

Structural changes of interfacial water upon fluid-ripple-gel Phase transitions of bilayers

4.1 INTRODUCTION

Cell membranes define the boundaries of live cells and assist in carrying out biological activities by allowing extracellular substances to enter and exit the cell. Water plays an instrumental role in assisting the membrane functioning [Gurtovenko and Vattulainen, 2005]. The hydration level defined by the number of water molecules per lipid head in a membrane, is a major determinant of membrane's fluidity. Using surface-specific vibrational spectroscopy, structure of air-water interface and bulk water are compared [Bonn *et al.*, 2015]. Despite the fact that water around bio and soft surfaces has been intensively studied [Srivastava and Debnath, 2018] with correlating their dynamical properties with phase transitions [Debnath *et al.*, 2013], the influence of water structure on the phase behaviour of membranes remains unattended so far.

Experimental studies have shown the structural origin of anomalous properties of liquid water [Nilsson and Pettersson, 2015] at different temperatures ranging from 328 K to 248 K. Water becomes anomalous not just in terms of thermodynamic response functions [Debenedetti and Stillinger, 2001], but also in terms of structures. The classic symmetric and the recently presented asymmetric models of liquid water offer a significant step toward a better understanding of the electronic structure of the hydrogen-bond network of water [Kühne and Khaliullin, 2013]. Similar structures are referred to as tetrahedral or locally favoured [Leetmaa *et al.*, 2008] or low-density liquid (LDL) and distorted or normal [Russo and Tanaka, 2014] or with a high degree of interstitials as high-density liquid (HDL) [Soper and Ricci, 2000] depending upon the properties observed or calculated in experiments or simulations. Interestingly, the terminologies HDL and LDL in the extreme supercooled regime have some historical ties to the liquid-liquid critical point (LLCP) hypothesis that water might exist as metastable macroscopic phases at a second critical point which is never observed for an one component fluid [Poole *et al.*, 1992]. A rough estimate for the LLCP type of behavior may be within the estimates obtained by decompression-induced melting of ice IV [Mishima and Stanley, 1998]. However, experimentally determining the extent of such tetrahedral local structures remains difficult and contentious [Skinner *et al.*, 2013].

Molecular dynamics (MD) simulations have been used in conjunction with the experiments to better understand the structural and dynamical features of water at the microscopic level [Srivastava *et al.*, 2019b]. The tetrahedral structure of bulk water (BW) molecules is supported by pair correlation functions (PCF) of oxygen atoms [Dhabal *et al.*, 2014]. The occurrence of a second peak in the O-O PCF at about $\sqrt{\frac{8}{3}}$ times the position of the first peak, which is roughly the second nearest neighbour distance of a tetrahedron, is regarded as the most recognised proof of the existence of tetrahedral structures in liquid water [Soper and Ricci, 2000]. Simulations with TIP4P/2005 water model demonstrate qualitatively compatible patterns with experimental data [Overduin and Patey, 2012; Wikfeldt *et al.*, 2011b,a]. The so-called first sharp diffraction

peak from MD, which is widely observed in silica and other tetrahedral liquids, results from the most protracted period of density wave associated with a tetrahedral water structure [Shi and Tanaka, 2020]. Although many research have been conducted on the structure of supercooled bulk water [Vega *et al.*, 2009; Jorgensen *et al.*, 1983], relatively no analyses has been conducted on biological water or interfacial water (IW) near the biological membranes.

To this objective, we conducted all-atom MD simulations of dimyristoylphosphatidylcholine (DMPC) bilayers which shows a fluid to ripple phase transition at 297 K and ripple to gel phase transition as 284 K. Radial distribution functions (RDFs), angular distribution distributions and potentials of mean forces of oxygen atoms in the hydration layers of lipid bilayers and in the BW are determined. The research examines the impact of the local structure of hydration water on the membrane phases. For a better understanding of the structure of the water, all of the calculations are compared to BW at each temperature. Our calculations reveal that the IW changes from distorted to tetrahedral arrangement upon lowering the temperature till 218 K which is 14 K below the homogeneous ice nucleation temperature [Mason, 1958]. Such a lower temperature is known as “no man’s land” since it is challenging to experimentally probe water in such a supercooled regime due to rapid ice crystallization. Such structural changes are strongly associated with membrane phase transitions.

4.2 SIMULATION DETAILS

All atom molecular dynamics simulations are performed using fully hydrated 128 DMPC molecules using CHARMM36 force fields solvated with 5743 TIP4P/2005 [Abascal and Vega, 2005] water molecules [Lopez *et al.*, 2004]. Initially an equilibrated bilayer is used from our previous work [Srivastava *et al.*, 2019b] at 308 K, from which simulation annealing is done in the NPT ensemble with a 0.02 K/ps annealing rate from 324 K to 284 K. A pre-equilibrated bilayer of 72 lipids at gel phase [Khakbaz and Klauda, 2018] is used to generate the gel phases with 128 lipids with same annealing rate from 273 K to 218 K and then twelve bilayers are equilibrated for 300 to 1000 ns in NPT ensembles. The bilayer undergoes a fluid to ripple phase transition at 297 K and a ripple to gel phase transition at 284 K (see figure 4.1). The pressure and temperatures are kept constant at 1 bar and respective temperatures using Berendsen pressure and temperature coupling [Berendsen *et al.*, 1984] methods with 0.5 ps coupling constants for all systems. Long-range interactions are rectified using the Particle Mesh Ewald (PME) approach with a grid size of 0.12 nm, while van der Waals and Coulombic interactions are cutoff at 1.2 nm. LINCS algorithm [Hess *et al.*, 1997] is used to constrain all bonds during the equilibration. Convergence in area per head group of the lipids (shown in figure 4.2), temperature and pressure indicate that the bilayers are well equilibrated and our results match well with previously done computational studies [Khakbaz and Klauda, 2018]. Next, NVT runs are carried out for 60 ns with a time step of 0.4 fs without constraints for all systems. Nosé-Hoover temperature coupling is used in NVT simulations. In all three directions, periodic boundary conditions are imposed. Trajectories are stored in every 0.1 ps.

