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Introduction

Water plays a vital role in carrying out life processes. Water is an essential component in all forms of life. Our planet has about two-third percentage of water. Water is considered as indispensable liquid in a way that most of the chemical reactions occur in presence of water and almost all biological functions of living organisms crucially depend on water. Water possesses a remarkable chemistry because of its various yet incompletely understood anomalous properties. The anomalous behaviors manifested by water have attracted researchers all over the globe to study origin of these anomalies. Over the past few decades, numerous experimental and theoretical studies are done for investigating various properties of water. For understanding the role of water in life processes, it is necessary to understand structure and dynamics of water in the vicinity of biological environment.

1.1 ANOMALIES IN WATER

Although water is the most abundant liquid in nature, it is not as simple as it seems by virtue of its anomalous behavior in comparison with other liquids [Ball, 2008]. The non-bonded interactions responsible for tetrahedrality in water are hydrogen bonds formed among themselves. Most of the anomalous behaviors manifested by water are due to presence of dynamic hydrogen bonds. Water anomalies are classified under two heads viz. thermodynamic and dynamic anomalies. Thermodynamic anomalies include density maximum, isobaric specific heat capacity (C_P), isothermal compressibility (κ_T) and coefficient of thermal expansion (α_P). Density anomaly is one of the oldest anomalies of water. For most of the simple liquids, density decreases upon heating while for water, at atmospheric pressure, water contracts at temperatures above 277 K (figure 1.1). The density anomaly plays a crucial role in numerous natural processes. One possible explanation for such an anomalous behavior can be inferred from coordination number [Bagchi, 2013]. Generally, at ambient temperatures, water forms 2 to 3 coordinated structures with the maximum probability of 3-coordinated structure. But at temperatures below melting point, the 3-coordinated structure is replaced by a 4 or 5-coordinated structure (figure 1.2 a) and b)) leading to emergence of water anomalies. C_P is the amount of heat required to raise the temperature of the system by one unit at constant pressure per unit mass. Generally, one would expect C_P to decrease with a decrease in temperature which is true for most of the simple liquids. For water, C_P increases on decreasing the temperature beyond 320 K. At a temperature below melting temperature, C_P diverges with a power law behavior as shown in figure 1.3 a). κ_T is a measure of volume change of the system with change in pressure at constant temperature. For simple liquids, κ_T is proportional to temperature. However, for water, κ_T diverges on decreasing the temperature (figure 1.3 b)). α_P provides a measure of change in volume of the system with respect to temperature at constant pressure. Volume of the system decreases with decrease in temperature for simple liquids while for water, α_P is negative on lowering the temperature (figure 1.3 c)). Like other thermodynamic properties, α_P tends to diverge with a power law.
For investigating bulk water anomalies, studies should be conducted beyond nucleation temperature ($-38°C$, temperature at which a new thermodynamic phase is formed due to self-assembly of the molecules). Beyond the nucleation temperature, the entire concept of liquid state is lost as water displays phenomenon of liquid polymorphism where it form clusters known as amorphous

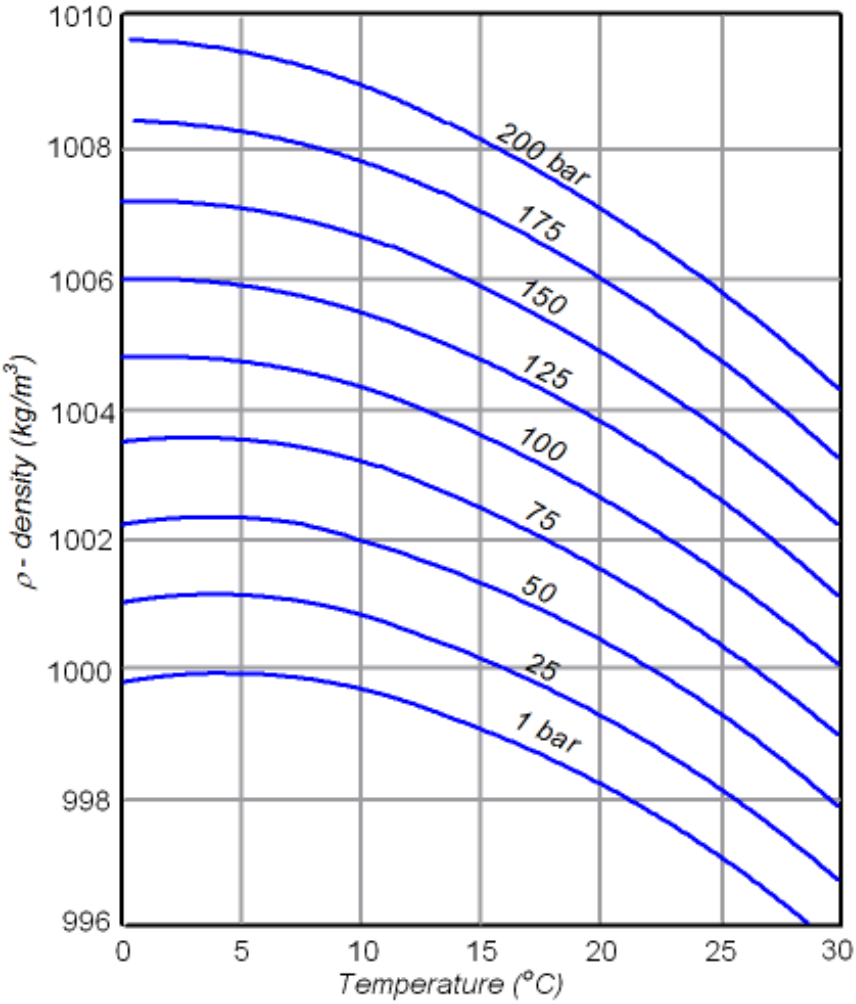

Figure 1.1: Temperature dependent density of liquid water at different pressures ranging from 1 bar to 200 bar. The figure is taken from [ToolBox, 2009].

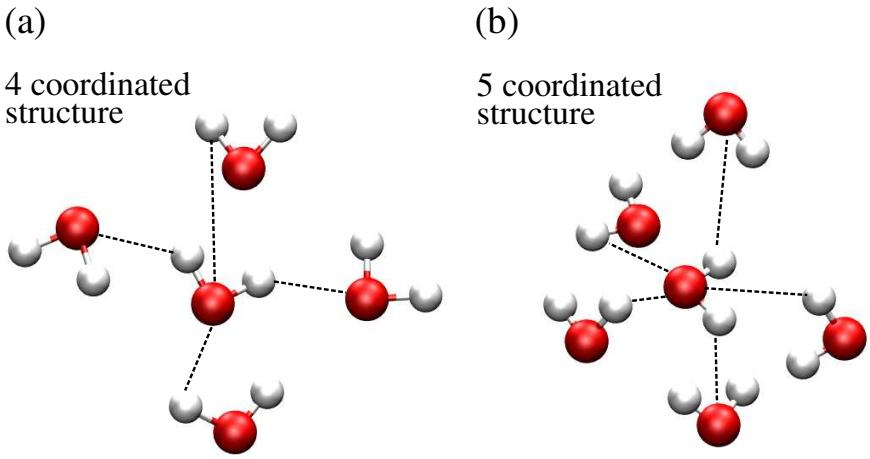

Figure 1.2: a) Four coordinated and b) five coordinated structures of water.

