

Membrane phase transition dictated heterogeneity length and time scales of interface water

5.1 INTRODUCTION

Lipid membrane provides the boundary of a cell and acts as a semi-permeable barrier between the extracellular and intracellular environments [Gutknecht and Walter, 1981]. Fluid (or liquid-crystalline) (L_α), ripple (P_β), and gel (L_β) are the three primary phases that exist in lipid membranes [Nagle and Tristram-Nagle, 2000]. At physiological temperature, membranes are at the fluid (or liquid-crystalline, L_α) phase. With lowering in temperature the fluid membrane undergoes a phase transition to a meta-stable ripple, P_β phase which quickly changes to a gel or L_β phase.

The membrane performs multitude of functions such as transport, pathogen entry, signal transduction, molecular trafficking and recognition, hosting other biological molecules, [Korade and Kenworthy, 2008; Pike, 2009; Simons and Toomre, 2000] and so on at the fluid or disordered phase and thus, the fluid phase is well studied [Needham and Evans, 1988; Kučerka *et al.*, 2006; Horta *et al.*, 2010]. Comparatively, the other two phases of the membrane remain less attended due to the inadequate understanding of their relevance to biological functions. Compartmentalization of the liquid ordered and disordered phases are often investigated to model lipid rafts or micro-domains, generally rich in cholesterol, sphingolipids and specific membrane proteins [Kaiser *et al.*, 2009; Schachter *et al.*, 2022]. Functionally important sterol-free rafts are found to be in the gel phase in living cells [Aresta-Branco *et al.*, 2011]. A co-existence in the gel and the fluid phases in membranes results in the creation of grain boundary defects which helps in the drug release and thus, crucial to control drug permeability [Smith and Klimov, 2018]. At supercooled temperature, the membrane is at the gel phase and functionally inactive. A clear understanding of the mechanism of the survival and functionality of the membrane at supercooled temperature will provide a fresh impetus to mimic cryo-preservation methods, which have a wide range of applications in food science, pharmacology, clinical medicine, and the preservation of human embryos for in vitro fertilization [Trounson and Mohr, 1983; Crowe and Crowe, 2000]. Despite such innumerable applications of different phase behaviors of membranes, the functional relevance of the dynamics of membranes across phase transitions remains unknown.

The P_β phase [Zasadzinski *et al.*, 1988] and L_β phase [Akabori and Nagle, 2015; Tristram-Nagle *et al.*, 2002] of the DMPC bilayers are characterized by the scanning tunneling microscopy and x-ray diffraction study respectively. A phase diagram of DMPC-DPPC and other PC lipid mixtures is constructed by various methods, such as, differential scanning calorimetry, electron spin resonance, and two proton nuclear magnetic resonance to detect transition temperatures [Jacobs and Oldfield, 1979; Mabrey and Sturtevant, 1976]. Using coarse-grained (CG) and all-atom (AA) molecular simulations, the phase behavior of model membranes have been investigated extensively [de Vries *et al.*, 2005b; Bennett and Tieleman, 2013; Högberg and

Lyubartsev, 2006; Lee and Larson, 2006; Khakbaz and Klauda, 2018; Leekumjorn and Sum, 2007; Uppulury *et al.*, 2015]. However, none of the work mentioned above focuses on the underlying dynamics across phase transitions.

Membranes are functionally active only at the full hydration state. Once dehydrated, the membrane undergoes a cross-over from the disordered to the ordered state induced by the drastic slow-down in structural relaxation times originated from the dynamical heterogeneity of the interface water molecules (IW) [Malik and Debnath, 2021] and this can cause losing the functionality. As the hydration of water decreases, a slowdown in orientational relaxation of water has been observed near artificial multibilayers of the dilauroylphosphatidylcholine lipids (DLPC) using ultrafast polarization selective vibrational spectroscopy [Zhao *et al.*, 2008]. As temperature reduces, a protein [Chen *et al.*, 2006a] or a cryoprotecting biomolecule undergoes a fragile-to-strong dynamical crossover accompanied by a dynamic transition of the IW strongly suggesting a correlation between their relaxation timescales [Iorio *et al.*, 2019; Abascal and Vega, 2005]. Neutron scattering experiments and molecular dynamics simulations reveal that the translational diffusion of the hydration water facilitates the functionally relevant large amplitude motions of the protein via the relaxation of the protein-water HB network at the supercooled temperature [Schiró *et al.*, 2015]. Thus a drastic slow-down in the relaxation time-scale of the hydration water is perceived as a signature of protein dynamical transition connected to its functionality. The interplay of crowding in the complex biological sea and the membrane electrostatics is found to govern the hydration dynamics relevant for membrane functions [Pal and Chattopadhyay, 2021]. The IW near a single component fluid membrane exhibit dynamical heterogeneity coherently with the local lipid heads due to chemical confinement [Srivastava *et al.*, 2019a, 2020, 2019b]. Since the relaxation times of the membranes can be scaled to that of the IW at room temperature due to their strong correlation [Srivastava *et al.*, 2020], IW is found to reflect the very slow membrane dynamics which is otherwise difficult to access. The thermodynamics and dynamics of the IW exhibit sharp cross-overs across the bilayer melting transition which demonstrates their correlation [Debnath *et al.*, 2013]. However, understanding the effect of dynamical heterogeneity of the IW in the laterally homogeneous single component model membrane across phase transitions is a long overdue.

Quantifying the heterogeneity in a bio-membrane using the current biochemical or biophysical tools is an incredibly challenging task due to the presence of unusual liquid like domains with microseconds to seconds time scale and nanometers to micrometers length scales having both short and long range orders. The scale-dependent transport is the key of the molecular encounters responsible for the cellular signaling [Lyman *et al.*, 2018] which remains unexplored yet. Dipalmitoylphosphatidylcholine (DPPC) bilayers exhibit dynamical heterogeneity in the gel phase having different mobility groups that produce dynamic clusters with characteristic time ($1 - 2 \mu\text{s}$) and length scales ($1 - 10 \text{ nm}$). These clusters can bind to membrane-based biomolecules and hence participate in a variety of membrane activities [Shafique *et al.*, 2016]. Molecular level characterization of such dynamical domains through experiments is still fragmentary due to the lack of the relevant atomistic trajectories and the unknown spatio-temporal correlations.

There is a disconnection between the single component model membrane to the multi-component complex biological membranes where lateral heterogeneity is accepted as a criteria for the membrane function, specifically in the context of a raft [Jacobson *et al.*, 2007]. Therefore, bridging the gap between characterizing the dynamically heterogeneous domains in a single component model membrane by the IW dynamics and the existing methods is a need of the hour. As a result, the current research focuses on understanding the applicability of recent simulation and numerical approaches on model membranes across phase transitions to capture the

underlying membrane dynamics through the IW that governs membrane organizations and phase transitions.

Complementary to the experimental techniques, the classical finite size scaling (FSS) approach can extract the length scale in critical phenomena. However, it requires lengthy simulations and extensive averaging to obtain reliable results for small systems to avoid artifacts caused by the suppression of density and composition fluctuations in small samples with periodic boundary conditions [Flenner and Szamel, 2010]. Recently, the block analysis approach is found to be successful in quantifying the growing dynamical correlation length scales by performing FSS with lowering in temperature for a realistic glass forming liquid [Karmakar *et al.*, 2009]. Earlier the block analysis is applied on the IW near the fluid membrane and the heterogeneity length scale is identified from the block size exhibiting a transition from a non-Gaussian to a Gaussian van Hove correlation function [Srivastava *et al.*, 2019a]. The length scale matches with the undulation of the bilayer. This opens a new possibility to correlate the heterogeneity length scale to the lateral organization of membranes. Furthermore, this strategy will aid in comprehending the domain length scale of a raft, which is quite difficult to gain from an experiment.

Therefore, all-atom MD simulations of dimyristoylphosphatidylcholine (DMPC) bilayers are performed at various temperatures to cover the three primary phases of the membranes. We provide the evidence of extensive dynamical heterogeneity in the hydration water when the membrane undergoes a fluid to a ripple to gel phase transition. Our results show a significant slow-down in relaxation time scales of the IW accompanied by similar slow-down in local lipids upon supercooling. The drastic growth in heterogeneity time-scale is governed by supercooling rather than the phase transition. The heterogeneity length scales of the IW is found to be dictated by the underlying lateral organization of the membrane where the temperature dependence disappears slowly from the fluid to the ripple phase due to the ripple curvature. The heterogeneity length scale of the IW can be scaled to the ripple wavelength adopting the signature of the ripple size. Thus the interface water dynamics can be used as an alternate probe to characterize any dynamical domain in membranes relevant for their functionality [Niethammer *et al.*, 2002]. The growth of the structural relaxation timescale of the IW follows an activated dynamical scaling only for the gel phase.

5.2 SIMULATION DETAILS

All-atom molecular dynamics simulations are set up using 128 DMPC molecules using CHARMM36 solvated in 5743 TIP4P/2005 [Abascal and Vega, 2005] water molecules in a fully hydrated state [Lopez *et al.*, 2004; Zhao *et al.*, 2008; Trapp *et al.*, 2010]. In the presence of the TIP4P/2005 water model, CHARMM36 [Klauda *et al.*, 2010; Zhuang *et al.*, 2014] force fields of the bilayer is found to reproduce the experimental properties of water while maintaining the membrane phase intact [Zhao *et al.*, 2008; Trapp *et al.*, 2010]. An equilibrated bilayer from our earlier work [Srivastava *et al.*, 2019b] is used as the initial configuration of the current work at 308 K which is cooled till 218 K, with an annealing rate of 0.02 K/ps to create initial configurations of bilayers at different temperatures. As the fluid to ripple to gel phase transition temperatures of DMPC are known [Klauda *et al.*, 2010], thirteen bilayers are built up at temperatures ranging from 324 to 218 K using simulation annealing to cover all three phases of the L_α to the P_β to the L_β phase. 300 – 500 ns long NPT runs are carried out to equilibrate each system at different temperatures where the pressure is kept constant at 1 bar using the Berendsen pressure coupling

[Berendsen *et al.*, 1984] technique and the Berendsen temperature coupling method with a 0.5 ps coupling constant. The Particle Mesh Ewald (PME) technique is used with a grid size of 0.12 nm to rectify long-range interactions, whereas van der Waals and Coulombic interactions are cutoffs at 1.2 nm. During the equilibration, the LINCS method [Hess *et al.*, 1997] is utilized to restrict all bonds. Next, NVT runs are performed for 60 ns with a time step of 0.4 fs for each systems without bond restrictions using nosé-Hoover temperature coupling [Evans and Holian, 1985]. The run-length of 60 ns is sufficient for obtaining appropriate sampling for the relaxations and dynamics discussed in this work. The temperature coupling, cutoff distances, and long-range interactions parameters remain unchanged from the NPT runs. Periodic boundary conditions are enforced in all three directions. Trajectories are saved at every 0.1 ps. The hydrated DMPC lipid simulation box-length is ~ 6.24 nm each in the x and y dimensions and 7.95 nm in the z-direction at 324 K. These systems are used to calculate bilayer properties, and the relaxation time scales.

Next, bilayers each with 1152 DMPC lipids are NPT equilibrated to achieve larger system size required for the block analysis. The calculations of intensity spectra at each temperatures. The box-lengths are ~ 18 nm² in both the x and y directions and 7.76 nm in the z-direction (table 5.1). Next 60 ns NVT runs are carried out with the same set of parameters as in the smaller system. To compare the dynamics of interfacial water with bulk water at different temperatures, 851 bulk water molecules (BW) are simulated. Total $\sim 11.55 \mu s$ all-atom molecular dynamics (MD) simulations are carried out for the current analyses. GROMACS-4.6.5 software is used for all simulations [Pronk *et al.*, 2013; Van Der Spoel *et al.*, 2005].

Table 5.1 : Details of the systems studied with total run-lengths of $11.55 \mu s$.