To compare the structure of the IW, 851 bulk water molecules (BW) are simulated without DMPC lipids at same temperatures of bilayers. A total of $\sim 9.45\mu\text{s}$ all-atom molecular dynamics (MD) simulations are carried out for the entire calculations in this study. GROMACS-4.6.5 software is used for all simulations [Van Der Spoel *et al.*, 2005].

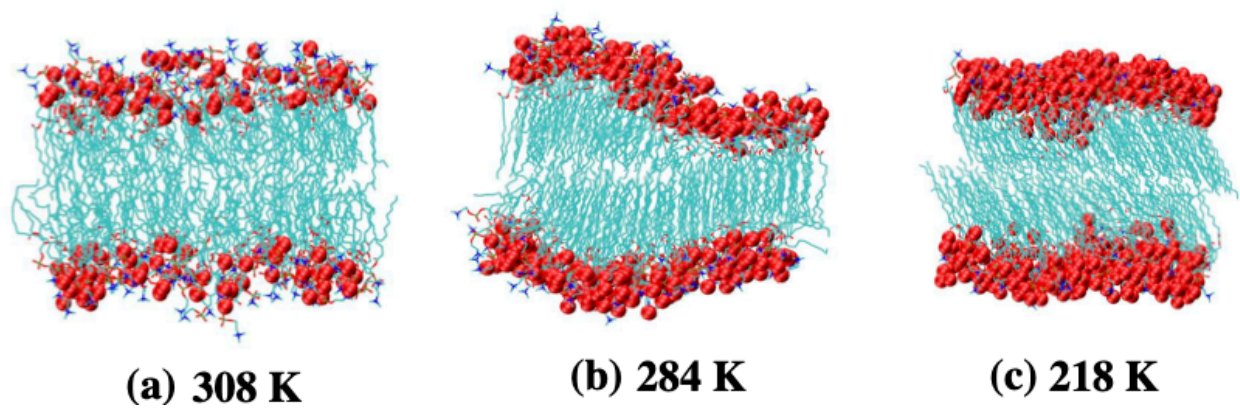


Figure 4.1 : Snapshot of interfacial water continuously residing in the vicinity of lipid headgroups a) Fluid Phase, b) Ripple Phase, and c) Gel Phase

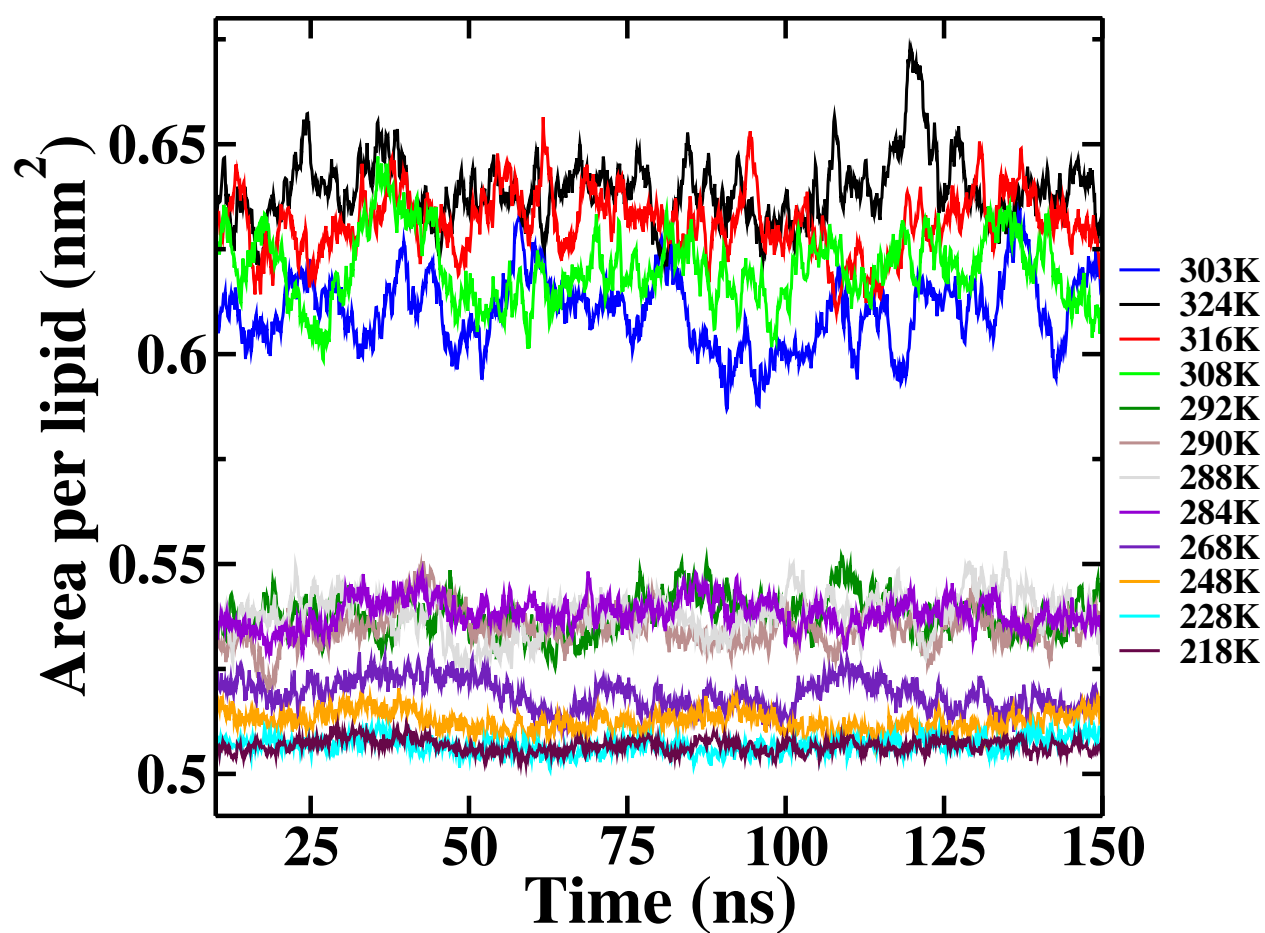


Figure 4.2 : Area per head group of lipids at different temperatures during production runs.