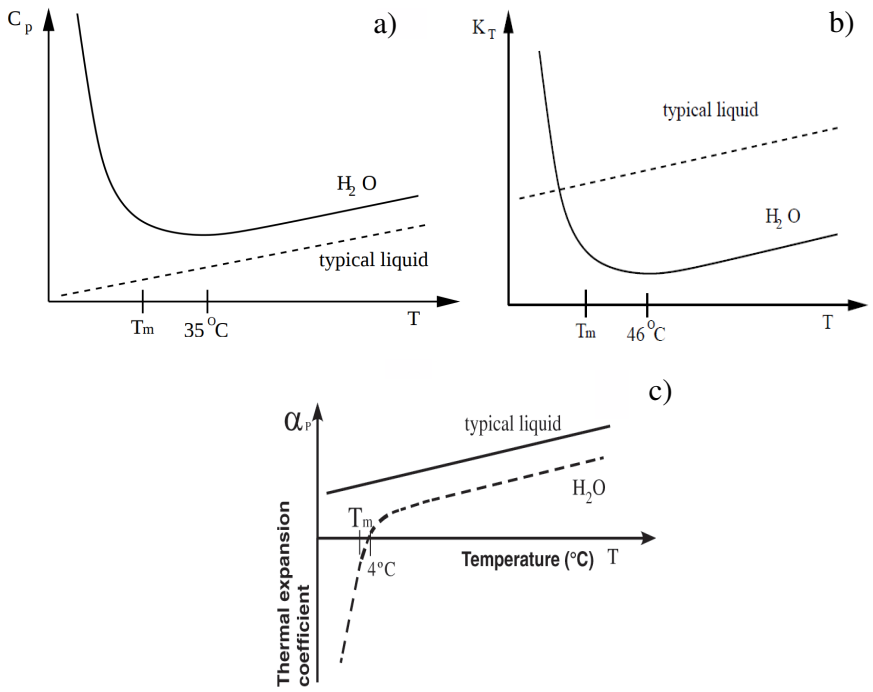

Figure 1.3: Thermodynamic anomalies of water a) specific heat capacity, b) isothermal compressibility and c) coefficient of thermal expansion of water. For contrasting anomalous behavior of water simple liquids are also shown. Figure is taken from [Kumar, 2008].

ice [Mishima and Stanley, 1998]. With a decrease in temperature, the hydrogen bond life time of these clusters start increasing thereby increasing the stability of the clusters. These clusters fall under two categories. First, high density amorphous ice (HDA) [O.Mishima *et al.*, 1984; Heide, 1984; O.Mishima *et al.*, 1985] which predominates at higher pressure and on further compression, it becomes high density crystalline ice [Tse and Klein, 1987; Hemley *et al.*, 1989]. Moreover, HDA has a structure resembling to a high pressure liquid water or a high pressure glassy water [BellissentFunel *et al.*, 1992; BellissentFunel and Bosio, 1995] Second, low density amorphous ice (LDA) [Burton and Oliver, 1935] exists at low pressure and exhibits an open hydrogen bonded network [Mallamace *et al.*, 2013]. On decreasing the temperature to $\sim$ 130 K, LDA transforms to a highly viscous liquid [McMillan and Los, 1965; Masayasu *et al.*, 1968; Johari *et al.*, 1987].

Dynamic anomalies in water include local structural changes in water at supercooled temperatures. Local structural changes are quantified by variations in the number of hydrogen bonds at high and low temperatures. At temperatures below supercooling, there is an increase in local tetrahedral arrangement of water as found in experiments [D'Arrigo *et al.*, 1981; Angell and Rodgers, 1984]. Temperature dependence of dynamic anomalies are governed by Arrhenius equation corresponding to the energetic barriers. A dynamic crossover from non-Arrhenius to Arrhenius behavior in terms of orientational correlation time of water is observed when approaching supercooled temperature regime. The orientational correlation time is associated with the change in number of hydrogen bonds. At sufficiently lower temperature, hydrogen bonds break and form new bonds resulting in formation of more number of tetrahedral networks. This increases activation energy of breaking of hydrogen bonds implying violation of Arrhenius behavior [Xu *et al.*, 2005; Kumar *et al.*, 2008, 2009].

Interestingly, water with several anomalies has few unique features which make it distinct from other simple liquids. First, its low molecular weight and size make it easy to confine itself near biomolecular surfaces such as membranes, grooves of DNA, active sites of enzymes and in the vicinity of protein chains [Cheng and Rossky, 1998]. Second, due to difference in electronegativity of oxygen

($\delta \approx -0.84e$) and hydrogen atoms ($\delta \approx +0.42e$), there is an emergence of partial charge separation which promotes hydrogen bond formation ability. Third, mid Infrared (IR) Laser spectroscopy has allowed to probe OH stretching vibrations over a wider range of water clusters ranging from dimers [Huang and Miller, 1988, 1989] to pentamers [Fröchtenicht *et al.*, 1996]. Terahertz laser vibration-rotation tunneling (VRT) spectroscopic experiments on water dimer and trimer reveal occurrence of intermolecular vibration at nearly same wavelengths [Pugliano and Saykally, 1992; Pugliano *et al.*, 1993]. Ab-initio quantum chemical calculations propose mechanisms for hydrogen bonding rearrangement in water dimer and trimer. For the water dimer, acceptor switching (AS) pathway has the lowest energy barrier for the hydrogen bonding rearrangement pattern. The AS pathway proceeds with flipping of the acceptor monomer which is followed by the rotation of donor monomer around its O-H bond axis. The pathway completes with a 180° rotation about the O-O bond of the complex formed by the two water monomers. The O-O distance between the water dimer is 2.952 Å. Similarly, for water trimer, AS pathway shows the minimum energy pathway where the O-O distance is 2.85 Å. The decrease in O-O distance of the trimer is due to the increase in hydrogen bonding strength due to cooperative effect of the three body forces. The proposed mechanism of hydrogen bonding rearrangement in water clusters can quantify hydrogen bonding rearrangement and dipole moments. Additionally, the detailed analysis of water clusters, in particular dimers and trimers, from these spectroscopic experiments can be used to deduce a universal liquid water force field [Keutsch and Saykally, 2001].