Temperature T(K)	Bulk water Run-length (ns)	DMPC (6 nm) Run-length (ns)	DMPC (18 nm) Run-length (ns)
324	100	420	245
316	100	435	160
308	100	700	160
303	100	400	—
292	90	550	200
290	90	630	210
288	90	730	190
284	90	330	160
273	100	380	—
268	120	630	160
248	110	830	160
228	120	1030	160
218	120	1030	160
	$1.33 \mu s$	$8.095 \mu s$	$1.805 \mu s$

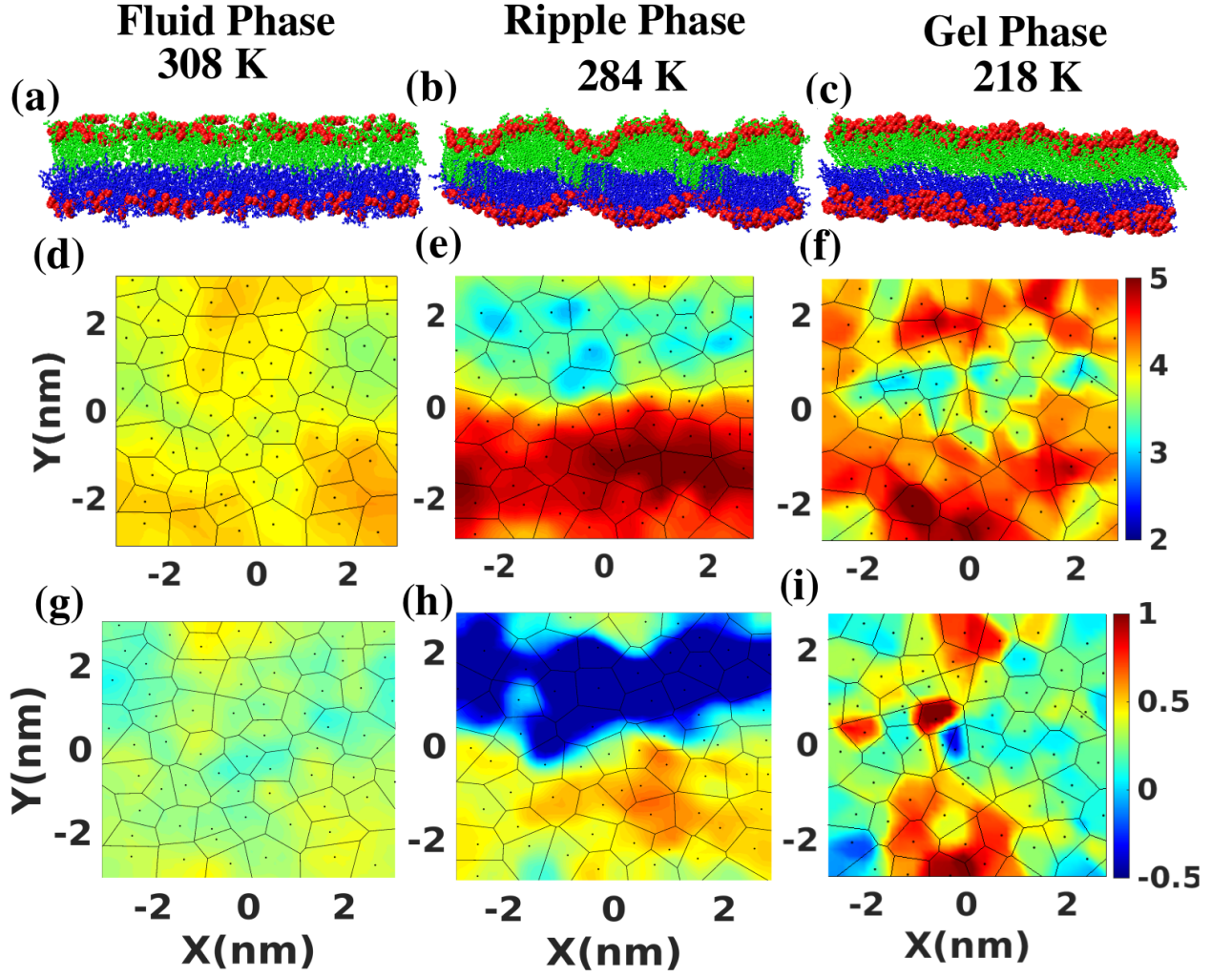


Figure 5.1 : Snapshots of bilayers at three phases (a-c), thickness (d-f) and interdigitation (g-i) superimposed on voronoi area per lipid head respectively. Color code with representation: DMPC: CPK representation in blue and green for two leaflets; IW water: VDW (van der Waals) representation in red.

5.3 RESULTS AND DISCUSSIONS

5.3.1 Structural characteristics of lipid bilayers upon phase transition

Snapshots of the equilibrated bilayers at three phases are shown in figure 5.1 (a-c) and the bilayers at the remaining temperatures are shown in figure 5.2 (considered for the calculations of structural relaxations). Area per lipid (a_h) for all cases are in good agreement with the previous simulations and experiments [Zhuang *et al.*, 2014; Khakbaz and Klauda, 2018; Kučerka *et al.*, 2011; Tristram-Nagle *et al.*, 2002] which validates our simulations (Table 5.2). The a_h drops abruptly from 308 K to 292 K and from 284 K to 268 K demonstrating the fluid (L_α) to the ripple (P_β) to the gel (L_α) phase transition temperatures (Figure 5.3).

The bilayer thickness and interdigitation are determined as a function of the bilayer surface, (x, y) , using the two equations respectively, $t(x, y) = \langle z_l(x, y) - z_u(x, y) \rangle$, and $I(x, y) = z_{l, end} - z_{u, end}$

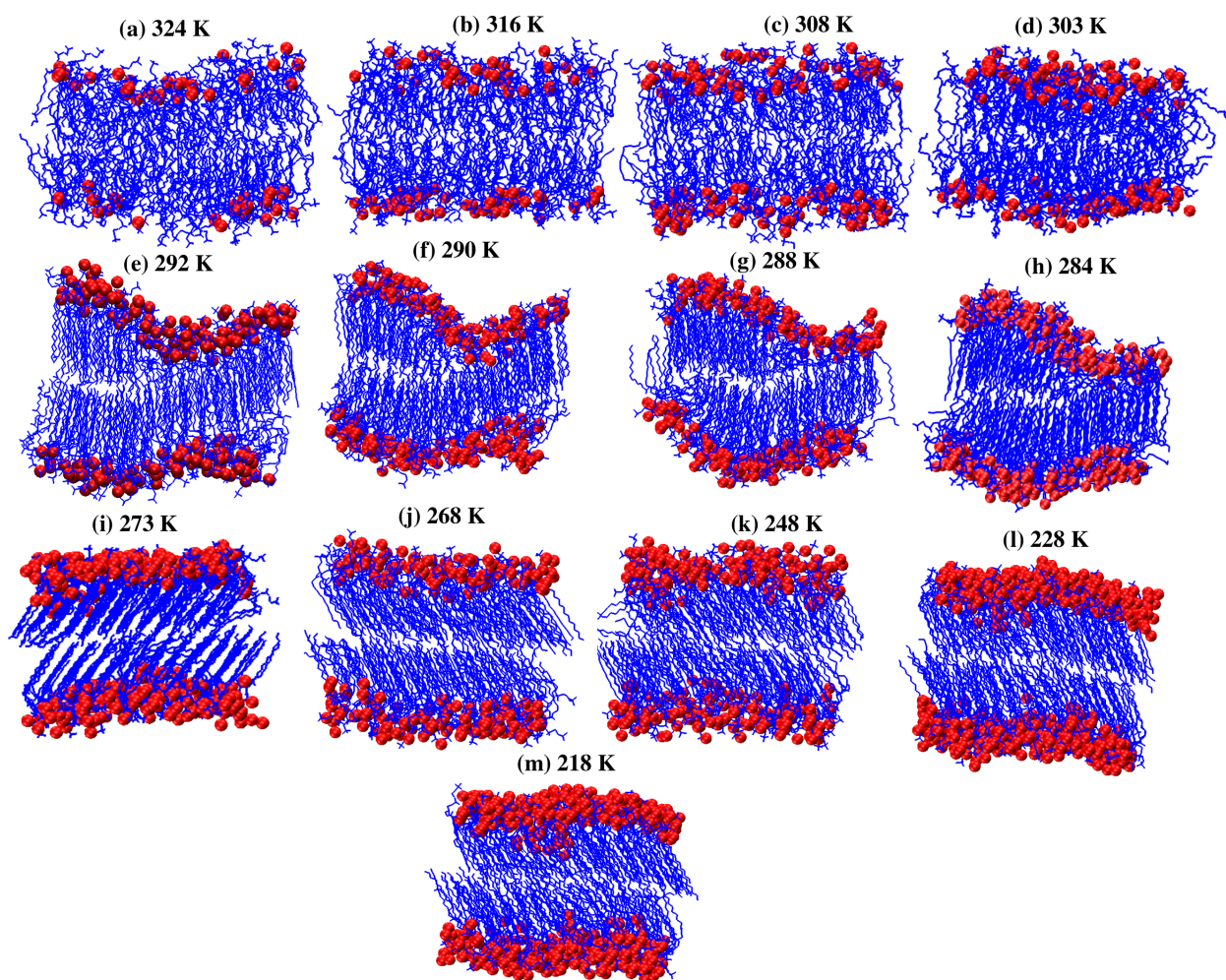


Figure 5.2 : Snapshot of bilayers at all temperatures at three phases shows the phase transition from the fluid-ripple-gel phase. (a-d) 324 K - 303 K: Fluid Phase; (e-h) 292 K - 284 K: Ripple Phase; (i-m) 273 K - 218 K: Gel Phase. All snapshots are generate with 6*6 nm box size.

Table 5.2 : Comparison of a_h at different temperature from our simulations with available literature.

Phase	T(K)	Our Simulation ($\text{\AA}^2$)	Literature	
			Simulation	Experiment
Fluid Phase	324	63.8		
	316	62.9		
	308	61.7	62.1 ± 0.1 [Malik and Debnath, 2021]	
	303	60.1	60.0 ± 0.2 [Zhuang <i>et al.</i> , 2014]	59.99 ± 0.12 Kučerka <i>et al.</i> [2011]
	292	54.0	58.6 ± 0.1 [Kučerka <i>et al.</i> , 2011]	
Ripple Phase	290	53.5		
	288	53.6	51.9 ± 0.9 [Kučerka <i>et al.</i> , 2011]	
	284	53.7	52.3 ± 0.4 [Kučerka <i>et al.</i> , 2011]	
	273	51.9	50.0 ± 0.3 Kučerka <i>et al.</i> [2011]	47.2 ± 0.5 [Tristram-Nagle <i>et al.</i> , 2002]
Gel Phase	268	51.8		
	248	51.44		
	228	50.7		
	218	50.4		

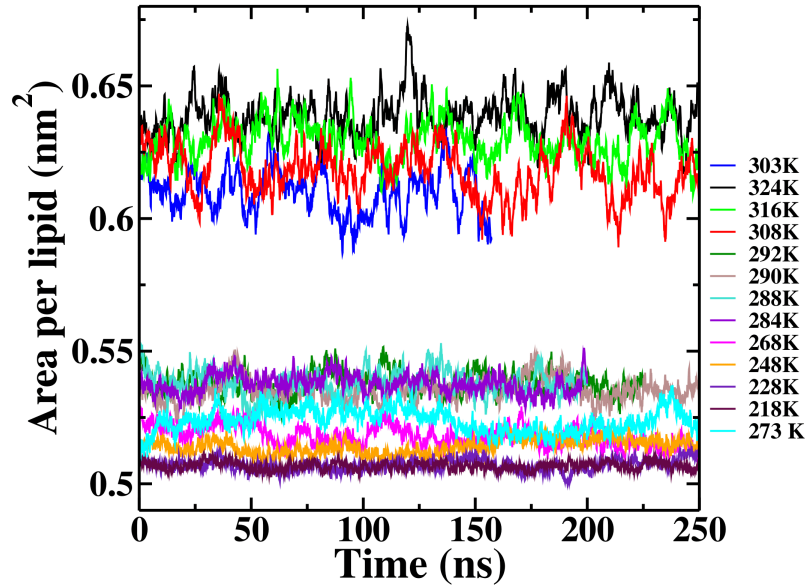


Figure 5.3 : Area per head group of lipids at different temperatures during production runs.

