

STRUCTURAL AND THERMODYNAMIC ANALYSES OF
PEPTIDE-BASED NANOMATERIALS VIA MOLECULAR
DYNAMICS SIMULATIONS

by

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A Thesis Submitted to the
Graduate School of Engineering
in Partial Fulfillment of the Requirements for
the Degree of

Doctor of Philosophy

in

Computational Sciences & Engineering

Koç University

October, 2011

Koç University
Graduate School of Sciences and Engineering

This is to certify that I have examined this copy of a doctoral dissertation by

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To my family and my spouse

ABSTRACT

Peptides are oligomers with aminoacids as building blocks. Discovery of naturally occurring functional peptides has led to a dramatic increase in research for both understanding of natural peptides as well as design of novel synthetic ones. As individual molecules, peptides serve a variety of purposes: such as drugs, antigens, ligands and antibiotics. They also act as building blocks for self-assembled nanofibers, nanotubes, micelles, and monolayers. Designing novel peptide-based materials with desired properties and functions can only be possible by understanding the link between sequence, structure and organization of these molecules.

This thesis work is composed of four main sections. In the first study, we analyzed structure and thermodynamics of small amphiphilic peptides that spontaneously form monolayers at the air/water interface. We accurately calculated the free energy of transferring peptides from bulk water to the air/water interface, and analyzed its composition. Next, in the light of information gathered from small amphiphilic peptides we analyzed folding of a carefully designed 24-residue amphiphilic peptide at the air/water interface. We calculated free energy of adsorption, and decomposed it into enthalpic and entropic contributions. We determined key elements required to adapt the targeted β -hairpin conformation and for adsorption at the interface via *in-silico* mutations. In addition, we also determined organization of β -hairpins within surface monolayers, which will help improve design strategies for manufacturing such 2-D structures. In the third study, we tried to understand self-assembly of tri-block peptides into nanofibers in bulk water. By substituting aliphatic residues with aromatic ones located in the central block of these peptides, we analyzed the stability and strength of these fibers. We also investigated possible nucleation mechanisms for fiber formation, which can be used to design stable and functional peptide nanofibers.

Investigation of material properties and bulk behavior of peptides requires more efficient computational techniques. In this regard, finally, we investigated the “transferability” of a recently developed solvent-free coarse-grained (CG) peptide model [1] that was shown to capture quantitatively structural and thermodynamic properties of a hydrophobic di-phenylalanine peptide (FF). By mimicking the CG mapping used in the FF CG model and transferring bonded and nonbonded interaction potentials to other hydrophobic di-peptides, namely valine-phenylalanine (VF) and isoleucine-phenylalanine (IF), we tested the generality of this CG model. We devised a general protocol to transfer the original CG model to other hydrophobic di-peptides, which are in the form of XF, where X represents an arbitrary amino acid. Hydrophobic di-peptides are the smallest molecules forming self-assembled hierarchical structures in aqueous solution. Therefore, development of a transferable CG model for simulation of such systems will help elucidate the driving forces important in the self-assembly of peptide-based materials and peptide aggregation.

ÖZETÇE

Peptitler yapıtaşları aminoasit olan oligomerlerdir. Fonksiyonel, sentetik olmayan peptitlerin keşfedilmesi, gerekse bu peptitlerin yapılarının araştırılmasıyla ilgili çalışmalarda gerekse yeni, sentetik peptit dizayn çalışmalarında çarpıcı bir yükselişe sebep olmuştur. Peptitler tek başlarına ilaç, antijen, ligand ve antibiyotik olarak kullanıldığı gibi, nanofiber, nanotüp, misel ve tek-tabakalı (monolayer) gibi kendiliğinden oluşabilen yüksek-yapılı nanoyapılar için de yapı taşları olarak görev yapar. Fonksiyonel ve istenilen özelliklere sahip peptit-bazlı nanomalzemelerin dizayn edilmesi ancak bu moleküllerin yapı, fonksiyon ve organizasyonları arasındaki bağlantıyı anlamakla mümkün olur.

Bu tez dört ana bölümden oluşmaktadır. Birinci çalışmada, küçük, amfifilik yapıya sahip hava/su yüzeyinde kendiliğinden monolayer oluşturan peptitlerin yapı ve termodinamiğini inceledik. Bu peptitleri su ortamından hava/su yüzeyine transfer etmek için gereken serbest enerjiyi ve bu serbest enerjinin ne tür bileşenlerden meydana geldiğini analiz ettik. Daha sonra, küçük, amfifilik peptitlerden elde ettiğimiz bilgiler yardımıyla beta-hairpin yapısına adapte olması amacıyla dizayn edilmiş 24 aminoasitten oluşan amfifilik bir peptidin hava/su yüzeyinde katlanmasını inceledik. Bu peptidin hava/su yüzeyinde adsorb olması için gerekli serbest enerjiyi hesapladık ve bu serbest enerjiyi entalpik ve entropik bileşenlerine ayırdık. Bilgisayar ortamında yapılan mutasyonlar aracılığı ile beta-hairpin yapısının oluşmasını ve peptidin yüzeyde adsorpsiyonunu sağlayan önemli aminoasitleri belirledik. Buna ek olarak, yüzeyde oluşmuş monolayer yapıları içinde bulunan beta-hairpin yapılarının birbirine göre nasıl organize olduğunu inceledik. Bu şekilde yapılan analizler iki boyutlu diğer monolayer yapılarının sentezlenmesinde başvuru dizayn stratejilerinin de iyileştirilmesine olanak sağlayacaktır. Üçüncü çalışmamızda, üçlü-blok yapılarından oluşan amfifilik peptit-

lerin su ortamında nasıl nanofiber yapılarını oluşturduğunu anlamaya çalıştık. Bu peptitlerin orta bloklarında bulunan alifatik aminoasitleri aromatik aminoasitlerle değiştirerek, oluşan nanofiberlerin kararlılığını ve kuvvetini inceledik. Aynı zamanda peptit nanofiberlerin oluşumu için öne sürülen mekanizmalardan hangilerinin daha olası olduğunu araştırdık. Bu çalışmadan elde edilen bilgiler kararlı ve fonksiyonel peptit nanofiberlerin sentezlenmesinde kullanılabilir.

Peptitlerin malzeme özellikleri ve kütle (bulk) halindeki hareketlerini incelemek daha etkili hesaplama tekniklerini gerektirir. Bu amaçla, son olarak, son zamanlarda geliştirilmiş, su içermeyen, daha az çözünürlükteki (coarse-grained) bir peptit modelinin diğer dipeptitler arasında ne kadar transfer edilebilir olduğunu araştırdık. Bu CG modelin, hidrofobik fenilalanin dipeptidinin (FF) yapısal ve termodinamik özelliklerini kantitatif olarak temsil edebildiği daha önceden gösterilmiştir. FF CG modelinde kullanılan CG haritalama, bağlı (bonded) ve bağlı-olmayan (non-bonded) etkileşim potansiyellerini valin-fenilalanin (VF) ve iso-leusin-fenilalanin (IF) gibi diğer hidrofobik di-peptitlere transfer ederek CG modelin diğer di-peptitlerin özelliklerini ne kadar temsil edebileceğini test ettik. Orijinal CG modeli, XF formunda (X, herhangi bir aminoasit) olan diğer hidrofobik di-peptitlere transfer edebilmek için genel bir protokol düzenledik. Hidrofobik dipeptitler su ortamında hiyerarşik, kendiliğinden oluşan yapıları meydana getirebilen en küçük moleküllerdir. Bu yüzden, bu tür sistemlerin simülasyonunu mümkün kılacak, transfer-edilebilir bir CG model oluşturabilmek hem peptit bazlı malzemelerin kendiliğinden oluşmasını sağlayan önemli kuvvetlerin aydınlatılmasına, hem de peptit yığılmasının (aggregation) anlaşılmasına yardımcı olacaktır.

ACKNOWLEDGMENTS

First of all, I would like to thank my advisor Assist.Prof. Mehmet Sayar for his guidance and support throughout my graduate study. Without his encouragement, it will not be possible to complete this thesis. Most of the time, I could not understand why he was so sensitive to tiny peaks on various time-line data and resolution of figures. However, it is now clear to me that all of these small details have helped me finish my PhD, and find a position for my Post-Doc studies.

I am also grateful to Prof. Burak Erman, Assist.Prof. Alkan Kabakçioğlu, Prof. Levent Demirel, and Prof. Canan Atılgan for their participation in my thesis committee as well as critical reading of my thesis.

Many many thanks go to Christine Peter, her husband Markus, and her lovely son Noah, for changing their route to Turkey during their holiday to participate in my thesis committee.

I would also like to thank Alessandra Villa, Berk Hess, Christine Peter and Jeffrey Hartgerink for their collaborations and fruitful discussions on various projects.

I would like to thank all my friends at Koç University who made the six years an enjoyable period of time for me: Cengiz Ulubaş, Sefer Baday, Emre Güney, Osman Yoğurtçu, Yasemin Oğuz, Fatih Toy, Zeynep Şimşek, Zeynep Akçay, Gözde Kar, Billur Engin, Ece Özbabacan, Engin Çukuroğlu, Güray Kuzu, Çiğdem Bayrak, Tuğba Özal, İlke Anaç, and Ayşe Küçükyılmaz.

Special thanks to Nurcan Tunçbağ, Besray Ünal and Bahar Öndül. We shared lots of things, and we have been next to each other during all of our good and bad times which is the basis of being close friends.

I would like to thank my research group friends and officemates, Beytullah Özgür, Cahit Dalgıçdır, Bahar Değirmenci, Derya Aydın, and Osman Eryurt for their friend-

ships.

Lastly, I would like to thank my grandparents Aysel & Seyfettin Yılmaz, Nefise & Basri Engin, my parents Nur Perran & İsmail, my lovely sister İlke, my spouse, my spouse's parents Emine & Muharrem, and his sisters Ash & Arzu, my aunt Meltem, my uncle Mehmet Emre, and my cousins Deniz & Ezgi for their endless support and understanding under all circumstances. My spouse, Aykut, has been like a source of light all the time when I have been lost in the dark. Without them, I definitely would not be here, where I am standing with my head looking forward to...

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NOMENCLATURE

MD	Molecular Dynamics
CG	Coarse-grained
S-MD	Steered Molecular Dynamics
MDP	Multidomain Peptides
PMF	Potential of Mean Force
FF	Phenylalanine Di-peptide
VF	Valine-phenylalanine Di-peptide
IF	Isoleucine-phenylalanine Di-peptide

Chapter 1

INTRODUCTION

Proteins are essential parts of organisms participating in almost all processes as structural, mechanical and/or functional units. Many proteins act as enzymes catalyzing biochemical reactions that are vital to metabolism. Some of them are motor proteins, such as actin and myosin, that display structural and mechanical functions for cell motility processes in muscle cells. Others are included in cell signaling, cell adhesion, and immune response processes. Protein molecules consist of amino acids, which are characterized by $(\text{NH})_2\text{CHRCOOH}$ structure. Nitrogen and two hydrogen atoms comprise the amino group, $-\text{NH}_2$, and the acid entity is the carboxyl group, $-\text{COOH}$. The **R** group corresponds to the variable radical group which gives each amino acid its unique chemical and physical properties.

There is another biological molecule that shares similar structural and functional properties with proteins: Peptides. Peptides also consist of amino acids that are joined by peptide bonds, but they are shorter in length compared to proteins. In fact, length only is not a differential feature between peptides and proteins. Structural flexibility should also be taken into account as a criterion to differentiate between the two. For instance, if we were to consider only the length as a criterion, long peptide, growth hormone releasing factor (GHRF) with forty-four residue, should be conceived as a protein; however, it has a considerable level of structural flexibility that is unusual to proteins. Another example is Trp-Cage protein (mini-protein), which consists of twenty residues, but has a well-defined tertiary structure that is encountered in proteins.[5]

It is not necessary for peptides to have long amino acid sequences to be functional.