1.2 HYDROGEN BONDS IN WATER

Hydrogen bonding is been used by the scientific community for almost a century. The importance of hydrogen bonding has been discerned in the areas of biology, chemistry, material science and so on. Hydrogen bond is defined as the weak electrostatic bond formed between a covalently bonded hydrogen atom of one molecule with a strong electronegative atom which has a lone pair of electrons [Pauling, 1941]. The strong electronegative atom is referred to as acceptor and covalently bonded hydrogen atom is referred to as donor. In particular, hydrogen bonding in water has a contribution from 90% electrostatic and 10% covalent interactions. Hydrogen bonds are formed from the complex combinations of several interdependent interactions. First, electrostatic interactions which include attractive forces between negative charges on oxygen atom of one molecule with the positive charges on the hydrogen atoms of the other molecule. Second, polarization interactions which include net attractive interactions between distortable electron clouds and nuclear charges prevail upto a length scale of 0.80 nm. Third, covalent attractions are associated with formations of molecular orbitals surrounding the nuclei of two or more water molecules. Covalent interactions are highly directional which depend on cooperative hydrogen bond formation. Fourth, dispersive interactions which have quantum mechanical origins prevail due to coordinated effects of the electron clouds formed by neighbouring water molecules. Fifth, electronic interactions which originate due to overlap of the electron clouds of oxygens of one molecule with hydrogens of the neighbouring molecule. Figure 1.4 show the geometric definition [A.Luzar and Chandler, 1996; Rey *et al.*, 2002; Lawrence and Skinner, 2003; Eaves *et al.*, 2005] of a hydrogen bond between two water molecules. The nature of hydrogen bonds are experimentally verified by 1H NMR [Dingley and Grzesiek, 1998] and Compton scattering x-ray experiments [Isaacs *et al.*, 1999] where the covalent nature of hydrogen bonding is observed in RNA and ordinary ice respectively.

It is now widely believed that the presence of hydrogen bonding in water makes it disparate from other simple liquids. Simple liquids exhibit fundamental properties of hydrogen bond breaking and formation which is still far from being completely understood. The thermodynamic and dynamic anomalies exhibited by water can be ascribed to hydrogen bonding. Thermodynamic anomalies can be accounted for the distinct behavior of the first and second coordination shells of liquid water at supercooled temperatures. First and second coordination shells are identified based on number of neighboring molecules around a central molecule. When there are 4 molecules situated closest to the Central molecule, it is referred to as the first hydration shell. Second hydration shell

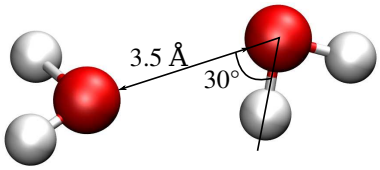

Figure 1.4: Snapshot showing geometric criteria for hydrogen bond formation in water where the distance between donor and acceptor (R_{HOO}) $< 3.5\mathring{A}$ and the angle between $\vec{OO}$ and $\vec{OH}$ (θ_{HOO}) $< 30^o$.

refers to the presence of $4-12$ closest neighbours around the central molecule. At physiological temperatures, rapid exchange of hydrogen bond partners leads to decrease in the population around central molecule (<4). At lower temperature, 4 nearest neighbours, constituting the first hydration shell, come close to the central molecule leading to contraction. Nearest neighbours between 4 and 12, constituting the second hydration shell, move away from the central molecule leading to a negative thermal expansion [Yan *et al.*, 2007; Cipcigan *et al.*, 2018]. On a similar note, hydrogen bonding are thought to be a plausible explanation for the density anomaly occurring at 4^oC. On decreasing the temperature, there is a gradual rearrangement of hydrogen bonds which forms an open tetrahedrally coordinated hydrogen bond network resulting in formation of water clusters. Formation of the water clusters result in increase in the density on decreasing the temperature. The interplay between 3 and 4 coordinated structures results in the density anomaly of water.

1.3 ROLE OF WATER IN BIOLOGY

Over the decades, water has gained major importance in studying living organisms. Some of the major functions of water in human body are regulating internal body temperature, metabolizing proteins and carbohydrates, insulating the brain, spinal cord thereby acting as a shock absorber. It is the principal solvent as it dissolve minerals, soluble vitamins and certain nutrients. In living organism water is not present in bulk form, rather, it reside in vicinity of substrates or filling small cavities, for instance, interior of the cells, surface of proteins, grooves of the DNA, etc. These water molecules are referred to as "biological-water" [Jungwirth, 2015]. Water has a large number of interactions with biomolecules which differentiates it from bulk. Understanding the relationship between functioning, structure and dynamics of biomolecules in water can help in developing and designing new drugs for enhancing various chemical pathways or by blocking them in order to prevent or cure various diseases. Water is an imperative entrant in protein functioning, maintaining its structure and stability. The assessment of water structure and dynamics near the proteins is necessary for understanding enzymatic activities of proteins, protein - enzyme interactions, etc. One of the foremost characteristics of proteins is its folding and unfolding nature requiring an adequate level of hydration served by water. Water protein interactions are paramount in understanding structure and dynamics of water under the influence of protein dynamics. This can help in understanding protein conformations and their interaction sites used as binding site for several drugs. Thus, water influence structure and dynamics of proteins thereby triggering protein functioning [Tarek and Tobias, 2002; Raschke, 2006; Volkhard, 2006; Chen *et al.*, 2008; Frauenfelder *et al.*, 2009]. Water present at the surface of a DNA plays a vital role in maintaining its stable conformation, DNA-protein function and DNA-ligand recognition. Water plays a major role in assisting conformational and thermodynamic changes of DNA occurring via interactions between DNA and water [Pal *et al.*, 2003]. Water present in major and minor grooves of DNA are of practical importance both in terms of its functionality and understanding the fundamental behavior of DNA. Water interactions at major and minor grooves of DNA significantly differ in dynamics thus indicating preferential motions of water in locale of these grooves. Water in minor grooves has

a slower dynamics depicting a stiff locale near minor grooves [Pal *et al.*, 2006]. Computational and experimental investigations regarding interactions of DNA with other biomolecules in presence of water unravels the functioning and stability of DNA [Lipscomb *et al.*, 1994; Korolev *et al.*, 2002; Maiti and Bagchi, 2006; Furse and Corcelli, 2008]. DNA plays a key role in gene expression and its regulation which is necessary in all living organisms. Protein-DNA complex formation is one of the most important steps in gene regulations in all living organisms. Water has major role to play in this complex process. Molecular dynamics simulation studies found the presence of a rigid thin layer of water between binding sites of the protein and DNA. The origin of rigidity of water layers at protein interaction sites is from the rapid breaking and formation of hydrogen bonds among water molecules. The protein DNA complex formation affects the longitudinal and transverse degrees of freedom of water [Sinha and Bandyopadhyay, 2011]. Apart from regulating membrane functionalities and assisting protein folding/unfolding, it plays a key role in carrying out physiological functioning of the brain by forming water channels known as aquaporin-4 (AQP4) which provides a passage for water movement across cell membranes of the brain cells [Borgnia *et al.*, 1999]. Intoxication of water channels formed in the brain can lead to several diseases such as meningitis, brain stroke, traumatic injury, Alzheimer's disease, etc.

Basic constituent of any living organism is cell which regulates most of their functioning. These cells have protective boundaries known as "cell membranes" which separate the cells from the extra-cellular environment [Rayleigh, 1891; Gennis, 1989; Gorter and Grendel, 2004]. Membrane-water interactions have gained a remarkable attention over the past few decades due to its role in several biological processes. Transportation of small molecules across the lipid membranes allow exchange of extra-cellular substances (such as ions) [Marrink and Berendsen, 1994, 1996], insertion of drugs and antibiotic in cells, maintaining osmotic balance, fusion events, etc. occur in presence of transient water pores formed during cellular processes. In particular, the transient pore formation is relevant for membranes as they maintain a cationic electrochemical gradient which is used for biochemical processes such as, ATP synthesis, transmitting electrical signals and transport of nutrients [Gurtovenko and Vattulainen, 2005]. Hydration water is considered as a crucial component in establishing a dynamical correlation between biological macromolecules with their surrounding environment. Molecular dynamics simulations performed with hydrated phospholipid bilayers show strong hydrogen bond formation of interfacial waters near lipid head-groups. Due to the presence of hydrogen bonds and chemical confinement of interfacial water near lipid heads, a slow down in orientational and translational dynamics is observed for interfacial waters [Bhide and Berkowitz, 2005, 2006].