where $z_l(x,y)$ and $z_u(x,y)$ are the z coordinates of the lipid head groups for the two leaflets. The time average is shown by angular brackets. The triangulation method is used to show Voronoi area per lipid which is superimposed to the thicknesses, $t(x,y)$, and interdigitation, $I(x,y)$, and shown for three bilayers at three phases in figure 5.1 (d-i). The data for the remaining temperatures are shown in figure 5.4 and 5.5. The homogeneous thickness and interdigitation of the fluid phase (324 K-303 K) (figure 5.1 (d) and (g)) become periodically heterogeneous for the ripple phase (292 K - 284 K) ((e) and (h)) which disappears for the gel phase (273 K - 218 K). The region with smaller thickness is correlated to the higher interdigitation at all phases. A fast Fourier transform of $t(x,y)$ and $I(x,y)$ provides the spectral intensity, $H_T(q)$ and $H_I(q)$ [Debnath *et al.*, 2013] respectively which are plotted on the xy plane of the membrane surface and shown in figure 5.6 (e)-(l) for 292 to 284 K, respectively. The q value corresponding to the highest peak in figure 5.6 (e)-(l) denotes the wave vector, q associated with the undulations of the ripple phase. The wavelength, ($\lambda = \frac{2\pi}{q}$) is calculated for the ripple phase, which are 4.27, 4.25, 4.3, and 4.26 nm from 292 to 284 K, respectively. At the ripple phase, there is a coexistence of highly interdigitated ordered domain with a fully expanded disordered domain (shown in figure 5.6) with a ripple wavelength of ~ 4.2 nm which matches with the λ value obtained from the intensity spectra of thickness and interdigitation fluctuations.

5.3.2 Dynamical heterogeneity in water and membranes upon supercooling and membrane phase transition

To start with, equation 5.1 is used to compute the survival probability ($S(t)$) of water molecules near bilayers at the highest and lowest temperatures,

$$S(t) = \sum_{i=1}^N \langle \prod_{t_k=t_0}^{t_0+t} P_i(t_k) \rangle \quad (5.1)$$

The angular bracket symbolises averaging over time origins $t = 0$, and N is the total number of molecules. At time t , $P_i(t) = 1$ if the molecule is present in the interface layer specified within ± 0.3 nm of the lipid phosphorous head; otherwise, $P_i(t) = 0$. Figure 4.3 (chapter 4) depicts the $S(t)$ of water near membranes surviving at the highest (324 K) and the lowest temperatures (218 K), respectively. At 218 K, $S(t)$ decays the slowest, suggesting that water has the highest chance to survive when the temperature is the lowest. As mentioned in the table 4.1 (chapter 4), the fastest residence time scale for the IW is 100 ps for 324 K. Thus, 100 ps is selected as the confinement lifetime of the IW at all temperatures.

Thus if a water molecule is continuously residing in the interface layer for 100 ps [Malik and Debnath, 2021, 2022], it is considered as an IW molecule. Figure 5.2 shows snapshots of the IW near bilayers at are temperatures ranging from 324 K to 218 K. Lateral mean square displacements (MSD_{XY}) of the BW, IW and lipid Phosphorous heads (P) heads are computed using equation 5.2 (figure 5.7 (a-c)).

$$\langle \Delta r(t)^2 \rangle = \frac{1}{N} \sum_{i=1}^N \langle [r_i(t+t') - r_i(t')]^2 \rangle_{t'}, \quad (5.2)$$

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_{XY} of the BW slows down with a decrease in temperature very similar to that found earlier [Sciortino *et al.*, 1996; Kawasaki and Kim, 2017]. MSD_{XY} of the P are the slowest compared to that of the IW as the lipids in the membranes are constrained than the IW. Due to the chemical confinement of the IW near the membrane, the IW exhibit slower MSD_{XY} compared to the free BW. As temperature reduces, the MSD_{XY} of the IW

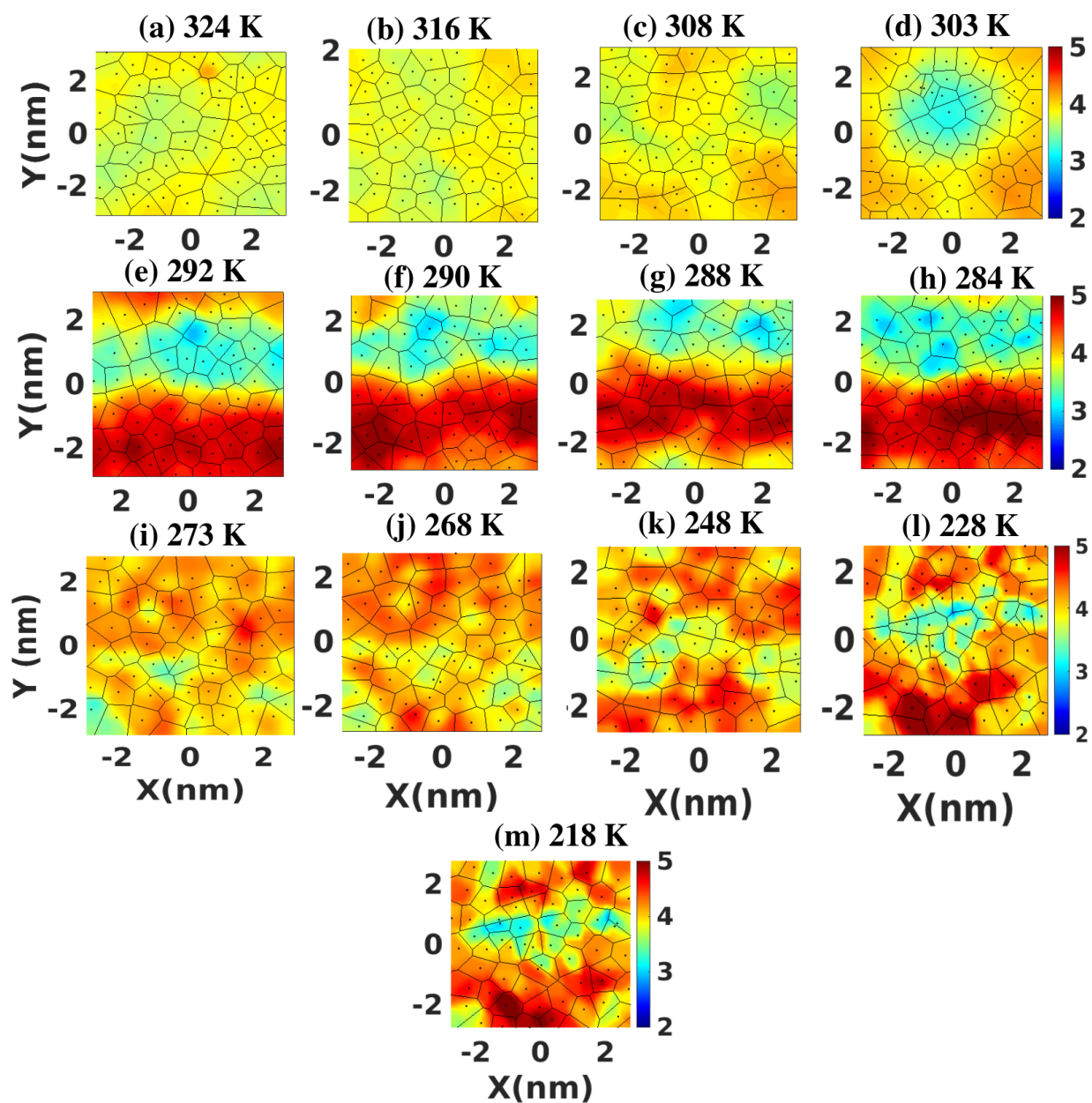


Figure 5.4 : Thickness superimposed on the Voronoi area per lipid head varying the temperature from 324 K to 218 K. The color bar is in nm. The bilayer undergoes a fluid-ripple-gel crossover upon lowering the temperature.

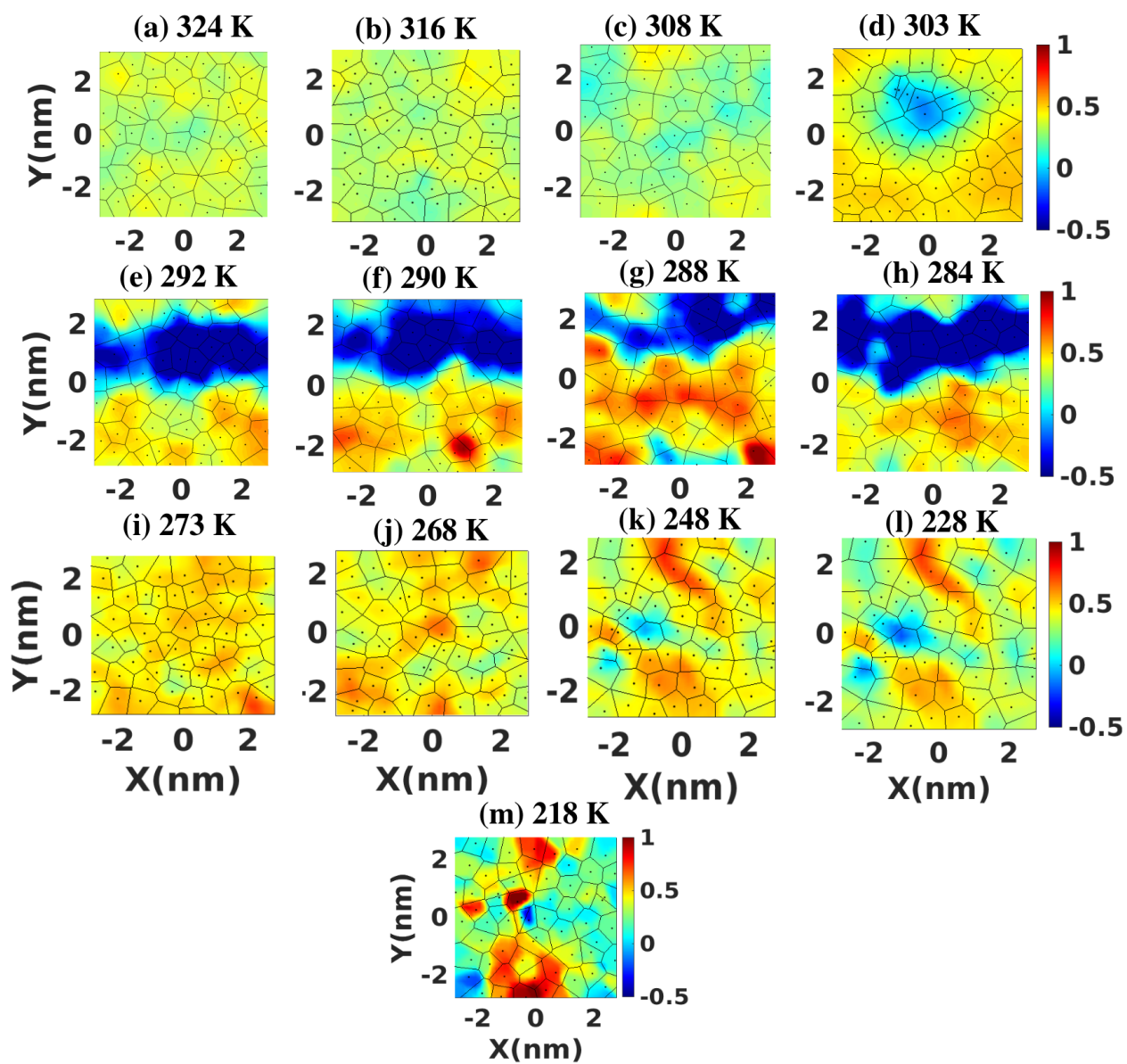


Figure 5.5 : Interdigitation superimposed in voronoi area per lipid head from temperature 324 K to 218 K.