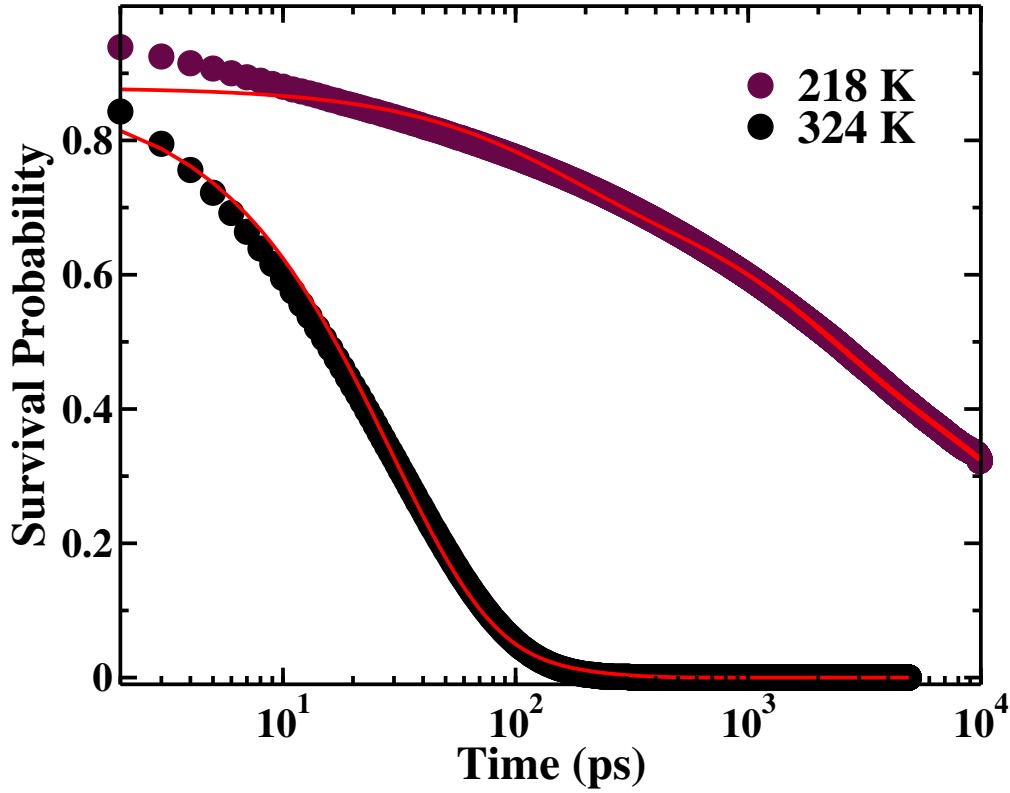


Figure 4.3 : Survival probability of water molecules for temperature 324 K and 218 K. Red solid lines show bi and tri-exponential fitting for 324 K and 218 K respectively.

4.3 RESULTS AND DISCUSSIONS

4.3.1 Survival Probability of IW

Survival probability ($S(t)$) of water molecules near bilayers are calculated at the highest and the lowest temperatures using the equation below,

$$S(t) = \sum_{i=1}^N < \prod_{t_k=t_0}^{t_0+t} P_i(t_k) > \quad (4.1)$$

The angular bracket denotes averaging over time origins $t = 0$, and N represents the total number of molecules. If the molecule is present in the interface layer defined within ± 0.3 nm of lipid phosphorous head at time t , $P_i(t) = 1$; otherwise, $P_i(t) = 0$. Figure 4.3 shows the survival probability of water near membranes at the maximum and the minimum temperatures, 324 K and 218 K respectively. $S(t)$ has the slowest decay at 218 K indicating that water has the greatest probability of surviving when the temperature is minimum. Fitting the normalised survival probability to bi or tri-exponential functions given by the following equation provides different residence time scales for the interface water.

$$y = A_f \exp(-t/\tau_f) + A_i \exp(-t/\tau_i) + A_s \exp(-t/\tau_s) \quad (4.2)$$

Since the fastest time scale at which water near membranes decay is 27.01 ps for 324 K (see table 4.1), 25 ps is chosen as the confinement lifespan of the interface water at all temperatures.

Table 4.1 : Fitted parameters to survival probability of water computed over 5 ns and 10 ns with a saving frequency of 1 ps for temperature 324 K and 218 K respectively.