Study of confined water has been a major topic of research over a past few decades. Advanced experimental techniques and computer simulations have explored various facets of confined water under different confinements. Water confinements have been extensively studied for exploiting properties of bulk water at supercooled temperatures as confined water ceases to freeze in crystalline state as manifested by supercooled water. Thus it is now believed that confined water can mimic structural and dynamical properties of supercooled bulk water provided that the confinement is strong enough to hold water molecules in forming tetrahedral structure as in ice [Bellissent-Funel, 2001; Rovere, 2004]. Theoretically, it can be achieved in three ways viz. water confinement in solid porous material, mixing water with other liquids or salts and hydrating biomolecules such as membranes, proteins, etc. Confined water in biological systems such as proteins, DNA, membranes, etc. have great importance in understanding biological phenomenon and developing drug for biomedical applications. Water confined in these biomolecular systems are generally termed as soft confined systems. There are restricted numbers of available literature on confined systems exhibiting universal slow relaxation time scales.

1.4 SUPERCOOLED WATER

When water is cooled below its freezing temperature (T_f), it crystallizes via first order phase transition to form ice. In supercooled conditions, water cools quickly in such a manner that it

remains in liquid state even at temperatures below its freezing point. Water existing in liquid phase beyond its freezing temperature is known as supercooled or glassy water [Mishima and Stanley, 1998; Handle *et al.*, 2017]. The anomalous behavior of water becomes more pronounced in supercooled regions. Glassy water has been a subject of intense research over the past few decades. The most interesting characteristic feature of supercooled liquids is the dynamic glass transition. When liquid water is cooled below a certain point beyond its freezing temperature, relaxation time increases drastically such that dynamical arrest starts emerging and the system cannot be equilibrated with reasonable experimental times [Cavagna, 2009]. An exhaustive investigation regarding supercooled water has been carried out using experiments and computer simulations on water anomalies near $\sim 4^oC$. Computer simulations show the evidences of structural arrest of water molecules due to cage-effect near this temperature which are in agreement with mode coupling temperature for supercooled liquids [Gotze and Sjogren, 1992; Sokolov *et al.*, 1995; Gallo *et al.*, 1996; Sciortino *et al.*, 1996]. Structural arrest of molecules in supercooled water leads to the formation of transient regions which have 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 heterogeneity are strongly correlated and fraction of molecules constituting these heterogeneities increase on 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].

The underlying mechanisms behind heterogeneous dynamics of supercooled water can be interpreted from predictions of the mode coupling theory (MCT). The mode coupling theory of glass transition [Götze, 2008] describes the idea that at lower temperature, microscopic dynamics of the particle drastically slows down leading to a cage-effect. The cage-effect is the phenomenon where a particle is temporarily confined by its surrounding particles and it takes some time to escape from the cage. The structural relaxation of particles diverge with a power law as $\tau \sim (T - T_C)^{-\gamma}$, where γ is the universal exponent and T_C is the mode coupling temperature. Mode coupling temperature is defined as the temperature at which there is a divergence in the relaxation rate and the system is no longer able to rearrange itself [Marzio *et al.*, 2017]. The cross-over from cage to diffusive regime is reported by various water models viz. ST2, SPC/E [Starr *et al.*, 1999] TIP5P [Xu *et al.*, 2005] and TIP4P/2005 [Marzio *et al.*, 2016]. The escape of molecules from the cage can be understood via hopping mechanism. For real liquid systems, at supercooled temperatures, the thermal fluctuations are so small that they cannot contribute to overcome the threshold required for diffusion. So, at longer times the molecules tend to hop from their respective cages and enter into diffusive regime. Since the hopping mechanism is an activated process, its dynamics is characterized by a strong Arrhenius dependence. Liquids obeying Arrhenius behavior with respect to temperature are known as strong liquids and which do not follow Arrhenius behavior with temperature are fragile liquids [Angell, 1985].

The caging of water molecules at supercooled temperatures can be quantified by computing time dependent displacement distribution or van Hove correlation functions. van Hove correlation function gives the probability of finding a particle at position r provided that the particle is present at the origin at time $t = 0$. Self part of van Hove correlation functions have the functional form [Hove, 1954; Hopkins *et al.*, 2010],

$$G_s(r,t) = \frac{1}{N}\Big\langle \sum_{i=1}^{N} \delta(r - r_i(t) + r_i(0)) \Big\rangle \tag{1.1}$$

where, $\delta(...)$ is Dirac delta function and $< ... >$ is the ensemble averaging. For supercooled water, below mode coupling temperature, van Hove correlation function develop sharp peaks for longer time scales signifying prolonged caging of molecules. With an increase in temperature, there is an emergence of second peak at longer times. This indicates the onset of escape mechanism of water from their respective cages [Marzio *et al.*, 2017] for diffusion.

The relation between translational diffusion constant (D) and shear viscosity (η) can be established by Stokes-Einstein (SE) relation [A.Einstein, 1905] where, $D \sim (\eta/T)^{-1}$, T is the temperature. At

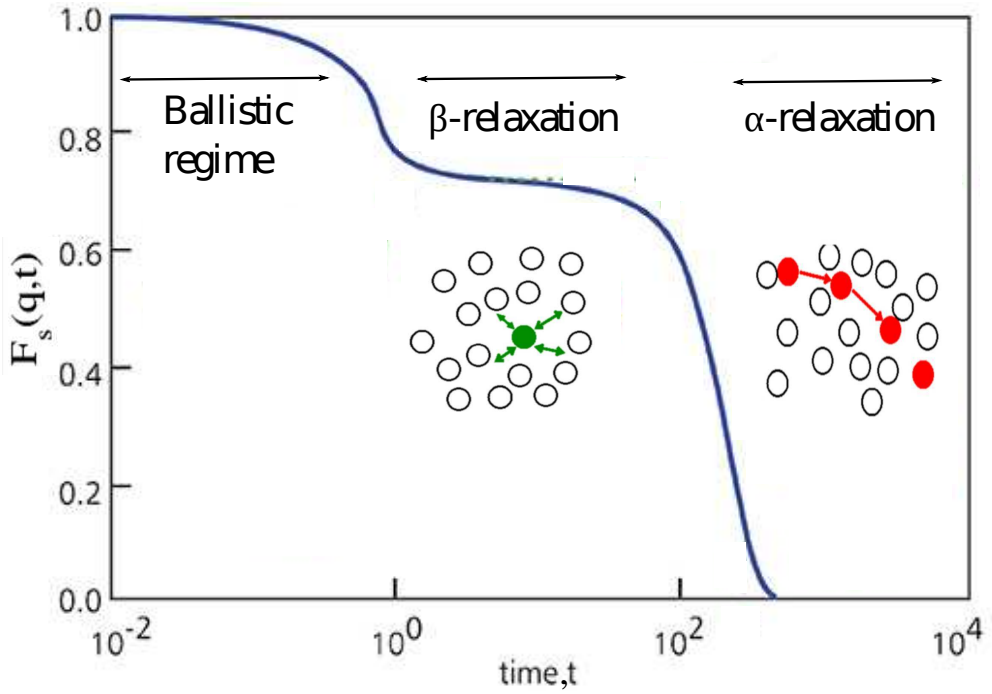

Figure 1.5: Self intermediate scattering function (F_s(q,t)) for bulk water at room temperature showing β and α relaxation regions. The pictorial representation of β and α relaxations are demonstrated by particles in green and red circles representing trapped and escaping molecules from its respective cage. The image is taken from [Janssen, 2018].

