Summary
The folding and unfolding of proteins is critical to a wide array of biological processes. Thus, not only is elucidating the relationship between the sequence of a protein and its structure is of fundamental importance in biology, so is understanding the mechanism by which proteins and peptides fold to their native conformations.

In particular, the focus of my PhD has been the investigation of the environmental effects on peptide and protein folding. To address this question, a series of molecular dynamics (MD) simulations in explicit water utilizing the GROMOS force field have been performed. It was found that several specific conditions might contribute to either promote or destabilize the protein folding.

1. **The effect of force field on the structure and dynamic properties of small peptides.**

Using a test set of 5 peptides and 36 proteins, we examined the ability of the GROMOS 43A1, 45A3 and 53A6 parameter sets, along with changes to the 53A6 parameter set proposed recently by Dr. Hao Fan and Dr. Bojan Žagrović, to reproduce the conformational preferences of a series of small peptides and proteins. It was shown that while the 45A3 parameter set is able to reproduce the balance between the helical and β-sheet propensities of a series of peptides to within approximately 10-20%, the 53A6 parameter set heavily favours β-hairpin conformations. In contrast, the changes proposed by Hao and Žagrović excessively favour α-helical conformations and led to a slight increase in the average RMSD for a set of 36 trial proteins. In particular, while the proposed corrections remove a spurious peak in the distribution in the φ angle, both the 53A6 parameter set and the proposed corrections lead to a shift in the centre of the distribution of ψ angles in β-sheet conformations from ~140 degrees (X-ray) to 120 and 110 degrees, respectively. This most likely accounts for the higher than expected RMSD in proteins simulated with the 53A6 parameter set. Finally, we analyzed the factors that determine the nature of the φ and ψ distributions and suggest a new set of dihedral potentials that give a better agreement between the simulations and the available X-ray data.

2. **Estimation the folding rates of small peptides: Comparing simulation to experiment.**
Using a test set of 5 peptides each simulated for a total period of 5 µs in explicit water we have examined the ability of the GROMOS 45A3 force field to reproduce the folding time and the secondary structure propensities for small peptides. It was found that although the secondary structure propensities compared well to experiment the folding times derived from the simulations were considerably shorter than that estimated from experiment. Possible reasons for this discrepancy are discussed.

3. Alternative approaches to promote protein folding.

Molecular chaperons assist proteins to achieve their native structure by modulating the process of folding and unfolding. Here we used an approach, which attempts to mimic the effect of the chaperon GroEL by modifying the polarity of the solvent environment has been investigated. Specifically, the effect of modulating the polarity of the solvent on the rate of folding of a series of small peptides was analyzed. The ability of an oscillating solvent environment to facilitate the folding of a small protein from thermally denatured states was also investigated. The simulations showed that mimicking the effect of the chaperon could facilitate the rate of folding of small peptides as well as a small protein from thermally denatured states.


Polyethylene Glycol (PEG) is used as an inert spacer in a wide range of biotechnological applications. In particular, PEG can be used to display peptide epitopes for diagnostic purposes such as micro array techniques. Using molecular dynamics (MD) simulation techniques, we investigated the influence of the PEG spacer on the conformational properties of the peptides to which it is attached. Here a series of five peptides with differing physical-chemical properties have been examined. Based on an analysis of backbone $\phi/\psi$ angles and the relative populations of alternative conformations, it was shown that, when isolated in solution, the PEG spacer had little effect on the conformation of the peptide to which it was attached. However, when constrained to a two dimensional lattice mimicking a peptide displayed on a surface, the PEG-peptide units aggregated dramatically, reducing the accessibility of the peptides to solvent.
5. The interfacial structure of self-assembled switchable peptide surfactants.

Recently a series of stimuli-responsive surfactant peptides that can self-assemble to form robust interfacial networks which can be switched between high- and low-elasticity states as a function of pH or metal ion concentration have been characterized. These surfactant peptides can reversibly control the stability of emulsions and foams, thus they have potential applications ranging from cosmetics to oil recovery. A combination of molecular dynamics simulations and neutron reflection studies were used to investigate the structure of two such stimuli-responsive peptides, AM1 and Lac21E, at an air-water interface. Both peptides are essentially unstructured in solution but fold cooperatively at an air-water interface to form an ordered monolayer of peptides. The separation between the hydrophobic and hydrophilic residues within these amphipathic peptides (0.55-0.3 nm and 0.49 nm for AM1 and Lac21E, respectively) predicted by the simulations, closely matched that inferred from neutron reflection experiments (0.75 ± 0.2 nm and 0.60±0.2 nm). However, the volume fraction of peptides normal to the interface calculated from the MD simulations of AM1 peptide either from helical or random conformation can both reproduce in detail the experimental neutron reflectivity measurement at different contrast variations suggesting the neutron reflection measurement is not sensitive enough to detect the conformational difference of this peptide at an air/water interface. Together the results suggest the peptides form highly ordered domains of the air/water interface and provide an atomistic model that can be used to explain the mechanical properties of the systems observed experimentally.
Samenvatting

Het onderzoek beschreven in dit proefschrift richt zich op het begrijpen van omgevingsinvloeden op de vouwing van peptiden en eiwitten. Door middel van moleculaire dynamica (MD) simulaties in expliciet water is in detail geanalyseerd welke omstandigheden eiwitvouwing bevorderen danwel hinderen. In eerste instantie is onderzocht in hoeverre het krachtenveld in staat is de vouwingsdynamica van een aantal korte peptiden in water te reproduceren. Uit deze simulaties bleek dat de conformatieele eigenschappen goed gereproduceerd worden maar dat de vouwingstijden in de simulaties aanzienlijk korter zijn dan de waarden die uit experimentele waarnemingen geschat worden. Als onderdeel van dit deel van het onderzoek werd een nieuwe set torsiepotentialen ontwikkeld die een betere overeenkomst tussen simulatie en gegevens uit Röntgenverstrooingsexperimenten opleveren. In het tweede deel van het onderzoek werd gepoogd het effect van de chaperonne GroEL op de vouwing van peptiden en eiwitten na te bootsen door de polariteit van oplosmiddel en verdere omgeving te veranderen. In de cel faciliteren chaperonnes zoals GroEL de vouwing van eiwitten en peptides op een energieafhankelijke manier. De simulaties lieten zien dat het mogelijk was de vouwingssnelheid van korte peptiden alsmede een klein eiwit vanuit thermisch ontvouwen toestanden te vergroten. Tenslotte is de invloed van grenslagen op het vouwen van peptiden onderzocht door te kijken naar het effect van polyethyleenglycol (PEG) spacers op de conformationele eigenschappen van korte peptiden en op de grenslaagstructuur van zelfassemblerende schakelbare peptidesurfactanten.