Temp (K)	A_f	τ_f (ps)	A_i	τ_i (ps)	A_s	τ_s (ps)
324	0.77	27.01	-	-	0.1	100.01
218	0.155	137.67	0.26	1895.93	0.46	28013

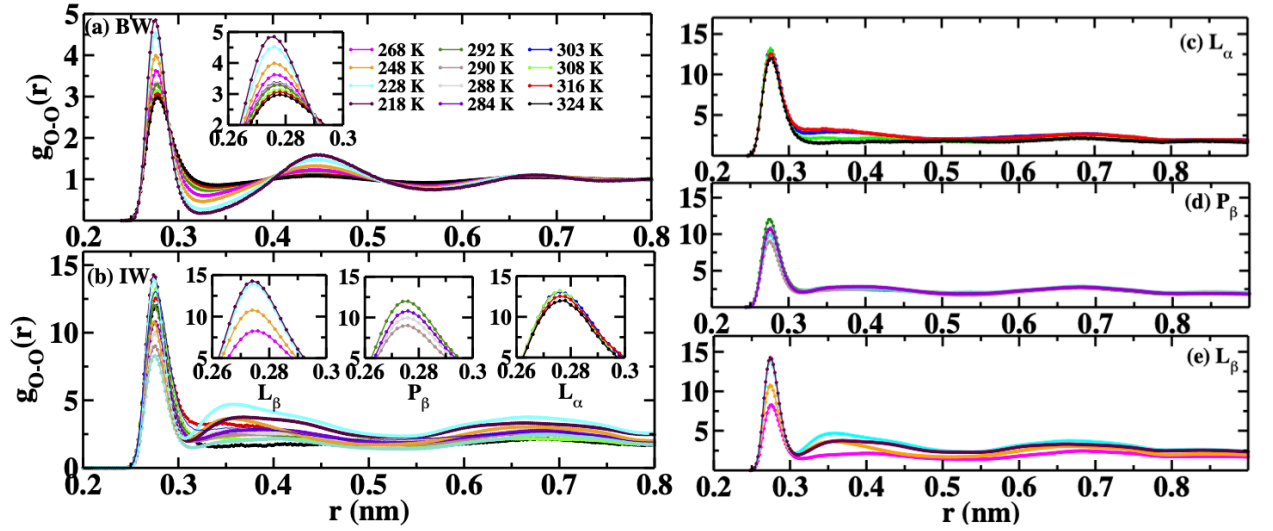


Figure 4.4 : RDFs ($g(r)$) of oxygen - oxygen (O-O) atoms of the a) BW and b) IW near all phases, c)-e) IW near L_α , P_β , L_β phases respectively. Insets in b) show first peaks of the IW near bilayers of respective phases.

IW molecules are distinguished from the rest of the water molecules using a condition where IW molecules reside in the interface layer [Srivastava *et al.*, 2020; Malik and Debnath, 2021] continuously for 25 ps. Figure 4.1 (a) and (c) represent the snapshots of the IW near bilayers at 324 K and 218 K respectively. All of our following analyses for the IW are performed after averaging over 100 sets.

4.3.2 Radial distribution functions

The radial-distribution functions (RDFs) assess pair correlations at various distances and possibly be compared with neutron scattering and x-ray diffraction investigations [Skinner *et al.*, 2013]. The RDFs ($g(r)$) between oxygen atoms of the BW and the IW at all temperatures are calculated using the following equation,

$$g(r) = \left\langle \frac{1}{\rho_N} \sum_{i=1}^N \sum_{j=1}^N \delta(r_{ij} - r) \right\rangle \quad (4.3)$$

where N denotes the total number of oxygen atoms in water. The bulk water density is ρ_N and the angular bracket indicates the average over time origins.

The RDFs of the BW molecules accord well with the results of prior investigations [Schmidt *et al.*, 2005]. Figure 4.4 a) demonstrates how the peak height of the BW grows and becomes sharper with a signature of enhanced tetrahedral structure when the temperature drops. At higher temperature,

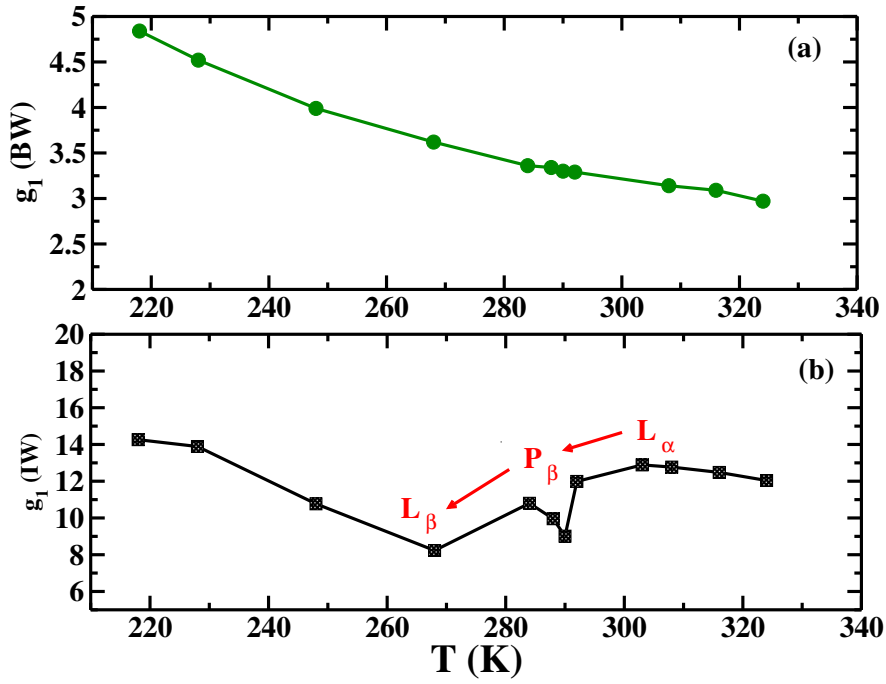


Figure 4.5 : Peak heights (g_1) of the first hydration shell of O-O RDF of the a) BW and the b) IW with respect to temperature.

the first peak is significantly lower and broader indicating that the first coordination shell is distorted, which is consistent with the previous work [Nilsson and Pettersson, 2015]. The peak heights of the first and the second hydration shells (g_1 and g_2 respectively) increase monotonically with lowering in temperature for the BW (figure 4.5 and 4.6).