physiological temperature, the relationship holds true for liquid systems with τ_α as the characteristic relaxation time associated with structural α-relaxation. However, at supercooled temperatures, the Stokes-Einstein relation no longer holds valid where structural relaxations of molecules becomes heterogeneous. Violation of Stokes-Einstein relation is attributed to an increase in non-Gaussianity from van Hove correlation functions for bulk water at supercooled temperatures [Kawasaki and Kim, 2017].

1.5 DYNAMICAL HETEROGENEITIES

The concept of dynamical heterogeneity emerges from experimental investigations of viscous liquids approaching glass transition temperatures. For systems approaching glass transition, structural disorderness prevail in the system implying formation of localized domains of particles each manifesting different dynamical behavior with different relaxation time scales. For quantifying dynamical heterogeneities, two and four point correlators are used. Two-point correlators include intermediate scattering function which is,

$$F_s(q,t) = \frac{1}{N}\sum_{i=1}^{N}\left\langle exp(iq.[r_i(t) - r_i(0)]\right\rangle \tag{1.2}$$

At higher temperatures, the correlation function decays to 0 in an exponential manner. However, when the temperature is lowered, the exponential behavior of the function is lost and it starts decaying non-exponentially. As the temperature approaches glass transition temperature, the decay proceeds in defined steps. After an initial ballistic decay, a slow relaxation (β-relaxation) occurs leading to an emergence of a plateau region where the correlation function is almost constant over the time scale. The plateau region is followed by a second step relaxation (α-relaxation) where the

correlation function decays to zero. The two relaxations are shown in figure 1.5 where pictorially it is shown that the particle (shown by green color in snapshot) gets trapped in a cage formed by its neighboring particles followed by escape mechanism where the particle leaves the cage and enters in diffusive regime (shown by red color in snapshot). Width of the plateau region is proportional to temperature and is also dependent on wave vector (q). The plateau region in the intermediate scattering function correspond to the Debye-Waller factor which quantifies the degree of caging for fluids persisting for the time scales which increase with decrease in temperature. The decay of the intermediate scattering function in α-relaxation regime is represented by the functional form,

$$f(t) = f_k \Phi(t^*) \tag{1.3}$$

where, $t^* \equiv \frac{t}{\tau_k}$, f_k denotes the plateau and $\Phi(t^*)$ is the universal scaling function. This scaling function is modelled by Kohlrausch-Williams-Watts (KWW) function given by,

$$\Phi(t^*) \approx exp\Big[- \left(\frac{t}{\tau}\right)^{\beta} \Big] \tag{1.4}$$

where, f(t) is the functional form of stretched exponential and β is the stretched exponent. For understanding the origin of two different kind of relaxations, it is beneficial to know size of the domains which are relaxing faster or slower, temperature dependency of localized domains, etc. Different domains prevailing in liquids relax in different manner with different rates thus producing a wide range of relaxation time scales, following non-exponential decay.

For fragile glass forming liquids, relaxation and correlation functions become increasingly non-exponential at glass transition temperature. The non-exponential behavior in fragile glass forming liquids have two different explanations. First, exponential relaxation of the particles in one environment may be non-exponential in the other which implies that the relaxation varies significantly in different domains of the same system. Second, supercooled liquids are homogeneous in nature where each particle has a relaxation which is non-exponential in nature. In this case, there is an increase in the cooperative movement of the particles with decrease in stretched parameter (β). Formation of different localized domains at supercooled temperatures raises several questions viz. nature of these localized domains, size of these domains, relation between their size distribution and relaxation time scales, classification of these domains, etc.

Several experimental studies addressed these questions where equilibrium length scale of dynamic heterogeneities are measured for poly (vinyl acetate) [Tracht *et al.*, 1998] and glycerol at supercooled temperatures [Reinsberg *et al.*, 2001] using multidimensional ^{13}C solid-state exchange NMR experiment. Spatially resolved NMR experiments are difficult and their approach for determining dynamic correlation length scale is indirect and only has been used on small number of liquids for a narrow temperature window [Ediger, 2000]. Experimentally, dynamical heterogeneities are measured from infrared experiments in molecular liquids [Sillescu, 1999; Ediger, 2000; Richert, 2002]. Experimental techniques for studying dynamical heterogeneities require further development where fluctuations can be resolved and which are not sensitive to the average behavior [Ediger, 2000]. Although, these experimental techniques give signatures of dynamical heterogeneities, they are not able to predict the underlying mechanism responsible for these heterogeneities nor they are able to determine their properties such as shape or sizes [Cicerone *et al.*, 1995].

In simulations, particles with pair potential functions have been used as models for investigating dynamical heterogeneities at supercooled temperatures where particles exhibit two characteristics. First, highly intermittent particles exhibiting long relaxations: these particles have rattling motion around well defined domains followed by sudden jumps. Waiting time of the particles before sudden jump are broadly distributed. Using molecular dynamics simulations, it is possible to identify particles as mobile and immobile particles. These immobile particles form clusters whose size depend on temperature. With a decrease in temperature there is a slow down in particle dynamics as they tend to cluster together. With further decrease in temperature, the population of the clustered particles increases and they tend to form clusters of bigger sizes. It is also observed that relaxation time for immobile particles is larger than mobile particles and the difference in relaxation time scales between immobile and mobile particles increases with a decrease in temperature. A

string like cooperative motion is observed in the liquids at temperature above glass transition. The average length of these strings increases on decreasing the temperature and their distribution is found to be exponential [Kob *et al.*, 1997; Donati *et al.*, 1998]. On a similar note, polymer model consisting of 400 bead spring polymers each having 20 monomers are also simulated at supercooled temperatures. It is observed that the time scales of high mobility clusters are associated with diffusive relaxation while low mobility clusters exhibit structural relaxation times. The difference between high and low mobility cluster time scales reveal decoupling of structural relaxation time scale with diffusion [Starr *et al.*, 2013]. Computer simulations have also been performed for bulk water at supercooled temperatures. Water models such as SPC/E [Gallo *et al.*, 1996; Sciortino *et al.*, 1996; Gallo *et al.*, 2000b], TIP4P/2005 [Marzio *et al.*, 2017; Kawasaki and Kim, 2017] are used for capturing origin of slow relaxations in bulk water at supercooled temperatures. Single particle dynamics of water at supercooled temperature follows a two-step relaxation process viz. β and α-relaxation which is in agreement with the mode coupling theory of liquids at supercooled temperatures. A crossover from q^2 to q dependency is observed at the time scale analogous to α-decay. These long-lived molecular cages and their associated slow relaxation dynamics can be inferred as the source of origin of anomalies in transport coefficients of water.
Study of dynamics of water in confined environment at supercooled temperatures has gained much attention over the past few decades. Two major effects contribute to the distinct behavior of confined water are its geometrical confinement and interactions with surface of the substrate. Molecular dynamics simulations of water confined in a silica pore at supercooled temperatures show two distinct relaxation time regimes. Free water molecules show bulk-like behavior and its dynamics is in accordance with mode coupling theory. Confined water residing in silica nano-pores manifests a drastic slow down in dynamics at room temperature [Gallo *et al.*, 2000b]. Similar molecular dynamics studies are conducted for aqueous solutions of trehalose and maltose at supercooled temperatures using SPC/E water model. Disaccharides maintain a favourable environment for water which is in contact with biomolecular surface even at lower water content or temperature. Among trehalose and maltose, trehalose does not form clusters in aqueous solutions thereby providing a wider interaction surface and offers a better choice as a bioprotectant [Magno and Gallo, 2011].