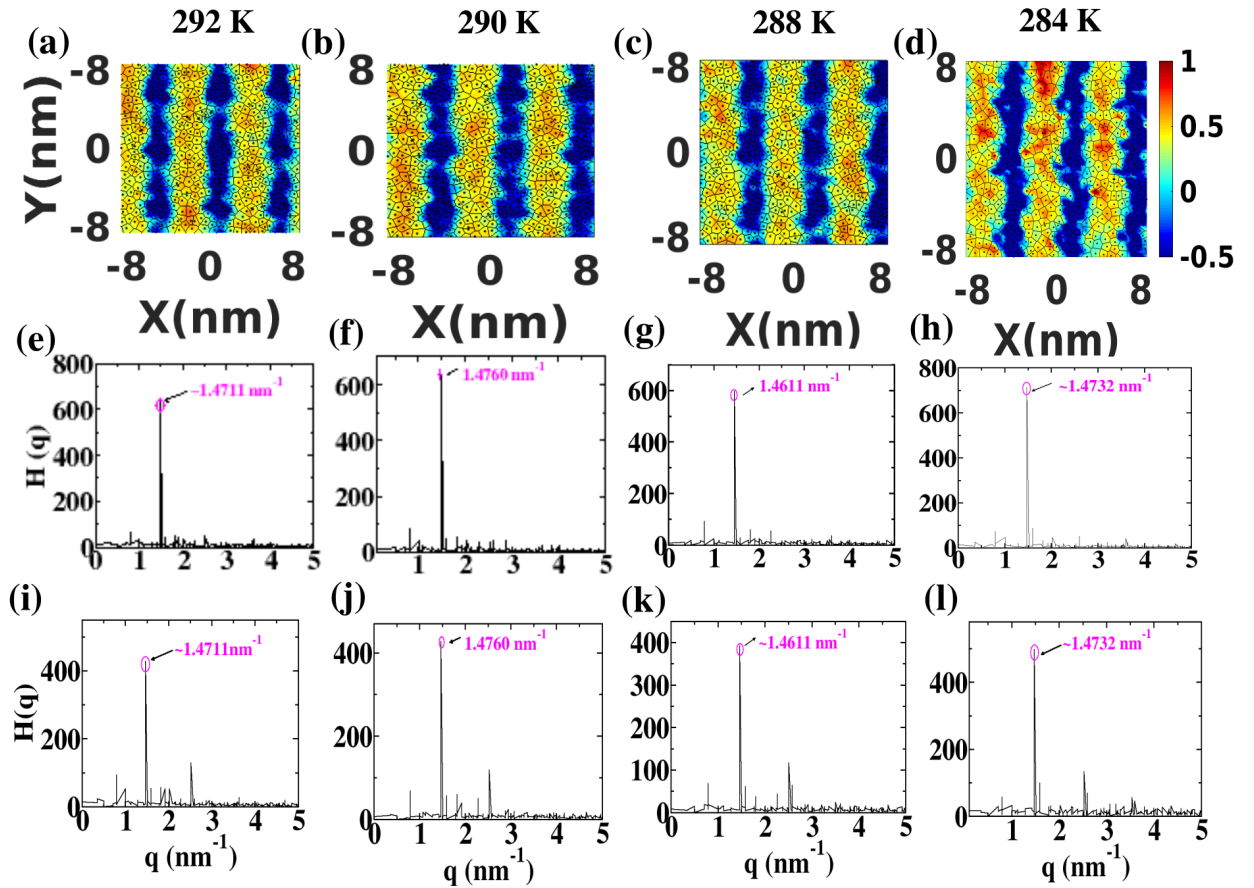


Figure 5.6 : Interdigitation for the ripple phase: a) 292 K, b) 290 K, c) 288 K and 284 K respectively. Spectral intensity from interdigitation fluctuations (e-h) and thickness fluctuations (i-l). Ripple wavelengths are 4.27, 4.25, 4.3 and 4.26 nm from 292 to 284 respectively. The size of each periodic patch in (a-d) close to 2 nm which is $\sim$ half of the ripple wavelength of 4.2 nm.

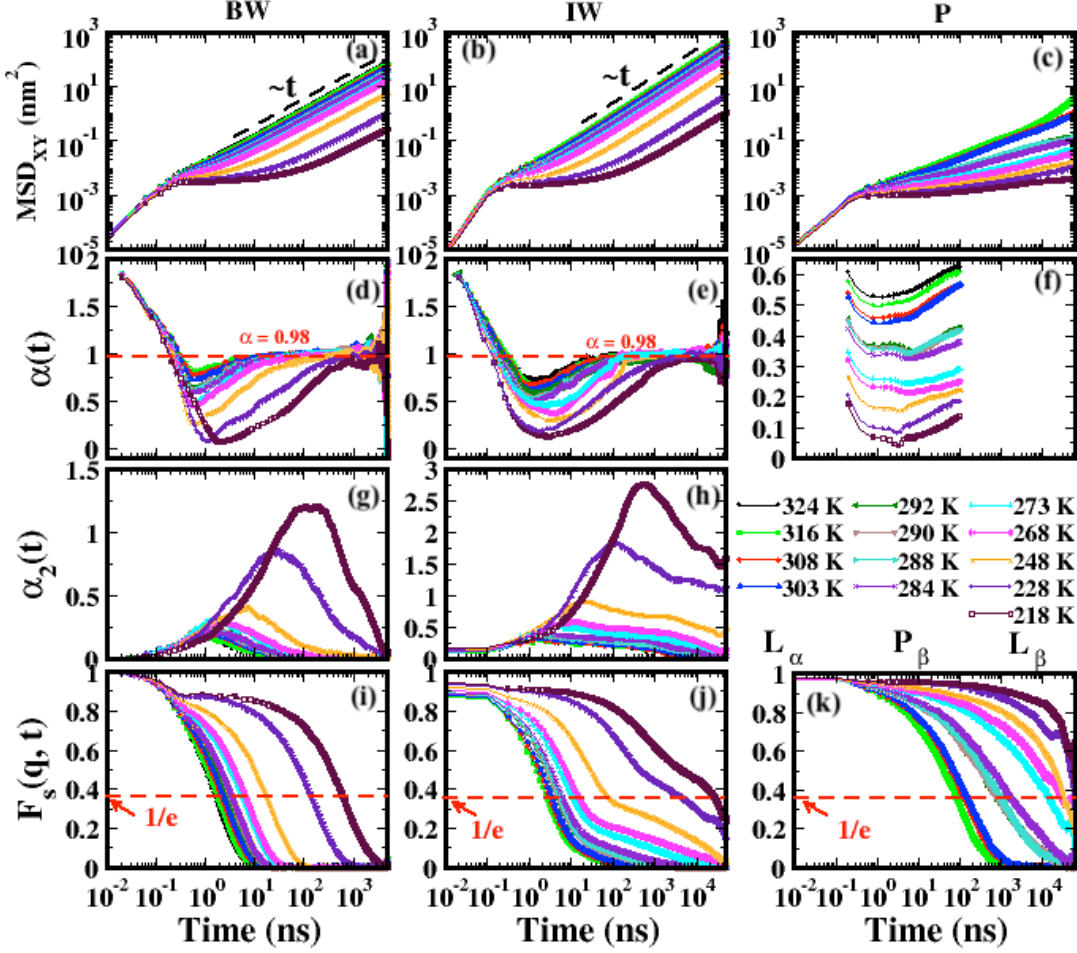


Figure 5.7 : Structural relaxations of the IW and the lipid heads upon phase transitions. Lateral mean square displacements, MSD_{XY} of a) BW, b) IW and c) phosphorous (P) heads of lipids; diffusion exponent, α of c) BW, d) IW and e) P; Non-Gaussian parameter, α_2 of f) BW, g) IW and h) P, and self intermediate scattering function, $F_s(q, t)$ of a) BW, b) IW and c) P heads respectively at $\lambda=0.5$ nm.

decreases, and at low temperatures where the ripple and gel phases occur, MSD_{XY} develop plateau due to their cage-like behavior commonly found near glass transition at supercooled temperature.

To quantify three different regimes of MSD_{XY} : an initial ballistic zone $MSD_{XY} \sim t^2$, a plateau $\sim t^\alpha$, and a diffusive regime, $\sim t$, diffusivity exponent, α is calculated as $\alpha(t) = \frac{d \ln \langle \delta r^2(t) \rangle}{d \ln t}$. Hence, a plot of the logarithmic derivative of the MSD_{XY} with time begins at 2, progresses to a minimum, and finally reaches 1, corresponding to the three separate regimes described above. Figure 5.7 (d - f) depicts the α for the BW, the IW, and the P heads respectively. The BW and the IW exhibit the initial ballistic regime, sub-diffusive regime, and finally diffusive regime. The P heads do not reach the diffusive regime within the same run-length due to their constrained dynamics [Srivastava *et al.*, 2020]. With a decrease in temperature, the α decreases signifying enhanced sub-diffusivity for all cases. The time-scale at which the $\alpha(t) \sim 1$ signifies the time required to reach a diffusive regime and is referred to as τ_D , which increases with lowering in temperature for the IW and the BW. The power law dependence of the values of the α of the P heads to the IW (figure 5.8 a) is a manifestation of the strong correlation between dynamical heterogeneity of the IW and the P heads

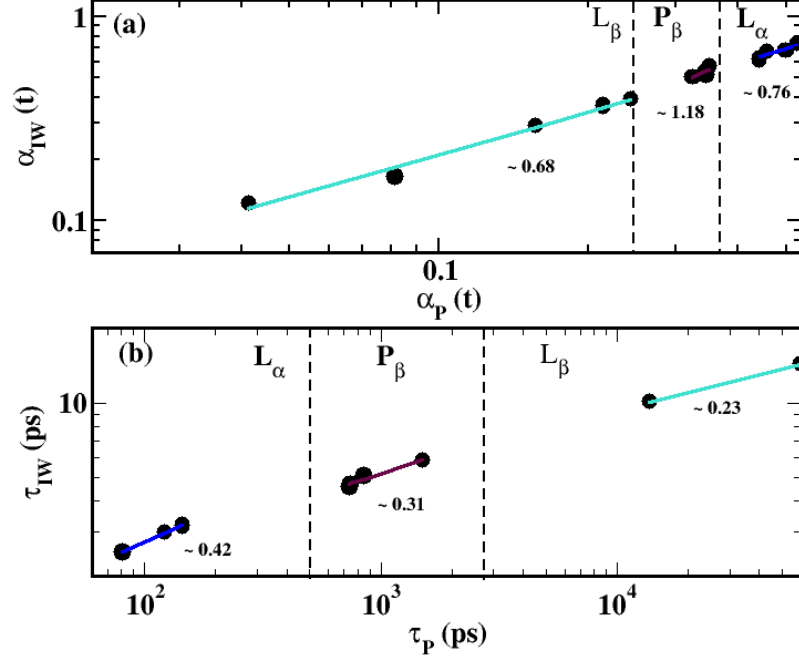


Figure 5.8 : Power law dependence of a) α and b) τ_α of the IW and the lipid heads showing their correlations at each phases of the membranes. Two dynamical cross-overs occur at the fluid-to-ripple and ripple-to-gel phase transitions.