Towards the lower temperatures (268 – 218 K) the slope of the g_1 and g_2 of the BW is steeper demonstrating an enhancement in tetrahedrality or low-density phase (similar to LDL like phase). Although similar monotonic increment in the g_1 are observed for the IW near fluid and gel phases (inset of figure 4.4 b) and figure 4.5, there are dips near phase transitions. In the case of IW, the second peak is not as sharp as the BW due to presence of interstitials. At the fluid phase, the IW features distorted tetrahedral arrangements of the oxygens with no long range order which is gradually developed at the gel phase. At the gel phase the g_2 of the IW increase with decrease in temperature and thus grow tetrahedral arrangements which provide more free space, and hence are classified as LDL. The locations of the first and second peaks of the RDFs of the BW decrease with decrease in temperature(figure 4.7 a) and b)), while the locations of the first peaks of the IW follow two cross-overs at the fluid to ripple and ripple to gel phase transitions confirming the correlation between the IW structure and the membrane phase transitions.

4.3.3 Angular distribution functions

The probability distribution of O–O–O angles within the first hydration shell can offer a straight forward estimate of tetrahedrality. For a random distribution of three oxygen atoms, the angular distribution is determined with varying the cut-off distances from first to second hydration shell, r_1 to r_2 .

A peak in the distribution at $\sim 109.5^\circ$ exists for a local tetrahedral coordination [Dhabal *et al.*, 2014]. Tetrahedral structures provide more free space, and hence are classified as a low-density

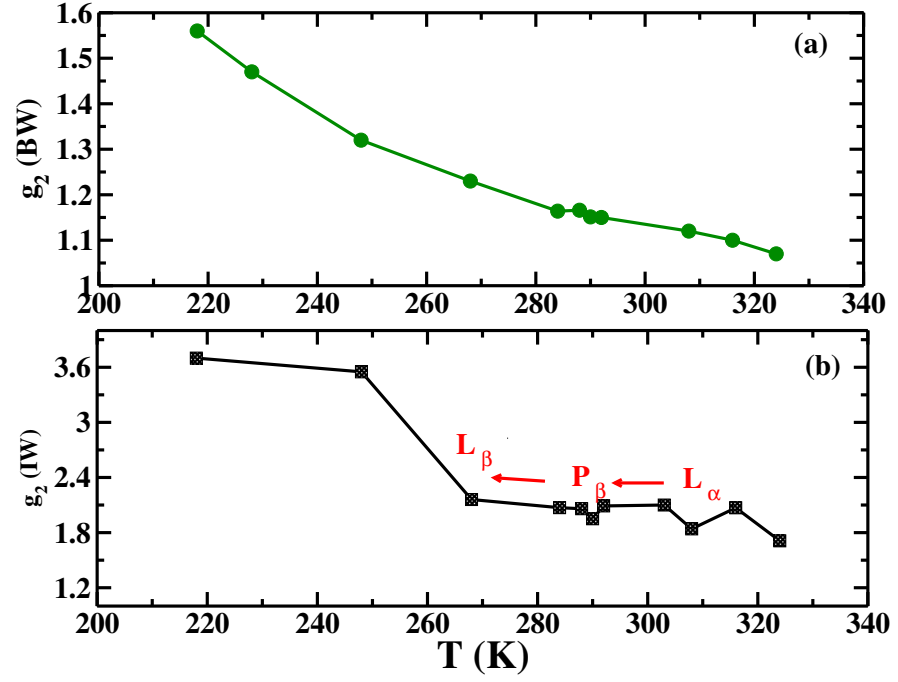


Figure 4.6 : Peak heights (g_2) of the second hydration shell of O-O RDF of the a) BW and the b) IW with respect to temperature.

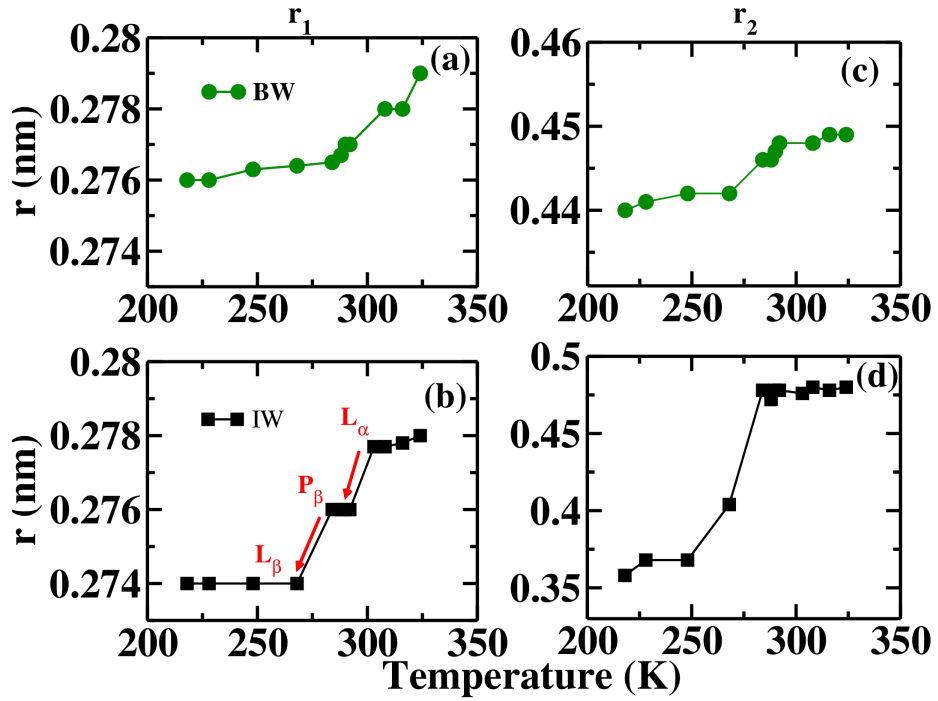


Figure 4.7 : Most probable locations of the first hydration shell (r_1) of the O-O RDFs of (a) BW, and (b) IW. Similarly, locations of the second hydration shell (r_2) of (c) BW, and (d) IW respectively as functions of temperatures.