One example is the vasopressin, which is a hormone that regulates blood pressure, but has only nine residues. Discovery of such naturally occurring functional small peptides have accelerated small-peptide design studies during the past decade. Various small peptide molecules with desired properties have been synthesized to serve a variety of purposes such as drugs [6, 7, 8], antigens [9], ligands [10], and antibiotics.[11]

Peptides can also self-assemble to form various higher-order nanostructures such as micelles, nanotubes, nanofibers, and monolayers [12, 13, 14, 15, 16, 17, 18] as shown in Figure 1.1 depending on the distribution and/or content of amino acid residues in the primary sequence. Segregation of nonpolar and polar/acidic/basic residues on the opposite sides of the peptide backbone leads to peptide adsorption and formation of 2-D monolayers in the presence of an interface, or formation of 1-D nanofibers in bulk water. Such nanostructures can also be obtained by synthetic

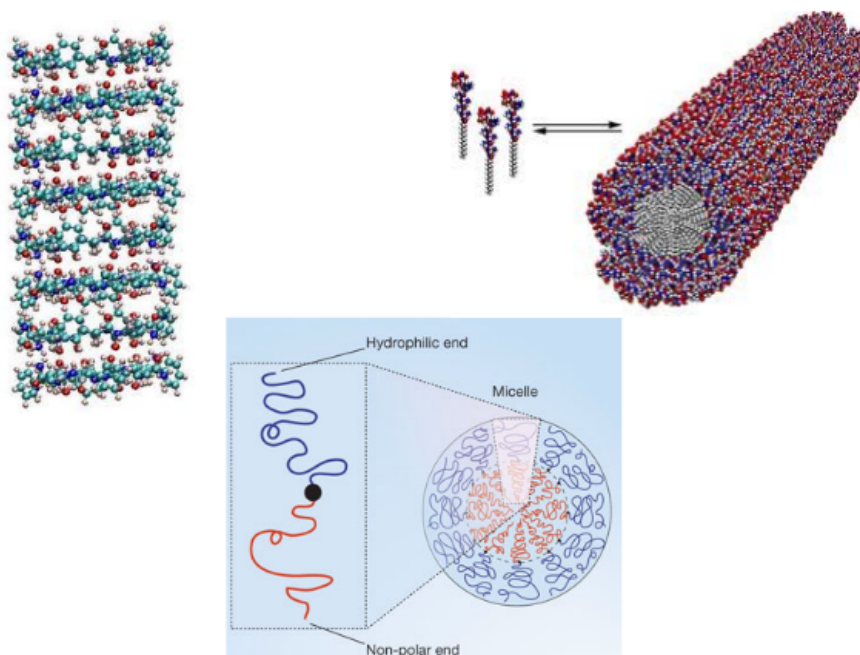


Figure 1.1: Examples of self-assembled nanostructures formed by amphiphilic peptides (nanotubes (left), nanofibers (right), and micelles (bottom)) are shown along with the corresponding building blocks.

polymers; however, biocompatibility and functionality of peptide-based nanomaterials make these systems more attractive over other synthetic systems especially in areas regarding health. Among them are, tissue engineering [19, 20], regenerative medicine [21], gene and drug delivery [22, 23], biosensors [24, 25], and hydrogels.[98, 99, 100] They have also been used in other areas such as microelectronics.[26]

Diversity in the building blocks (amino acids) makes the rational design of new peptide sequences (as well as peptide-based nanomaterials) challenging. Depending on the distribution of amino acids within the primary sequence they can adapt to various secondary structures, which, in turn, determine the final structure of the assembly whose examples are given above. In addition, the same peptide sequence can adapt to different secondary structures depending on the surrounding environment.[27] Design of new functional peptide sequences as well as peptide-based nanomaterials becomes possible by understanding the link between the sequence and structure of naturally occurring peptides. There are various experimental and theoretical studies performed to establish this link. NMR [28], FT-IR [27], CD spectroscopy [27, 29], and X-ray crystallography [30] are the most commonly used experimental techniques. They have some advantages and disadvantages with respect to each other. Although X-ray crystallography gives a more detailed information on the structure of the system, it does not correspond to that observed under physiological conditions because the sample is in the crystal form. On the other hand, remaining techniques do not give structural details as that of the X-ray crystallography gives; however, samples analyzed via these techniques are embedded in solution, which is closer to physiologic conditions.

Molecular dynamics (MD) simulations; however, can provide atomistic details for systems that are found under physiological conditions. They can be used to elucidate atomistic details underlying macroscopic behavior observed in experiments. However, results obtained from MD simulations are highly dependent to the force field and the water model used.[31] Although all-atom MD simulations can give atomistic details for small length- and short time-scales, it becomes insufficient for simulation of processes occurring at longer time-scales (for instance, formation of monolayers by amphiphilic β -

hairpins at the air/water interface). Simulation of such big systems and/or long time-scales becomes possible “in the expense of atomistic details” via coarse-grained (CG) MD simulations, where the reference atomistic system is represented with smaller number of CG beads. However, CG modeling still remains challenging for peptide systems due to heteropolymer design with 20 different amino acid residues, with their own specific chemistry and level of conformational flexibility.[32]

This dissertation primarily focuses on structural and thermodynamic analyses of amphiphilic peptide-based nanomaterials via MD simulations. The outline of this dissertation is as follows:

Chapter 2 presents a general overview on the properties, application areas of peptide-based nanomaterials as well as difficulties for simulation of such systems in an effort to emphasize our motivation for studying these systems.

Chapter 3 focuses on determination of driving forces for adsorption of small amphiphilic peptides adapting extended secondary structures at the air/water interface.

In Chapter 4, we use information gathered from smaller amphiphilic peptides presented in Chapter 3 to investigate folding, adsorption and packing of a carefully designed amphiphilic β -hairpin peptide at the air/water interface. We determine key features responsible for formation of the β -hairpin structure and adsorption at the interface. Finally, we investigate packing behavior of individual β -hairpins within preformed peptide monolayers.

In Chapter 5, we investigate structure and stability of preformed nanofibers formed by amphiphilic peptides in bulk water. We study how aliphatic to aromatic residue substitution affects the stability of peptide fibers. We also investigate possible nucleation mechanisms for self-assembly of nanofibers.

In Chapters 3,4,5 we investigate structural and thermodynamics analyses on the preformed self-assembled structures such as monolayers and nanofibers. In order to study self-assembly of these systems from scratch an efficient CG model is required. There is already one such CG model [1] developed to model self-assembly of hydrophobic dipeptides in water. In chapter 6, we test the “transferability” of the CG model

among other hydrophobic dipeptides.

In Chapter 7, we discuss the results, and emphasize major conclusions of the study by unifying all the work presented in this dissertation.

Chapter 2

LITERATURE REVIEW

Our bodies are composed of a range of self-assembling nanoscale systems based on lipids, nucleic acids, and amino acids. They form highly organized structures with well defined properties by spontaneous arrangements of disordered mixtures. These self-assembled higher-order structures perform a wide range of functions. For instance, lipids primarily act as barriers within cell membranes, polynucleic acids as information storage units, and proteins as the machinery of life. Inspiration by such self-assembling systems has led to an increase in manufacture of nanomaterials from natural building blocks. There have been two approaches taken to manufacture these nanomaterials: top-down and bottom-up. The former uses traditional microfabrication methods, such as photolithography and inkjet printing, to cut and shape materials into the desired shape and order of organization. In contrast to that, chemical properties of individual molecules (usually building blocks of higher-order assemblies) are used to direct self-assembly process into higher-order assemblies in the latter as shown in Figure 2.1. In principal, bottom-up approach, which is inspired by nature, is more efficient, cost effective and robust compared to top-down methods for systems, where the system size and the complexity are relatively smaller.

Among these naturally occurring functional systems, peptide-based nanomaterials have become more popular due to small size, ease of synthesis, and chemical and biological modularity of building blocks. They display less toxicity and inflammation, and have less clearance issues compared to synthetic polymers due to recognition by biological environment. Advantage of these systems over many single and multi-component synthetic systems is that self-assembly can be controlled on demand due to responsiveness of the building blocks (amino acids) to various stimuli such as

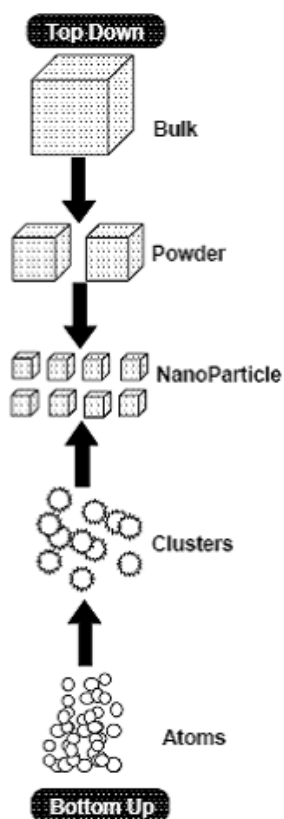


Figure 2.1: Top-down and bottom-up approaches for manufacturing nanomaterials

pH, ionic strength, enzymes, and light. Another property of these building blocks is their diversity which makes rational design of peptide-based nanomaterials challenging compared to synthetic homopolymers such as polystyrene, polyvinyl chloride or even heteropolymers such as styrene-acrylonitrile. For instance, even a short peptide of 4 amino acids has 20^4 or 160000 possible sequences. However, it is not guaranteed that all of these sequences will have a biologic functional activity. Therefore, it is crucial to find the rules that link the peptide sequence to structure to manufacture nanomaterials with desired functional properties.

Peptide-based nanomaterials have been used in a wide-range of areas for both biological and non-biological applications as shown in Figure 2.2. Biological applications include, but are not limited to, tissue engineering such as regeneration of the cavernous nerve by aligned peptide amphiphile nanofiber noodles as shown in Figure 2.3, rapid

bone regeneration [3, 47, 48, 49, 50], drug and cell delivery [51, 23, 52] such as delivering angiogenic factors to pancreatic islets, in treatment of cancer [53], and as scaffolds [54]. On the other hand, non-biological applications include nano-electronics such as construction of nanowires [55, 56], and nanoreactors.[57] Solubilisation of membrane-bound proteins in aqueous environment can be given as an example to both biological and non-biological application. One such system proposed by Zhang *et.al* [58] is usage of amphiphilic peptides with a structure similar to lipids. For instance, A₆D sequence has been shown to be able to stabilize the G protein-coupled receptor bovine rhodopsin in combination with traditional lipids.

There have been numerous experimental and theoretical studies in the literature focusing on understanding the structural, functional properties and thermodynamics of peptide-based nanomaterials which will guide future studies that aim to synthesize new functional peptide-based systems with desired properties. Experimental studies have dominantly focused on elucidation of structure, function of synthesized peptides and/or their interactions with other assemblies at the macroscopic level. Among many experimental studies, the study of Signor *et al* [33] focused on the function of a small amphiphilic peptide, bombolitin III. They analyzed the interaction between the peptide and phospholipid monolayers and liposomes via Langmuir film balance technique and fluorescence microscopy to determine how the activity of phospholipase A2 is altered. In a similar study, Rajangam *et al* [34] analyzed the interaction between a peptidic amphiphilic gel and the heparin molecule to investigate the effect of the peptidic gel on the angiogenic bioactivity of the heparin. In a study of Vocht *et al* [35], the structures of hydrophobin molecules as monomers and after self-assembly were characterized via spectroscopic techniques at hydrophobic/philic interfaces. In an another experimental study, Tsonchev *et al* [36] analyzed both the structure and stability of peptidic assemblies as a function of pH and salinity of the solution. The relation between the structural properties and function of peptides was analyzed systematically by Phillips *et al*. [37] They determine how the side chain, chain length and sequence affected the amphiphilic peptide adsorption at hydrophilic and hydropho-

bic surfaces via sum-frequency generation vibrational spectroscopy and quartz crystal microbalance technique. In a similar study, Krejchi *et al* analyzed the effect of the chemical sequence on the β -sheet assembly in macromolecular crystals of periodic polypeptides via vibrational spectroscopy, NMR and wide-angle X-ray diffraction.[38] Although all of these experimental studies give important insights into structural properties and thermodynamics of peptide-based systems at the macroscopic level, to understand the link between the sequence, structure and organization alternative techniques, such as MD, which can give more detailed atomistic information, should also be used to complement experimental results.

