neutron diffraction and neutron scattering. Both the fluids display comparable patterns on increased hydrogen bonding strength and oxygen-oxygen distance in the first hydration shell. These results demonstrate that supercooled bulk water may be studied using restricted water as a model [Ricci *et al.*, 2009]. Biological confinement and supercooled water both show comparable patterns in a slowdown in local structural relaxations, according

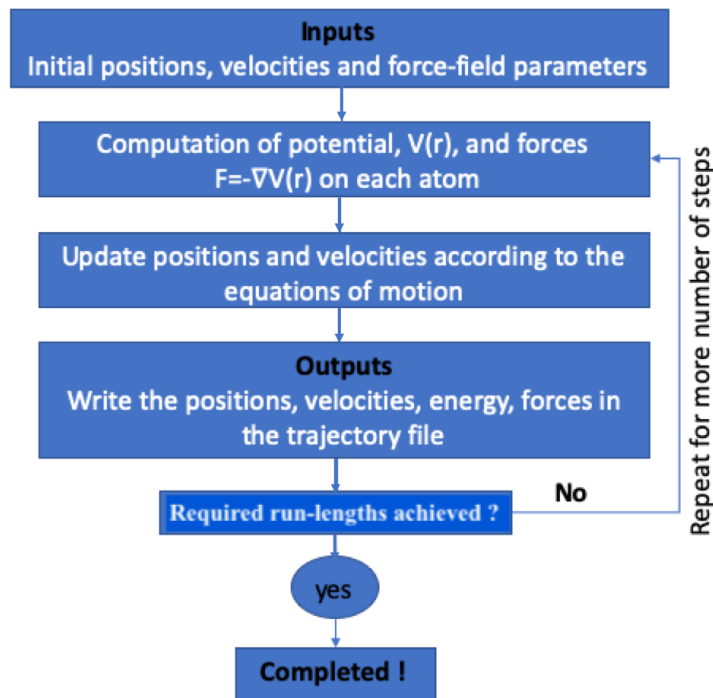


Figure 1.7 : Flowchart illustrating the steps followed in the MD simulations.

to computer models. The hydrogen bonding network rearrangement in both fluids is seen to be slowed down over a comparable time period [Capponi *et al.*, 2019]. Lipids and proteins, as essential components of biological cell membranes, exhibit a significant degree of freedom for different kinds of motions including lateral long-range mobilities. Compared to the bulk water, the diffusion of the confined water is retarded by a factor of four to seven [Knapp and Muegge, 1993; Sedlmeier *et al.*, 2011]. Dynamical heterogeneity controls the microscopic process of lipid organisation by dehydration [Malik and Debnath, 2021].

1.3 WATER SUPERCOOLING

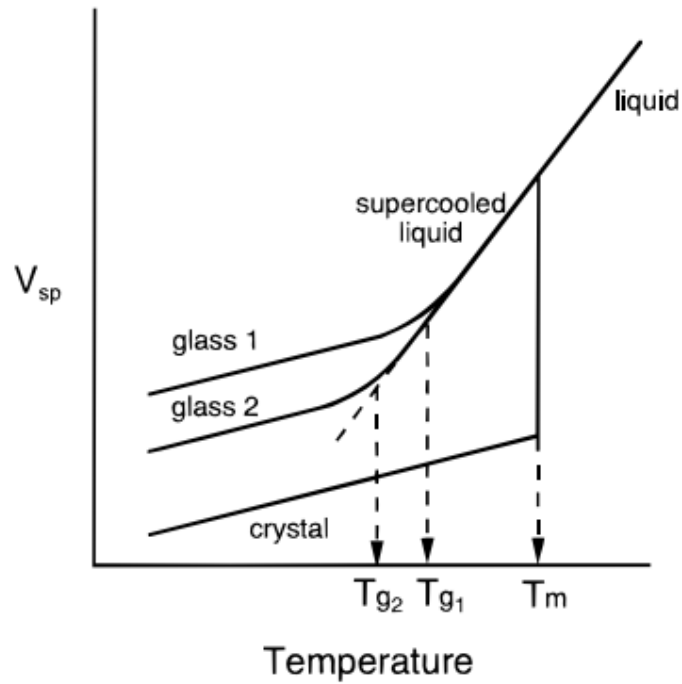


Figure 1.8 : Specific volume (V_{sp}) vs Temperature of a liquid that can crystallise and turn into glass. The cooling rate affects both the thermodynamic and dynamic features of a glass; glass 2 was made at a lower cooling rate than glass 1. T_g can be determined by extending the V_{sp} in the glassy state back to supercooled liquid. T_g is influenced by the rate of cooling [Ediger *et al.*, 1996]

Figure 1.8 shows the specific volume V_{sp} of a typical liquid as a function of temperature; when a liquid is cooled below its freezing temperature (T_f), it crystallizes via a first-order phase transition to form ice. In supercooled conditions, water cools quickly to remain liquid even at temperatures below its freezing point. Water existing in the liquid phase beyond its freezing temperature is known as supercooled or glassy liquid [Mishima and Stanley, 1998; Handle *et al.*, 2017].

