Hydration layer dynamics and association mechanisms of food and antifreeze proteins

A Molecular Dynamics and Transition Path Sampling study

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Chapter 4

Correlation between water structure and dynamics in the hydration layer of a type III ocean pout anti-freeze protein

We report on a molecular dynamics study on the relation between the structure and (orientation and hydrogen bond) dynamics of hydration water around the ocean pout AFP III anti-freeze protein. We find evidence for an increasing ice-like structure from the area opposite to the ice binding site (IBS) towards the protein IBS, with the strongest ice-like structure around the THR-18 residue of the IBS. This ice-like structural signal correlates with increased reorientation decay times. Moreover, we find anti-correlation for several key residues that are not part of the IBS but are in its vicinity. These effects are enhanced at lower temperature. Finally, as AFP III anti-freeze protein is binding to ice crystal planes through a predominantly hydrophobic patch, we investigate the ice-like structure and dynamics of waters at partially dehydrated IBS. We find that upon dehydration the IBS becomes even more ice-like for the wild type, and that the water reorientation time becomes longer, but less so for the mutant T18N, which also has a higher hydration at the IBS. These results are in agreement with water-air VSFG spectroscopic experiments showing a reduced ice-like signal upon mutation at the IBS.

4.1 Introduction

The structure and dynamics of a protein’s hydration layer is crucial for its functioning and conformational dynamics. Hydration plays an active role in biological processes such as protein folding, ligand binding, and protein recognition [1–3]. Protein-
Solvent interactions are especially important in antifreeze proteins (AFP), as they have to specifically recognize and bind — through their Ice Binding Site (IBS) — nucleating ice crystals in the excess of liquid water and prevent further growth of ice [4]. In spite of this seemingly tough goal, AFPS show a large structural diversity in nature and are seen in many organisms such as fungi, bacteria, fish, insects, where their major function is to help these organisms survive at subzero temperatures [5]. In addition to their biological role, AFPS also have a variety of applications as ice avoiding agents, from organ preservation [6] to texture enhancers in food [7]. Many AFPS are characterized by an IBS consisting of regular \( \beta \)-sheets or spirals, commensurate with the ice-crystal planes. A counterexample is the ocean pout AFP III that does not exhibit a regular \( \beta \)-sheet ice binding site, raising the question of how the binding to ice is achieved instead.

Both simulations [8–10] and experiments [11–13] indicate that water is more structured at the IBS with respect to other non IBS sites of the AFP III protein. The type of interactions necessary for the affinity and specificity of the IBS to ice crystals have been addressed by mutation studies, X-ray crystallography [13, 14] and simulations [8, 9, 15]. There is an increasing consensus on the combined role of both polar groups (able to form hydrogen bonds) and apolar groups in ice binding by matching to ice-lattice oxygens as well as by hydrophobic ice-IBS interactions. AFP III’s ice-binding-site is relatively flat and hydrophobic, thus serving as a great candidate for surface sensitive interface experiments. In particular, VSFG studies [16] have shown that a single point mutation of a core IBS amino acid, from THR-18 into ASN-18, causes a loss in anti-freeze activity of the ocean pout AFP. The effect of this mutation on the water structure and dynamics is not known in full detail. Here, we employ all atom molecular dynamics (MD) simulations to obtain microscopic insight in the tetrahedral structure and reorientation dynamics of the protein hydration layer for the wild type and the T18N mutant. As these experiments were surface sensitive, we mimic the exposure of the protein to the water-air surface by studying a partially dehydrated IBS. For a \( \beta \)-helical anti-freeze protein, Nutt et al [17] pointed out that not only the IBS is important for ice recognition but also the non-IBS residues, which distort the ice-like water structure. Therefore, we also investigate here the correlation between reorientation and structure in different parts of the protein, and interpret this in terms of tetrahedral structure-making and -breaking ability of the residues.