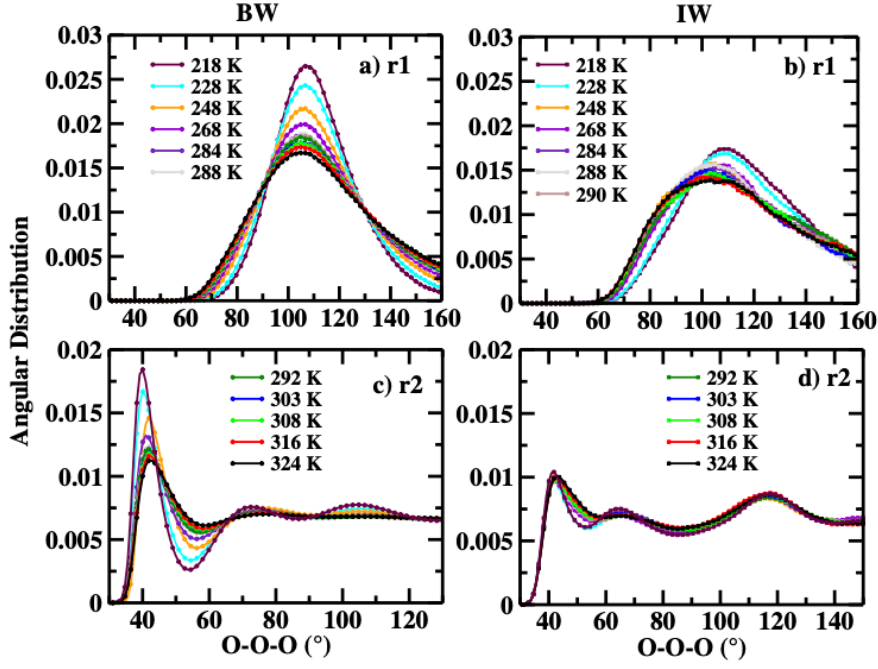


Figure 4.8 : Angular distributions of nearest oxygens within r_{cut} of a) r_1 nm b) r_2 nm for BW, and c) r_1 nm d) r_2 nm for IW. The most probable angle is found to be $\sim 42^\circ$ at r_2 combination. Circle symbol represents the BW data whereas square symbol represent the IW data.

phase[Soper and Ricci, 2000]. For the BW, the peak at $\theta \sim 109^\circ$ becomes sharper with decrease in temperature (figure 4.8 a)). For the IW of the ripple phase, the distributions develop a shoulder near $\theta \sim 90^\circ$ which change to clear peak near $\sim 112^\circ$ at the gel phase (figure 4.8 b)). This reveals that tetrahedrality in the BW is more probable for all temperatures, however in the IW cases, the geometry is deformed [Milischuk and Ladanyi, 2011]. The angular distributions of the BW and the IW at r_2 show more long range order at lower temperatures. The most probable angular distributions are $\sim 40^\circ$, $\sim 70^\circ$ and $\sim 104^\circ$ for the BW and $\sim 42^\circ$, $\sim 65^\circ$ and $\sim 117^\circ$ for the IW. The peaks $\sim 70^\circ$ and $\sim 65^\circ$ respectively for the BW and the IW are characteristic angles of low-density water (LDW) reported earlier[Soper and Ricci, 2000]. Our findings suggest that the BW and the IW at 218 K are similar to low-density water (LDW). Since the peak disappears at higher temperature, both the BW and the IW behave more like the high density water (HDW) or a combination of HDW and LDW. The peak height $\sim 42^\circ$ reduces from the BW to the IW indicating the intensity of the interstitial oxygen atoms are less in the IW compared to the BW. Therefore, upon lowering the temperature, HDW-like water or mixture of HDW and LDW are converted into the LDW-like phase.

4.3.4 Potential of Mean Force

The potential of mean force is calculated by Boltzmann inversion [Srivastava *et al.*, 2019b] of the probability distribution functions (figure 4.9 of oxygens separated by a distance of r_{cut} and concertedly producing an O-O-O angle using the equation below,

$$U(r, \theta) = -k_B T \ln(P(r, \theta)) \quad (4.4)$$

where k_B is Boltzmann constant, T is temperature.

The PMF reveals the most stable local arrangements of oxygen atoms in the IW and the BW.