across the phase transitions. The plateau in the MSD_{XY} represents the emergence of dynamical slowing down and cage-like behavior corroborated with a minimum in $\alpha(t)$ which are distinctly different across the three phases of the membranes (figure 5.7 f). Next, the 2D non-Gaussian parameter (NGP, $\alpha_2(t)$) [Rahman, 1964] is calculated using equation 5.3 to investigate the presence of dynamical heterogeneity. The 2D non-Gaussian parameter (NGP) is given by,

$$\alpha_2(t) = \frac{\langle \Delta r(t)^4 \rangle}{2 \langle \Delta r(t)^2 \rangle^2} - 1 \quad (5.3)$$

if NGP is truly Gaussian, then $\alpha_2(t) = 0$. The NGPs of the BW and the IW initially increase, reach maxima and subsequently decrease. The time-scale corresponding to the maximum of the NGP is known as β to α relaxation and is referred to as τ_{α_2} which signify the cross-over from the cage to the diffusive time-scales and thus represent the correlation time-scale of spatially heterogeneous dynamics for all temperatures, as shown in figure 5.7 g)-h). The τ_{α_2} of the BW is in good agreement with the previously reported time scale for the TIP4P/2005 water model [Srivastava *et al.*, 2019b; Kawasaki and Kim, 2017] and neutron scattering experiments [Arbe *et al.*, 2016]. At room temperature, the BW molecules exhibit Gaussian dynamics and with lowering in temperature, peak heights of the NGP increase, indicating the enhancement in dynamical heterogeneity. Compared to the BW, the IW molecules have higher peak heights and slower τ_{α_2} due to the stronger structural arrest in the cage for a considerable longer period when the temperature is reduced. Another ubiquitous manifestation of dynamical heterogeneity is the self intermediate scattering function (SISF) calculated using equation 5.4 in two-dimensions,

$$F_s(q, t) = \frac{1}{N} \left\langle \sum_{i=1}^N \cos(\mathbf{q} \cdot [\mathbf{r}_i(t) - \mathbf{r}_i(0)]) \right\rangle \quad (5.4)$$

where, $q = \frac{2\pi}{\lambda}$ and λ is the wavelength. Since the initial peak of $g(r)$ of the IW and the phosphorous heads are at 0.3 nm and 0.6 nm respectively, $\lambda = 0.50$ nm is used for the SISF calculations. Figure

Table 5.3 : Comparison of E_a (kJ/mol) at different temperatures from our simulations with available literature.

System	Phase	Temp.- Range (K)	Our Simulation E_a (kJ/mol)	Literature	
				Experiments	Simulation
BW		303 – 273	17.62	14.6 Laage and Hynes [2008] (300 – 260 K)	19.2 Kawasaki and Kim [2017]
		268 – 218	38.62		37.6 Kawasaki and Kim [2017] (250 – 190 K)
IW	L_α	324 – 303	20.12	20.2 Kausik and Han [2011] (DPPC bilayer)	
	P_β	292 – 284	26.64		
	L_β	273 – 218	42.56	42.41 Kausik and Han [2011] (DPPC bilayer)	10.41 Debnath <i>et al.</i> [2013] (350 – 283 K)

5.7 i)-k) show the SISF of the BW, IW, and P head at different temperatures. The initial decay of the SISF is due to the ballistic or cage motion followed by the emergence of Boson peaks at lower temperatures (228 – 218 K), as observed commonly in supercooled liquids [Hansen and McDonald, 2013]. The time at which $F_s(q, t) = 1/e$, is referred to as the α relaxation time-scale, τ_α . With lowering the temperature, τ_α start growing for the BW, the IW and the P heads. The growths are significant for the IW and the P once the temperature goes below 273 K and the membrane is at the gel phase.

5.3.3 Quantification of relaxation time scales and their behaviors across membrane phase transitions

Studying the Stokes-Einstein (SE) relationship between the translational diffusion constant D and relaxation times, (τ), obtained from the α , α_2 and $F_s(q, t)$ across all temperatures is crucial for identifying anomalous behavior of supercooled BW and IW. Relaxation time scales, τ_D , τ_{α_2} , and τ_α , are extracted from the diffusion exponent, α , the NGP, α_2 , and the SISF, $F_s(q, t)$ (from figure 5.7) respectively. As $D \sim \tau^{-\nu}$, $\nu \sim -1$, the SE relation is valid and the liquid is known to exhibit the Brownian motion and $\nu < -1$ denotes a breakdown in the SE relation due to “fragile-to-strong” cross-over in the BW and emergence of dynamical heterogeneity [Chen *et al.*, 2006b; Mallamace *et al.*, 2010; Staley *et al.*, 2015; Sengupta and Karmakar, 2014; Viot *et al.*, 2000; Xu *et al.*, 2006; Rössler, 1990]. Interestingly, the SE relation is preserved for the BW (see in figure 5.9 a)) and the IW (figure 5.10 a) for τ_D and τ_{α_2} even at supercooled temperatures. In contrast, the SE relation is violated once τ_α is considered as the relaxation time scale for the BW and the IW .

The ν changes from 1 to 0.8 to 0.6 as the membrane changes from L_α to P_β to L_β phase. Similar breakdown in the SE relation is found using NMR and Quasi-Elastic Neutron Scattering spectroscopies for supercooled confined water near a nano-pore or protein, or methanol diluted bulk water [Chen *et al.*, 2006b; Mallamace *et al.*, 2010].

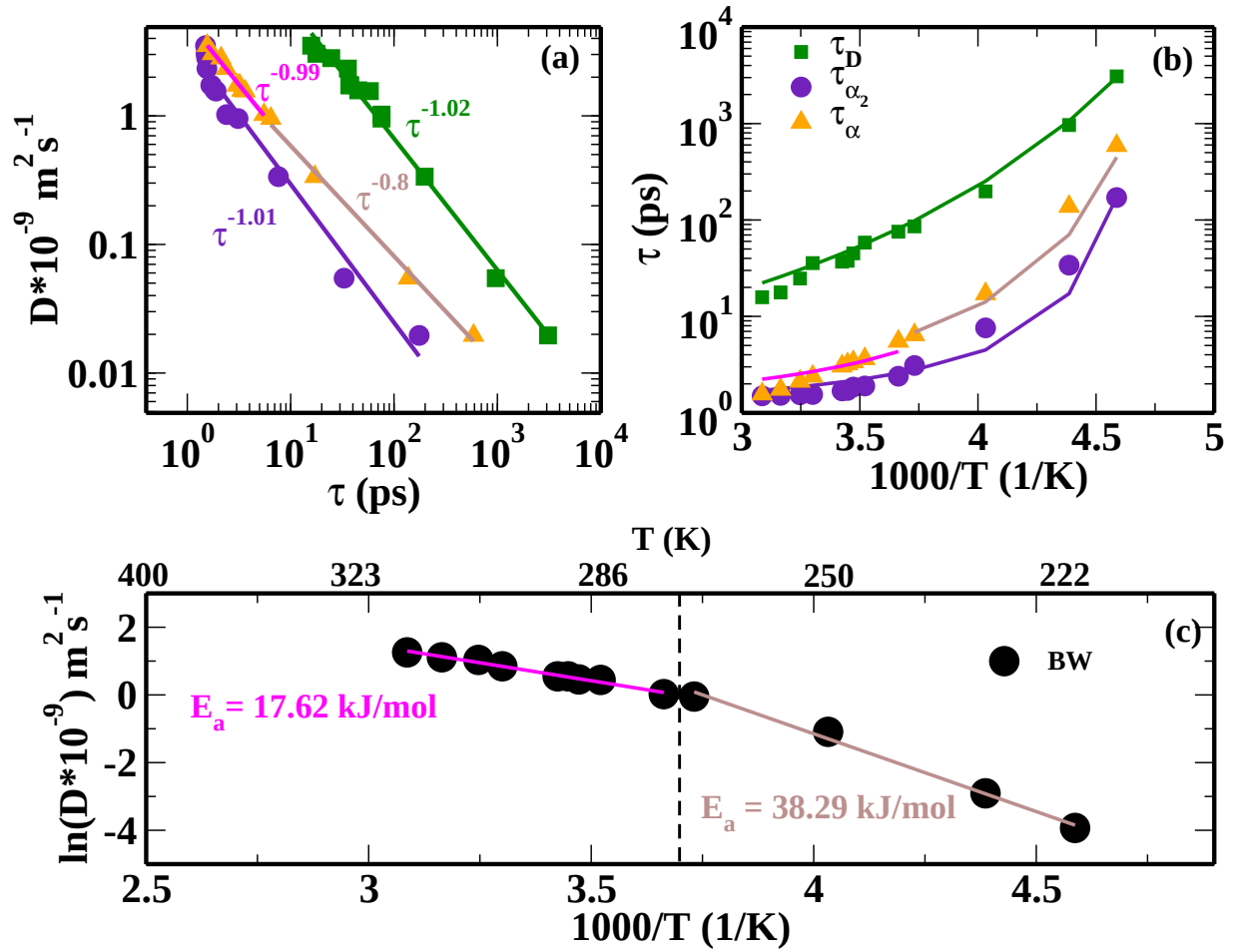


Figure 5.9 : a) Diffusion constant, D , vs τ of the BW. The SE relation is preserved for the τ_D and τ_{α_2} even at lower temperatures. The relation is violated for the τ_α reflecting a dynamical crossover in between 268 – 272 K. b) Changes in relaxation time scales, τ , with respect to $1000/T$. The τ_D and τ_{α_2} are fitted with one VFT-like equation where the τ_α is fitted with two VFT-like equations. The fitted parameters are presented in the table 5.4. The fragility index for the BW is lower than the IW signifying less fragile nature of the BW compared to the IW at the same temperature. c) Diffusion constant, D vs $\frac{1000}{T}$ for the BW exhibits Arrhenius behavior with a crossover within 268 – 218 K. The activation energies are in good agreement with previous simulations and experiments (table 5.3).

Table 5.4 : VFT fitting parameters for the BW and the IW.

System	BW		IW	
	T_0	B	T_0	B
τ_D	151	3.55	117	8.5
τ_{α_2}	205.6	0.31	191.5	0.95
τ_α	211	0.43	203	0.5
	195	0.72	200	0.71
			196	1.09