The remainder of the paper is organized as follows. The next section describes the simulation setup, and analysis methodology. Subsequently, we present the analysis of the results, and discuss these in the light of the VSFG (and other) experiments. We end with conclusions.
4.2 Methods

4.2.1 Simulation setup

All molecular dynamics simulations were performed with the Gromacs 4.5.4 package [18]. The ocean pout wild type monomer structure was obtained from PDB 1HG7 [19] and the mutated ocean pout structure (THR-18 to ASN-18) was obtained from PDB 1JAB [20]. Each structure was solvated with (SPC/E or TIP4P/2005 molecules, resulting in solutions of approximately 5% w/w. The choice of two different water models serves, not so much as a comparative study of the two models, but as a consistency test. It is known that SPC/E water model is known to better reproduce the dielectric constant of water [21] than the TIP4P/2005. Therefore, in the main text, we report the results for the SPC/E water model unless stated otherwise. The TIP4P/2005 water model is being reported in the Appendix.

The atomic interactions were defined by the amber99sb-ildn force field [22]. The protonation state of the amino acids corresponds to pH between 4.25 and 10.53, since glutamic acid side chain is deprotonated (pKa=4.25) and the Lysine side chain is protonated (pKa=10.53). No ions needed to be added to the solution after assigning hydrogens to the pdb structures since the total charge of both structures was zero. After energy minimization, both water force-fields systems were equilibrated for 1 ns at a constant pressure of 1 atm and at several temperatures. These temperature were 298 K, 285 K, 270 K, 255 K, 225 K and 1 atm for the SPC/E systems and 298 K, 285 K, 270 K, 255 K for the TIP4P/2005 systems. All bonds were constrained with the Lincs algorithm. A cutoff of 1 nm was used for the non-bonded Lennard-Jones interactions.

The Particle Mesh Ewald method was used to calculate the electrostatic interactions with a Fourier spacing of 0.12 nm and a 1 nm cutoff for the short range electrostatic interactions. Neighbour lists were updated every 10 fs with a cutoff of 1 nm and the time step was 2 fs [22]. The leap-frog algorithm was used for integrating Newton’s equations of motion. In the NPT simulations the v-rescale thermostat[23] with a coupling time constant of 0.2 ps controlled the temperature, while the Parrinello-Rahman barostat[24] with a coupling time constant of 1.0 ps kept the pressure constant.

For each temperature 10 frames corresponding to the average energy and volume were selected from a 100 ns long MD trajectory in the NPT ensemble. From each frame a 1 ns NVE run was conducted. This approach eliminated any unwanted influence from the thermostat or barostat on the dynamics. To prevent energy drift we used a switching function for the non-bonded interactions from 0.8-1.0 nm. The pair lists were updated every 5 fs with a cutoff of 1.2 nm and the time step was 1 fs. The frequency of the energy calculation was 10 fs, and the frames were saved every 100 fs in order to obtain sufficient data for the analysis.
In this paper the above systems of wild and mutant type water solutions for different temperatures will be denoted as fully hydrated systems. The effect of water dehydration on the water structure and dynamics was investigated by analyzing partially dehydrated systems. For each combination of temperature, water force field, and WT/mutant, we took the last frame of the 100 ns NPT MD trajectory and reduced the water content by about a factor of 10, leaving only 722 protein surface water molecules. We ran another 100 ns NVT at the same temperatures as the fully hydrated systems. From each of these NVT trajectories, 10 frames corresponding to the average energy and volume were selected, from which a 1 ns NVE run was conducted. The other parameters for the NVT and NVE of the dehydrated systems were identical to those of the fully hydrated systems.