However, the anomalous behavior of water becomes more pronounced in supercooled regions. An exhaustive investigation on water anomalies near $\sim 4^{\circ}\text{C}$ has been carried out using experiments and computer simulations. Hydrogen bonds that develop between one another

cause the tetrahedral structure of water. Thermodynamic anomalies and dynamic anomalies are the two categories under which water anomalies fall. Isothermal compressibility (κ_T), density maximum, coefficient of thermal expansion (α_P), and isobaric specific heat capacity (C_P) are examples of thermodynamic anomalies of water. When cooled from room temperature, most liquids undergo a consistent densification process. However, liquid water achieves its maximum density at roughly 4°C, below which it expands and becomes less dense as it is cooled further. From the coordination number, it is feasible to deduce one of the potential explanations for such an unusual behavior of water [Bagchi, 2013]. At room temperature, water often forms 2 to 3 coordinated structures, with 3 coordinated arrangements having the highest probability. However, below melting point temperatures, the 3-coordinated structure is replaced by a 4 or 5-coordinated structure, which causes water anomalies to appear. Thus variations in the number of hydrogen bonds at high and low temperatures quantify local structural alterations. An increase in the local tetrahedral structure of water is found from experiments on supercooling [D’Arrigo *et al.*, 1981; Angell and Rodgers, 1984]. When approaching the supercooled domain, a dynamic crossover from non-Arrhenius to Arrhenius behavior in terms of the orientational correlation time of water is seen.

When liquid water is cooled below a certain point beyond its freezing temperature, relaxation time increases drastically, such that dynamical arrest emerges, and the system cannot be equilibrated with reasonable experimental times [Cavagna, 2009]. Computer simulations show the structural arrest of water molecules due to the cage-effect at supercooled temperature which is in agreement with mode coupling theory [Sokolov *et al.*, 1995; Gallo *et al.*, 1996; Gotze and Sjogren, 1992]. The structural arrest in supercooled water leads to the formation of transient regions with different relaxation time scales [Sillescu, 1999; Ediger, 2000]. Computer simulations reveal that the rotational and translational dynamics become spatially heterogeneous, which is more pronounced on lowering the temperature. Both rotational and translational heterogeneities are strongly correlated, and a fraction of molecules constituting these heterogeneities increases with further decrease in temperature. The correlations between translational and rotational heterogeneities are due to the defected hydrogen bond networks manifested at supercooled temperatures [Sciortino *et al.*, 1991, 1992; Mazza *et al.*, 2006]. A supercooled liquid becomes more viscous and its molecules move more slowly as it is cooled to lower temperatures as shown in figure 1.9. The molecules may move so slowly at some temperatures that they do not have time to meaningfully reorganise themselves before the temperature is reduced much further. The glass transition temperature, or T_g , is the point at which a supercooled liquid turns into a glass. T_g varies depending on the cooling rate. Regardless of its dependency on cooling rate, T_g is an important material attribute; when specified consistently, it is the one quantity most useful for evaluating the mechanical properties of a polymeric material. It is essential to note that the glass transition is not a first-order phase transition, but rather a kinetic phenomena or second-order transition.

1.4 DYNAMICAL HETEROGENEITY

Experimental studies of viscous liquids reaching the glass transition temperatures upon supercooling lead to the conceptualization of dynamical heterogeneity. Dynamical heterogeneity is ubiquitous in nature. From molecular systems to biological cells and tissues, dynamical heterogeneity is present in a wide range of natural processes [Angelini *et al.*, 2011; Bi *et al.*, 2016, 2015; Nnetu *et al.*, 2012]. Dynamic heterogeneity has been the subject of extensive study over the past 10 years and is now recognised as a key component of structural relaxation in disordered materials with slow dynamics. In the past decade, studies and computer simulations have demonstrated that a range of glassy systems exhibit the clusters of comparable mobility as depicted in figure 1.10. The behavior of glass-forming materials during a phase transition from the

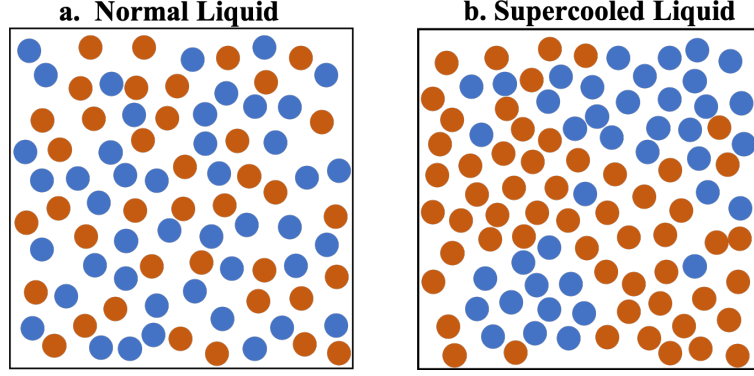


Figure 1.9 : Dynamic heterogeneity in supercooled liquids shown. a) Normal liquids (Throughout particle motion is uniform), and b) Supper cooled liquid (Particle motion is heterogeneous among groups of particles that are travelling together). Blue and orange color: Used to differentiate the movement of the different particle.

liquid state to the glassy state is referred to as dynamical heterogeneity.

