Hydration Layer Dynamics and Association Mechanisms of Food and Antifreeze Proteins: A Molecular Dynamics and Transition Path Sampling Study

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Summary

By the time the reader reads this line, billions of protein association events just occurred in our body, such as the ones regulating cell communication, signalling pathways, or in initiating a self-assembly processes, such as tissue fabrication, etc. The timescale of such transitions is slow, compared to atom vibrations and such events are termed rare, the reason being that protein or/and solvent interactions have to be disrupted and reformed in order for the transition to occur. Having an atomistic insight into rare transitions and their respective important interactions is pivotal for understanding and experimentally controlling such processes. Water is an important agent on its own in facilitating protein folding, recognizing ice crystal planes (anti-freeze proteins) and in mediating protein association. The aim of this thesis is threefold. First to better understand the role of water at the hydration shell of single proteins in terms of structure and dynamics, secondly to understand the association and first steps of self-assembly mechanisms of food and anti-freeze proteins, and thirdly to understand the role of water during the association mechanism. By performing Molecular Dynamics, we are able to investigate the H-bond structure and dynamics of water around hydrophilic and hydrophobic protein groups, as well as the effect of unfolding on water dynamics. We are able to correlate water reorientation dynamics with the H-bond structure at the hydration shell of anti-freeze proteins. Moreover, by employing Transition Path Sampling and Molecular Dynamics we study how anti-freeze peptides self-assemble into nanotubes, as well as their stability as a function of size. We further study the dimerization mechanism of globular proteins, the important interactions playing a role during the transition as well as the role of water. In order to do so, since the dimerization transition is rare, and the transition barrier asymmetric, we develop and employ a novel Transition Path Sampling shooting scheme that efficiently samples rare transitions with asymmetric barriers which simultaneously gives access to the transition state region.

Chapter 3: Dynamics of hydration water around native and misfolded α-lactalbumin. At first we investigate water dynamics around bovine α-lactalbumin by combining molecular dynamics simulations with polarization resolved femtosecond infrared (fs-IR) spectroscopy. We identify slowly reorienting surface waters and establish their hydrogen-bond lifetime and dynamical orientation relaxation dynamics, which we compare to the experimentally measured anisotropy decay. The calculated number of slow surface waters is in reasonable agreement with the results of fs-IR experiments. Slow waters form fewer hydrogen bonds compared to the bulk. At concave sites the protein-water hydrogen bonds break preferably via translational diffusion rather than

via a hydrogen-bond jump mechanism. The reorientation of water molecules residing at these concave sites is slower than at convex water exposed sites. Protein misfolding leads to an increased exposure of hydrophobic groups, inducing relatively faster surface water dynamics. Nevertheless, the larger exposed surface slows down a larger amount of water. While for a native protein hydrating water is slower near hydrophobic residues than at hydrophilic sites, mainly due to stronger confinement, misfolding causes hydrophobic water to reorient relatively faster because the exposure of hydrophobic groups destroys concave protein cavities with a large excluded volume.

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.

Chapter 5: Stability and growth mechanism of self-assembling anti-freeze cyclic peptides. Cyclic peptides (CPs) that self-assemble in ice-binding nanotubes are great candidates for use as anti-freeze proteins. Based on cyclic peptide sequence, cyclo-[(L-LYS-D-ALA-L-LEU-D-ALA)$_2$], which can stack into nanotubes, we propose an anti-freeze cyclic peptide (AFCP) sequence, cyclo-[(L-LYS-D-ALA)$_2$-(L-THR-D-ALA)$_2$] which contains THR-ALA-THR ice binding motifs. Using molecular dynamics simulations we investigate the stability of cyclic peptides and their growth mechanism. We find that dimers of the AFCP sequence dissociate more frequently and are less stable than dimers of the original CP sequence, while nanotubes consisting of more than two peptides are stable. This sudden increase in stability of nanotubes of the AFCP sequence may be explained by the formation of H-bonds between Threonine side-chains. The Threonine distances in the ice-binding motifs are similar to those in the ant-freeze protein of Christoneura fumiferana, suggesting good ice lattice matching, and a potential for depression of the freezing point. In addition, we investigated the nanotube growth process, i.e. the association/dissociation of a single CP to an existing AFCP

nanotube, by Transition Path Sampling. We found a general dock-lock mechanism, in which a single CP first docks loosely before locking into place. Moreover, we identified several qualitatively different mechanisms for dissociation, involving different meta-stable intermediates, including a state in which the peptide was misfolded inside the hydrophobic core of the tube. We also find evidence for a mechanism involving non-specific association followed by 1D diffusion. Under most conditions, this will be the dominant pathway. The results yield insight in the mechanisms of peptide assembly, and might lead to improved design of self-assembling anti-freeze proteins.

Chapter 6: Spring shooting, a novel efficient Transition Path Sampling move. We present a novel transition path sampling shooting algorithm for efficient sampling of complex (biomolecular) activated processes with asymmetric free energy barriers. The method employs a fictitious potential that biases the shooting point toward the transition state. The method is similar in spirit to the aimless shooting technique by Peters and Trout [B. Peters and B. L. Trout, J. Chem. Phys. 125, 054108 (2006)], but is targeted for use with the one-way shooting approach, which has been shown to be more effective than two way shooting algorithms in systems dominated by diffusive dynamics. We illustrate the method on a 2D Langevin toy model, the association of two peptides and the initial step in dissociation of a β-lactoglobulin dimer. In all cases we show a significant increase in efficiency.

Chapter 7: Elucidating the mechanism and role of solvent for β-lactoglobulin dimerization using Transition Path Sampling. Dimerization of proteins is a fundamental process in nature. While conceptually simple, the underlying association mechanism and the role of the solvent are poorly understood. Here we resolve these issues for the dimerization of β-lactoglobulin using Transition Path Sampling of all atom molecular dynamics trajectories. The association process is found to occur via (at least) three distinct mechanisms: 1) aligned association to the native dimer interface, 2) misaligned association at non native sites followed followed by hop towards the native state and 3) misaligned association followed by sliding of the protein towards the native state. We find that the native dimer state is stabilized by hydrogen bond bridging waters. Interestingly, water at the native interface can be found in two dynamical hydration states, a glassy one and a tetrahedral one. The crevice introduced upon binding increases the glassy populations as well as increases the average tetrahedrality of water, mainly at the vicinity of hydrophobic residues.