4.2.2 Analysis

Hydrogen bond lifetimes

Throughout this paper hydrogen bonds will be referred to as H-bonds. An H-bond is defined present if the distance between H-bond donor $D$ and acceptor atom $A$, $R_{DA} \leq 0.35$ nm, and the angle between the $DH$ and $HA$ vectors $\theta_{ADH} \leq 30^\circ$ [25, 26]. Here, the donor or acceptor heavy atom can belong to either the protein or to water. The H-bond lifetime was defined as the time it takes for the bond to break and to form a stable bond with another acceptor (exchange), or to remain dangling (translational diffusion). If the bond reforms within 200 fs, it is considered not broken, in order to avoid counting fast recrossings events.

The recrossing time $\Delta \tau = 200$ fs was based on the fact that the vibrational characteristics of the water water H-bond occur in the far infrared and around $200 \text{ cm}^{-1}=166$ fs [27, 28].

Water reorientation dynamics

Reorientation dynamics can be represented using the time correlation function [29, 30]

$$C_2(t) = \frac{2}{5} \langle P_2[u(0) \cdot u(t)] \rangle$$ (4.1)

where $P_2$ denotes the second Legendre polynomial, $u$ is the unit vector characterizing the orientation of an OH group in a given frame and the angular brackets denote an ensemble average over all water molecules and all time origins. This correlation function can easily be related to anisotropy curves obtained from polarization-resolved femtosecond infrared spectroscopy and to orientation relaxation times from magnetic relaxation techniques [29].
From MD simulations we compute the reorientation dynamics for each individual molecule. We are particularly interested in the anisotropy decay of water molecules hydrating different protein sites. The reorientation dynamics of individual water molecules was investigated by following the dynamics of both OH bond vectors of each molecule \( j \). For each bond unit vector \( \mathbf{u}_{jk}(t) \), we computed the time correlation function

\[
C_j^m(t) = \frac{1}{5\ell} \sum_{t' = t_0}^{t_0 + \ell} \sum_{k=1}^{2} P_2[\mathbf{u}_{jk}(t') \cdot \mathbf{u}_{jk}(t' + t)]
\]

where the sum over \( k = 1, 2 \) refers to the two OH bond vectors, and the time correlation is summed over the \( m \)th time interval \( t_0^m < t' < t_0^m + \ell \Delta t \) that the molecule is within the hydration layer of the protein (defined as the water oxygen being within 4.4 Å from the protein heavy atoms). We used a buffer time of 2 ps to avoid counting fast non-essential excursions inside and outside the hydration layer [31]. For each curve \( C_j^m(t) \) we extracted the reorientation decay time \( \tau_j^m \) by fitting \( C_j^m(t) \) to a single exponential fit in the interval \( 0 < t < 10 \) ps. Each estimate \( m \) was viewed as a separate measurement, since the water molecule can change its dynamics when leaving or entering the hydration layer. For each water molecule \( j \) and for each \( m \)th interval of length \( \ell \) the decay time \( \tau_j^m \) was histogrammed with a weight \( \ell \), leading to a probability distribution of decay times. The weight \( \ell \) follows from the fact that the correlation in eq. 4.2 occurs \( \ell \) times in eq. 4.1. The average decay time \( \tau \) from these histograms thus should be close to the overall decay time of eq. 4.1. The decay times of the individual water molecules allow us to establish the relation between the water structure and dynamics. We therefore divide the waters into several categories.

For each water molecule \( j \) and for each \( m \)th interval \( \ell \) the decay time \( \tau_j^m \) was histogrammed with a weight \( \ell f_{\#AA} \) in the residue \#AA reorientation distribution. Here \( f_{\#AA} \) refers to the fraction of frames in the \( m \)th interval \( \ell \) in which the oxygen of water \( j \) is within 4.4 Å of any of the heavy atoms of residue \#AA.