1.4.1 Manifestation of Dynamical Heterogeneity

Self intermediate scattering function, $F_s(q, t)$, is one of the universal characteristics of dynamical heterogeneities.

$$F_s(q, t) = \frac{1}{N} \left\langle \sum_{i=1}^N \cos(\mathbf{q} \cdot [\mathbf{r}_i(t) - \mathbf{r}_i(0)]) \right\rangle \quad (1.1)$$

where, $q = \frac{2\pi}{\lambda}$ and λ is the wavelength. The correlation function decays exponentially to zero at higher temperatures. When the temperature drops, the function begins to decay non-exponentially. Following an initial ballistic decay, there is a steady relaxation (β -relaxation), which causes a plateau to appear where the correlation function is nearly constant across the time scale. The width of the plateau is dependent on the temperature and the wave vector (q). The Debye-Waller factor, which measures the degree of caging for fluids, lengthens with decreasing temperature and corresponds to the plateau area in the intermediate scattering function. Finally the correlation function decays to zero following a second step relaxation (α -relaxation) [Berthier and Biroli, 2011; Johari and Goldstein, 1970; Karmakar, 2016] that occurs beyond the plateau phase shown in figure 1.11. The α -relaxation can be fitted by a stretched exponential or Kohlrausch-Williams-Watts (KWW) function [Kohlrausch, 1854; Yang, 2014], $F_s(q, t) \sim \exp(-\frac{t}{\tau})^\beta$. The exponent β falls somewhere between $0 < \beta < 1$ and depends a lot on both the wave vector and the temperature. Near T_g , the relaxation becomes progressively non-exponential at extremely low temperatures.

The dynamics of a single particle can be measured by the mean square displacement (MSD), $\langle [\Delta r_i(t)]^2 \rangle$.

$$\langle \Delta r(t)^2 \rangle = \frac{1}{N} \sum_{i=1}^N \langle [r_i(t+t') - r_i(t')]^2 \rangle_{t'} \quad (1.2)$$

N is the total number of molecules. t' denotes the time origin, and t implies the time gap. Ensemble average is represented by an angular bracket. The MSD follows $\sim t^2$ scale in the ballistic regime

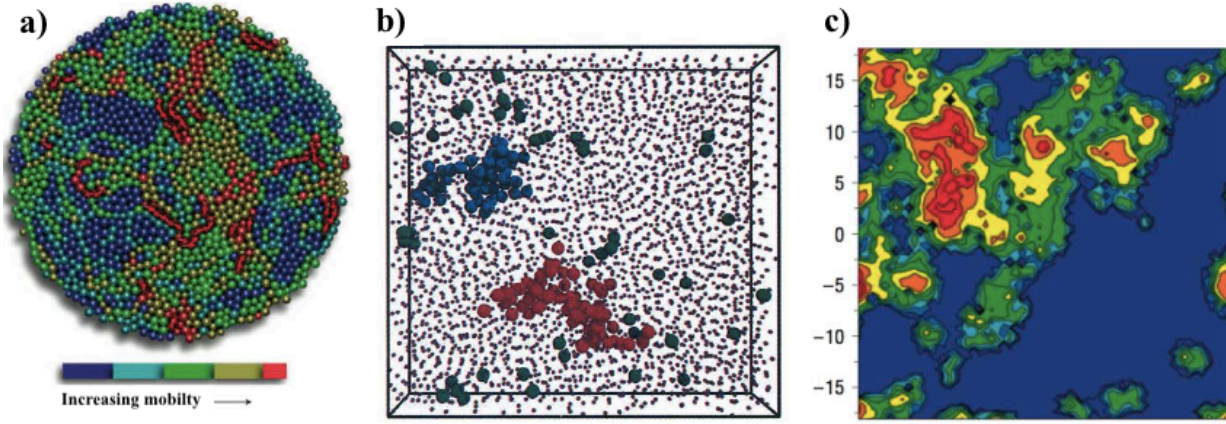


Figure 1.10 : Three illustrations of dynamical heterogeneity. In all cases, the figures highlight the clustering of particles with comparable mobility. (a) A colour scale depicting a range of mobility increasing from blue to red in the granular fluid of ball bearings [Keys *et al.*, 2007]. (b) Colloidal hard sphere suspension, with the majority of moving particles highlighted [Keys *et al.*, 2007]. (c) A two-dimensional system of repellant discs as simulated by a computer. The colour scheme, which ranges from blue to red, shows the presence of particles whose motion is reproducibly immobile or mobile [Widmer-Cooper *et al.*, 2008].

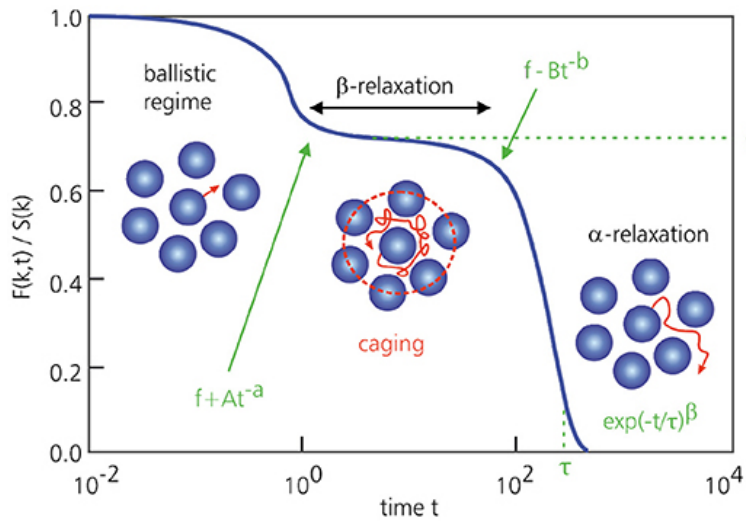


Figure 1.11 : $F_s(q, t)$ of a supercooled liquid as a function of time. At very short times, particles undergo ballistic motion. At intermediate times, particles become transiently trapped in cages (β -relaxation) and $F_s(q, t)$ correspondingly remains approximately constant. Only at sufficiently long times particles become free and full relaxation takes place (α -relaxation) [Janssen, 2018].