Many theoretical studies on peptide-based systems have focused on understanding the stability of pre-formed peptide assemblies, or the self-assembly process itself starting from randomly distributed self-assembling units. Adsorption phenomenon and self-assembly of monolayers become the subject of studies, where hydrophobic/hydrophilic interfaces and amphiphilic peptides are of concern. Moreover, effective CG models have also been developed in an effort to simulate the self-assembly process itself to get insights into the mechanism of self-assembly. Among various theoretical studies, Velichko *et al* [39] constructed a CG model to study self-assembly of peptide amphiphiles that consist of a hydrophobic alkyl tail, a hydrophobic peptide block, and a bioactive epitope head group. In their model, three different bead types, namely H, P, and E, are used to represent different blocks of the peptide. They also introduced H-bonding via an angle-distance dependent potential. In another study, mechanism of electrostatically-directed self-assembly of cylindrical peptide amphiphile nanostructures was studied via Monte Carlo simulations by Tsonchev *et al*. [40] According to their theory, in the absence of directional electrostatic interactions between the headgroups the peptide amphiphiles self-assemble into spherical micelles; however, introduction of electrostatic interactions leads to formation of cylindrical nanostructures in accordance with experimental findings. In a study of Lu *et al*, thermodynamics and dynamics of amyloid peptide oligomerization were analyzed with respect to different peptide sequences by using the OPEP [41] CG force field.[42] Wei *et al* ana-

lyzed the early oligomeric species on the aggregation pathway of the β 2-Microglobulin NHVTLSQ peptide using the OPEP [41] CG force field with implicit water.[43] They showed that β -barrels with small inner diameters may represent a common intermediate during the early steps of aggregation. Instead of simulating the self-assembly of randomly distributed monomers from scratch, Lee *et al* [18] performed atomistic MD simulations on the self-assembled cylindrical nanofiber, which is composed of 144 peptide amphiphile molecules, to analyze the equilibrium structural properties of the assembly by using explicit water having a physiologic ion concentration.

In another series of theoretical studies, structure and stability of amphiphilic peptide systems as well as thermodynamics of adsorption at hydrophilic/hydrophobic interfaces were analyzed. Knecht analyzed the β -hairpin folding by a model amyloid peptide in solution and at an interface to determine conformational distribution of the peptide at the microscopic level.[44] In another study, mechanism of binding of hydrophobin SC3 molecule to a hydrophobic/hydrophilic interface was modeled via atomistic MD simulations by Fan *et al*.[45] Miller *et al* [46] performed detailed all-atom MD simulations to study the surface activity of amphiphilic helical β -hairpin molecules. They decomposed the adsorption free energy into its enthalpic and entropic contributions. Although these theoretical studies act to complement experimental results by giving insights into structure, stability, and thermodynamics of peptide-based systems at the microscopic level, there are still some issues remained untouched regarding both the structure and thermodynamics of peptide-based nanomaterials. Among these are detailed analyses of thermodynamics of adsorption at hydrophilic/hydrophobic interfaces with amphiphilic peptides that adapt a specific secondary structure. Other issue can be development of a transferable CG model to model the self-assembly of peptide-based nanomaterials. Here, “transferability” can refer to both thermodynamic and structural “transferability”.

Large system size and long time-scale requirement (formation of higher-order structures) make simulation of peptide-based nanomaterials difficult via all-atom MD simulations. In order to circumvent these, alternative strategies have been devel-

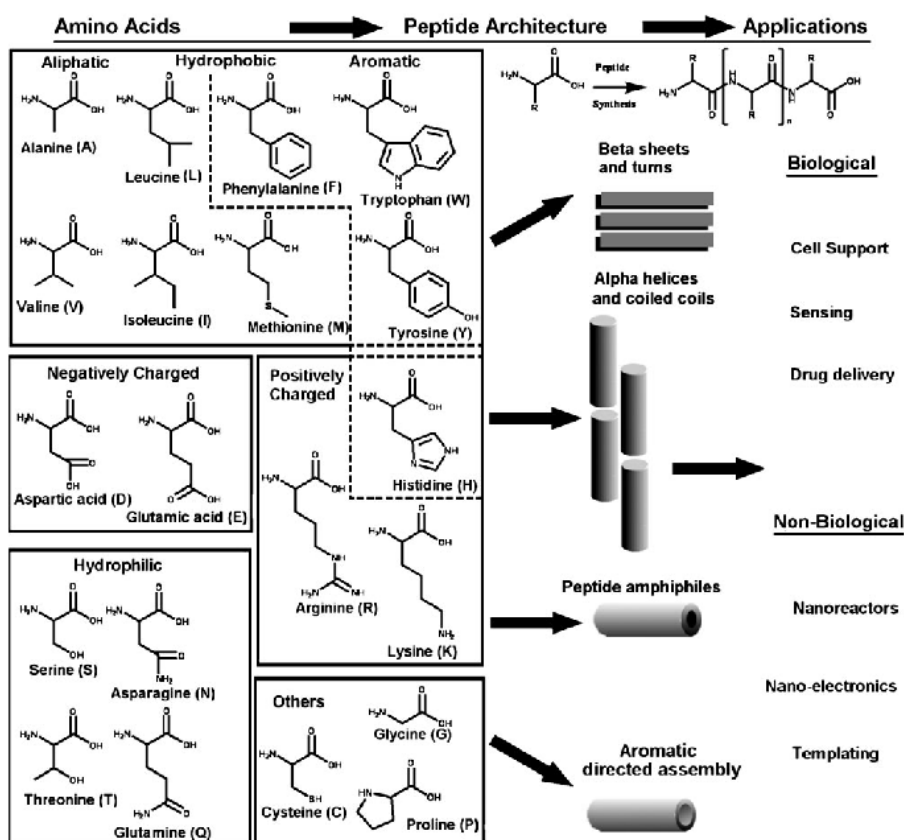


Figure 2.2: Amino acid structures that are commonly used in peptide sequences are given. Four main classes of possible architectures that peptides can adopt are shown along with their potential applications. [2]

oped. If atomistic details of the self-assembling units are important, then, preformed structure of the self-assembled system can be used as the reference structure, and the stability and/or dynamics around the reference structure is analyzed.[18, 59] However, it is crucial to have some *a-priori* experimental data on the structural organization of the system for such simulations. As another strategy, for simulations where investigation of self-assembly itself is more crucial than inclusion of some of fine atomistic details, CG models [39, 60, 61, 42, 62, 63] can be used, instead of all-atom representations, due to long time-scale characteristic of these processes. It is common to use CG models with MD simulations; however, different simulation methods can also be

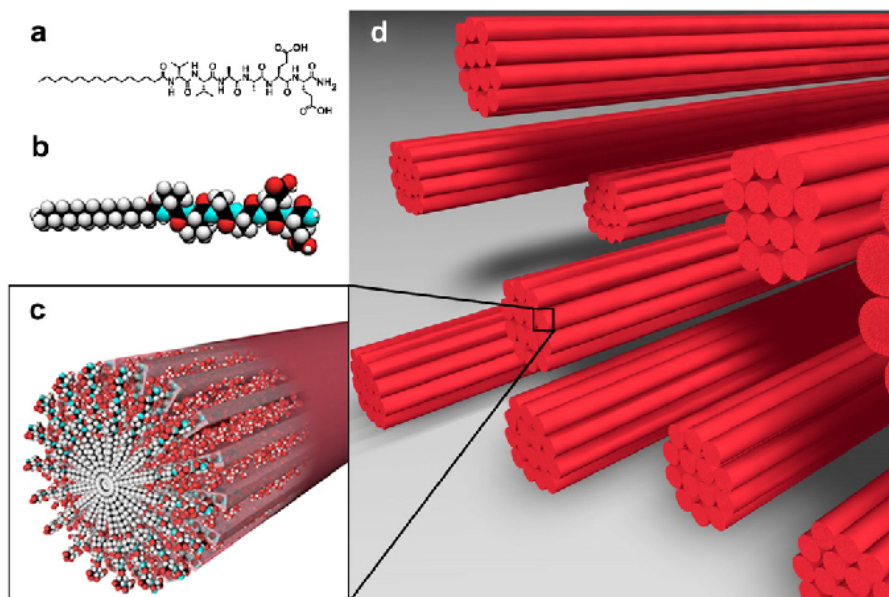


Figure 2.3: Chemical structure of the $(C_{16})\text{-V}_2\text{A}_2\text{E}_2\text{-(NH}_2\text{)}$ peptide amphiphile forming monodomain noodle gels (a), molecular graphics representation of the peptide amphiphile (b), self-assembly of peptidic amphiphile molecules into a nanofiber (c), and schematic representation of nanofiber bundles within the noodle gel.[3]

used such as Brownian Dynamics [64], and Monte Carlo [65], etc. Although these two methods make simulation of long-time motion and large-scale sampling possible they have the disadvantage of having limitations to systems with small inertia and displaying difficulties to devise structural perturbations, respectively.

Despite their success in representing atomistic systems with fewer degrees of freedom, hence covering long time-scales, development of a generic CG model for peptide-based systems remains as a challenging task.[32, 66] In general, CG models are constructed to be used for a specific thermodynamic regime or a specific phase of the system as it is for atomistic force-fields meaning that parameters in CG models are optimized against a specific temperature, pH, ionic strength or a specific phase of the system. In terms of “structural transferability” it is still challenging due to their heteropolymer design with 20 different amino acid residues, with their own specific chemistry and level of conformational flexibility. The heterogeneous nature of pep-

tides results in a vast number of primary, secondary, and tertiary structures depending on the surrounding environment.

Chapter 3

DRIVING FORCES FOR ADSORPTION OF AMPHIPHILIC PEPTIDES TO THE AIR/WATER INTERFACE

Understanding the structure and organization of amphiphilic peptide-based nanomaterials at the air-water interface is of interest for various research fields, from materials science to biology as we already discussed in Chapter 2. The air/water interface provides a well-defined two dimensional environment to study and control molecular self-assembly. In addition, it may also be used to mimic hydrophobic/hydrophilic interface of cellular membranes. This simplifies the study of protein-like systems that are surface active and/or self-assemble within membranes.[45, 46, 67] Experimentally, these systems can be studied at interfaces by Langmuir techniques, spectroscopic measurements and by atomic force microscopy.[68, 69, 70] Nevertheless, these techniques can not provide direct information on the molecular conformation at the interface or on the specific thermodynamic contributions to the adsorption free energy. Therefore, we studied the partitioning of small amphiphilic peptides with ϕ - ψ angles correspond to extended secondary structure at the air-water interface via extensive MD simulations to give a detailed picture of the adsorption of amphiphilic peptides to the air/water interface. The free energy of adsorption from bulk to interface was calculated by determining the potential of mean force (PMF). In particular, we aimed to analyze different contributions to the free energy difference and understand if there is a correlation between peptide length and the driving force for adsorption. We chose model peptides with an alternating sequence of hydrophobic valine and hydrophilic asparagine residues. The simulated peptides are capped and differ in the number of Val-Asn blocks: Ace-(Val-Asn) $_n$ -NME where blocks of $n = 1, 2, 3$ and 5 were used,

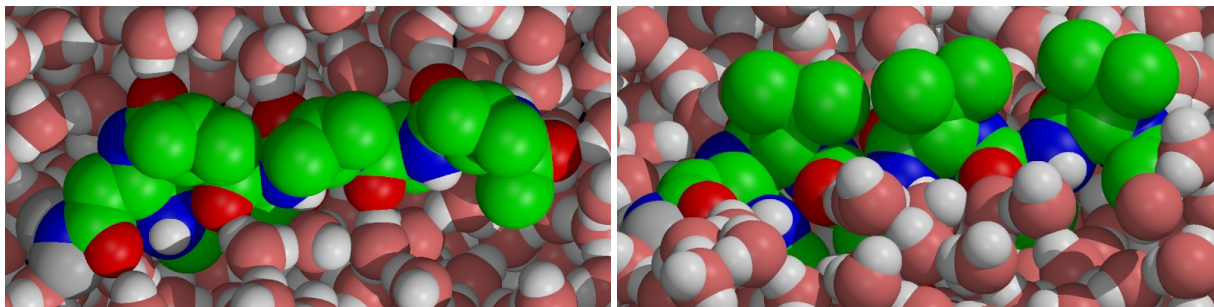


Figure 3.1: Top and side view of a typical conformation of the hexa-peptide at the interface with SPC/E water. The alternating sequence of hydrophobic and hydrophilic residues enables robust partitioning of the molecule at the interface, with all three valine side-chains sticking out into the air.

which we refer to as di-, tetra-, hexa- and deca-peptide in the manuscript, respectively. A method was also introduced to restrain or constrain the center of mass of a group of molecules in a periodic system.