**Water structure and dynamics at different areas of the protein.**

Based on mutation studies by Graether et al. [20] we define six different parts of the protein surface, the THR-18 and ASN-18, the IBS, the vicinity and the opposite face, the non-IBS residues (everything but the IBS), and the entire hydration shell which consists of the IBS and non-IBS (see for an illustration Fig. 4.2.1a,b). The vicinity and opposite face residues were selected to include key surface residues identified in Ref. 20. They include vicinity residues 39,42,46,47,61 and opposite residue 29. Figure Fig. 4.2.1a,b shows which residues of the protein surface belong to the IBS, the vicinity and the opposite group. Rendered in cyan are the IBS residues 9,10,12,
Figure 4.2.1: (a), (b): Partitioning of the protein into three distinct areas. The area IBS (denoted by cyan), vicinity (denoted by magenta) and opposite (denoted by green). THR-18 is denoted by black. (c) $\theta$ angle

13,14,15,16,18,19,20,21,44. Depicted in magenta are the vicinity residues within 0.4 nm of the IBS (8, 23, 37, 39, 42, 43, 46, 47, 48, 50, 51, 61). The green opposite site residues are 1.5 nm away of the IBS and include residues 0, 1, 2, 26, 27, 28, 29, 30, 39, 56.

**Water-water angular distribution function**

Sharp and coworkers [9, 32, 33] introduced a method to identify the tetrahedral structuring of water around amino acids based on the distribution of a water-water angle. In a given frame one computes for each amino acid the minimum water-water OOH angle $\theta$ (see Fig. 4.2.1c for a graphical definition) for all water-water pairs within 3.5 nm from each other and solvating that amino acid. The distribution $P(\theta)$ of these angles shows a bimodal distribution with a minimum at $30^\circ$ (see Fig. 4.3.3a), distinguishing between tetrahedral water population (angles lower than $30^\circ$) and a perturbed H-bond network, mostly occurring around hydrophilic groups (angles higher than $30^\circ$). The tetrahedral structure parameter $S$ is now defined as the integral of $P(\theta)$ up to $\theta = 30^\circ$.

It turns out that water around hydrophobic groups have a larger $S$ due to smaller H-bond angles $\theta$, inducing stronger water-water bonds, with bigger energy fluctuations and therefore a positive heat capacity of the solvating water. Reversely, the introduction of a hydrophilic group around water strains the water-water H-bond angle and shifts the angle distribution to higher values, and hence a lower $S$, thus decreasing the water-water bond energy and fluctuations which decreases the heat capacity of solvation [9, 32, 33].

Throughout the text we will associate tetrahedral/ice-like/structured water with a large $S$ value (high tetrahedral water population) and unstructured water with a low $S$ value (low tetrahedral water population). Unstructured water coinciding with slow reorientation dynamics will be labeled as glass-like water. Residues solvated by ice-
like tetrahedral water will be labeled as *tetrahedral structure makers* and the ones by glass-like waters, *tetrahedral structure breakers*.

**Structural parameter - water reorientation time correlation**

As mentioned above, each water $i$ surrounding one amino acid for an $m$th interval of length $\ell$, has a reorientation decay time $\tau_{i,m}$ associated with it. Additionally in this $m$th interval we calculated the distribution $P(\theta_{ij})$ of the angle $\theta_{ij}$ of waters $j$ within 3.5 of $i$ and hydrating the same amino acid. From the normalized distribution $P(\theta_{i}) = \sum_j P(\theta_{ij})/\sum_{i,j} P(\theta_{ij})$ we obtain the structural parameter $S_{i,m}$ for each water $i$ hydrating a particular amino acid, by integrating from $0^\circ$ to $30^\circ$. We bin the pair $\tau_{i,m}$ and $S_{i,m}$ for each residue in a 2D histogram, in order to investigate possible correlation between water tetrahedral structuring and reorientation dynamics. The $S - \tau$ correlation is quantified using Pearson’s product-moment correlation coefficient $R$.