and $\sim t$ at the diffusive regime on a longer time scale. As the temperature goes down, the motion of the particles slows down due to caging and a plateau shows up at the intermediate time scale before the transition to the diffusive regime. As the system gets closer to the glass transition

temperature, the plateau starts to grow. The diffusion constant (D) can be used to calculate the slowing down of dynamics. The diffusion constant (D) falls when the temperature is lowered. The Stokes-Einstein (SE) relation [A.Einstein, 1905] may be used to establish the relationship between D and shear viscosity (η), where $D \sim (\frac{\eta}{T})^{-1}$ and T represents the temperature. The SE relation is no longer true at supercooled temperatures, because the movements of the particle remain in the sub-diffusive region for longer time.

The distribution of particle displacements characterises the underlying dynamics. At supercooled temperatures, the caging of water molecules may be characterised by calculating time dependent displacement distributions or van Hove correlation functions.

$$G_s(r, t) = \frac{1}{N} \left\langle \sum_{j=1}^N \delta[(r - r_i(t) + r_i(0))] \right\rangle \quad (1.3)$$

where, $\delta(\dots)$ is Dirac delta function and $\langle \dots \rangle$ is the ensemble averaging. If a system follows a Gaussian diffusive process (e.g. a simple liquid or any system at high temperatures), then the van Hove function is Gaussian in nature as given by $G_s(r, t) \sim \exp(-\frac{|r|^2}{4Dt})$. The aforementioned distribution, however, dramatically departs from its Gaussian character on the time scale of the structural relaxation period for super cooled liquids or glassy systems. The non-Gaussianity in van Hove function is reflected in an exponential tail [Chaudhuri *et al.*, 2007]. The range of the exponential tail is determined by the diffusivity of local motion and the waiting time distribution for succeeding hopping motions.

2-Dimensional non-Gaussian parameter, α_2 is another metric that measures the non-Gaussian behaviour of the van Hove function given as

$$\alpha_2(t) = \frac{\langle \Delta r(t)^4 \rangle}{2 \langle \Delta r(t)^2 \rangle^2} - 1 \quad (1.4)$$

$\alpha_2(t)$ is zero if $G_s(r, t)$ is Gaussian. The non-Gaussian parameter increases with time for the systems having dynamical heterogeneity and reaches a maximum and then decreases to zero after long time. The time when the $\alpha_2(t)$ has a maximum, is referred to as τ_{α_2} .

1.4.2 Theories of Dynamical Heterogeneity

Near the glass transition, there is a predominance of structural disorder in the system due to small, localised domains of particles, each of which exhibits a unique dynamical behaviour with a unique relaxation time scale. An important area of study is the occurrence and characterisation of dynamical heterogeneity in various dynamical processes, notably in the dynamics of glass-forming liquids near the glass transition [Berthier and Biroli, 2011; Weeks *et al.*, 2000; Ediger, 2000; Berthier *et al.*, 2011; Widmer-Cooper *et al.*, 2004]. In critical phenomena, one sees a diverging timescale while approaching a second order phase transition which is somewhat similar to slowing down of dynamics for glass-forming liquids. As in second order phase transition, this is associated with diverging length scale, one is often tempted to understand similar growth of correlation length in glass-forming liquids. Thus existence and subsequent growth of a correlation length scale in glass transition will be of much importance in understanding the puzzles of glass transition. This led to a lot of intense research in the past couple of decades and has become one the most sought after problems in the theory of glass transition. Although success in this field of research is somewhat limited, yet over-all progress to identify length scales in supercooled liquids approaching glass

transition is very intriguing and indeed contributes to our understanding of the glass transition problem. There exists a large number of theories developed to explain glass transition. However none of these theories can fully resolve all glass phenomenology and also, there are controversies between the connection of different theoretical approaches. We will briefly discuss some of these theories in the following paragraphs. In general, most theories applied to supercooled liquids assumes that the glass transition arises from a dynamical transition and it is not controlled by any thermodynamical transition. One of the most difficult problems in condensed matter science that has yet to be resolved is the understanding of the mechanics of glass transition. There are number of theories to understand the glass transition, such as Adam–Gibbs theory Adam and Gibbs [1965], Random first order transition theory (RFOT) [Freed, 2014], Mode coupling theory (MCT) [Götze, 2009; Das, 2004], Free volume theory of glasses, and Kinetically constrained models (KCM) [Garrahan *et al.*, 2011] etc.