The results obtained from this study give an insight into the thermodynamics of adsorption at the hydrophobic/hydrophilic interface that will guide and improve design of novel peptide-based nanomaterials with desired properties.

3.1 Methods

One of the most important choices that has to be made for simulations in general, and especially for simulating partitioning of molecules between different environments, is the force field. Hydration free energy of hydrophobic side chains is one of the main driving forces to push the amphiphilic peptides to the air-water interface. Therefore, we chose the GROMOS G53A6 force field, which has been parameterized to reproduce the hydration free energies of amino acid analogs.[71] For the water molecules two different models are used: SPC [72] and SPC/E.[73] The former has been used for the parameterization of the GROMOS G53A6 force field and the latter has been shown to provide accurate hydration free energies.[74]

Hydration free energy and its partitioning into enthalpic and entropic contributions for the analogues of the amino acid side chains of the two residues present in the

model peptide are available in the literature.[74] Results for both SPC and SPC/E water models, and the experimental data (for comparison) are given in Table 3.1. The calculated hydration free energy for propane is fairly good, while for acetamide it is slightly underestimated. It is seen that the composition of the free energy is strongly affected by the water model. SPC overestimates the hydration enthalpy and entropy of propane, whereas both water models slightly overestimate the hydration entropy of acetamide. The hydration enthalpy and entropy have large, compensating solvent contributions. SPC/E does better for both propane and acetamide mainly because it better reproduces hydration of hydrophobic solutes. In SPC/E the more unfavorable hydration of the methyl group is compensated by stronger hydrogen bonds between acetamide and water. We expect that the chosen force field will perform well at 298 K. We do not have data for an analogue of the backbone, but since the backbone as well as the force field for it are quite similar to acetamide, this should also have good partitioning properties. The capped end groups are composed of similar building blocks to the ones considered so far, therefore we expect a similar level of accuracy.

Table 3.1: Hydration thermodynamic properties of the analogues of the amino acid side chains of valine and asparagine at 298 K in kJ/mol.

force field	propane			acetamide		
	ΔG	ΔH	$T\Delta S$	ΔG	ΔH	$T\Delta S$
G53A6 + SPC [74]	7.8	-10.5	-18.3	-43.1	-66.9	-23.8
G53A6 + SPC/E [74]	8.9	-13.8	-22.7	-42.3	-67.2	-24.9
precision	0.2	0.8	0.8	0.3	0.8	0.8
experiment [75, 76]	8.3	-13.7	-22.0	-40.5	-67.0	-26.5

All simulations were performed with the Gromacs 4.0 molecular simulation package.[77] The particle mesh Ewald (PME) method [78] was used for the electrostatics with a real space cut-off of 1.0 nm and a grid spacing of 0.13 nm. The Lennard-Jones (LJ) interactions were determined with a twin-range cut-off scheme of 1.0 and 1.4 nm with the long-range interactions updated every 10 steps. The time step was 2 fs. The

temperature was coupled to 298 K using the Berendsen thermostat.[79] Bond lengths were constrained using SETTLE [80] for the water and P-LINCS for the peptide.[81]

For bulk simulations the peptides were solvated in cubic unit cells ranging from 3.1 nm (1000 H₂O) for the di-peptide to 5.8 nm for the deca-peptide (6336 H₂O). For the interface simulations the height of the box was increased by a factor of 3 to create a vacuum of twice the size of the water slab. A slab correction was applied for PME.[82] In the PMF simulations the distance in the z -dimension between the centers of mass of the water slab and the peptide was constrained. PMF calculations were also performed for acetamide.

A common problem that can occur with this setup is that water molecules can escape from the bulk water phase and enter the vapor phase. Once this happens a molecule will travel at (near) constant speed and will join the bulk water phase on the other side through the periodic boundary in the z -direction. If the center of mass of the water molecules is simply determined as the center of mass within the unit-cell, the jump of a molecule over the periodic boundary will result in discontinuities in the constraint force. Apart from introducing a small error in the integration of the equations of motion, this also complicates the analysis. These events become more common as simulation time and size increases. One can ignore (part of) the problem by filtering out the spikes in the constraint force, but we decided to resolve this issue by designing a constraining procedure that takes periodicity properly into account, which is described in the Appendix.

For obtaining the PMF curves, the data points were sampled with 0.1 nm spacing. The mean force was accumulated over 20 ns for acetamide, the di- and tetra-peptides and over 40 to 80 ns for the hexa-peptide. Additional simulations of 50 to 160 ns were performed in bulk water and of 500 to 1750 ns at the interface to determine the bulk and interface properties. Interface properties were determined from additional unconstrained interface simulations or from the constrained simulations through Boltzmann averaging with the PMF values. Standard error estimates were determined using block averaging taking into account possible long time correlations.[83]

3.2 Results and Discussion

3.2.1 Water surface tension

Since reduction of the surface tension of water is one of the main forces driving amphiphiles to an interface, we calculated the surface tension of SPC and SPC/E under the conditions used for the peptide simulations. The combined tension γ of both air-water interfaces is given by the difference of the normal and lateral pressure multiplied by the height of the unit cell ℓ_z :

$$2\gamma = \left[P_{zz} - \frac{1}{2}(P_{xx} + P_{yy}) \right] \ell_z \quad (3.1)$$

To this end we performed simulations of a slab of 1728 water molecules (3.7 nm in each dimension) for 500 ns to determine the surface tension of the air-water interface. The surface tension was determined as 52.2 ± 0.05 mJ/m² for SPC and 59.5 ± 0.05 mJ/m² for SPC/E. The experimental value at 298 K is 72 mJ/m².^[84] An analytical correction for the dispersion interactions beyond the cut-off distance can be made, assuming a flat surface and a uniform dispersion density within the slab. The correction for both water models beyond the cut-off distance of 1.4 nm is 1.9 mJ/m². For a peptide at the interface the correction might change slightly, because the interface may deform to some extent close to the peptide. In water the dispersion correction is small relative to the long range electrostatic forces, for a thorough analysis see for instance ref. ^[85]. Since the PMF simulations were performed with the Berendsen thermostat, we used the same thermostat for comparing the surface tension of pure water. We additionally performed 500 ns simulations with the velocity-rescale thermostat,^[86] which, unlike the Berendsen thermostat, guarantees a canonical ensemble, and the surface tension for both water models is 0.1 mJ/m² higher. This difference is very small, but just above the statistical accuracy.

Both water models significantly underestimate the surface tension, with the error for SPC being twice as large as for SPC/E. The absence of dispersion interactions beyond the interface accounts for part of the discrepancy between experimental and computed values. This term will not change when a peptide is transferred to the

interface, because the dispersion density of peptides is equal to that of water. It is difficult to quantify this term, since in water the effect of different potential terms can not be separated easily. From the calculated surface tension values, we expect that the free energy difference for transferring an amphiphilic peptide from bulk to the air-water interface will be significantly larger with SPC/E compared to SPC. However, simulations with both water models probably still underestimate the free-energy differences compared to the real value.

3.2.2 Potentials of Mean Force

The PMF curves for the di-, tetra- and hexa-peptides in SPC and SPC/E water are shown in Figures 3.2 and 3.3, respectively. The deca-peptide switches between extended and collapsed conformations on a time scale of tens of nanoseconds in bulk. When (partially) at the interface, these transitions are further slowed down. Since these two states show quite a different mean force, obtaining a converged PMF turned out to be computationally too expensive for the deca-peptide. The origin along the z-axis was chosen as the location of the water interface, which we defined as the point where the water density is at half the bulk density. Note that for both water models the minima in PMF curves are slightly below the interface, which is consistent with the fact that a larger portion of the peptides is hydrophilic. This can also be confirmed with the snapshot for hexa-peptide shown in Figure 3.1, which represents a typical conformation of the peptide at the PMF minimum.

Although all our interface simulations were performed in a constant- NVT ensemble, we will still refer to the free energy as G and to enthalpy instead of energy, since experiments are usually performed at constant pressure and the volume changes are so small that the $p\Delta V$ term is always smaller than the statistical accuracy of the simulations. Defining a free energy for partitioning between the bulk and the interface is not straightforward, since it will depend on the amount of bulk that is present below the interface. To obtain a definition that does not require a reference length scale of the bulk, the free energy for constraining a particle is required. This involves the mass

of the molecule and Dirac's constant (see e.g. ref.[87] for details). The dependence on mass is inconvenient for interpreting the change in ΔG values for different peptide lengths, because the mass does not play a role in the thermodynamics of partitioning. We therefore decided to determine the free energy with respect to a reference thickness of the bulk layer ℓ :

$$\Delta G^\ell = -k_B T \log \left[\frac{1}{\ell} \int_{\text{well}} \exp \left(-\frac{W(z)}{k_B T} \right) dz \right] \quad (3.2)$$

where W is the PMF with a reference value of 0 in the bulk. The integral is performed over the well at the interface. The precise extent of the well is not critical; since the minimum is several $k_B T$ deep, varying the boundaries has a negligible effect. The free energy difference can be easily transformed to another reference length h by adding a term $-k_B T \log \ell/h$. The dependence on a reference bulk layer thickness drops out since we are interested in the change in ΔG between different peptides.

In Table 3.2 we report ΔG^ℓ obtained from the integration of PMF curves in Figures 3.2 and 3.3 based on a reference length of 1 nm. This length corresponds to 4 times the average width of the distribution at the interface (0.25 nm) measured as 2 times the standard deviation. The first thing to notice is that the free energy depends linearly on the peptide length when going from 2 to 6 residues, both in SPC and SPC/E. One can see that the free-energy difference between bulk and interface is around 30% larger for SPC/E than for SPC. As stated above, this is expected based on the surface tension of the water models. The effect can also be seen in the hydration free-energies of the small molecules in Table 3.1. We will analyze this in more detail later.

Another useful quantity is the ensemble averaged work $\langle W \rangle$ for transferring a peptide to the interface. It can be computed as W averaged over the interface region with the Boltzmann factor of W :

$$\langle W \rangle = \int_{\text{well}} W(z) \exp \left(-\frac{W(z)}{k_B T} \right) dz \quad (3.3)$$

This measures the free energy difference for inserting a peptide in the bulk and at the interface; it is ΔG without the entropic contribution of the center of mass of

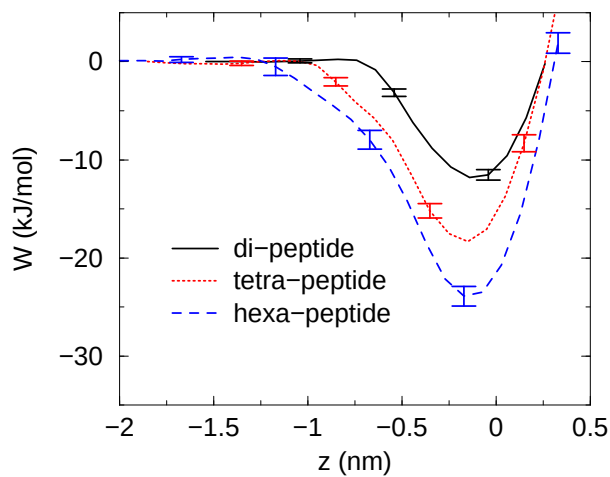


Figure 3.2: The PMF of the di-, tetra- and hexa-peptide in SPC water as a function of the distance from the center of mass of the peptide to the interface. Note that points are spaced by 0.1 nm, while error estimates are only plotted every 0.4 nm.