### 4.3 Results and Discussion

![Figure 4.3.1: Anisotropy decay of a) all water and b) the hydration shell water of the wild type and mutant SPC/E systems respectively.](image)

**4.3.1 Water reorientation dynamics slows down at surface**

From the MD simulations trajectories we calculated the anisotropy correlation function $C_2(t)$ using eq. (4.1) for all water molecules in the system and for the hydration shell water molecules only, for both wild type and mutant at different temperatures. Fig. 4.3.1 shows these correlation functions for the SPC/E model. As expected the
Figure 4.3.2: a) Water reorientation time, b) protein water H-bond lifetimes of hydration water for SPC/E systems of a wild type (WT), and mutant (T18N) at room temperature. Color coding as in Fig. 4.2.1a,b

anisotropy for all waters decays within a few ps for ambient conditions, but decays slower with decreasing temperature. This agrees with the work of Stirnemann et al [34], who found that the decay time becomes longer upon cooling. For the hydration water this effect is even more striking.

**4.3.2 Water reorientation dynamics differs locally**

Strikingly, the difference in overall hydration water reorientation dynamics between mutant and wild type is small (see Fig. 4.3.1). This indicates that the effect of the mutation on the water reorientation dynamics is either small and/or local, since the number of waters hydrating THR-18 and ASN-18 is small compared to the total amount of waters in the hydration shell, washing out any local effect. To study whether THR-18 and ASN-18 differ significantly in local water dynamics we therefore categorized the hydration water by their local environment (see Methods section 4.2.2). In Figure 4.3.2a, we plot the water orientation decay time for the six different parts of the protein. Xu et. al [35] discovered a small acceleration of the H-bond dynamics upon mutating ALA-16 to HIS-16. Here we find that water reorients significantly slower around THR-18 than around other regions, and also slower than at the mutated site ASN-18. This effect is enhanced at lower temperatures (see Fig. 4.A.1). However, the point mutation does not drastically accelerate the reorientation dynamics of the IBS, but only has a local effect. This local acceleration of water reorientation dynamics around ASN-18 coincides with the less stable water-ASN-18 hydrogen bonds, compared to the water-THR-18 ones (see Fig. 4.3.2b). This effect is again enhanced at lower temperatures (see Fig. 4.A.2).

X-ray crystallography mutation studies by Graether et al [20] and Neutron scatter-
4.3.3 Hydration water structure

Following Sharp and Gallagher [9, 33] and other authors [10], we characterize the water structure by computing the OOH angle distribution for several parts of the protein (see Fig. 4.3.3a), from which we obtain the average water structural parameter S. Figure 4.3.3b (see also Fig. 4.A.3), shows the average water structural parameter S for the overall hydration shell, the IBS, the vicinity and the opposite region, and residue 18, for the WT and T18N systems. Interestingly, there seems to be a monotonic increase of water structure along the protein, i.e. the water structural parameters obey the relation $S_{\text{opp}} < S_{\text{vicinity}} < S_{\text{IBS}}$. This effect is slightly more pronounced at the wild type system, compared to the mutant system. Both the absolute values of S and the difference $S_{\text{IBS}} - S_{\text{opposite}}$ increases slightly upon cooling (see Fig. 4.A.3). This behaviour holds for both force fields.
Figure 4.3.4: Structural parameter $S$ as a function of water reorientation decay time for selected residues of a) IBS (WT), b) IBS (T18N), c) THR-18 (WT), d) ASN-18 (T18N), e) ARG-39 (WT/vicinity), f) ALA-48 (WT/vicinity) at 298 K.

Water is more structured around the IBS and in particular residue 18 of the wild type, compared to the mutant, especially at low temperatures (see Fig. 4.A.3). Waters around residue 18, which reorient slower for the wild type compared to the mutant, have also a more ice-like structure for the wild type compared to the mutant. These findings partially agree with the VSFG experiments [16], which showed a drastic increase in ice like water signal for the wild type compared to the mutant system. While we observe a reduction of ice structure around THR-18 upon mutation, the reduction in the IBS ice-like water going from the wild type to the mutant is not as great as experiments suggest.