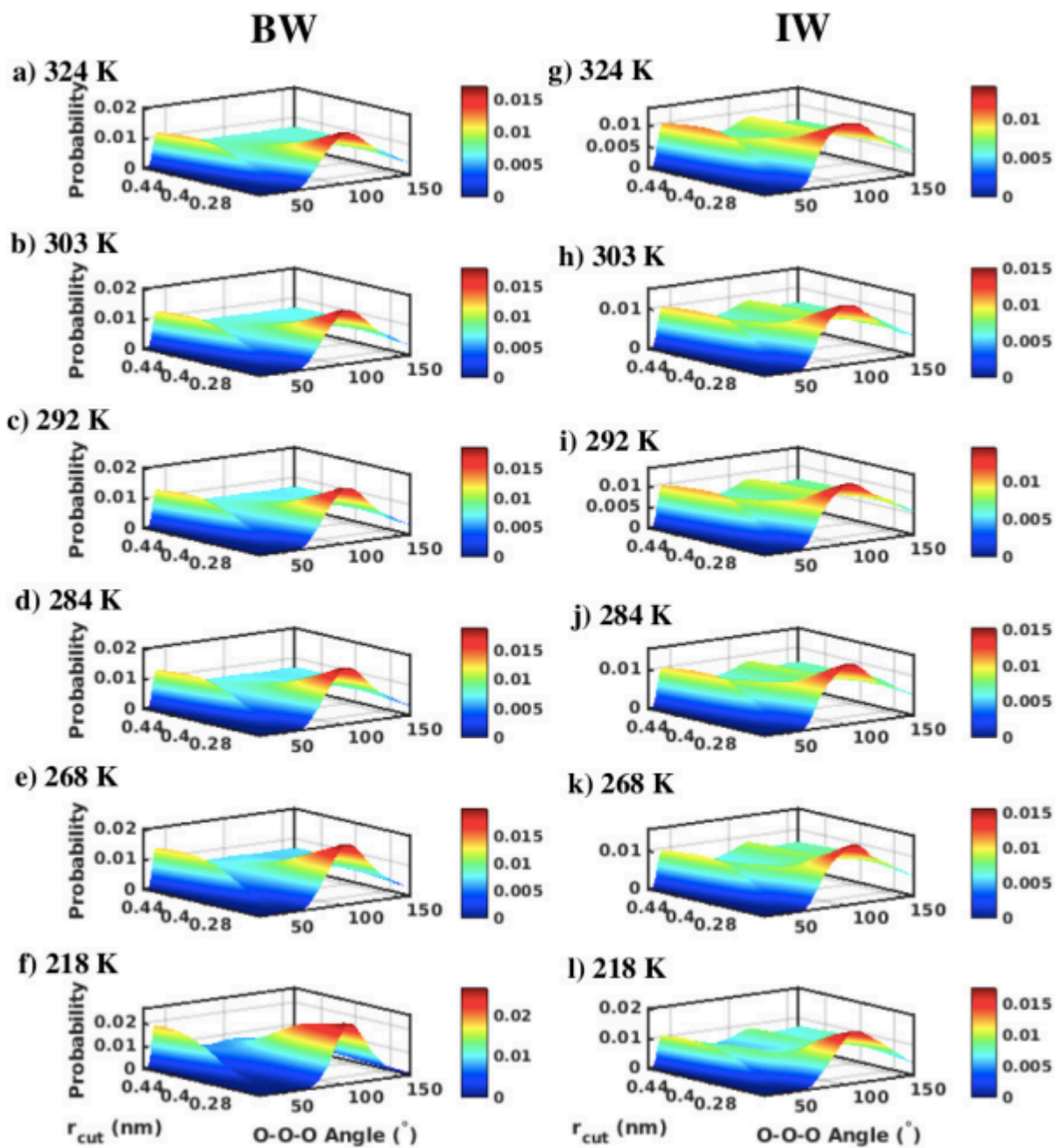


Figure 4.9 : O-O-O angular distributions with varying r_{cut} for a)-f) BW (left panel), g)-i) IW (right panel)

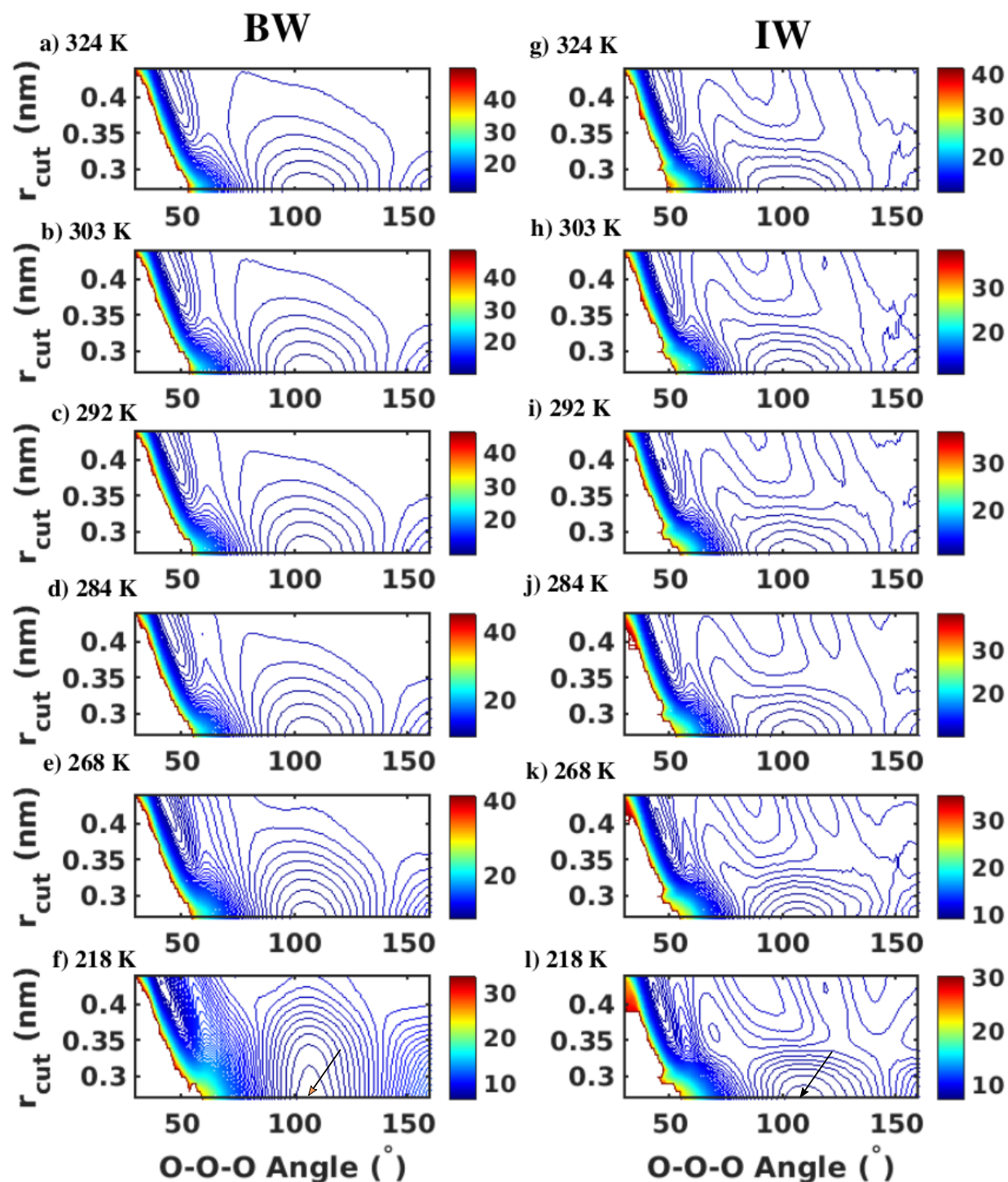


Figure 4.10 : Contour map of potential of mean force (PMF) for BW (left panel) (a-f), IW (right panel) (g-i) with respect to the distance between nearest neighboring oxygens and angle between O-O-O.