The earlier experimental findings strongly support the existence of cooperative methods in glass-forming liquids, which further supports the existence of expanding length scales that describe the spatial extent of correlated or "cooperatively rearranging regions" (CRR), a term coined by Adam and Gibbs in their predictive description of glassy dynamics [Adam and Gibbs, 1965]. The main contention is that for a liquid to remain mobile as its temperature drops, its atoms or molecules must reorganise themselves in an increasingly cooperative manner. Adam–Gibbs theory predicts CRR size grows with lowering in temperature. As a result, the Adam–Gibbs theory establishes a connection between relaxation duration and an increasing size or length scale, in addition to a link to thermodynamics through the configurational entropy as given by

$$\tau(T) \propto \exp \left[\frac{C}{TS_c(T)} \right] \quad (1.5)$$

Here, S_c is configurational entropy, τ is relaxation time, T is temperature.

The mode coupling theory (MCT) of glasses was first developed by Götze and others [Bengtzelius *et al.*, 1984; Mezard and Alcaraz, 2016; Leutheusser, 1984]. MCT provides a somewhat realistic explanation of relaxation dynamics, in particular, the intermediate scattering function $F_s(q, t)$ and the self-diffusion for liquids and colloids that form glasses. The striking features of MCT is that the full dynamics of supercooled liquid is completely determined from the static structural quantities as input. As equation of motion is obtained from a self consistent dynamical equation, a large change in the dynamics is possible with just a small change in structure factor which is in agreement with simulation results [Berthier and Tarjus, 2010]. The solution of full MCT equation predicts a divergence of relaxation time or viscosity at a temperature, T_c , that is generally much higher than normal glass transition temperature T_g . The relaxation times τ scales as [Bengtzelius *et al.*, 1984; Götze, 1999],

$$\tau(q, t) = (T - T_c)^{-\gamma} \quad (1.6)$$

This implies that when $T \rightarrow T_c$, relaxation time τ diverges as a power law with an exponent γ . The most notable failure of MCT is the prediction of glass transition temperature at $T = T_c$ which is much higher than even the calorimetric glass transition temperature, T_g . Violation of Stoke-Einstein relation, which connects η , D and T as $\eta \sim (\frac{T}{D})$ can not be explained by MCT.

Free volume theory of glasses, proposed by Cohen and Turnbull [Cohen and Turnbull, 1959] in 1959, suggests that most liquids undergo a glass transition by very rapid cooling if crystallization does not intercede. In molecular liquids, the glass transition with decreasing temperature is

manifested by a discontinuous change in specific heat, viscosity and thermal expansion. The idea is that in a dense liquid a molecule is confined in a cage by its nearest neighbours and the free volume v_f is free part of the cage that exceeds the volume taken by a molecule. The distribution of free volume is exponential and they are uncorrelated. The main assumption is that the molecular transport or diffusion mechanism happens when local free volume (v_f) is greater than some critical value v_c and no energy is required for the redistribution of free volume (v_f). Within the above assumption, the free volume (v_f) varies linearly with temperature $v_f \propto (T - T_0)$ and it recovers the Vogel-Fulcher-Tamann (VFT) [Fulcher, 1992] equation for viscosity,

$$\eta = \eta_0 e^{\frac{B}{T-T_0}} \quad (1.7)$$

Despite its noticeable success, the most unclear and unanswered question is that why the free volume vanishes at some non zero temperature (T_0) and also, how the theory is related to other recent theories of glasses.

The Random first order transition theory (ROFT) theory for structural glasses is first introduced by Kirkpatrick, Thirumalai and Wolynes [Kirkpatrick and Thirumalai, 1987; Kirkpatrick and Wolynes, 1987]. This theory is inspired by the theory of mean field for “generalized spin glass”. It merges different theories of glass forming liquids such as spin glass theory, Adam Gibbs theory [Adam and Gibbs, 1965] and Mode coupling theory (MCT) [Adam and Gibbs, 1965] in a common framework. In “random first order transition” theory, Kirkpatrick et. al. establish an intense analogy between the supercooled liquids and the mean field spin glass [Young, 1998]. This theory manifests two transition temperatures. The first one is dynamical or MCT like transition temperature T_{MCT} where ergodicity is broken and the system is confined in a metastable state. The lifetime of that state is infinite (in mean field limit). The second transition happens at low temperature T_K , corresponding to a thermodynamic phase transition to a disorder state (which is not universally accepted) and shows a discontinuity in the order parameter with no latent heat (a transition known as “random first order”). In between T_0 and T_{MCT} , there exist an exponentially large number of metastable free energy states which are statistically similar to each other.

The kinetically constrained models (KCM) [Garrahan *et al.*, 2011] are simple lattice models to understand dynamics of supercooled liquids [Glarum, 1960], glasses [Palmer *et al.*, 1984], and other systems with kinetic constraints. This perspective assumes that most of the interesting properties of glass forming systems are dynamical in origin, while thermodynamics plays a very limited role [Garrahan and Chandler, 2002]. KCM tends to have simple and uninteresting thermodynamics, typically that of a non-interacting lattice gas. The complex dynamics arises from some simple kinetic rules which leads to dynamical frustration in systems. Indeed, KCM predicts the non-exponential relaxation time and breakdown of Stokes-Einstein relation at high density and low temperature as a result of dynamical heterogeneity. Also, different multi-point correlation functions, susceptibility and its scaling behavior [Toninelli *et al.*, 2005; Whitlam *et al.*, 2005] can be studied within the framework of these models. The slowing down of dynamics in a supercooled liquid arises from kinetic constraints that are either self generated by the system or are externally imposed. These views are very different compared to RFOT [Lubchenko and Wolynes, 2006; Freed, 2014] where thermodynamics is the sole player.