4.3.4 Structure - reorientation time correlation

We investigate the correlation between the water reorientation dynamics and tetrahedral structure by histogramming the hydrating waters as function of their $S_ℓ$ and $τ_ℓ$ in which we weigh each entry with a weight $ℓ$ (see Methods section). Figures 4.3.4 and 4.3.5
show the 2D histograms of $S_\ell$ and $\tau_\ell$ of the IBS and of selected amino acids for, respectively, 298 K and 275 K for the wild type and mutant SPC/E system. At room temperature (Fig. 4.3.4a) the IBS of the wild type shows clearly a positive correlation between tetrahedral structure $S$ and water reorientation time ($\tau$). While this correlation is not perfect, a more structured water is more likely to exhibit a longer reorientation time. Upon mutation (Fig. 4.3.4b) the IBS $S - \tau$ distribution shifts to slightly lower $S$ and $\tau$, and the correlation becomes less pronounced. More strikingly, water hydrating the THR-18 (WT system) shows a higher values for $S$ and $\tau$ as well as a stronger $S - \tau$ positive correlation compared to ASN-18 (T18N system). (see Figs. 4.3.4c and 4.3.4d respectively). Clearly, more ice-like waters also reorient much slower. These positive correlations can be contrasted with the behavior of other key residues in the vicinity region of the IBS. For instance, the charged and hydrogen bond donating residue ARG-39 exhibits no or even a negative correlation ($R=-0.55$ at 275 K) between structure and dynamics (with a lower peak at S compared to THR-18). Here, a less structured water shows a longer reorientation time. Interestingly, water around ALA-48 is able to exhibit
Table 4.3.1: Pearson’s correlation coefficient between S and τ for water hydrating different IBS amino acids of WT and T18N at 275 K.

<table>
<thead>
<tr>
<th>#AA</th>
<th>WT</th>
<th>T18N</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN 9</td>
<td>0.05</td>
<td>-0.38</td>
</tr>
<tr>
<td>LEU 10</td>
<td>0.39</td>
<td>-0.03</td>
</tr>
<tr>
<td>PRO 12</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>ILE 13</td>
<td>0.34</td>
<td>0.21</td>
</tr>
<tr>
<td>ASN 14</td>
<td>0.08</td>
<td>-0.05</td>
</tr>
<tr>
<td>THR 15</td>
<td>0.51</td>
<td>0.18</td>
</tr>
<tr>
<td>ALA 16</td>
<td>0.55</td>
<td>-0.18</td>
</tr>
<tr>
<td>THR/ASN18</td>
<td>0.56</td>
<td>0.22</td>
</tr>
<tr>
<td>LEU 19</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>VAL 20</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>MET 21</td>
<td>0.46</td>
<td>-0.08</td>
</tr>
<tr>
<td>GLN 44</td>
<td>0.30</td>
<td>0.13</td>
</tr>
</tbody>
</table>

both ice like and glass-like structure. ALA-48 is next to ARG-47, which is a charged amino acid capable of donating hydrogen bonds.

Upon cooling the systems to 275 K, the WT (S-τ) correlation at the IBS becomes stronger (R=0.45) compared to room temperature (R=0.19) (Fig. 4.3.5a). Since the S-τ correlation signals at 275 K are enhanced, we can more clearly distinguish between strong tetrahedral structure making and subtle tetrahedral structure making residues at the WT-IBS. Interestingly enough, as shown in Fig. 4.A.4 for the WT-IBS residues, there exist amino acids that show a very strong positive S-τ correlation (LEU-10, PRO-12, ILE-13, ALA-16, THR-15, THR-18, MET-21), as well as amino acids that show a subtle S-τ correlation (GLN-9, ASN-14, LEU-19, VAL-20 and GLN-44). At 275 K, the point mutation results in a weaker S-τ correlation for water around the T18N-IBS (R = 0.14) (see Fig. 4.3.5b). As shown in Fig. 4.3.5c,d, Fig. 4.A.4 and Fig. 4.A.5 and summarized in Tab. 4.3.1, the origin of the decreased (S-τ) correlation in the T18N IBS compared to the WT IBS, is predominantly attributed to the decreased (S-τ) positive correlation of GLN-9, LEU-10, PRO-12, THR-15, ALA-16, ASN-18, MET-21. Again, as shown in Fig. 4.3.5e, the charged and hydrogen bond donating residues ARG-39 of the vicinity exhibit negative correlation (with a peak at lower S compared to THR-18), and water around ALA-48 exhibit both ice like and glass-like structure (Fig. 4.3.5e).