1.6 COMPARISON OF SUPERCOOLED WATER AND CONFINED WATER NEAR BIOLOGICAL SURFACES

Study of confined water has gained a remarkable importance in the areas of biology, chemistry, geology, etc., where the system properties are highly dependent on water concentration, its structural and dynamical properties. Another reason for focussing on confined water is the possibility of exploring numerous structural and dynamical properties of water in deeply supercooled regime. The confinement of water should be strong enough to prevent water molecules from forming a tetrahedral network. Experimentally, it is a tideous task to study supercooled water as the anomalies become more pronounced making it cumbersome to investigate structural, thermodynamical and dynamical properties. It has been proposed that water confined in small volumes such as in protein-binding pockets, narrow membrane pores, membrane protein channels [Rasaiah *et al.*, 2008; Li *et al.*, 2013], etc. can assist in gaining deeper insights on the behavior of bulk water in deep supercooled regions. This is because confined water can surpass crystallization process under confinements [Capponi *et al.*, 2011; Cerveny *et al.*, 2016]. Experimental techniques such as neutron diffraction and neutron scattering have been employed for comparing supercooled bulk water and water confined in silica substrate. Both the waters are found exhibiting similar trends in terms of enhanced hydrogen bonding strength and oxygen-oxygen distance in the first hydration shell. These findings show that confined water can be used as a model for studying supercooled bulk water [Ricci *et al.*, 2009]. Computer simulations have also reported that biologically confined and supercooled water exhibit similar trends in slow down in local structural relaxations. Hydrogen bonding network rearrangement is observed to be slowed down at a similar time scale for both waters [Capponi *et al.*, 2019].

1.7 EXPERIMENTS

There are several experimental techniques which can measure dynamics and structure of confined water. These are Quasi Elastic Neutron Scattering (QENS) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, Vibrational Sum Frequency Generation (VSFG), Infrared spectroscopy (IR), etc. The advantage of these techniques is that they can probe long time β-relaxation processes which can be distinguished from short time α-relaxation. QENS can probe self-particle and all particle motions for incoherent and coherent scattering respectively. The length scale dependency of relaxation time scales can be probed using QENS. However, QENS is limited by its narrow range of frequencies. NMR spectroscopy can not only probe the relaxation time scales but also accounts for molecular reorientation. Time dependent correlation functions obtained from NMR can yield the information regarding angular jumps of reorientation mechanisms. QENS and NMR spectroscopic techniques are widely employed for studying structure and dynamics of lipid bilayers and the mechanisms of water orientational relaxation rates.

NMR spectroscopy is a widely accepted technique for probing hydration water on biological surfaces. These high resolution spectroscopic experiments can probe hydration water in proximity of membranes and membrane proteins. Hydration level plays a major role in functioning of the membranes. The membranes can modulate their phases in response to the level of hydration [Ulrich and Watts, 1994]. Upon decrease in hydration water concentration, N^+ end of the phosphocholine dipole of lipid bilayer move closer towards the hydrophobic region. The driving force behind the changes in alignment of the phosphocholine group is the electrostatic interaction between water and phosphocholine dipole [Bechinger and Seelig, 1991]. ^{13}C NMR shows location of hydration water near polar head-group as well as near carbonyl and glycerol moieties of the lipid membrane exhibiting the lifetime of the order of 100 ps [Volke *et al.*, 1994; Volke and Pampel, 1995]. Studying membrane phases are significant from biological and biophysical view point. 1H NMR spectroscopy characterizes phase transition of membranes over a wide range of temperature which can help in developing membrane-protective cryoprotectants and studying freezing tolerant organisms [Wolfe and Bryant, 1999; Mandal and van der Wel, 2016]. In addition to the phase transitions, the relaxation rates of lipid membranes are also dependent on hydration levels. This gives new insights on role of water in determining structural dynamics and viscoelastic properties of membranes [Molugu *et al.*, 2018]. Dynamics of hydration water near DMPC lipid bilayers are studied using ^{2}H-NMR spectroscopy at subzero temperatures. The hydration level for DMPC bilayer is 8 water molecules per lipid. It has been observed that about $1-2$ water molecules associated with phosphate group of DMPC lipid bilayer freeze at $-70°C$ and $5-6$ water molecules near choline group aggregate to form water clusters and remain unfrozen at $-70°C$ [Hsieh and Wu, 1997].

QENS is one of the powerful experimental techniques for probing water dynamics near lipid bilayers. Planar DMPC lipid bilayer fully hydrated with ~ 9 water molecules per lipid is investigated to study microscopic dynamics using QENS. Water and lipid dynamics are separately investigated using selective deuteration approach where self and collective dynamics are probed separately. Further, it is possible to probe molecular motions parallel and perpendicular to the bilayer by varying orientation of the samples with respect to incident neutron beam. At noticeably lower temperatures (260 K), water dynamics is dominated by rotational motions where near phase transition temperature (gel to liquid, 295 K), a cross-over from rotational to translational motion is observed which is well described by jump-diffusion model. Because of the experimental limitations in terms of time and length scales, isotropic translational diffusion is observed for water where the hydrated system is anisotropic [Swenson *et al.*, 2008]. Quasi elastic scattering data for hydration water near DMPC membranes show distinct relaxation time scales well described by KWW functions [Williams and Watts, 1970] as mentioned in equation 1.4. The relaxation time scale of hydration water show sub-diffusive and anisotropic diffusion of hydration water spanning over nanometer distances. Bulk water shows intrinsic sub-diffusion of the order of several pico-seconds probably due to caging effects [Toppozini *et al.*, 2015]. Dynamics of confined water in aqueous solution of poly vinyl methyl ether shows diffusive-like motions at higher temperatures while confined behavior is observed on decreasing the temperature revealing presence of a strong

dynamical asymmetry in the solution. At temperature near 225 K, water starts exhibiting confined behavior which is due to the freezing of the polymer surrounding the water molecules. Similar crossover from an Arrhenius (lower temperature) to non-Arrhenius (higher temperature) behavior is observed for confined water [Cerveny *et al.*, 2005] due to the freezing dynamics of poly vinyl methyl ether. Vibrational density of states for poly vinyl methyl ether are strongly affected by presence of water in glassy state. Non-Gaussian dynamics is observed for both confined water and poly vinyl methyl ether [Capponi *et al.*, 2011].