1.5 HETEROGENEITY LENGTH SCALES

A prevalent notion in the physics community regarding the glass transition is that along with the dramatic slowdown associated with increase in dynamical heterogeneity, the length scale should also grow in proportion to the increasing time scale [Berthier and Biroli, 2011]. The discovery for such a length scale took decades of research [Donth, 2001; Shi *et al.*, 2011].

The length scale, known as the dynamical correlation length, ξ_d , estimates the spatial correlations of the local inhomogeneous dynamics of glass-forming liquids. Lots of questions remain about how these length scales will behave as the transition to glass approaches. The drastic slow dynamics near the glass transition does not allow the system to cross the phase transition being at equilibrium. Moreover, the nature of the glass transition can not be understood with the help of traditional Ginzburg-Landau theory since the choice of order parameter, correlations, dynamics are unknown. So it's critical to have techniques for measuring these length scales precisely in simulations and experiments. Using finite size scaling (FSS) analysis, phase transition is estimated from a parameter which has temperature and system size dependencies [Berthier, 2003]. At the glass transition temperature, the dynamical correlation length, ξ_d , diverges [Ediger, 2000]. For a model glass forming liquid, ξ_d is measured from a four point time dependent structure factor obtained from the mobility correlation [Karmakar *et al.*, 2010]. ξ_d measured from the finite size scaling of dynamic susceptibility grows with decrease in temperature [Karmakar *et al.*, 2009].

In addition to the dynamic length scale ξ_d , another length scale, known as static length scale, exists, which too is accompanied by the dynamic slow down in relaxation time. The length scale can not be obtained from the pair density correlation which does not change significantly with the drastic slow down of the structural relaxation. Supercooled systems with very similar structural features might show very distinctive dynamical behaviors. Thus, several theories have been proposed to calculate such a nontrivial static length which provides an estimate of the spatial extension of some local arrangement. However, very few theories remain useful to pin down the length scale. Thus, measuring such length scale remains an open problem for long. One of the popular theories to calculate such length considers “point-to-set” (PTS) correlation lengths, ξ_{PTS} . Generally, the dynamic slow-down near the phase transition is expected to be related with the thermodynamic phase transition where the long range order begins to become important. Since glasses have no specific boundary conditions due to their random nature similar to the bulk amorphous state, equilibrium configurations are used to define their boundary in the similar line of RFOT. In an equilibrium configuration, the boundary is imposed by introducing a cavity of radius R filled up with mobile particles and the particles outside are kept frozen and thermodynamics of the mobile particles are studied. A local overlap at the centre of the cavity is defined in presence of the boundary imposed. The decay of the overlap changes due to the growth of ξ_{PTS} with decreasing temperature from the decay, ξ_{PTS} is extracted. Using this approach, evidence of a qualitative thermodynamic signature of a transition. From a high temperature to a deeply supercooled liquid is found [Biroli *et al.*, 2008]. Within the RFOT theory, the supercooled phase is composed of glassy spheres with interfaces. Near the glass transition temperature, these glassy spheres diverges with an exponent of $\nu = 2/d$ [Kirkpatrick *et al.*, 1989]. ξ_{PTS} is quantitatively connected to the growth of relaxation times [Hocky *et al.*, 2012]. Upon supercooling, the static ξ_{PTS} grows much slower than the dynamical length ξ_d at the onset of nontrivial dynamics [Charbonneau and Tarjus, 2013]. Another approach to calculate the static length scale is the length where the lowest eigen value of the Hessian matrix starts sensitive to disorder. Both the approaches from the PTS and the Hessian matrix reduce to identical length scale [Biroli *et al.*, 2013].

Experimental studies of length scales in glass-forming liquids have been limited due to the difficulty in accessing particles' trajectories. Using confocal microscopy a direct imaging of structural relaxation is obtained for colloidal glasses and supercooled fluids [Weeks *et al.*, 2000]. Direct experimental evidence of dynamical heterogeneities is found from the analysis of two dimensional trajectories of colloidal hard spheres using confocal microscope [Kegel and van Blaaderen, 2000]. The prediction by RFOT on the growing amorphous order in the form of ξ_{PTS} on approaching glass transition is verified by freezing a wall of particles into two dimensional colloids by optical tweezer [Hima Nagamanasa *et al.*, 2015]. A non monotonic dependence of dynamic correlation length on area fraction has been observed. The non-monotonicity results directly from the change in structure of cooperatively rearranging regions (CRR). The morphology of CRR changes from a string-like to compact near the mode-coupling temperature [Singh and Ganapathy, 2015]. The non monotonicity in dynamical correlation length is unexpected in the literature of glass dynamics with an exception of numerical results from Kob *et al.* [Kob *et al.*, 2012]. Cage formation of two dimensional colloidal suspensions has been identified using video microscopy upon approaching glass transition [Li *et al.*, 2020]. The cage formation is related to the appearance of domains with cooperative movements.

