Hydration layer dynamics and association mechanisms of food and antifreeze proteins

A Molecular Dynamics and Transition Path Sampling study

Brotzakis, Z.F.

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- **Chapter 4**

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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.

Samenvatting

Tegen de tijd dat de lezer deze regel heeft gelezen, zijn er miljarden eiwit associatie processen gebeurd in ons lichaam, zoals bijvoorbeeld het reguleren van de communicatie van de cel, signaal transductie of het initiëren van zelf-assemblage processen, zoals het maken van celtissue. De tijdschaal van dit soort transities is langzaam, vergeleken met de vibraties van moleculen and zulke transities worden dus ook als zeldzaam beschouwd. De reden hiervoor is dat eiwit interacties moeten gebroken worden of gevormd voor de transitie om plaats te vinden. Atomair inzicht in zulke zeldzame transities is belangrijk voor het begrijpen van zulke processen. Water is een belangrijk op zich zelf in het faciliteren van eiwitvouwing, zoals het herkennen van kristal oppervlaktes (antifreeze eiwitten), and in het begeleiden van eiwit associatie. Het doel van deze thesis is drievoud. Ten eerste, het beter begrijpen van de rol van water in de hydratieschil van enkele eiwitten in termen van structuur en dynamica, ten tweede om de associatie en eerste stappen in de zelf-assemblage mechanisme van voedsel en antivrieseiwitten te begrijpen en ten derde de rol van water te bestuderen in dit mechanisme. Door het uitvoeren van moleculaire dynamica simulaties, zijn we in staat om de waterbrug netwerk en dynamica van water rond hydrofiele en hydrofobe eiwitgroepen, zowel als het effect van ontvouwing op de dynamica van water. We zijn in staat de water reorientatie dynamica van water te correleren met het waterstofbrug netwerk van de hydratieschil van antivrieseiwitten. Bovendien, door het gebruiken van Transition Path Sampling en moleculaire dynamica bestuderen we hoe antivries eiwitten zelf-assembleren in nanotubes en ook de stabiliteit als functie van de grootte de nanotubes. Verder, bestuderen we de dimerisatie mechanisme van globulaire eiwitten, de interacties die een belangrijke rol spelen in deze transitie alsook de rol van water. Om dit te doen, aangezien dit proces zeldzaam is, en de transitie barrière asymmetrisch, hebben we een nieuwe Transition Path Sampling techniek ontwikkeld wat efficient reactieve paden genereert voor systemen met een asymmetrische barrière.

Hoofdstuk 3: Dynamiek van hydratie water rond native en verkeerd gevouwen α-lactalbumin. Ten eerste hebben we de dynamica van water bestudeerd om dierlijk α-lactalbumin door het combineren van moleculaire dynamica simulaties en polarisatie opgeloste femtoseconde infrarood (fs-IR) spectroscopie. Wij identificeren langzaam oriënterende oppervlakte water moleculen en meten de levensduur van deze waterstofbruggen en de dynamische orientatie relaxatie dynamica, die we vergelijken met de gemeten anisotr-opieverval experimenten. De uitgerekende aantal langzame waters is in goeie overeenkomst met de resultaten van fs-IR experimenten. Langzame waters vormen minder waterstofbrugggen vergeleken met bulk water. Bij concave sites

breken eiwit-water waterstofbruggen eerder via translationele diffusie dan via waterstofbrug sprong mechanisme. De reorientatie van water moleculen zittend op deze concave is langzamer dan convexe water blootgelegde sites. Eiwit misvouwing leidt tot een verhoogde blootstelling van hydrofobe groepen, wat leidt tot relatief snellere water dynamica. Desondanks, de grotere blootgestelde oppervlakte vertraagt een grotere hoeveelheid water. Terwijl voor native eiwit hydraterende water langzamer is dichtij hydrofobe residuen dan bij hydrofiel sites, voornamelijk door sterkere opsluiting, misvouwing zorgt ervoor dat hydrofoob water relatief sneller reorienteert doordat de blootstelling van hydrofobe groepen de concave eiwit porien met een grote uitgesloten volume.

Hoofdstuk 4: Correlatie tussen water structuur en dynamica in de hydratie laag, van een type III amerikaanse puitaal antivries eiwit. Wij geven een moleculair dynamica studie over de relatie tussen de structuur en (oriëntatie en waterstofbrug) dynamica van gehydrateerd water rondom de Amerikaanse puitaal AFP III antivries eiwit. We vinden bewijs voor een verhoogde ijsachtige structuur van het gebied tegenover de ijsbindend site (IBS) naar het IBS van het eiwit, met de sterkste ijsachtige structuur rondom de THR-18 residue van het IBS. Dit ijsachtig structuur signaal correleert met verhoogde reorientatie vervaltijden. Bovendien, wij vinden een anticorrelatie voor verschillende sleutel residuen die niet deel uitmaken van het IBS maar dichtbij zitten. Deze effecten zijn verhoogd bij lagere temperaturen. Ten slotte, als het AFP III antivries eiwit bindt met het ijsvlakte door voornamelijk hydrofobe stukken, bestuderen we de ijsachtige structuur en dynamica van water bij partieel gehydrateerd IBS. We vinden dat het IBS nog meer ijsachtig wordt in het wild-type wanneer dehydratie plaatsvindt, en dat de water reorientatie tijd groter wordt, maar minder zo voor het T18N mutant, welke ook een hogere hydratatie heeft dan het IBS. Deze resultaten komen overeen met water-lucht VSFG spectroscopie experimenten wat een gereduceerd ijsachtig signaal bij mutatie van het IBS laat zien.