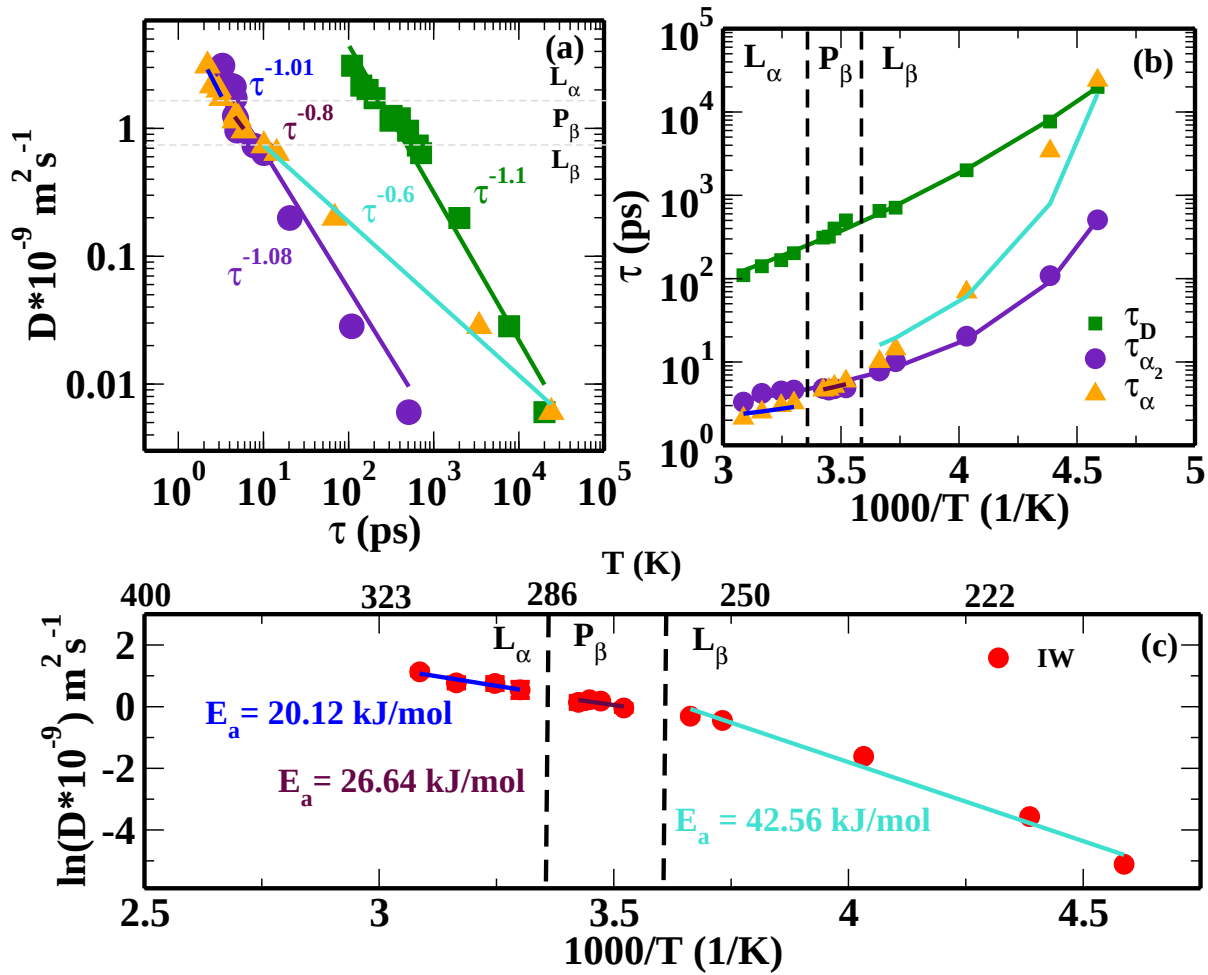


Figure 5.10 : a) Diffusion constant, D , vs τ of the IW showing that the SE relation is conserved upon lowering the temperature for the τ_D and τ_{α_2} which is no longer valid for τ_α , b) Relaxation time scales, τ_D , τ_{α_2} , and τ_α , with respect to the temperatures, $1000/T$, and c) Diffusion constant, D vs $\frac{1000}{T}$. The crossovers are associated with the fluid-to-ripple-to-gel phase transitions.

The growing τ_α of the IW and the P heads are strongly correlated to each other at all three phases of the membranes even at supercooled temperatures (figure 5.8 b). Arrhenius behavior of all relaxation time scales is examined for the BW and the IW in figure 5.9 b) and figure 5.10 b) respectively. All relaxation times are fitted by the Vogel-Fulcher-Tamman (VFT) [Cammarota and Biroli, 2012; Kob and Coslovich, 2014; Li *et al.*, 2015] equation, $\tau \propto \exp\left(\frac{BT_0}{T-T_0}\right)$, where T_0 is the VFT temperature or ideal glass transition temperature, and B is the fragility index (table 5.4). Each of the τ_D and τ_{α_2} of the BW and the IW can be fitted to one VFT equation where the fragility index of the IW is higher than the BW indicating more Arrhenius like behavior of the IW than the BW. In contrast to the τ_D and τ_{α_2} , the τ_α s of the BW and the IW are fitted with multiple VFT equations. τ_α of the BW is fitted with two VFT equations which provides two transition temperatures of 211 and 195 K. Similar behavior is found earlier for the BW [Saito *et al.*, 2018] where the higher transition temperature signifies a crossover from the non-Arrhenius to weak Arrhenius-like behavior associated with a strong change in response functions. The lower transition temperature may not reflect any thermodynamic anomaly [Saito *et al.*, 2018]. τ_α s of the IW near the three phases of the membrane are fitted to three VFT equations where the fragility index increases from the fluid to the ripple to the gel phase. The transitions of the IW are attributed to fragile-to-intermediate-to-strong crossovers accompanied by the fluid to the ripple to the gel phase transitions of the membrane. Such dynamical cross-overs of the IW near the three phases of the membranes are attributed to the structural changes from the high-density liquid (HDL) or a distorted tetrahedral structure to the low-density liquid (LDL) or a mixture of HDL-LDL like structure of the IW at the gel phase [Malik and Debnath, 2022].

5.3.4 Temperature dependence of diffusion coefficient upon phase transition

To find out the temperature dependence of the diffusion constants of the IW near all three membrane phases, the diffusion coefficient (D) is computed from the long time limit of MSD_{XY} by following Einstein's relation, $D = \lim_{t \rightarrow \infty} \langle r^2(t) \rangle / (4t)$. Two cross-overs are found for the the IW occur at the two-phase transition temperatures (figure 5.10 c). A single cross-over in D in the Arrhenius behavior is observed at 273.15 K for the BW (figure 5.9 c)) and the activation energies of the BW and the IW are extracted using the relation, $D = D_0 e^{-E_a/RT}$. The E_a s of the IW are 20.12 kJ/mol, 26.64 kJ/mol, and 42.56 kJ/mol for the L_α , P_β , and L_β phases respectively and 17.62 kJ/mol and 38.29 kJ/mol above and below the cross-over temperature of the BW. The values of the E_a of the BW and the IW (table 5.3) from our calculations are consistent with the E_a obtained from earlier experiments [Laage and Hynes, 2008] and computational studies [Kawasaki and Kim, 2017; Piskulich *et al.*, 2017]. The E_a of the IW near the L_α phase is slightly higher than that of the BW (2.5 kJ/mol) above 273K. Therefore the local water diffusion near the L_α lipid membrane is slightly restricted than the free diffusion of the BW which is shown earlier for the DPPC bilayer [Kausik and Han, 2011]. The activation energy of the IW diffusion near the L_β phase (42.56 kJ/mol) is almost twice that compared to the one near the fluid phase (20.12 kJ/mol) due to the higher number of hydrogen bonds present in the IW at the gel phase (figure 5.11). The greater activation energy may induce an enhanced coupling between the IW and the lipid heads in the gel phase.

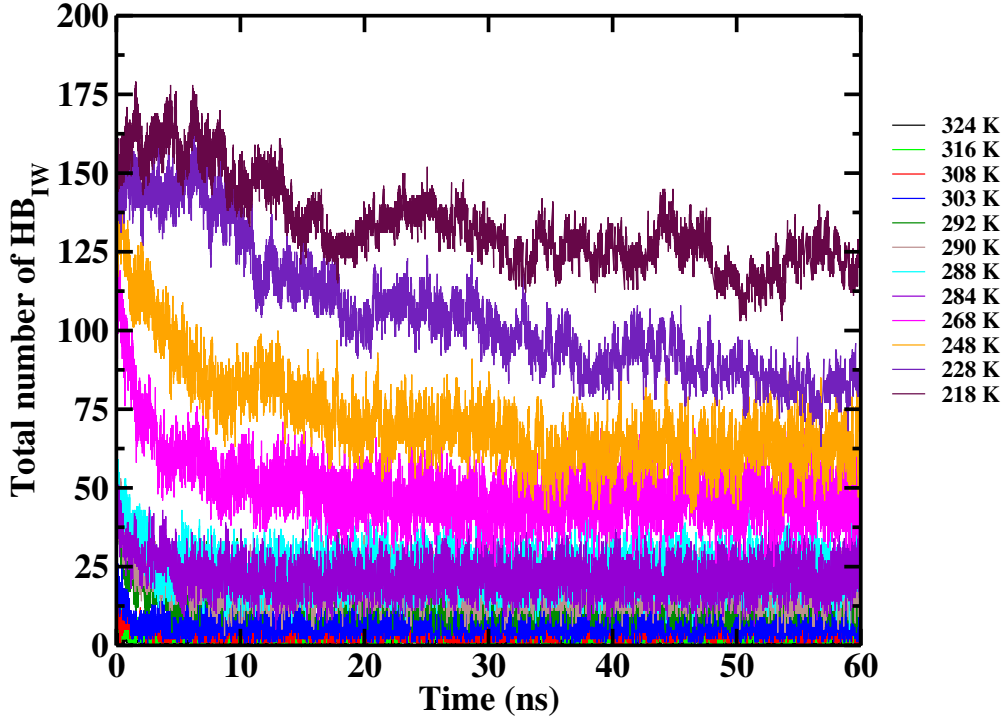


Figure 5.11 : Time evaluation of the total number of hydrogen bonds. The number increases upon the decrease in the temperature.

5.3.5 Quantification of heterogeneity length scales upon phase transitions

Block analysis is a recently developed finite-size scaling approach to extract the heterogeneity length scale of spatially heterogeneous glassy systems [Karmakar *et al.*, 2009; Chakrabarty *et al.*, 2017a] which has been employed on the membrane interface to quantify the dynamical correlation length of the IW upon membrane phase transitions. The membrane interface is split into blocks of length L_B . $(L/L_B)^d$ represents the total number of blocks to be examined, L means the total box length, and d represents the spatial dimension. To access the length scale associated with dynamical heterogeneity [Srivastava *et al.*, 2019a], larger bilayers with $18 \times 18 \text{ nm}^2$ surface have been simulated (table 5.2). Coarse-grained displacement of one block is computed by

$$\Delta x_j^B(\tau) = \frac{1}{n_j} \sum_{i=1}^{n_j} [x_i(\tau) - x_j(0)] \quad (5.5)$$

The number of IW molecules in the j^{th} block is given by n_j . $G_s^B(x, \tau)$ (equation 5.6) is computed from the coarse-grained displacement (using equation 5.5) of the IW at each block for $\tau = \tau_\alpha$ at all temperatures shown in figure 5.13. The van Hove correlation function is defined as follows:

$$G_s^B(\mathbf{r}, \tau) = \frac{1}{N_B} \left\langle \sum_{j=1}^{N_B} \delta[x_i(\tau) - x_j(0)] \right\rangle \quad (5.6)$$

The non-Gaussian van Hove distributions become more and more Gaussian as the L_B increases, as shown in figure 5.12 a), d), and g) for the fluid, ripple, and gel phases, respectively. To determine the deviation from the Gaussian distribution, binder cumulant of the distribution has