Hydrogen bonding interactions in water are of great importance in biological and chemical applications. In biological environments, water is found in crowded regions where it hydrates the closest membranes and large molecules. In chemical environments, water plays a major role as a polar solvent where it is found residing at the interfaces. Potential of water to form hydrogen bonds at the chemical interfaces makes it distinct from bulk water. Dynamics of hydrogen bonding rearrangement is of the order of pico seconds, therefore femto second time resolved IR spectroscopy can probe dynamics of water occurring at pico-second time scales. A pioneering 2D infrared (2D-IR) spectroscopy experiment on water directly characterizes the mechanism of hydrogen bond network rearrangement [Woutersen and Bakker, 1999; Cowan *et al.*, 2005] by probing the OH frequency time evolution. However, the 2D-IR cannot distinguish between the formation of a hydrogen bond with and without allowing the change of partners. Vibrational relaxation time in HDO:D_2O solution shows anomalous temperature dependence which increases with temperature. O-H bond vibrational lifetime for ice is observed to be of the order of sub-pico seconds spanning over a temperature range of $33 - 270$ K. For liquid water, it is comparatively higher than ice as shifting from 270 K to 363 K. This increase in vibrational lifetime reveals temperature dependent vibrational relaxation anomalies of water. Vibrational relaxation in liquid water is higher than other hydrogen bonded systems as water allows fast dissipation of energy [Nienhuys *et al.*, 1999]. Orientational relaxation time scales of water at neutral and ionic surface probed by IR spectroscopy are $\sim$ 13 and 18 ps respectively which are higher than bulk. Presence of biological or chemical interface affects hydrogen bond dynamics more as compared to the nature of the interface (ionic or neutral) [Fenn *et al.*, 2009; Moilanen *et al.*, 2009]. On a similar note, two distinct vibrational lifetime are found for water residing at DMPC bilayer interfaces when OD vibrations of water are probed via femto second mid IR spectroscopy. These two distinct vibrational lifetimes are associated with phosphate and choline moiety of lipids and are identified as fast ($\sim$ 0.60 ps) and slow ($\sim$ 1.90 ps) vibrational lifetimes respectively. OD stretching vibrational lifetime of water associated with choline moiety manifests a slow down in vibrational relaxation with a decrease in temperature. Phase of the bilayer have a vital role in governing vibrational dynamics of water residing in the vicinity of lipid head groups. For instance, in gel phase, the tight packing of lipids restrict other molecules near choline moiety to interact with water molecules associated residing near choline moiety. This suggest preferred interactions of biomolecules towards choline moiety of membrane head-groups [Kundu *et al.*, 2016a, 2017] during adsorption.

Vibrational sum frequency generation (VSFG) spectroscopy experiments are realized for obtaining molecular level information of water present at membrane interfaces. The high sensitivity of VSFG allows to obtain vibrational spectra of water at lipid interface which can give information on structure of interfacial water. VSFG spectrum shows three different type of water orientation at lipid-water interface based on the orientation of hydrogen bonds of water. If the vibrational spectra of OH stretch has a positive sign, it is referred to as H-up orientation and vibrational spectra with negative sign correspond to H-down orientation. The interfacial water orientations are identified based on the orientation of water residing near hydrophilic and hydrophobic regions. Water residing near choline moiety have weak hydrogen bonding with H-down orientation and water residing near phosphate moiety are strongly hydrogen bonded and have H-up orientation. Water residing near hydrophobic chains have weak interactions and also have H-up orientation [Mondal *et al.*, 2012]. The orientation of water at lipid interface is dependent on the charge of the head-group moiety [Chen *et al.*, 2010; Nojima *et al.*, 2017].

Despite robustness of NMR, QENS, femto second time resolved IR and VSFG spectroscopy, they come with some limitations which can be addressed using molecular dynamics simulations. NMR

have inherently low intensity signals which are due to low magnetic moment of nuclei. Therefore compounds present in micromolar concentrations cannot be detected by NMR. Most of the NMR equipments are motion sensitive which leads to signal distortions on quantitative measurements [Chatham and Blackband, 2001; Emwas, 2015; Spiess, 2017]. Ideally, cell-NMR is a powerful tool for studying dynamics of cell membranes at atomistic levels, however there are few limitations. First, there are only few proteins available which can provide high quality NMR spectra inside the cells. Second, life-span of cells inside the NMR tube is not enough for gathering high quality relaxation data. Third, contamination of relaxation time scales by protein interactions give erroneous results [Li and Liu, 2013]. Forth, quantification of hydrogen bond dynamics of water in spectroscopic experiments exhibits non-Condon effect. The Condon principle states that in a liquid system, dipole moment associated with electronic transition is independent of vibrational coordinates of all molecules present. For liquid water, OH stretch vibrational transitions for different clusters exhibit different transition dipole moments [Huggins and Pimentel, 1956] signifying dependency of vibrational coordinates on electronic transition dipole moments indicating violation of Condon principle [Schmidt *et al.*, 2005]. For studying structure and dynamics of water, it is imperative to include these effects in order to quantify any frequency dependent time-correlation property [Schmidt *et al.*, 2005]. Vibrational relaxation time scales of water are of the order of $\sim$ 1.8 ps which cannot be captured in spectroscopic experiments. Thus it becomes difficult to probe water dynamics precisely [Yamada *et al.*, 2017].

1.8 COMPUTER SIMULATIONS

Atomic level elucidation of membrane water interface is necessary for understanding many important bio-chemical phenomenon. The foremost requirement of membranes for proper functioning is the level of hydration. Hydration level in membranes plays an important role in membrane functionality [Killian, 1998; Ueda and Yoshida, 1999]. Structural properties of membranes such as area per lipid, order parameter, diffusion, electron densities, reorientation, etc. are strongly affected by the level of hydration. In low hydration levels, membranes tend to approach towards a more ordered state where its acyl chains are stiffened and orientation of lipid head-group becomes parallel to plane of the membranes. Under low levels of hydration, the phase transition temperature shifts towards a higher temperature region [Trapp *et al.*, 2010]. Interestingly, lipid dynamics is greatly affected by decrease in hydration levels while structural properties are moderately affected [Högberg and Lyubartsev, 2006; Zhao *et al.*, 2008]. Many other environmental factors such as temperature, ionic strength, pH, salt concentration, etc. affect the properties of membranes. 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 several ions (Na^+, K^+) [Papahadjopoulos *et al.*, 1972], small molecules (steroids, glycerols, drugs) [Orbach and Finkelstein, 1980; Majumdar *et al.*, 2004], peptides [Rezai *et al.*, 2006], etc. are strongly affected by local orientational structure of water near membranes [Yang and Hinner, 2015]. At membrane surfaces water reorientation is distinct than bulk water. Interfacial water residing near membrane head-group regions show preferred orientation towards lipid heads. Water residing near hydrophobic regions form small hydrogen bonded clusters. Distortion in tetrahedral arrangement of water is found near lipid head regions [Mezei, 2001]. Cholesterol induced stiffening of membranes weakens the water-membrane interaction thereby reducing the number of interfacial water [Päslack *et al.*, 2019b].