Hoofdstuk 5: Stabiliteit en groei mechanisme van zelf-assemblerende antivries cyclishe peptiden. Cyclische peptiden (CP) die zelf-assembleren in ijsachtige nanotubes zijn kandidaten om te gebruiken als antivries eiwitten. Gebaseerd op de cyclische peptide sequentie, cyclo-[(L-LYS-D-ALA-L-LEU-D-ALA)$_2$], welke kunnen stapelen in nanotubes, stellen wij een antivries cyclische peptide (AFCP) sequentie voor, cyclo-[(L-LYS-D-ALA)$_2$-(L-THR-D-ALA)$_2$] welke een THR-ALA-THR ijsbinding motief bevat. Door moleculaire dynamica te gebruiken bestuderen wij de stabiliteit van cyclische peptiden en het groei mechanisme. Wij vinden dat dimeren van de AFCP sequentie dissociëren vaker en zijn minder stabiel dan dimeren van het originele CP sequentie, terwijl nanotubes met meer dan twee peptiden stabiel zijn. Deze plotse sprong in stabiliteit van nanotubes van het AFCP sequentie kan worden uitgelegd door de formatie van waterstofbruggen tussen Threonine en de zijketens. De Threonine afstanden in de

ijsbinding motieven zijn gelijk aan die in antivries eiwitten van Chrisoneura fumiferana, wat een goeie ijs-rooster matching oppert, en de mogelijkheid om het vriespunt te verlagen. Daarnaast bestuderen we het nanotube groeie proces, i.e. de associate en dissociate van een enkele CP bij een reeds bestaande AFCP nanotube met Transition Path Sampling. We vinden een algemene dock-lock mechanisme, waar een enkele CP eerst licht bindt voordat het compleet bindt. Bovendien, we identificeren verschillende kwalitatieve verschillende mechanismes voor dissociatie, met verschillende meta-stabiele intermediaire toestanden, inclusief een toestand waar het peptide misvouwt binnen de hydrofobe kern van de tube. We vinden ook bewijs voor een mechanisme wat een nonspecifieke associatie inhoudt gevolgd door 1D diffusie. Onder de meeste condities, zal dit het dominante pad zijn. De resultaten bieden inzicht in de mechanismes van eiwit assemblage, en kunnen ook leiden tot verbeterde ontwerp van zelf-assemblerende antivries eiwitten.

Hoofdstuk 6: Spring shooting, een nieuw efficient Transition PAth Sampling move. We presenteren een nieuwe transition path sampling shooting algoritme voor efficient samplen van complexe (biomoleculair) geactiveerde processen met asymmetrische vrije energie barrières. Deze methode gebruikt een fictief potentiaal wat het shooting punt biased naar het transitiepunt. De methode is vergelijkbaar in geest met de aimless shooting move techniek van Peters en Trout [B. Peters en B. L. Trout, J. Chem. Phys. 125, 054108 (2006)], maar is gericht op het gebruik van one-way shooting, wat effectiever is gebleken dan de two-way shooting algoritmes in systemen die gedomineerd zijn door diffusie. We illusteren de methode met een 2D Langevin model, de associatie van twee peptiden en de initiële stap in de dissociatie van β-lactoglobulin dimeer. In elke geval laten we zien dat de efficientie door het nieuwe spring shooting move verhoogt wordt.

Hoofdstuk 7: Mechanisme en rol van het oplosmiddel voor β-lactoglobulin dimerisatie door middel van Transition Path Sampling. Dimerisatie van eiwitten is een fundamenteel proces in de natuur. Al hoewel simpel, de onderliggende associatie mechanisme en rol van het oplosmiddel is niet volledig begrepen. Hier geven we verklaringen voor deze vragen over de dimerizatie van β-lactoglobulin door middel van Transition Path Sampling van moleculaire dynamica trajectoriae. Het associatie proces vindt plaats via (ten minste) drie verschillende mechanismes: 1) directe associatie naar de native dimeer, 2) associatie via non native sites gevolgd door hops naar de native toestand en 3) associatie gevolgd door het schuiven van het eiwit richting de native toestand. We vinden dat de native eiwit toestand gestabiliseerd wordt door waterstofbrug gebrugde waters. Waters dichtbij de native interface wordt gevonden in twee verschillende dynamische hydratatie toestanden, een glasachtige en een tetrahedrale. De kloof geïntroduceerd bij de binding verhoogt de glasachtige populatie alsook de gemiddelde tetrahedrale karakter van het water, voornamelijk in de buurt van hydrofobe structuren.

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Σα βγείς στον πηγαιμό για την Ιθάκη,
να εύχεσαι να᾽ ναι μακρύς ο δρόμος,
γεμάτος περιπέτειες, γεμάτος γνώσεις

...

Τους Λαιστυγόνας και τους Κύκλωπας,
τον ἀγριο Ποσειδώνα δε θα συναντήσεις,
αν δεν τους κουβαίνεις μες στην ψυχή σου,
αν η ψυχή σου δεν τους στήνει εμπρός σου.

...

Κι αν πτωχική την βρείς, η ιθάκη δεν σε γέλασε.
Ἐτσι σοφός που ἐγινες, με τόση πείρα,
ἠδη θα το κατάλαβες οι Ιθάκες τι σημαίνουν.