We can conclude that vicinity residues such as ARG-39 and ALA-48 reduce the structure of water, while their charges induce stronger protein-water hydrogen bonds, which slow down the water (reorientation) dynamics. Such residues can be labeled
Figure 4.3.6: Starting frame of dehydrated NVT system (left) and a dehydrated protein at a frame after 100 ns (right). Protein color coding as in Fig. 4.2.1a,b

water 'tetrahedral structure breakers’. It follows that there exist "tetrahedral structure making" (hydrophobic) and “tetrahedral structure breaking” residues (charged/polar, H-bond makers) which influence the structure and dynamics of water near the IBS and at the vicinity of the IBS. The tetrahedral structure making residues should have a stronger affinity for ice, while the tetrahedral structure breaking residues would have a lower affinity to ice crystal. The point mutation reduces the $S-\tau$ correlation at the IBS and therefore the affinity of the IBS for ice. We speculate that water at the vicinity around the IBS should exhibit tetrahedral structure breaking behaviour in order for the Ice to bind only to the IBS and not to the surrounding parts, thus prohibiting crystal growth around the entire protein.

4.3.5 Effect of dehydration

The VSFG experiments that inspired this investigation probe the air water surface, where the symmetry of the system is broken. It is reasonable to assume that the T18N point mutation is more soluble than the wild type, due to its lower free energy of solvation [36] (10 kT lower), compared to THR-18. According to the hydrophobic character of residues [37], THR is a more hydrophobic residue, than ASN, and therefore the IBS surface of the WT will most likely be (part of) the more hydrophobic side of the protein, and therefore more likely to be adsorbed at the air-water interface. Hence, it is conceivable that the wild type AFP proteins have a different preferred orientation at the air water surface than the mutant, resulting in less hydration of the IBS for the wild
Figure 4.3.7: a) Water reorientation time, b) protein water H-bond lifetimes, and c) structural parameter $S$ of hydration water for SPC/E systems of a fully hydrated wild (wtf), mutant (mtf) and for dehydrated wild type (wtd) and mutant (mtd) at room temperature.

type compared to the mutant IBS. To understand the effect of preferential adsorption at the air-water interface, we need to investigate the protein-water dynamics at a reduced hydration degree. We therefore also performed MD simulations at a lower hydration degree, by reducing the water content to 10% of the fully hydrated system. The remaining 722 waters initially hydrate the entire protein surface, but quickly lead to exposure of some parts to the gas phase due to the hydrophobicity of the surface. In particular the hydrophobic patch of the protein surface near the IBS became exposed (see Fig. 4.3.6 for snapshot). It is thus likely that this part of the surface becomes exposed at the air water interface. Nevertheless, the IBS is not completely water free, and we can analyze our system in the same way as for the fully hydrated system. Therefore, we measure again the structural parameter $S$, the reorientation time $\tau$ and the hydrogen bond life time $\tau_{HB}$, and compare them in Fig. 4.3.7). Clearly, the dehydrated WT shows a strong increase both in water structure and reorientation time at the THR-18, although not in $\tau_{HB}$. 