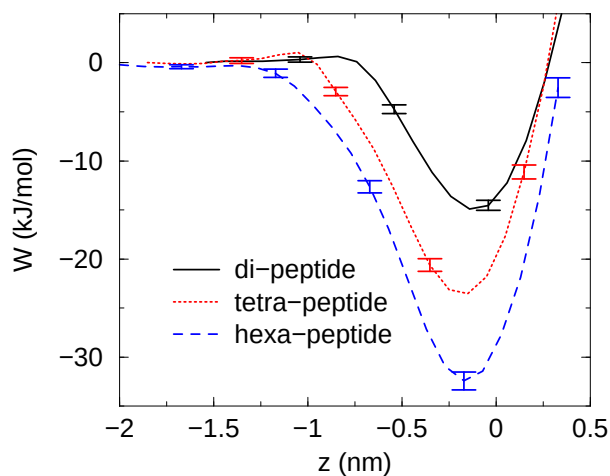


Figure 3.3: As Fig. 3.2, but in SPC/E.

the peptide. This quantity is useful for interpreting different contributions to ΔG , because it measures only the contributions due to differences in environment (note that this still includes changes in peptide conformational states). Unlike ΔG , it does not depend on the amount of bulk below the interface. Results for $\langle W \rangle$ are also reported in Table 3.2. The difference between $\langle W \rangle$ and ΔG^{1nm} is 2.2 kJ/mol on average and are within error margins, which shows that the entropy loss of the center of mass of the peptide at the interface is the same for all cases.

In order to determine the enthalpic and entropic contributions to the free-energy, we also performed simulations of 40 ns for the di-peptide in SPC/E, and measured the average potential energy. By subtracting the enthalpy from the PMF the entropic contribution can be obtained. The results are shown in Figure 3.4, where the PMF is denoted by W . As the peptide moves from bulk to the interface, there is a minimum in the enthalpy at -0.4 nm. The entropy does not show a significant change at this distance. Here most of the time the peptide is completely in the solvent, except for the side chain of valine (see Figure 3.1). The enthalpy change of 12 kJ/mol is close to what one would expect based on the value for propane in Table 3.1. The entropy change, however, is negligible and can not be explained as a simple de-solvation of the side chain of valine. Apparently the entropy gain of the water molecules that loose contact with the hydrophobic side-chains is compensated by, among other things, an entropy loss of conformational degrees of freedom of the peptide. As the peptide moves further beyond the interface, both ΔH and ΔS increase, as one would expect from the solvation properties of the propane and acetamide moieties. Eventually one would expect that these contributions converge to the values of a di-peptide in vacuum. However, even at the largest distance analyzed here, there is still a water meniscus which bridges the peptide to the bulk water, and therefore we do not observe such a behavior.

In the following section we will analyze different contributions to the free energy change.

Table 3.2: The ensemble average of the work ($\langle W \rangle$) and the free energy for moving a peptide and acetamide from bulk water to the air-water interface (ΔG^{1nm}).

	molecule	$\langle W \rangle$	ΔG^{1nm}
SPC	acetamide	-3.9 ± 0.2	-2.0 ± 0.2
	di-pep.	-11.0 ± 0.5	-9.1 ± 0.5
	tetra-pep.	-17.4 ± 0.8	-15.2 ± 0.8
	hexa-pep.	-23.1 ± 1.0	-20.7 ± 1.0
SPC/E	acetamide	-4.9 ± 0.2	-2.9 ± 0.2
	di-pep.	-14.1 ± 0.5	-12.1 ± 0.5
	tetra-pep.	-22.8 ± 0.7	-20.4 ± 0.7
	hexa-pep.	-31.6 ± 0.9	-29.0 ± 0.9

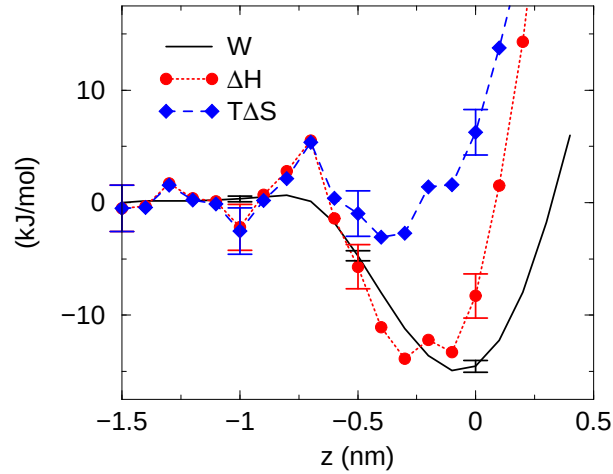


Figure 3.4: The PMF, enthalpy and entropy differences for the di-peptide in SPC/E as a function of distance to the interface.

3.2.3 Decomposition of the Free Energy

The free energy of transferring an amphiphilic molecule from bulk water to the air-water interface can be decomposed into three main contributions. Two contributions can be attributed to the solvent, although they are caused by changes in the interface between peptide and water: the de-solvation of hydrophobic groups and the decrease

of the air-water interface area. A third contribution can be attributed to the peptide, although it is caused by changes in the solvent environment: entropy changes due to changing weights of peptide conformational states, this can in turn be decomposed into internal and center of mass degrees of freedom.

We will start with the first contribution. The backbone as well as the asparagine sidechains are similar to acetamide, with one or more hydrogens replaced by other moieties. The asparagine sidechains remain solvated during all the simulations and we therefore do not expect that they contribute significantly to the free energy difference. But the backbone is partially dehydrated at the interface. The $\langle W \rangle$ value for acetamide is 1 kJ/mol lower in SPC/E than in SPC (Table 3.2), which is exactly opposite to the hydration free energy behavior (Table 3.1), which is due to stronger hydrogen bonding and more unfavorable hydrophobic solvation. Because in the peptide the backbone is less solvent accessible than acetamide it is difficult to make a quantitative estimation of its contribution to $\langle W \rangle$ of the peptide. We will come back to this later.

At the minimum of the PMF most of the peptide is in contact with water, only the hydrophobic side-chains of valine are completely de-solvated. For a very short peptide, such as a di-peptide, hydrophobic side-chains are always solvent exposed in bulk water. Longer peptides might be able to partially shield hydrophobic groups by forming intra-molecular hydrophobic contacts. This can be quantified by determining hydrophobic solvent accessible surface (SAS) areas, which are shown in Table 3.3 (a probe radius of 0.14 nm was used). In bulk the hydrophobic surface area increase upon addition of a valine (and an asparagine) is around 1 nm². All values are less than the SAS area of 1.6 nm² of a propane molecule. The difference is caused by the fact that the side chain is connected to the C_α and therefore part of the side chain is not solvent accessible. A similar difference is observed in the number of water molecules within the first solvation shell. If we define the first solvation shell as all water molecules which have their oxygen atom within 0.5 nm from any of the propane carbons, we obtain 22.5 for propane and around 17 for the valine side chains

of the peptides in bulk water. The amount of screening increases only slightly when the peptide size increases, due to some single atom pair contacts between adjacent valine side-chains. This is consistent with the fact that we mainly observe extended conformations in the bulk. The peptide lengths in consideration are too short for the formation of a stable hydrophobic core. Only for the deca-peptide a slight deviation from the linearly increasing trend of SAS areas is seen. Investigation of the simulation trajectory did not reveal the presence of a stable hydrophobic core.

Table 3.3: Thermodynamics of transferring propane from bulk to vacuum and valine side-chains in peptides from bulk to the air-water interface, V_{bulk} and $V_{\text{interface}}$ are the potential energy for the solute-solvent interactions in bulk and at the interface, respectively.

molecule		$-\Delta G_{\text{solv}}$ (kJ/mol)	V_{bulk} (kJ/mol)	$V_{\text{interface}}$ (kJ/mol)	SAS_{bulk} (nm ²)	γ_{prop} (mJ/m ²)
SPC	propane	-7.8 ± 0.2	-29.5 ± 0.0		-1.59 ± 0.00	38.9 ± 0.2
SPC/E	propane	-8.9 ± 0.2	-30.5 ± 0.0		-1.59 ± 0.00	41.2 ± 0.2
SPC	di-pep.		-21.4 ± 0.2	-8.3 ± 0.1	-1.07 ± 0.00	
	tetra-pep.		-41.1 ± 0.1	-13.3 ± 0.1	-1.97 ± 0.02	
	hexa-pep.		-60.3 ± 0.4	-20.4 ± 0.3	-2.83 ± 0.01	
	deca-pep.		-96.9 ± 0.9	-32.2 ± 1.3	-4.53 ± 0.05	
SPC/E	di-pep.		-22.6 ± 0.1	-7.3 ± 0.2	-1.07 ± 0.00	
	tetra-pep.		-41.2 ± 0.2	-13.3 ± 0.2	-1.95 ± 0.02	
	hexa-pep.		-60.0 ± 0.4	-18.8 ± 0.3	-2.79 ± 0.02	

When a peptide is transferred to the air-water interface, part of the peptide will no longer be in contact with water. The valine side chains avoid solvation in water and have an average of 5 water molecules within 0.5 nm and 90% of time between 1 and 8 water molecules. Although there is some contact with water, this does not seem to affect the structure and hydrogen bonding of the water at the interface. The orientation of the valine side chains fluctuates slightly over time and now and then

the side chains touch several water molecules at the interface. These interactions are probably favorable, since they add attractive Lennard-Jones interactions, while not perturbing the hydrogen bonding of the water.

To rationalize the contribution of the desolvation of the side chains to the free energy, we need to make several assumptions. The first is additivity of the different side chains. Since the conformations are mainly extended, there are very few water molecules that are in contact with more than one valine side chain. Furthermore, the conformations of the peptide in bulk and at the interface are very similar, therefore the side chains will not loose or gain any significant amount intra-peptide of interactions upon transfer to the interface. With this assumption each side chain can be considered separately and the contributions added. To quantify the free-energy change upon transfer of one side chain to the interface, we can use propane as a reference system, since for propane we know the solvation free energy. There are two types of contributions to the hydration of a hydrophobic solute. One is the ordering of water around the solute, as it can not make hydrogen bonds with the solute; this term is proportional to the SAS area of the solute, although for small solutes it is proportional to the volume.[88] A second contribution comes from the direct Van der

Table 3.4: Estimated transfer free energies of the side chains from the interface to the bulk

	molecule	$-\Delta G_{\text{Val}}^*$ (kJ/mol)
SPC	di-pep.	-11.8 ± 0.4
	tetra-pep.	-18.4 ± 0.4
	hexa-pep.	-26.3 ± 0.5
	deca-pep.	-41.4 ± 2.8
SPC/E	di-pep.	-11.3 ± 0.3
	tetra-pep.	-20.5 ± 0.5
	hexa-pep.	-28.0 ± 0.7

Waals interactions (LJ in the force field) between the solute and the solvent, for small solutes this term is not proportional to the SAS area. Therefore we first have to subtract the solute-solvent interaction energy from ΔG_{solv} to obtain an effective surface tension for the water-propane interface. We determined the water-propane interface area from additional simulations of propane in SPC and SPC/E. The resulting effective surface tension γ_{prop} is given in Table 3.3. Note that the LJ energy is much larger than ΔG_{solv} . These surface tensions are about two thirds of those at the air-water interface, but the difference between SPC and SPC/E is much smaller in the case of propane solvation. If we assume that the effective surface tension is proportional to the SAS, we can multiply the SAS in the peptide systems with the surface tension and add the side-chain-water LJ energy differences to obtain an estimation for the transfer of the side chains from the interface to the bulk (ΔG_{Val}^*). The values are given in Table 3.4; note that the error estimate contains all statistical errors, but it does not contain errors due to approximations of additivity and linear dependence of the surface area. Since this term is large and involves two important approximations, there is some uncertainty in the values. But we can safely state that these side-chain dehydration contributions are equal in magnitude to $\langle W \rangle$ and form the main driving force for transfer of the peptides to the interface.