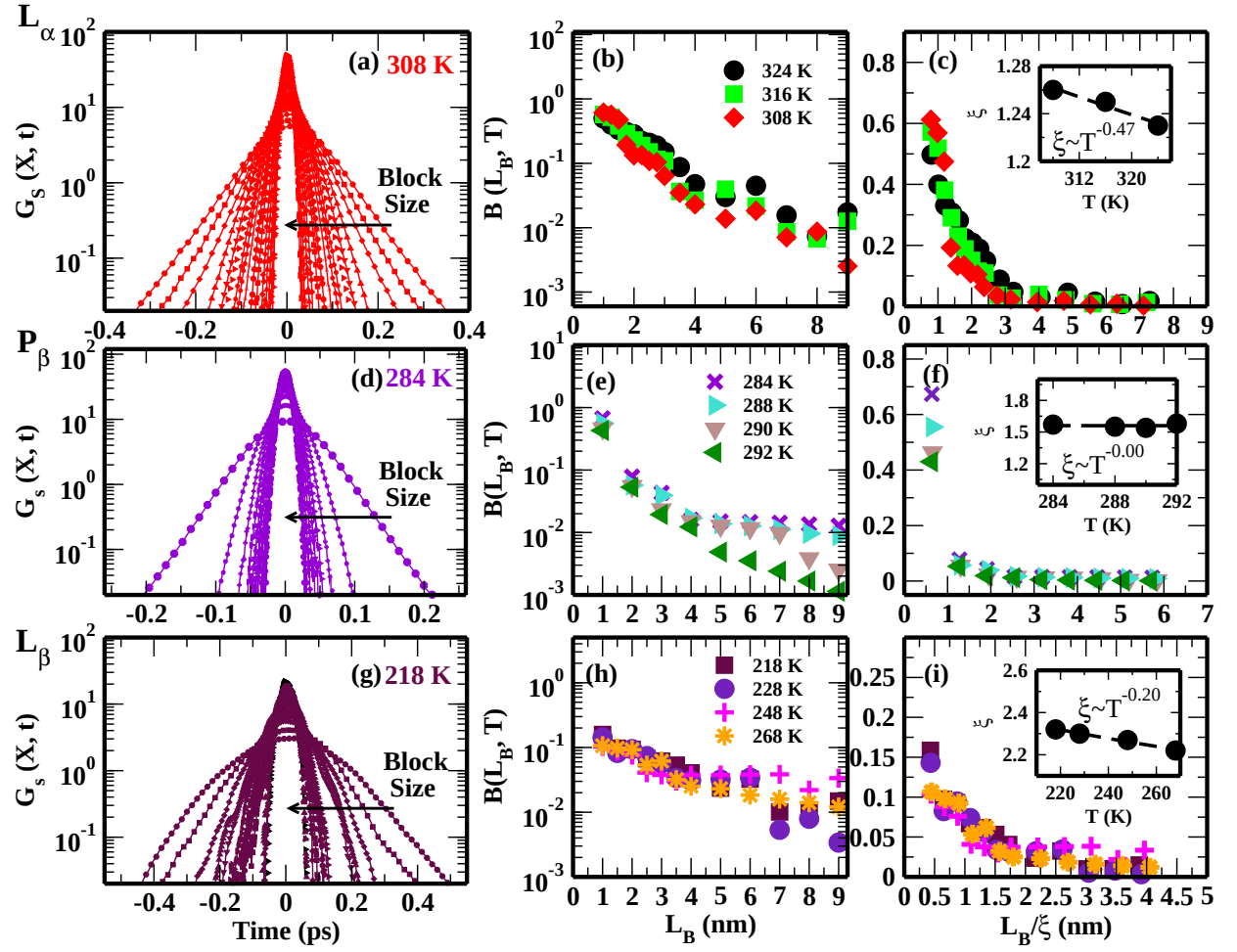


Figure 5.12 : van Hove function for different block lengths varying from 9 nm to 1 nm for a) 324 K, d) 284 K and g) 218 K at the fluid, ripple and gel phases respectively. Binder cumulant of the blocked van Hove function at previously mentioned three temperatures in b), e) and h) respectively. The deviation from the Gaussianity increases with decreasing block size. Finite-size scaling of the binder cumulant by data collapse at three temperatures c), f), and i). Inset shows the temperature dependence of the heterogeneity length scale, ξ , obtained from the data collapse of the binder cumulant for the fluid, ripple, and gel phase, respectively

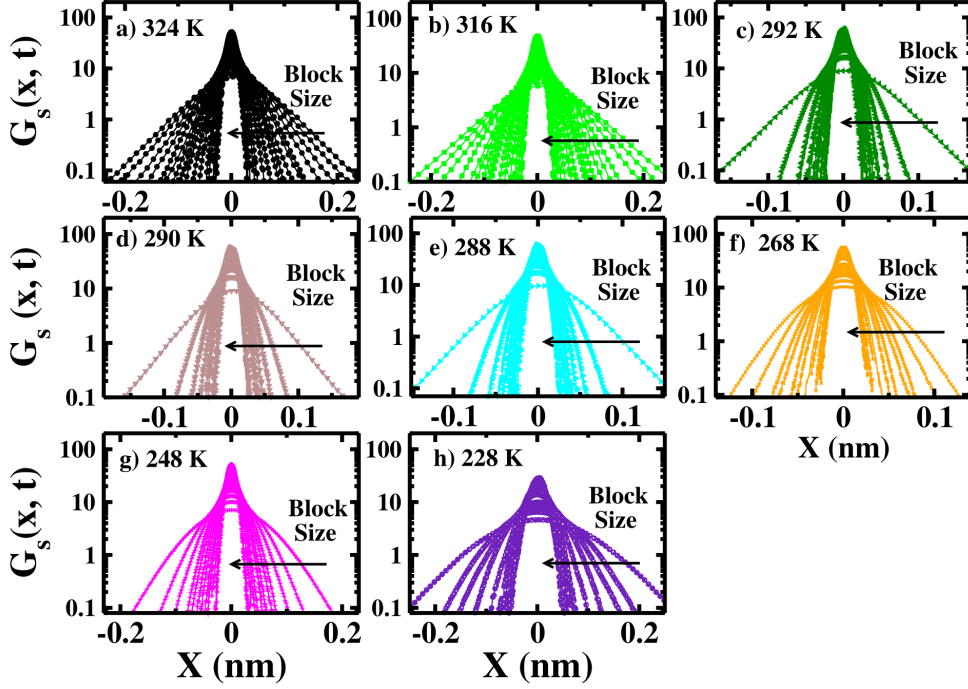


Figure 5.13 : van Hove correlation function as a function of displacement for different coarse-graining block length, L_B at different temperatures.

been computed using equation 5.7,

$$B(L_B, T) = \frac{\langle x^4 \rangle}{3\langle x^2 \rangle^2} - 1. \quad (5.7)$$

[Bhowmik *et al.*, 2018; Chakrabarty *et al.*, 2017c; Karmakar *et al.*, 2009]. $B(L_B, T) = 0$ for higher temperatures and larger blocks as the underlying distribution is Gaussian. With decreasing block size, the values of the $B(L_B, T)$ increase at all temperatures due to larger deviations from Gaussianity seen in figure 5.12 b), e), and h) for three phases of the membrane. As the binder cumulant is a scaling function of the underlying correlation length, it is assumed to follow the relation of $B(L_B, T) = f[L_B/\xi(T)]$. Thus, $B(L_B, T)$ at different temperatures are subjected to a data collapse and plotted in figure 5.12 c), f) and i) for the fluid, ripple, and gel phases, respectively. The insets of figure 5.12 depict the temperature dependence of the scaling length scale, ξ . For the fluid L_α phase, ξ strongly varies with T . The dependence disappears for the ripple P_β phase unlike the glassy liquids and thus can be scaled to the ripple wavelengths at all temperatures within the given ripple phase (figure 5.14). The average value of ξ in the ripple phase is 1.6 which is close to the size of the patches obtained in the ripple phase (in figure 5.6). This signifies that the domain size of the ripple of the membrane can be captured by the heterogeneity length scale of the IW which do not vary with temperature within the same phase. The T dependency of the ξ is very weak at the gel L_β phase. Thus the heterogeneity length-scale ξ of the IW is strongly coupled with the lateral organizations of the membranes and thus does not change significantly within a given phase. The amplitude of the first peak of the radial distribution function of the IW, g_1 [Malik and Debnath, 2022] is strongly correlated to ξ for all three phases (figure 5.15 a), b) and c)) suggesting that the correlation length scale is structure dominated. The change in ξ is minor within a given phase but the length scale significantly grows once there is a phase transition (figure 5.15 d). Figure 5.15 e) shows that, within a given phase, there is a drastic slow-down in the τ_α with decreasing temperature without a significant change in ξ for L_α , P_β , and L_β respectively. Similar to the the

ROFT [Young, 1998] theory, an activated dynamic scaling of τ_α is obtained for the L_β phase but not for the other two phases (shown in figure 5.16). Thus the heterogeneity time scales are slowed down mainly by a reduction in temperature where the major changes in the heterogeneity length scales are dominated by the membrane phase transitions since the length scales are controlled by the underlying structures.

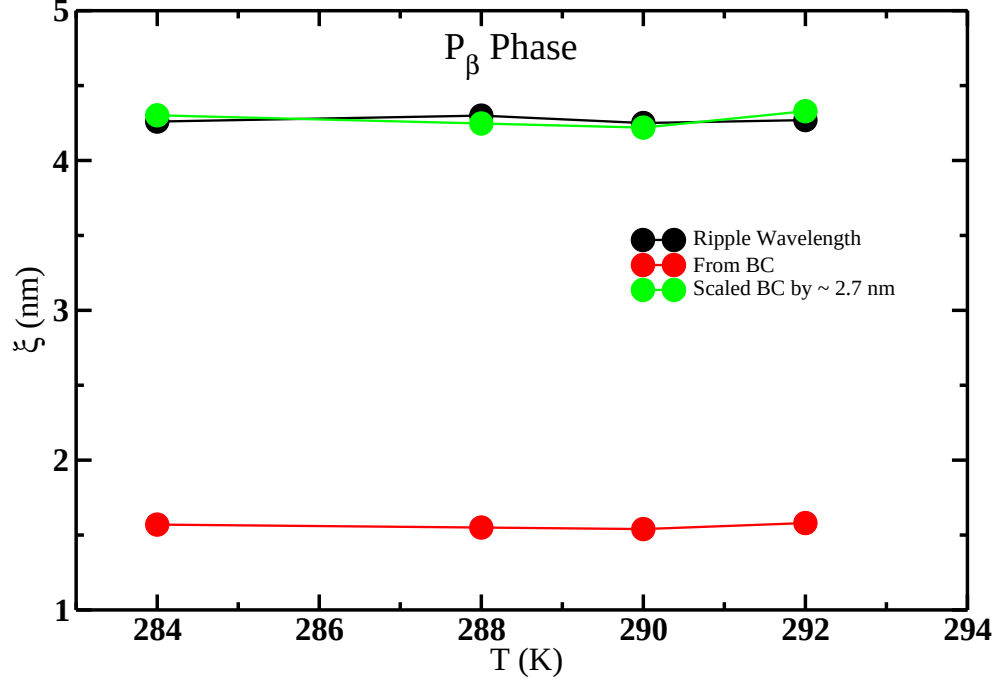


Figure 5.14 : Dynamical length (ξ) versus temperature for the IW near the ripple phase. Red: from binder cumulant, black: ripple wavelength from the spectral intensity plot, and green: scaled length from the binder cumulant by ~ 0.27 nm.