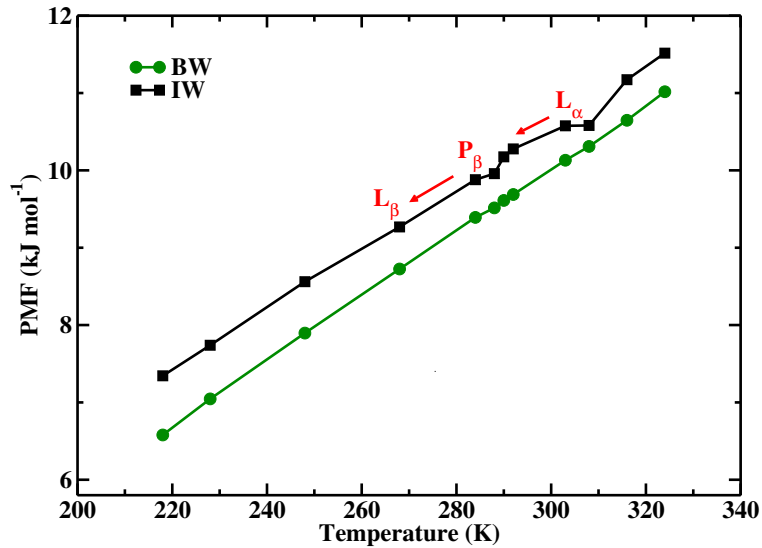


Figure 4.11 : Minimum energies (derived from figure 4.10) of BW and IW with respect to temperatures.

Figure 4.10 illustrates a contour diagram of PMF for the IW and the BW at temperatures ranging from 324 K to 218 K (six plots out of twelve are presented from each phase for clarity), with the color bar representing the PMF values with varying the r_{cut} from r_1 to r_2 . The regions with the lowest PMF are identified at angles of $\sim 85^\circ$ – 117° , $\sim 90^\circ$, $\sim 112^\circ$ for the fluid, ripple and the gel phases respectively within the first hydration shell (r_1). The PMF is rather broad for the fluid phase which becomes sharper at the ripple to gel phase. The most stable configurations for the BW occur at an angle $\sim 109^\circ$ for temperatures at which gel phase exists for the bilayer, confirming their tetrahedral structures within the first hydration shell (r_1). The lowest PMF is found at r_1 (see figure 4.7) and at θ where the peaks of respective angular distributions are found for all temperatures and are shown in figure 4.11. Generally, the PMF of BW is lower than the IW demonstrating more stability in case of the BW. This is probably due to the presence of lipid molecules at the interface creating defective hydrogen bond network [Srivastava *et al.*, 2019b]. Although the PMF of the BW is linear with respect to temperature, the linearity breaks for the IW near the fluid to ripple to gel phase transitions. This exhibits a significant correlation between the structure of the IW and the bilayer phase transitions which do not exist for the BW. In addition to the most stable PMF described earlier, three local minima are found at r_2 near $\sim 40^\circ$, $\sim 70^\circ$ and $\sim 104^\circ$ for the BW and at $\sim 42^\circ$, $\sim 65^\circ$ and $\sim 117^\circ$ for the IW at 218 K. The occurrences of minima at $\sim 70^\circ$ and $\sim 65^\circ$ for the BW and the IW respectively at r_2 confirm the presence of LDW-like phases both for the IW and the BW at the lowest temperature.

4.4 CONCLUSIONS

Using all-atom MD simulations, we studied the structural characteristics of the IW near DMPC bilayers at the fluid to ripple to gel phases. The phase transitions of the bilayers are confirmed from the area per head group which agree well with the previously published results [Khakbaz and Klauda, 2018]. The bilayer undergoes from a fluid (L_α) to a ripple (P_β) phase transition at 297 K which goes to a gel (L_β) phase near 284 K. Water molecules residing continuously for the entire time period of the fast residence time are identified as the IW.

When temperature drops, the first hydration peak of O-O RDFs of the BW rises and becomes sharper, with a characteristic of enhanced tetrahedral structure. However, peak heights of the RDFs of the IW rise monotonically with decreasing temperature in the fluid and gel phases of the bilayer, but not at the ripple phase. The locations of the first (r_1) and second (r_2) peaks of the O-O RDFs decrease with decrease in temperature with a clear transition in r_1 near phase transition temperatures of bilayers demonstrating correlation in IW structure and bilayer phase transitions.

Upon lowering the temperature, the O-O-O angular distribution functions show peaks at $\sim 109^\circ$ and $\sim 112^\circ$ for the BW and the IW respectively within r_1 . This clearly depicts a significant enhancement in tetrahedrality upon decrease in temperature for the BW which is distorted for the IW. In comparison to the BW, the IW have higher potential of mean force due to the presence of lipid molecules breaking the hydrogen bond network. The transitions in the PMF of the IW are correlated with the bilayer fluid to ripple to gel phase transitions. Thus, our results clearly show that the structure of the IW near the gel bilayer is more tetrahedral indicating the presence of LDL like phase which transforms to a mixture of LDL and HDL like phases at higher temperature. Our analyses provide insights on the coupling of the bilayer phase transitions and the structure of the IW.

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