Another contribution is conformational freedom. Since at the interface the peptide is to a large extent restrained to a two-dimensional space, there will be a loss of conformational entropy of the peptide. For larger molecules, which have more conformational freedom, the loss of entropy at the interface might be higher. To quantify this, we calculated the Shannon entropy s of the peptides:

$$s(x) = - \sum_{i=1}^n p(x_i) \log p(x_i) \quad (3.4)$$

where $p(x_i)$ is the probability of the i^{th} outcome of x . Shannon entropy can be related to thermodynamic entropy [89] (by multiplying with the Boltzmann constant) and it has been widely used in studies in order to quantify the entropies of the peptides. The absolute value of the Shannon entropy depends on the number of bins used. But when calculating entropy differences this dependency drops out when the same number of

bins is used. The conformational degrees of freedom can be split into two types: intra-molecular and molecular orientation. In principle these two sets of degrees of freedom are coupled, but we expect the coupling to be small, so we can analyze them separately to keep the calculation tractable, with the additional advantage that we get two separate numbers. We determined Shannon entropies for the dihedrals of the di-, tetra- and hexa-peptides using bins of 30° for each dihedral, both in bulk water and at the interface. In Table 3.5 one can see that the intra-molecular degrees of freedom indeed loose entropy, but, surprisingly, the amount is negligible. For the orientational entropy we determined the two Euler angles that rotate the peptide out of the interface by least squares fitting to the reference structure which has the smallest sum of root mean square deviation to all other structures. We determined the Shannon entropy using 326 bins of equal size on a sphere (using 732 bins changes the entropy by less than 0.1 kJ/mol for all systems). In Table 3.5 that the orientational entropy loss is significant. For longer peptides the length dependence decreases, as one would expect. The entropy loss of 1 to 3 $k_B T$ means that the peptide still has quite some orientational freedom, but part of this might be because the air-water interface is not a rigid, flat surface, but rather a fluctuating interface.

Table 3.5: Dihedral and orientational entropy change in kJ/mol upon transferring the peptides from the bulk to the interface.

	pep.	$T\Delta S_d$	$T\Delta S_{ori}$
SPC	di	-0.1	-2.8
	tetra	-0.2	-5.5
	hexa	-0.1	-6.7
	deca		-7.9
SPC/E	di	-0.0	-3.3
	tetra	-0.2	-5.5
	hexa	-0.1	-6.7

If we assume that we have now treated the major contributions, we can make a

decomposition of the free energy of transferring a peptide from the bulk to the air-water interface. We have made several approximations and neglected several effects, but we should have captured all the major contributions. We can add up the contributions and determine the difference with $\langle W \rangle$. Since the backbone dehydration is too complex to quantify directly, we simply scaled it by a constant to make the total $\langle W \rangle$; this constant turns out to be a quarter. Note that the number of acetamides in this calculation is equal to the numbers of residues plus one, since there are that many peptide bonds. The "rest" terms (see Table 3.6) comply with the statistical error of around 2 kJ/mol. However, the rest term is systematically positive for SPC and negative for SPC/E. This indicates that a (small) water model dependent contribution is missing or underestimated. We can look at water model dependence more accurately by determining the $\Delta\Delta G$ for hydration between SPC and SPC/E.

Table 3.6: All the calculated contributions to $\langle W \rangle$ as well as the rest term in kJ/mol.

	pep.	$\langle W \rangle$	$-T\Delta S_d$	$T\Delta S_{ori}$	$-\Delta G_{Val}^*$	$\frac{1}{4}\langle W_{ace} \rangle$	rest
SPC	di	-11.0	0.1	2.8	-11.8	-2.9	0.8
	tetra	-17.4	0.2	5.5	-18.4	-4.9	0.0
	hexa	-23.1	0.1	6.7	-26.3	-6.8	3.2
	deca			7.9	-41.4	-13.0	
SPC/E	di	-14.1	0.0	3.3	-11.3	-3.7	-0.4
	tetra	-22.8	0.2	5.5	-20.5	-6.1	-1.9
	hexa	-31.6	0.1	6.7	-28.0	-8.6	-1.8

3.3 $\Delta\Delta G$ Calculations

The difference in hydration free energy between the two water models, both in bulk water and at the air-water interface, can tell something about the nature of the free-energy differences upon transfer to the interface. Determining a ΔG of hydration of a peptide is a computationally expensive task, especially at the interface where long

sampling is required. It turns out to be computationally much cheaper to change SPC water into SPC/E. Using Bennett’s acceptance ratio method [90, 91] this can be done in 10 ns with just one intermediate state for water and the di-peptide system. The error was estimated by block averaging using 5 blocks of 2 ns. The result is a $\Delta\Delta G$ of 3.3 ± 0.3 kJ/mol for bulk water and a $\Delta\Delta G$ of -0.3 ± 0.3 kJ/mol at the interface. The difference between these numbers nicely matches the $\Delta\langle W \rangle$ obtained from the PMF calculations in SPC and SPC/E. For the hexa-peptide we only performed the calculation in bulk water, with three intermediate states; the result is 14.3 ± 1.2 kJ/mol. The difference between SPC and SPC/E matches the sum of the difference of the hydration free energies of the components listed in Table 3.1, assuming that the backbone matches acetamide and the difference for the terminal methyl groups is negligible. For the di-peptide the difference vanishes at the interface. The di-peptide $\Delta\Delta G$ between bulk and interface, between SPC and SPC/E is -3.6 kJ/mol. This is two to three times as much as our free energy decomposition gave with the hydration free energy difference of propane plus $3/4\Delta\langle W \rangle$ of acetamide. This again shows there is a slightly larger water model dependence than our analysis provides, probably due to an oversimplified analysis of the backbone contribution and ignoring the effect of the termini.

3.4 Surface Tension

Reduction of the surface tension is also a key element in driving the peptides to the air-water interface. To determine the surface tension reduction when the interface is covered with peptides, we solvated 40 randomly placed tetra-peptides in an interface of 2000 SPC/E water molecules. Using a cubic initial volume turned out to produce quite stable clusters of peptides in the bulk in addition to peptides at the interface. Therefore, we started with a slab of 6 by 6 by 2.5 nm, which we slowly deformed to a slab of 3.8 by 3.8 by 6 nm. The final state has 19 peptides on the top and 21 peptides on the bottom interface, where a representative snapshot from the top surface is shown in Figure 3.5. After equilibration the surface density of the peptides

is around 1.2 peptides per nm^2 .

The dehydration of valine side chains, which provides the largest contribution to the transfer free energy, does not contribute significantly to the surface tension reduction. Although a lot of free energy is gained by moving these sidechains out of bulk water, valine side chains are too small to cover the surface completely. In fact, valine side chains remain completely isolated from the rest of the system. On the other hand, the peptide backbones shield water molecules by forming a continuous layer connected via hydrogen bonds.

The surface tension is difficult to estimate accurately, since fluctuations are larger and convergence is slower compared to pure water. If we ignore the first 100 ns as equilibration, we obtain $41 \pm 2 \text{ mJ/m}^2$ for the final 100 ns. This is a reduction of the surface tension by 30%, or a reduction of $9 \pm 2 \text{ kJ/mol}$ per peptide. Note that the free-energy difference between peptides dispersed in bulk and a layer of peptides at the interface not only involves the free energy difference of transfer of a single peptide from bulk to the interface, but also the favorable free energy contributions of the interactions between two or more peptides at the interface.

3.5 Concluding Remarks

We have accurately determined the free energy of transferring peptides from bulk water to the air-water interface, for the given force field and two water models. The $\Delta\Delta G$ between the two water models has been verified with free energy transformations using Bennett's acceptance ratio. The minimum of the PMF is deep: between 5 and $11 k_B T$. Absolute free energy values can not be given, since there is an entropic term in the free energy that depends on the height of the bulk water phase below the interface. The shape of the PMF is the same for all peptides and water models with one deep minimum with a (thermal) width of a few Ångstroms. We have shown that the ΔG of amphiphilic peptides is dominated by one favorable contribution: the desolvation of hydrophobic side chains. For these peptide lengths two smaller terms are the partial dehydration of the backbone and the loss of orientational entropy, which

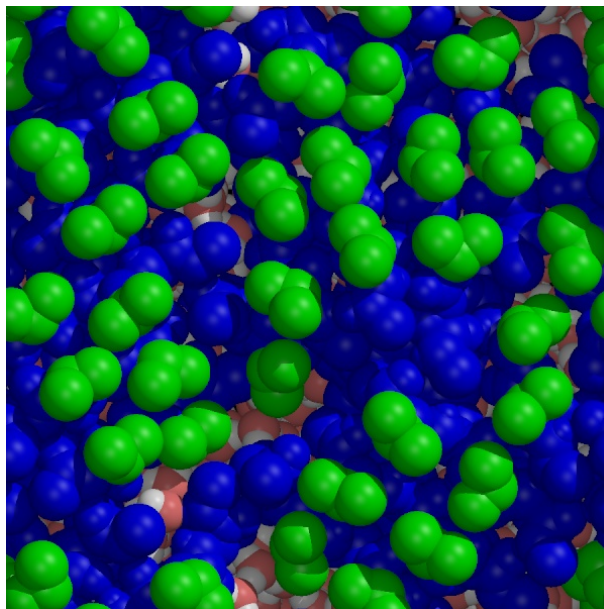


Figure 3.5: Top view of 19 tetra-peptides (in blue) at the air-water interface. Note that the valine side chains (in green) are nearly all separated from the rest of the system.

is the only significant unfavorable term. In SPC/E ΔG is 30% larger than in SPC. This difference is caused by the stronger hydrophobic effects in SPC/E, which appear in the dehydration of both the backbone and the valine side chains. This means that for quantitative modeling the choice of water model is critical. SPC/E is a better choice than SPC, since it reproduces hydrophobic hydration thermodynamics much better than SPC. This is also reflected in the surface tension of the water models. However, SPC/E still underestimates the surface tension by 15%. It remains to be seen what effect this discrepancy has on the partitioning of amphiphilic molecules to the interface.

There are also contributions that disfavor the transfer of a peptide to the air-water interface. The largest one is the restriction of the orientation of the peptide at the interface. This term is $1 k_B T$ for the di-peptide up to $3 k_B T$ for the deca-peptide. We also expected some entropy loss of the internal degrees of freedom of the peptide, but this does not seem to be the case. The net effect of the three main contributions

produces a free energy difference which is linear for the chosen sequence at least up to hexa-peptides, but probably also up to deca-peptides. At a certain length this linear increase will level off, since a long peptide will be able to form contacts between hydrophobic side chains in bulk water, thereby shielding hydrophobic groups from the solvent. For the deca-peptide we see this happening to a minor extent. An effect which might play a role in experiments, but which is beyond the scope of this work, is the clustering of peptides in bulk water. At high concentrations peptides will come together to shield off hydrophobic groups from the solvent. At the interface the peptides cluster through intramolecular hydrogen bonds between backbones. The resulting layer lowers the surface tension by 30% compared to a pure SPC/E air-water interface.

The three main contributions to the free energy difference depend strongly as well as differently on the length and the composition of the molecule, therefore it is difficult to predict the partitioning behavior of general peptide sequences at the air-water interface. The behavior of complete proteins at the interface varies widely with sequence (see e.g. ref [92]). However, we have shown that the thermodynamics for peptides with alternating hydrophobic/hydrophilic sequences is relatively simple. The driving force is the reduction of hydrophobic interface area with water, both for hydrophobic side chains and the hydrophobic air interface. The only significant opposing force is the loss of orientational entropy of the peptide at the interface, but this term is relatively small. Therefore alternating hydrophobic/hydrophilic sequences will partition strongly to a hydrophobic/hydrophilic interface, without frustration.