It was already mentioned, FSS has been crucial in the determination of both ξ_d and ξ_s [Karmakar *et al.*, 2009, 2012; Chakrabarty *et al.*, 2017a; Biroli *et al.*, 2013]. Studies of the equilibrium behaviour of systems with PBC and various sizes that are equivalent to the values of the pertinent length scales are part of the traditional FSS technique. Due to the difficulty of performing experiment on such systems, this approach cannot be used. Therefore, it is crucial to create alternate FSS techniques that may be used in research. Additionally, the traditional FSS technique has certain drawbacks such as the requirement of lengthy simulations and considerable averaging in order to produce accurate findings for tiny systems and artefacts [Flenner and Szamel, 2010]. It would be really nice to use FSS methods without these issues. The traditional FSS strategy may be used to extract the length scale in important phenomena as a complement to experimental methods. Recently, the block analysis method is used for a realistic glass-forming liquid by executing FSS with a decrease in temperature and the approach is found to be effective in measuring the dynamical correlation length scales [Berthier and Witten, 2009]. Most quantitative investigations of dynamical heterogeneity length scale are based on multipoint correlation functions [Dasgupta *et al.*, 1991] and associated susceptibilities. As a continuous phase transition approaches, the rise of the peak value of $\chi_4(t), \chi_4^p(t) = \chi_4(\tau_4)$ grows similarly to the growth of the order-parameter susceptibility, showing the presence of an increasing correlation length. The length scale connected to the increase of χ_4^p , however, must be categorized as a dynamical length scale because it depicts behavior with a finite time horizon. At temperature T_c , inhomogeneous MCT forecasts power-law divergences of the four-point susceptibility χ_4^p and a corresponding dynamical correlation length, ξ_d . A novel technique for doing FSS that avoids the issues of standard FSS analysis, has been developed recently and known as block analysis [Chakrabarty *et al.*, 2017b]. Blocks of differing sizes are implanted in the system, and several parameters of interest, including χ_4 and the relaxation time, τ , are measured for individual blocks. A FSS study of the dependency of χ_4 and the corresponding Binder cumulant on the block size yields the length scale ξ_d associated with dynamic heterogeneity. The extent of the underlying correlation length for the diverse local dynamics can only be determined by data collapsing a block size-dependent Binder cumulant. The static PTS length scale, ξ_s is determined by the FSS analysis on the dependency of the variance of the α relaxation time of individual blocks on the block size. Another way to calculate the dynamical length scale, ξ_d is using van Hove block analysis [Bhowmik *et al.*, 2018] in which the block size demonstrating a change from a non-Gaussian to a Gaussian van Hove correlation function is used to identify the heterogeneity length scale. This approach is successfully used on the IW near the fluid membrane where the length scale matches with the size of the undulation membrane [Srivastava *et al.*, 2019a]. This offers a fresh opportunity to link the lateral arrangement of membranes to the heterogeneity length scale. This approach may

be useful to identify the domain length scale of a raft, which is otherwise difficult to learn from an experiment.

The connection with critical phenomena explains in part why we expect relaxation times to expand in lockstep with length scales. The mode-coupling and renormalization group theories are successful in explaining most of the available experimental data near the critical point of binary mixtures or pure fluids. The significant slow down during the phase transitions often follow the rules of traditional dynamic scaling. The relaxation time grows exponentially with the correlation length and deviates at the transition point given by,

$$\tau = \xi^z \quad (1.8)$$

Here, z is referred to as the dynamic critical exponent. This relation is motivated by the theory of dynamic critical phenomena [Hohenberg and Halperin, 1977]. In some disordered systems such as random field Ising model [Young, 1998], the rise of the relaxation period near the phase transition is thought to obey a different pattern called as activated dynamic scaling,

$$\tau = \exp(A\xi^z/T) \quad (1.9)$$

where, A is a fixed value [Young, 1998].

1.6 PROBLEM STATEMENT

The research work presented in the thesis mainly contemplates the structure and dynamic correlation of the IW to the lateral organization of the cell membrane to answer few fundamental questions.

1. How significant is the coupling between the dynamics of IW and the membrane?
2. What is the governing mechanism of dehydration induced membrane ordering?
3. How does the structure of IW change upon membrane phase transition?
4. Are the suggested length scales derived from the above mentioned theories and numerical techniques of glass transitions general enough to characterize the heterogeneity length scale of the IW across membrane phase transitions? How do the length scale behave with temperature?
5. Is there a connection between any of the postulated length and time scales that occur close to the membrane phase transition?

In order to get the answers to these problems, one must primarily rely on simulations of model membranes because the time and length scales relevant to these questions are generated from quantities that are difficult to measure in experiments. Thus, the objectives of the thesis are the following:

- Quantifying the extent of correlation of the IW and the membrane dynamics at physiological temperatures
- Insight on the dynamical origin of the dehydration induced membrane ordering
- Elucidate the structural changes of the IW upon membrane phase transitions

- Identifying and characterizing the heterogeneity length and time scales of the IW across membrane phase transitions