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Comparing the 100 ns NVT simulations of the SPC/E dehydrated wild type and mutant systems at room temperature, on average there are 44 water molecules within 4.4 Å of the wild type IBS, compared to 50.41 for the mutant IBS. There are on average 4.6 water molecules present around THR 18 and on average 5.3 water molecules around ASN-18 of the mutant, indeed suggesting an increase of solvation upon mutation. Therefore, to assess whether differences in hydration contribute to the strong ice-like signal in VSFG experiments, one needs to compare the dehydrated wild type IBS, THR-18 water structure to the fully hydrated mutant IBS, ASN-18 water structure. Indeed, the dehydrated wild type $S_{IBS,18}$ is significantly higher than the relevant structural parameter $S$ of the fully hydrated mutant (see Fig. 4.3.7c).

### 4.4 Conclusions

Antifreeze proteins (AFPs) are believed to prevent ice formation by binding to specific ice crystal planes and blocking their growth. Their ice-binding recognition sites have remarkable solvation properties. Spectroscopic experiments[16] and analysis of crystal structures [20] of the ocean pout AFP III anti-freeze protein indicated that upon mutating THR 18 to ASN-18 water locally to the mutation reduces its tetrahedral structure dramatically, suggesting that THR18 plays a crucial role in the ice binding site, and is able to structure water, leading to an increased AFP III affinity to ice. The structure of a protein’s hydration layer is tightly related to its dynamics, but this relation has not been elucidated for AFPs. To investigate this relation we performed molecular dynamics simulations of AFP III. We investigate both dynamics and structure of the ice-binding surface and compared these to the non-ice-binding surfaces. We find that while the hydrogen bond dynamics is not remarkably altered, the water reorientation relaxation around THR18 is slower than that of the non-ice-binding surface. This local slow reorientation relaxation is correlated with longer lived H-bonds between THR-18 and its hydrating water compared to ASN-18 in the mutant. Moreover, by comparing structural signatures we found, in agreement with experiments[16] and predictions [20], that upon replacing THR18 with ASN-18, water around the point mutation reduces its tetrahedral structure. Indeed, the THR18 water tetrahedral structure, exhibits a positive correlation with reorientation relaxation time, whereas this positive correlation is significantly reduced for the mutant as is for the entire IBS of the mutant system. This reduction of the S-$\tau$ correlation at the IBS of the mutant, enhanced at lower temperatures, could explain the smaller affinity of the mutant IBS to ice. In contrast, we find that ARG-39, a positively charged H-bond donating residue in the vicinity of the IBS, shows a negative correlation of water tetrahedral structure and reorientation while some others, can show both a positive and negative correlation (ALA-48). Overall the wa-
tetrahedral structure seems organized in a gradient fashion towards the IBS. In the VSFG experiments the proteins adsorbed at the surface contribute most to the signal. As these proteins experience a lower degree of hydration we also investigate the effect of hydration. We find that dehydration leads to a significant increase in the tetrahedral nature of water, as well as its reorientation dynamics.

Our findings show that amino acids can act as tetrahedral water structure makers and breakers. This may explain the mechanism of pout AFP to prevent engulfment within ice, since the charged residues such as ARG break the ice structure to a more glassy one around the IBS thereby preventing ice from covering the whole protein, which would make it inactive.
Appendix

4.A Water structure and dynamics for different temperatures and forcefields.

Figure 4.A.1: Reorientation time of hydration water for all fully hydrated systems of study, a) SPC/E and b) TIP4P/2005 water forcefield, wild (left) and mutant (right)
Figure 4.A.2: Hydrogen bond lifetimes of hydration water for all fully hydrated systems of study, a) SPC/E and b) TIP4P/2005 water forcefield, wild (left) and mutant (right)
Figure 4.A.3: Structural parameter $S$ of hydration water for all fully hydrated systems of study, a) SPC/E and b) TIP4P/2005 water forcefield, wild (left) and mutant (right).
Figure 4.A.4: Reorientation decay time of versus structural parameter $S$ for selected residues of WT at 275 K.
Figure 4.A.5: Reorientation decay time of versus structural parameter $S$ for selected residues of T18N at 275 K.


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