Chapter 4

FOLDING, ADSORPTION AND PACKING OF AN AMPHIPHILIC PEPTIDE AT THE AIR/WATER INTERFACE

Small peptide molecules with a well-defined β -hairpin secondary structure have gained an increased attention over the past decade. These molecules provide ideal models for studying amyloid formation, which triggers diseases such as Alzheimer's,[93, 94] Huntington's [95] and diabetes.[96, 97] In addition, they have been successfully used in a wide range of areas including tissue engineering,[19] regenerative medicine,[21] gene and drug delivery [22, 23], and as biosensors.[24, 25] Molecular precision and specific control of inter-molecular interactions among these molecules brings up the opportunity to design hydrogels,[98, 99, 100] filaments, fibrils,[101, 102, 103, 104] and cylindrical micelles.[105] In response to different environmental stimuli assemblies of these peptides can adopt different structures such as micelles,[106, 107] nanotubes,[105, 108, 109] or monolayers.[110] A key requirement for many of these application areas is that these peptide molecules should exhibit a well-defined and stable equilibrium conformation under stringent conditions and self-assemble to enable bottom up design strategies.[104, 105, 111, 112, 113] In addition to above-mentioned properties, β -hairpins can also be considered as model folding motifs for globular proteins.[114, 115] In this respect, they provide important insights into the protein folding problem and also help improve secondary structure prediction algorithms with a "fragment-based approach" as already shown in the study of Ho and Dill.[116] Similarity with globular proteins also provides the opportunity to improve peptide design: survey of structures in the Protein Data Bank enables identification of novel sequences for loop and strand residues in β -hairpin structure.[117] Another important feature

of these molecules is their ability to fold below microsecond timescales, which makes these systems accessible via molecular dynamics simulations. In previous computational studies, key determinants of β -hairpins, such as turn,[118, 119, 120, 121, 122] hydrophobic [123, 124, 125, 126, 122, 127, 128] and polar residues,[129, 122, 127, 128] as well as their relative weights on the stability [129, 130, 131, 132] were investigated. Close similarities to amyloid formation have guided most of the earlier computational studies to investigate folding mechanisms and energy landscapes of different β -hairpins.[133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 44, 145, 146, 147]

In this study, we studied the folding and adsorption of a carefully designed 24-residue amphiphilic peptide (Figure 4.1) via extensive MD simulations using information gathered from smaller amphiphilic peptides (see Chapter 3). The molecule exhibits several key design features: The peptide sequence has an alternating array of hydrophobic and hydrophilic residues, which gives the molecule an amphiphilic character. D Pro-Gly residues in the center of the sequence create a kink and strongly favor formation of a β -hairpin secondary structure. The charge distribution of the molecule is chosen such that the first half of the sequence carries mostly positive, whereas the second half carries mostly negatively charged residues.

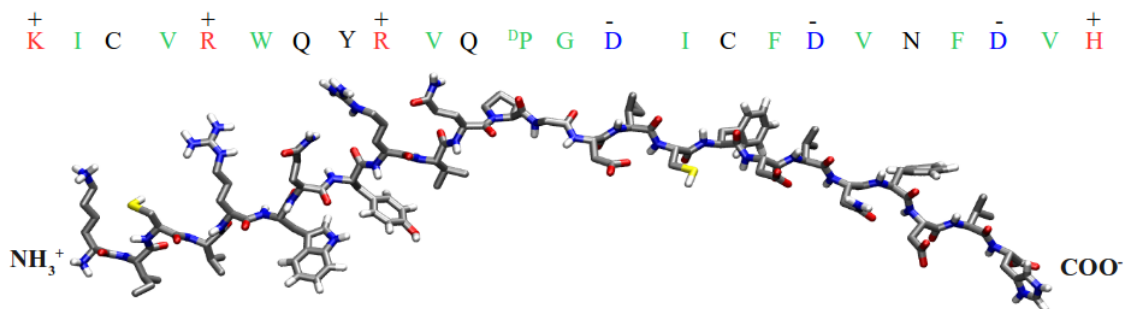


Figure 4.1: Sequence of the peptide under study. Residues are labeled with one-letter coding. Positively and negatively charged residues are shown in red and blue, whereas non-polar and polar residues are shown in green and black. D Pro-Gly residues located in the center lead to a kinked conformation.

We highlighted the role of key design features required for the formation and stabil-

ity of the β -hairpin conformation via *in-silico* mutations. Furthermore, by calculating the PMF among two peptide molecules at the interface, we investigated packing of these molecules into surface monolayers.

4.1 Methods

Molecular dynamics simulations are performed with Gromacs 4.0 simulation package.[148] Temperature is kept constant at 298 K via Berendsen thermostat with a coupling time of $\tau_T = 0.1$ ps. For constant pressure simulations Berendsen barostat [79] is used with a coupling time of $\tau_P = 0.5$ ps.

GROMOS G53A6 force field,[149] which is parametrized to reproduce hydration free energies of amino acid analogs, is used for peptides. Water molecules are modeled with SPC/E,[73] which is known to provide accurate hydration free energies. Bond lengths for water molecules and peptides are constrained via SETTLE [80] and P-LINCS [150] algorithms.

The particle mesh Ewald method (PME) [151] is used for electrostatic interactions with a real space cut-off of 1.0 nm and a grid spacing of 0.12 nm. A slab correction is applied for PME. Lennard-Jones interactions are calculated with a twin-range cut-off scheme of 1.0 and 1.4 nm with neighbor list updated every 10 steps. A time step of 2 fs is used for the integration. Prior to production MD runs, systems are energy minimized via steepest descent algorithm to remove all possible steric clashes.

Ionization state of each residue is assigned according to pH 7.0 resulting in a total charge of +1 per peptide. Peptide termini are in zwitterionic state in accordance with experimental conditions. One counter-ion is added for each charged residue of the peptide to maintain neutrality.

DSSP algorithm [152] implemented in Gromacs 4.0 package is used for secondary structure analysis. Mutations are done via WHATIF program.[153] For hydrogen bond (H-bond) analysis angle and distance criteria of 30° and 0.35 nm are used, respectively. Visual molecular dynamics program [154] is used in the preparation of all molecular graphics.

Single peptide simulations in the presence of the air/water interface are performed in the canonical ensemble. In order to create the air/water interface an equilibrated water box is expanded to three times the height of the original box along the z-direction. The peptide in its kinked conformation (Figure 4.1) is used as starting conformation, except in simulations where the turn residues are mutated. The molecule is placed in the middle of the water slab at the beginning of the simulations. Simulations are repeated at least twice to check for dependence on initial conditions.

The adsorption free energy of a single peptide to the air/water interface is calculated via constraint pulling MD simulations. The center-of-mass of the peptide is constrained at 40 different points, with 0.1 nm spacings from the center of the water slab to the upper interface. The position of the interface is defined as the z-coordinate where the density of water drops to half of its bulk value. Average force measured at each constraint distance is integrated by the trapezoidal rule to calculate the potential of mean force (PMF) for peptide adsorption. Standard error estimates are obtained by block averaging,[155] taking into account possible long time correlations. On average 40 ns simulations are performed for each constraint point.

In order to calculate the association free energy between a pair of peptides placed at the air/water interface umbrella sampling method [156, 157] is used. The peptides are pulled away over 400 ps until the center-of-mass distance between the two peptides is 4.0 nm. The pulling force is confined to the interface (x-y plane) and a spring constant of $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ and a pull rate of 0.01 nm/ps is used. Snapshots from these trajectories are used to generate starting configurations for the umbrella sampling windows. The windows contain configurations with increasing center-of-mass spacings of 0.1 nm increments up to 2.5 nm, and 0.2 nm increments beyond. A total of 32 windows is used and a 10 ns MD simulation is performed for each. Results are analyzed with the weighted histogram analysis method (WHAM).[158] Error analysis are performed using the bootstrap method.[159]

4.2 Results and Discussion

4.2.1 Single peptide molecule in a water slab

The peptide molecule under study is designed with the goal of obtaining an amphiphilic molecule with a hairpin structure. Hence, we begin our analysis by looking at the conformational behavior of a single peptide molecule in a water slab by performing molecular dynamics simulations under constant volume and temperature. A water layer of 5 nm thick is centered in a box of length 15 nm so that two interfaces are present in the system. The remaining part of the box is left as vacuum. The peptide molecule is placed in the center of the water slab in a kinked conformation (Figure 4.1).

As the simulation proceeds, the peptide molecule is driven strongly towards the interface due to its amphiphilic nature. It only takes 8-9 ns for the molecule to adsorb to the upper air/water interface as seen from the time evolution of the center of mass of the molecule with respect to the center of the water slab (Figure 4.2 upper left plot). Presence of the interface dramatically accelerates adoption of the β -hairpin structure and also stabilizes it. In our earlier analysis, we have shown that adsorption of the molecule to the interface reduces the conformational space of the molecule compared to bulk.[160] Simultaneous with the adsorption to the interface, the molecule forms a β -hairpin structure as displayed by the DSSP analysis (Figure 4.2 bottom). A representative conformation of the molecule at the interface can also be seen in Figure 4.2, at the top. Here, sideview of the molecule is shown in a typical conformation at the interface and water molecules are not shown for visual clarity. The molecule is displayed both in ball-and-stick and cartoon representation, which is colored according to the secondary structure of the molecule. The red section in cartoon view represents the portion of the molecule which adopts the β -hairpin structure. The middle of the molecule adopts a bend/turn conformation or no conformation at all as seen in DSSP data. The hairpin structure is favored not only by formation of intra-molecule hydrogen bonds. The strong partitioning of the hydrophobic and hydrophilic side

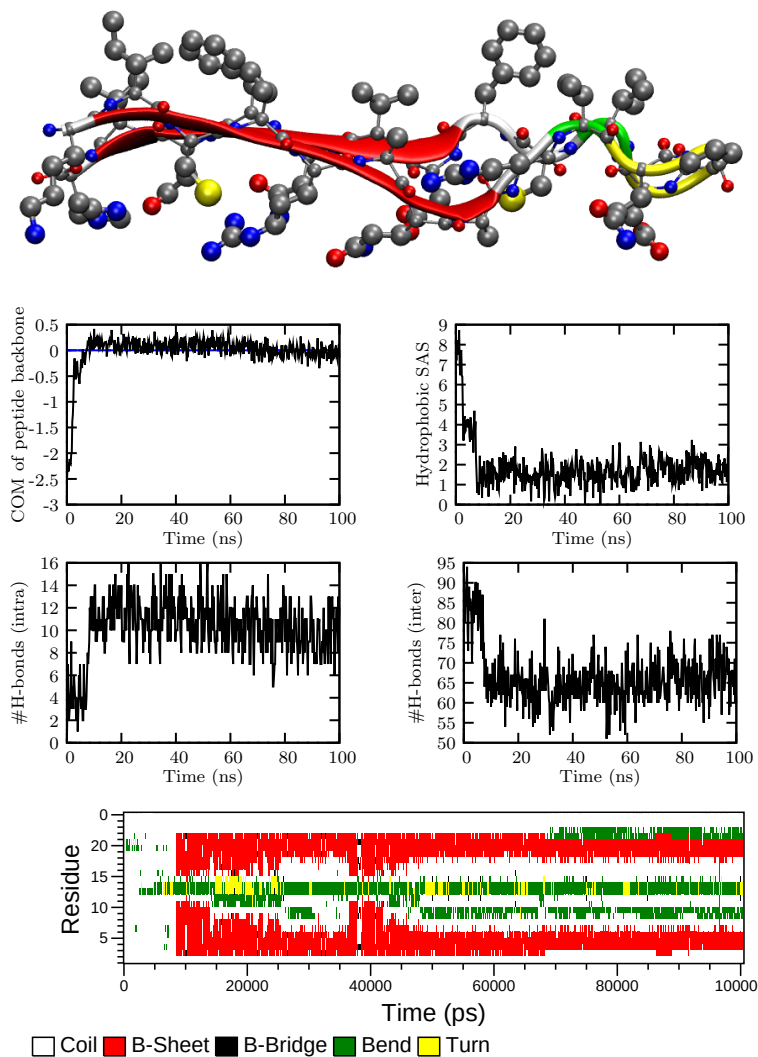


Figure 4.2: Adsorption of the peptide molecule to the air/water interface. Starting from the center of the simulation box, the molecule adsorbs to the interface within 10 ns as seen from the time evolution of the distance of the center-of-mass of the peptide from the upper air/water interface (top-left plot). Upon adsorption, the hydrophobic side groups are pushed out of water as evidenced by the reduction of the hydrophobic SAS (upper-right plot) and seen in the sideview of the molecule at the interface (top). As the molecule adsorbs to the interface it adopts a β -hairpin secondary structure (see secondary structure evolution data at the bottom obtained by DSSP algorithm). Adsorption and secondary structure formation lead to the replacement of peptide-water hydrogen bonds with intra-peptide ones (middle left and right plots).

chains between vacuum and water also favors β -hairpin structure, as evidenced by the dramatic reduction in the solvent accessible surface area (SAS) of the hydrophobic parts of the molecule (see Figure 4.2 upper-right plot). The number of hydrogen bonds made within the peptide and with water are also shown in Figure 4.2. Upon adsorption, the rise in intra-peptide H-bonds is a direct result of the formation of the β -hairpin structure at the interface.