5.4 DISCUSSION

When the temperature is reduced, the MSD_{XY} of both the IW and lipid heads exhibit more sub-diffusive behavior with a plateau which indicates the emergence of dynamical heterogeneity upon supercooling. The ballistic, sub-diffusive, and diffusive regimes from the MSD_{XY} and the values of diffusion exponents, α are consistent with each other. The decrease in α with a lowering in temperature for all cases confirms the enhancement in sub-diffusive nature upon supercooling. Our calculations show that the lipid heads do not reach the diffusive regime within the simulated run-length while the IW takes a longer time compared to the BW to reach the diffusive regime. Similar slow dynamics of the lipid heads and the IW compared to the BW is found earlier at room temperature from the probability distributions of their logarithmic displacements which are attributed to the structural arrest of the lipids heads in their neighboring cages and the intermittent dynamics of the IW due to their hydrogen bonding to the lipid heads [Srivastava *et al.*, 2020]. However, $\alpha(t)$ of the IW and lipid heads are correlated by a power law with two cross-overs across the fluid-to-ripple and ripple-to-gel phase transitions. When the temperature is reduced, the amplitude of the non-Gaussian parameter, α_2 , increases with a drastic slow-down in the β to α relaxation times, τ_{α_2} due to a strong structural arrest of the IW at the cage

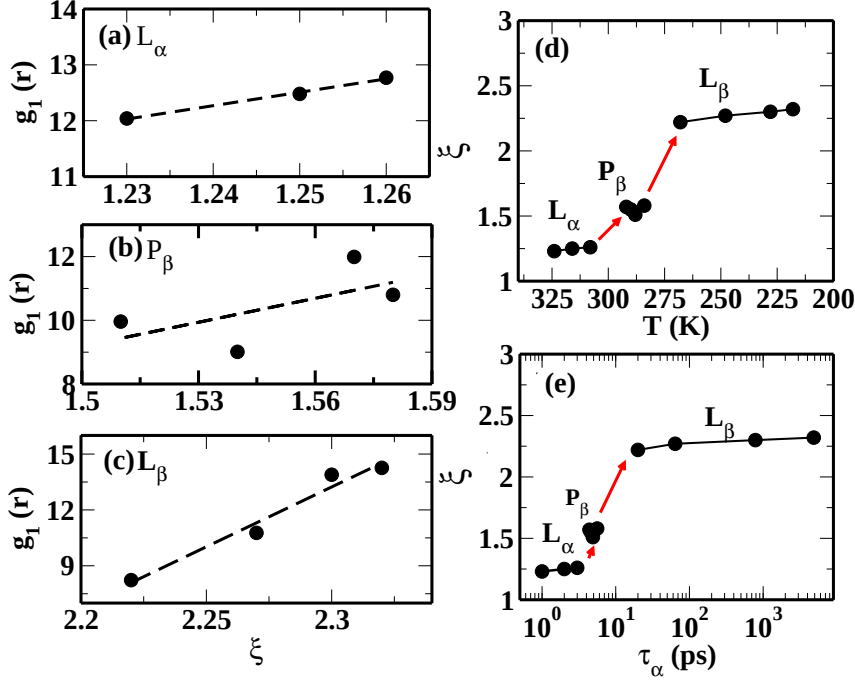


Figure 5.15 : A plot of g_1 versus ξ for a) L_α , b) P_β , and c) L_β phase. g_1 follows power law with ξ where the exponents are 2.41, 3.66, and 13.24 for the L_α , P_β , and L_β phases respectively. d) Temperature dependence of ξ of the IW near three phases of membranes showing drastic growth in ξ at the phase transition temperatures. e) Dependence of ξ on τ_α shows drastic slow-down in τ_α with decreasing temperature and drastic growth in ξ with phase transitions. τ_α do not exhibit standard finite-size scaling with ξ .

manifesting the enhancement in dynamical heterogeneity. A similar slow-down in τ_α relaxation is observed with the emergence of boson peaks in the $F_s(q, t)$ for the BW, the IW, and the lipid heads as the temperature is dropped. The SE-relation is preserved for the IW and the BW even at lower temperatures as $D \sim \tau^{-1}$ if τ is derived from the diffusion exponent and the NGP. On the contrary, the SE relation is violated for the IW near the ripple and gel phases and the BW if τ is obtained from the SISF. The violation in the SE relation at supercooled temperatures have been found earlier using τ_α for the BW [Kawasaki and Kim, 2017] which shows that the τ_α does not characterize the translational diffusion constant, D at lower temperatures and the violation is correlated with the growing dynamical heterogeneity. Similar preservation and violation of the SE relation from different relaxation time scales are reported for the supercooled BW [Kawasaki and Kim, 2017] and for model glass-forming liquids [Adhikari *et al.*, 2021; Das *et al.*, 2020; Malik and Debnath, 2021]. The break-down in the SE relation of the IW across the fluid-to-ripple and ripple-to-gel phase transitions demonstrates that the slow-down in the α relaxation times of the IW is correlated with the membrane phase transitions. The τ_D and τ_{α_2} are fitted to one VFT equation for the BW and the IW near three membrane phases. The higher fragility index of the IW than the BW indicates more Arrhenius-like behavior of the IW. On the contrary, τ_α is fitted to three-VFT equations for the IW near three phases of the membranes. The same behavioral differences between τ_α to the τ_D and τ_{α_2} in terms of temperature dependence and the SE preservation or violation are observed for the IW, the BW and the glass formers [Kawasaki and Kim, 2017; Adhikari *et al.*, 2021; Das *et al.*, 2020; Malik and Debnath, 2021]. This suggests that this behavior is universal for systems exhibiting dynamical heterogeneity irrespective of their physical nature. Three transition temperatures of the IW from the fluid to the ripple to the gel membrane is an indication of fragile-to-intermediate-to-strong cross-overs similar to the

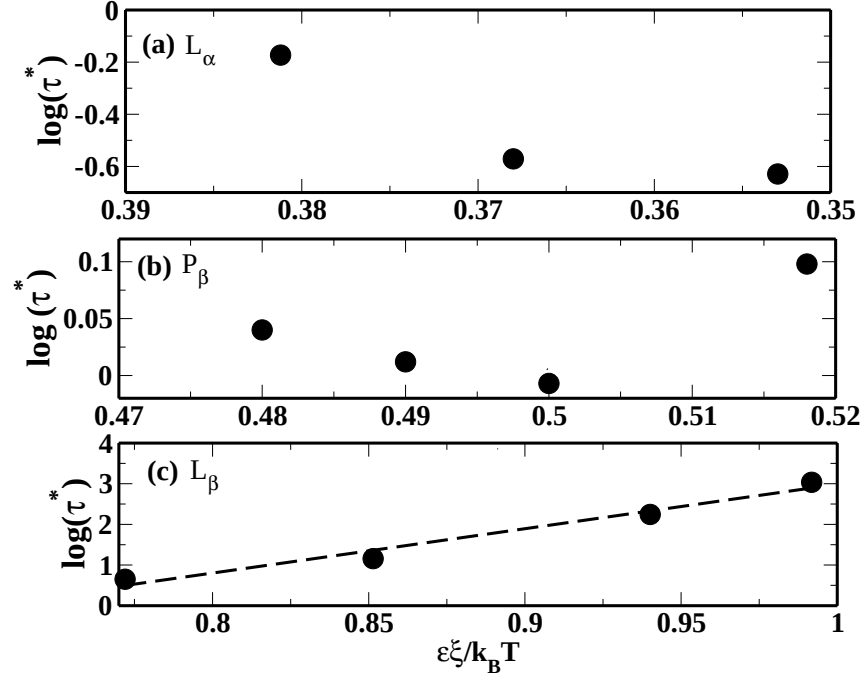


Figure 5.16 : Dependence of $\log \tau^*$ a) L_α , b) P_β Phase, and c) L_β Here, $\tau^* = \tau_\alpha \sigma^{-1} \sqrt{\epsilon/M}$. σ, ϵ , and M are LJ parameters and mass respectively and considered for oxygens of water molecules. The IW near the L_β phase exhibit activated dynamic scaling.

fragile-to-strong crossovers for the BW associated with a HDL to LDL like structural change. Our earlier study shows that the IW undergoes a structural transition from the HDL to a mixture of HDL-LDL or LDL like phase once the membrane undergoes a phase transition from the fluid to the ripple to the gel phase [Malik and Debnath, 2022]. Thus the fragile-to-intermediate-to-strong cross-overs of the IW are attributed to the structural transitions from the HDL to a mixture of HDL-LDL or LDL like phase. However, a detailed analysis is required to provide molecular level evidence on IW structural change. The diffusion constant of the IW follows the Arrhenius trend with two dynamical changes at both phase transition temperatures. The differences in the activation energy between the BW and the IW near three phases of the membranes from our simulations are in good agreement to the previous experiments for the DPPC bilayers [Kausik and Han, 2011]. The IW near the gel phase has higher activation energy due to the higher number of hydrogen bonds than the ones near the other two phases.

The length scales of spatially heterogeneous dynamics of the IW are quantified using a block analysis technique, and then correlated to the lateral organizations of the membranes across phase transitions. One-dimensional van Hove correlation functions of the IW shows a shift from the non-Gaussianity to the Gaussianity once the block size increases at each phase of the membrane. The deviations from the Gaussianity are estimated by Binder cumulant which increases with lower block size at all temperatures. Since Binder cumulant is a scaling function of the underlying correlation length scale, the values of the Binder cumulant at all block sizes have been collapsed into a single scaling for the IW near each of the three phases. The scaled length has been identified as the heterogeneity length scale, ξ , which increases with a decrease in temperature at the fluid phase similar to the supercooled glass-forming liquids [Karmakar *et al.*, 2009]. The temperature dependence of the ξ disappears for the IW near the ripple phase. Hence, the heterogeneity length-scale of the IW near the ripple phase can be scaled to the ripple wave length calculated from

the intensity spectra of the thickness and interdigitation fluctuations which too remain unchanged with temperature within the ripple phase.

5.5 CONCLUSIONS

In conclusion, the paper uses $11.55\mu\text{s}$ all-atom molecular dynamics simulations to correlate and quantify the spatio-temporal heterogeneities of the IW to the DMPC lipid membranes upon fluid-to-ripple-to-gel phase transitions. Bilayer properties such as area per lipid, thickness, and interdigitation imposed on Voronoi from our simulations are in good agreement with previous computational and experimental studies [Khakbaz and Klauda, 2018] validating our simulations at all phases. To comprehend the role of water in such phase transitions of membranes, IW molecules are identified for their continuous residence period within the first hydration layer of the lipid heads. All dynamical properties such as MSD_{XY} , τ_α , α_2 , and $F_s(q, t)$ show signatures of dynamical heterogeneity for the IW, BW, and lipid heads upon supercooling. The relaxation time scales are extracted for the IW, BW from τ_α , α_2 , and $F_s(q, t)$ and are referred to as τ_D , τ_{α_2} , and τ_α respectively. The SE relation is preserved for τ_D and τ_{α_2} even at supercooled temperatures. The relation is not valid for τ_α for both the BW and the IW. Single transition is obtained for both τ_D and τ_α upon single VFT fitting whereas two transition temperatures are obtained for τ_α from two VFT fittings for the IW. This is attributed to the fragile to intermediate to strong cross-over due to the structural changes from the HDL to a combination of HDL and LDL or LDL like phases of the IW near the gel phase. The activation energy of the IW near the gel phase is almost twice to the one near the fluid phase due to maximum number of the H bonds. The heterogeneity length scale extracted from the finite size scaling of the binder cumulant follows an inverse power law with temperature for the fluid phase. The length scale becomes temperature invariant and is similar to the size of the periodic domain in the ripple phase as the heterogeneity length scale is governed by the underlying structure. The heterogeneity length scale is very similar to the value of the half wavelength of the ripple or the size of each patch in the ripple membrane.

Thus, the size of the rippling of the membrane can be obtained from the heterogeneity length scale of the IW signifying that the dynamical lengths of the IW is controlled by the rippled domain of the membrane. The temperature dependence of the length scale is very weak at the gel phase. The heterogeneity length scale follows a power law behavior with the amplitude of the first peak of the radial distribution functions of the IW at each of the three phases of membranes demonstrating a strong coupling between the heterogeneity length scale and the structure of the IW. The length scale does not change significantly with temperature within a given phase but exhibits two drastic growths at the fluid-to-ripple and ripple-to-gel phase transitions of the membranes. Thus the growth in the heterogeneity length scale is dominated by the membrane phase transitions, not by the temperature. On the contrary, the drastic growth in the relaxation time scale is governed by supercooling. The slowdown in relaxation time of the IW follows an activated dynamical scaling in similar line to the ROFT theories. This is not observed for the IW near the fluid or the ripple phase. Thus, determining the dynamic cross-over in the heterogeneity length scale of the IW from block analysis will provide a new avenue to identify domain size which can be critical for their functions. Since probing nano-domains in biological membranes are extremely challenging and no current biophysical tool is available yet to this end [Jacobson *et al.*, 2007], our method opens up a new direction to bridge the gap between the available biophysical tools and theoretical methods to characterize functionally relevant nano-domains of membranes. As per our knowledge, for the first time, a systematic analysis is performed to estimate the heterogeneity time and length scales of the IW and membranes across phase transitions. The spatiotemporal scales of the hydration water are correlated to the dynamical properties and lateral organizations of the membranes across phases relevant to physiological and low-temperature conditions. The findings

can help us to better understand bio-protection processes under extreme conditions and will aid future research on domain-associated transport and signaling in biomembranes at low temperatures.

Publication:

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