Lipid bilayers are vital parts of cell membranes because they act as semi-permeable barriers between the extracellular and intracellular environments. The cell membrane is functional when it is in its fluid state and is fully hydrated. Understanding the role of water in the membrane phase transitions is crucial to control the function of the membrane under physiological and low-temperature conditions. Our results showing the coupling between the interface water and lipid relaxation times suggest that relatively simple localized dynamics of hydration layers may be used to measure inaccessible perturbations at the membrane-water interface that are important for drug or protein binding or molecular recognition. Understanding biological cell membranes under anhydrous conditions is of tremendous biological importance for different reasons. There is a broad range of species that can live for a longer period under anhydrous conditions without dissociation which is poorly understood so far. Additionally understanding dry membranes are relevant for the cryo-preservation techniques applicable in food science, clinical medicine, and the preservation of human embryos for in-vitro fertilization. Our results show that dehydration induces dynamical heterogeneity in water which is the origin of disorder-order structural cross-overs in bilayers. Thus, the dry-ordered membrane loses its functionality once fluidity disappears. Thus, by measuring the impact of the interface water dynamics on the phase transition, our investigations advance the fundamental knowledge of dehydration-induced bilayer ordering. Using block analysis approach, we have captured the heterogeneity length scales of the interface water near three primary phases of membranes. The length scale drastically grows across the phase transitions and does not change significantly within a given phase. Remarkably the length scale is similar to the size of rippling which opens up the possibility of capturing ripple size or curvature-induced domain size through interface water dynamics. The length scale follows activated scaling with the relaxation timescale only at the gel phase. The understanding from our study can be extended to complex bio membranes under supercooled conditions to understand their survival strategy in the future.

1.7 ORGANIZATION OF THE THESIS

The thesis presented here focuses on the fundamental study of dynamical heterogeneity of the IW upon membrane (1,2-dimyristoyl-sn-glycerol-3-phosphocholine (DMPC)) phase transitions using all atom molecular dynamics simulations.

Chapter 2 attempts to gain insight into membrane dynamics and understand its influence on the hydration layer. Interface water (IW) molecules are categorized based on their constant physical proximity to certain lipid head groups. Multiple relaxations are observed for the spatially resolved IW and the physically close lipid heads from the self intermediate scattering functions. Both fast and slow relaxations of the IW and the lipids are strongly correlated. From the tails to the heads of the lipids, deviations from Gaussianity in van Hove correlation functions increases depicting the existence of dynamical heterogeneity near the membrane interface. The regional dynamics of lipid heads are strongly reflected in the dynamics of IW molecules which are physically adjacent to the heads. These findings suggest that, in the future, spatially resolved IW dynamics might serve as a sensitive reflector of regional membrane dynamics for a variety of critical biological processes at physiological temperature.

Chapter 3 investigates the effects of dehydration on membrane structure to understand membrane functions linked to domain formation and cell fusion in anhydrobiosis. As dehydration

increases, the lipid area per head group reduces, along with an increase in bilayer thickness and lipid order parameters, showing a bilayer ordering. The IW display notable signs of dynamical heterogeneity at the onset of bilayer ordering, as evidenced by non-Gaussian parameters and one-dimensional van Hove correlation functions. IW follows a Stokes-Einstein (SE) relation while fully hydrated. However, when dehydrated, the relationship breaks, revealing glass dynamics. Due to non-diffusive dynamics, the bilayer order is accompanied by an increase in the fraction of caged lipids spread over the bilayer surface and a reduction in the fraction of mobile lipids. These findings show that dynamical heterogeneity governs the microscopic process of lipid ordering by dehydration. The mechanistic insights from this chapter will be useful to enhance the fundamental understanding on dehydration induced membrane ordering in membrane.

Chapter 4 investigates the effect of fluid to ripple to gel phase transition of the membrane on the structure of the IW. Changes in the radial and angular distribution functions of the IW indicate a transition from a distorted to a mixture of distorted and tetrahedral structure to a tetrahedral structure near the fluid, ripple, and the gel membranes respectively. Unlike the BW, potential of mean forces of the IW follow two cross-overs near both phase transitions. These findings indicate the presence of a high density liquid (HDL)-like phase in the IW near the fluid membrane. This slowly turns into of a combination of low density liquid (LDL) and HDL like phase near the rippled membrane that converts to a LDL-like phase near the gel membrane. According to these studies, the structural features of the IW is highly correlated with the bilayer phase transitions.

Chapter 5 elucidates the influences of membrane phase transitions on the dynamics of the IW. DMPC lipid membranes are performed to cover three (fluid, ripple and gel) primary phases of membranes relevant for bio-activity under physiological and supercooled conditions. As temperature drops, structural relaxations of the IW and the lipids originating from dynamical heterogeneity are significantly slowed down. At both fluid-to-ripple-to-gel phase transitions, diffusion constants of the IW undergo dynamic crossings, where the IW near the gel phase exhibits the highest activation energy due to the highest number of hydrogen bonds at the gel phase. Interestingly, the Stoke Einstein (SE) relation is conserved for the IW near all three membrane phases for the time scales derived from the diffusion exponents and the non-Gaussian parameters. However, the SE relation breaks for the time scale obtained from the self-intermediate scattering functions (SISF). This observation is universal for the IW, BW and other glass forming liquids. The dynamical heterogeneity length scale is extracted from the FSS analysis of block size dependent Binder cumulants. The dynamical length of the IW has inverse power law dependence on temperature for the fluid phase. Remarkably, it becomes temperature invariant for the ripple phase as the ripple wavelength controls the underlying heterogeneity length scale. The dependency is very weak for the gel phase where the growth of the relaxation follows activated dynamical scaling in similar line to the ROFT theory. Within a given phase, the length scale is monotonically dependent on the first peak of the radial distribution function of the IW near all phases indicating that the heterogeneity length scale is structure dominated. Our analyses, for the first time, provide estimations of the spatio-temporal scales of the IW across membrane phase transitions. The work establishes an alternate method to probe and characterize functionally relevant domains in membranes through the cross-overs in heterogeneity time and length scales of the IW.