Membranes have diverse functionalities relevant from fundamental processes of cell biology such as viral infections, fertilization, etc. Membrane fusion is among one of the most important processes of membranes. It is mixing of two different membranes into a single continuous layer. The fusion processes are catalyzed by various proteins which initiate the process of initial recognition of membranes followed by initiating mixing of lipids [Jahn *et al.*, 2003]. It is now a well established fact that water residing near lipid heads exhibits a slow down in its dynamics. During fusion process, the slow down in water dynamics is further amplified as water dynamics becomes glassy

and a dynamic coupling prevails between water layer and the approaching leaflets of the membrane. The dynamic coupling between bilayers is mediated by hydrogen bonding between lipid-water-lipid. These hydrogen bonds start dominating over water-water hydrogen bonding at the leaflet interface [Pronk *et al.*, 2015]. Investigating interactions of lipid bilayers with macromolecules in presence of water serves as a tool in various applications such as drug deliveries, biomedical applications, etc. One such macromolecule is caffeine which is amphiphilic in nature. It is known for promoting attentiveness in human beings, additive for enhancing anelgesics and acting as a psychoactive drug [Nathanson, 1984; Nehlig *et al.*, 1992]. Membrane caffeine interaction has a significant effect on interfacial water residing near membrane head-group regions. Caffeine has an attractive electrostatic interaction with interfacial water leading to an increase in local density of water at the lipid head-group interface. This increase in local density of water increases the bilayer thickness thereby decreasing its fluidity [Khondker *et al.*, 2017]. Unlike caffeine which is amphiphilic in nature, curcumin is another macromolecule which is hydrophobic in nature and is found to partition in lipid membranes causing thinning of the bilayer leading to alteration in bilayer properties [Ingolfsson *et al.*, 2007; Hung *et al.*, 2008]. It is mostly found in turmeric and is known for its anti-oxidant and anti-Alzheimer properties. Computer simulations of lipid-curcumin interaction in hydrated and dehydrated state shows a protecting mechanism exhibited by curcumin. Curcumin interacts with lipid via two mechanisms. First, under dehydrated state, it lies flat on the membrane surface and acts as a layer for protecting the membranes from rupturing by forming a steric barrier. Second, under hydrated state it penetrates in the membranes and stiffen the acyl chains thereby hindering peptide insertion [Alsop *et al.*, 2017].
For investigating interactions among DMPC lipid head-groups and their associations in presence of water, molecular dynamics simulations are carried out at physiological temperatures where it is found that water acts as a cross-linking bridge between negatively charged groups by simultaneously forming hydrogen bonds with two DMPC molecules. It is reported that 76% of the DMPC molecules are hydrogen bonded with water molecules forming a cross-bridging network. DMPC lipid membrane contains positively and negatively charged groups although the overall molecule is neutral. These positively and negatively charged groups form stable charge associations which constitutes 93% of the linking of DMPC molecules. The overall percentage of lipid-lipid associations comes from water bridging networks and stable charge associations which is $\sim$ 98% [Pasenkiewicz-Gierula *et al.*, 1999]. Extended water network at surface of the lipid membranes affect dynamics of both lipids and water. To some extent, the dynamics of water and lipids are correlated. A cooperative molecular motion persists between hydration water and lipid molecules indicating presence of dynamic coupling between hydration water and lipids.

1.9 OBJECTIVE

The research work presented in the thesis mainly contemplates the study of dynamical properties of water residing in the vicinity of cell membranes. The structural stability and functioning of the cell membranes is slaved by hydration water which resides at the cell membrane surface. The objectives of the thesis are the following:

- Finding the suitable combination of lipid force fields and water models to study hydration dynamics in computer simulations keeping membrane phase intact as in experiments.
- Finding the influence of chemical confinement on dynamics of water near membranes and getting insights on thermodynamic stability of membrane-water interface relevant to biological systems.
- Quantification of slow relaxations of chemically confined water and length scale of dynamical heterogeneities in chemically confined water.
- Understanding the mutual influence of hydration water and bilayer dynamics so that water

dynamics can probe regional membrane dynamics for several vital biological functions at physiological temperatures.

1.10 THESIS OUTLINE

The thesis presented here focuses on dynamics of hydration water residing in the locale of lipid bilayers using molecular dynamics simulations. **Chapter 2** discusses a comparison of dynamics of water with different water models in presence of lipid bilayers using CHARMM36 and Berger force fields. The choice of water model to be used in conjunction with lipid force fields is a challenging task till date and a general consensus has not been yet established. Bilayer properties in particular, area per head-group, bilayer thickness, order parameter and lateral diffusivity are calculated which confirmed the fluidic phase of the bilayer as in experiments. Dynamical properties such as anomalous diffusion exponent, velocity auto correlation function and non-Gaussian parameter are calculated for interfacial water which reveal TIP4P/2005 water model apprehend hydration water dynamics at lipid interface at fluid phase more accurately than TIP3P water model implying its applicability to other biological systems as well. **Chapter 3** investigates the influence of chemical confinement on dynamics of hydration layers of a lipid membrane. Interfacial water molecules are classified on the basis of hydrogen bonding between interfacial water and oxygens of different lipid head moieties by decoupling bulk and interfacial water interactions with lipids. Several dynamical properties such as translational mean square displacement, reorientational auto correlation function and hydrogen bond auto correlation function are computed showing influence of lipid head-groups on hydration water. Kinetics of hydrogen bond breaking and formation are investigated employing reactive flux analysis. This indicates the importance of lipid head-groups on activation energy of hydrogen bond breaking mechanism. Our calculations show that interfacial water continuously hydrogen bonded to different moieties of lipid head results in bridging network of interfacial water with lipid head-groups. This bridging network formed between interface water and lipid head moieties revealed water mediated lipid-lipid associations. **Chapter 4** describes the investigation of slow relaxations of interface water near the bilayer. The slow relaxations are found to be universal irrespective of the nature of chemical confinement as the underlying source is dynamical heterogeneities at physiological temperature. Further investigations reveal three time scales of water relaxations under the influence of chemical confinement. Longer time relaxations of interfacial water are in agreement with their respective hydrogen bonding lifetimes irrespective of the nature of chemical confinement and confinement lifetime. Crossover from non-Gaussianity to Gaussianity in one dimensional van Hove correlation functions is observed for interfacial waters using block analysis approach. Dynamical heterogeneity length scale is found to be comparable to the wave-length of small and weak undulations of the membranes. **Chapter 5** elucidates coupling of hydration layer with lipid membranes which is important for understanding membrane dynamics and its functionality towards various biological processes. Chemically confined interfacial water and lipid membranes exhibit distinct relaxation rates manifesting heterogeneous dynamics. Fast relaxation time scale for interfacial water and their associated lipid moieties are strongly coupled and slow relaxation rates are strongly correlated by a scaling factor. Chemically confined interfacial water show Fickian yet intermittent behavior leading to glassy dynamics at physiological temperature. Intermittency in interfacial water arises due to the caging of molecules hydrogen bonded to their respective lipid moieties. Our calculations quantify the correlation between relaxation time scales of interface water and hydrogen bonded lipid moieties to find a sensitive reflector of regional membrane dynamics in the future.