After adsorption, the peptide remains at the interface during the course of the 80 ns simulation, which suggests that the adsorption free energy is much larger than the thermal energy. In order to quantify the free energy of adsorption at the interface we performed constraint pulling MD simulations. The starting conformation at each constraint point is chosen as a β -hairpin, otherwise the calculated free energy would have included energetic terms related to peptide folding as well. We did not put any constraints on the peptide to maintain the β -hairpin conformation during simulations. H-bonds formed between the strands are stable enough that once the peptide adopts the β -hairpin conformation, it does not unfold again at 298 K. In fact the β -hairpin conformation is stable up to 500 K.

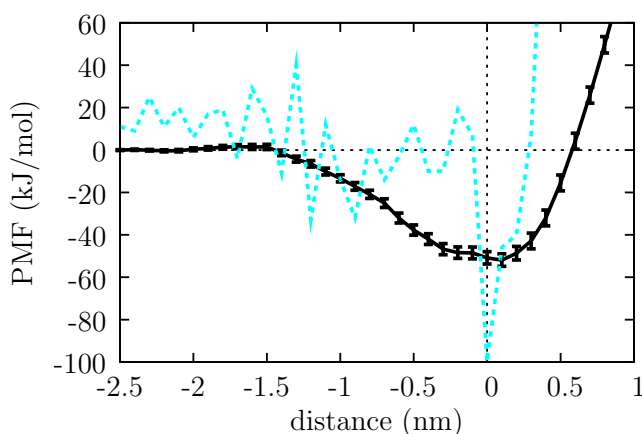


Figure 4.3: PMF (solid line) for adsorption of a single peptide molecule to the air/water interface as a function of the distance from the upper air/water interface. The enthalpic contribution, measured as the change in the average potential energy of the system, is also shown with dashed lines.

The PMF during adsorption is shown in Figure 6.5. PMF is measured as a function of the distance of the peptide center-of-mass from the top air/water interface. Flat region of the PMF corresponds to bulk water, where there is no preference for the orientation of hydrophobic/philic groups of the peptide. Beginning from -1.5 nm, the hydrophobic side chains of the peptide start to feel the presence of an interface, hence the molecule is drawn towards that direction. At the PMF minimum, the partitioning of the hydrophobic and hydrophilic sidechains is optimized with respect to the interface. As the molecule is dragged above the interface into the air, a sharp increase in the PMF is observed, since hydrophilic side chains strongly oppose this desolvation. Furthermore, pulling of the hydrophilic groups out of water leads to a dramatic disruption of the interface, with several water molecules bridging the gap between the molecule and bulk water. Eventually, when the molecule is completely in vacuum, one would expect a flat PMF profile. However, we did not drag the molecule so far out into vacuum.

Also shown in Figure 6.5 is the change in the internal energy of the system (dashed line). The zigzag pattern is closely related to the discrete nature of the hydrophobic sidechains of the molecule (see supporting information). Due to the rigid nature of the hairpin conformation, peptide molecule tries to optimize its conformation under the given constraint distance. Hence a fluctuating internal energy profile is observed. Interestingly, the internal energy more or less fluctuates around zero for distances as close as 0.1 nm to the interface, despite a large reduction in PMF beyond -1.5 nm, when the molecule touches the interface first time. Hence, we conclude that, reduction in PMF upon contact with interface should be mainly due to the increase in the entropy of the whole system. A sharp reduction in the internal energy is only seen, when the molecule's hydrophobic/philic parts are completely partitioned right at the interface.

Free energy of adsorption, ΔG^ℓ , is obtained via Equation 4.1 by integrating the PMF in Figure 6.5 based on a reference length of 1.5 nm. This length corresponds to the point where the PMF starts to decrease from its bulk value. Defining a free

energy for partitioning between the bulk and the interface is not straightforward since it depends on the amount of bulk that is present below the interface as we discussed in our previous study.[161] We determine the free energy with respect to a reference thickness of the bulk layer ℓ :

$$\Delta G^\ell = -k_B T \log \left[\frac{1}{\ell} \int_{\text{well}} \exp \left(-\frac{W(z)}{k_B T} \right) dz \right] \quad (4.1)$$

where $W(z)$ is the PMF with a reference value of $W(0) = 0$ in the bulk. The integration is performed over the PMF well. The precise boundaries of the well are not critical, because of the exponential weighting. This calculation yields $\Delta G^{1.5nm} = -50.29 \pm 2.94$ kJ/mol, showing that the adsorption of the amphiphilic peptide at the air/water interface is highly favorable.

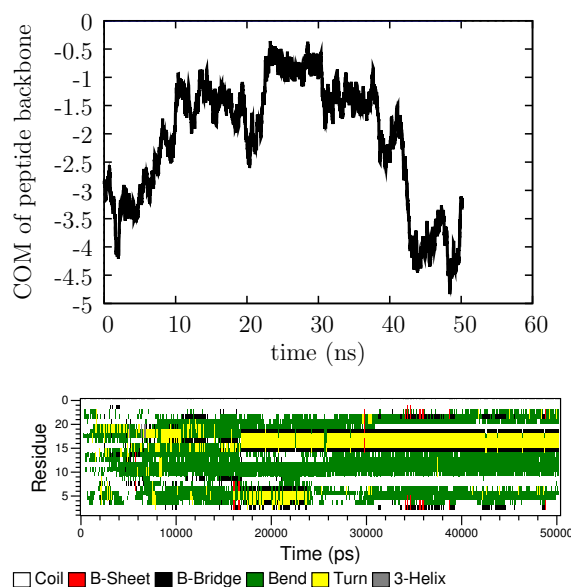


Figure 4.4: Mutation of the hydrophobic residues to glycine prevents adsorption to the interface, hence the center-of-mass of the molecule randomly diffuses within the water slab (top). This mutation also prevents formation of a stable secondary structure and molecule randomly switches between different conformations as seen in DSSP data (bottom).

4.2.2 Conformational Stability Against Mutations

We analyzed the importance of hydrophobic and turn residues on both the adsorption process and the formation/stability of the β -hairpin structure via *in silico* mutation experiments.

In order to investigate the influence of the alternating hydrophobic/hydrophilic residue sequence of the original molecule we mutated all the hydrophobic residues to glycine. As seen from the time line evolution of the center-of-mass of the peptide molecule (Figure 4.4), removal of the hydrophobic residues prevents adsorption of the peptide to the air/water interface. In addition, the molecule cannot adopt the β -hairpin conformation, even though the kinked geometry of the molecule is not disrupted. This is in agreement with our earlier observation that the stability of the β -hairpin structure is only achieved via confinement at the air/water interface.

The presence of the turn residues, ^DPro-Gly, is another key feature of the peptide's design. Mutation of these residues to alanine disrupts the kinked geometry of the peptide. Since the molecule still maintains its amphiphilic nature, the peptide adsorbs to the air/water interface within 8 ns as shown in Figure 4.5 top. However, in the absence of turn residues, the peptide cannot maintain the "ideal" β -hairpin structure, but samples a large number of conformations. Two of the possible conformations are also shown in Figure 4.5. Compared to the "ideal" β -hairpin structure, these conformations lack the stability and perfect registry of the original molecule's intramolecule hydrogen bonding.

4.2.3 Molecular Packing at the Interface

The magnitude of the adsorption free energy for the peptide molecules suggests that these molecules can form a monolayer film at the interface and thereby reduce the surface tension significantly. β -hairpin secondary structure and confinement to the interface significantly reduce the conformational flexibility of the individual molecules. Even in this fairly restricted conformational space, predicting the packing of peptide molecules within a surface monolayer is quite challenging. Strong adsorption free en-

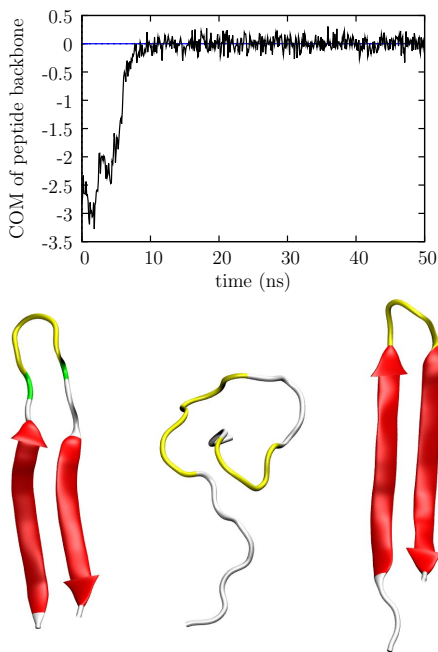


Figure 4.5: Mutation of the turn residues $^D\text{Pro-Gly}$ with two alanines lowers the stability of the β hairpin structure. The molecule still adsorbs to the interface within 8 ns as seen in the time evolution of the distance of the center-of-mass of the peptide from the air/water interface (top). β -hairpin structure of the original (bottom-left) molecule and representative structures obtained from the mutated molecule are shown together with the original (bottom middle and right).

ergy rules out conformations where hydrophobic side of the β -hairpin faces towards water. In trial runs we observed that starting from such a flipped conformation leads to an immediate back flipping of the molecule so that hydrophobic/philic partitioning with respect to the interface is restored (see supporting information). If one assumes that the neighboring molecules should be aligned with respect to each other, maximizing the inter-peptide hydrogen bonds, parallel and antiparallel orientations (Figure 4.6) remain as possible targets.

In order to compare parallel and anti-parallel orientations we calculated the potential of mean force (PMF) among two molecules at the air/water interface. In these calculations a pair of peptide molecules is placed at the air/water interface and the mean constraint force is calculated for fixed center-to-center spacing among them.

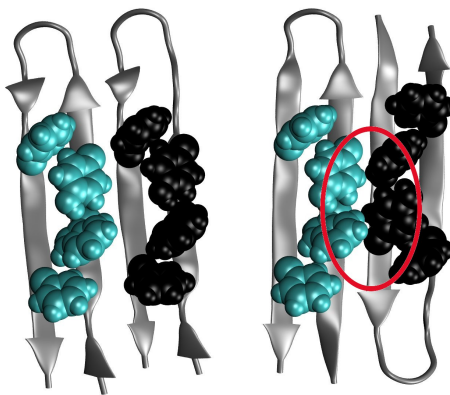


Figure 4.6: Parallel and anti-parallel packing of molecules at the interface (looking down from the air side). Aromatic sidechains of the peptides are shown with their Van der Waals radii.

The surface area for the interface is chosen large enough to ensure that PMF calculation is not biased by the influence of the periodic images. The constraint distance is increased until the peptides are completely isolated from each other and PMF curve is obtained by integrating over the constraint distance.

As seen in Figure 4.7 the parallel and antiparallel orientations lead to optimum spacings (PMF minimum) of 0.98 and 1.05 nm, respectively. Despite the larger spacing, the anti-parallel orientation is energetically more favorable compared to parallel one, with association free energy of 103.70 ± 0.05 kJ/mol for anti-parallel and 93.76 ± 0.02 kJ/mol for parallel orientation. The larger spacing of the antiparallel orientation is a result of the charge-charge interactions and packing of the aromatic sidechains. Due to the design of the peptide molecule the N-terminus arm is positively and C-terminus arm is negatively charged. Parallel orientation places the peptides such that a negative arm is adjacent to the positive arm of the neighboring peptide. In the anti-parallel orientation, however, the like-charged arms are placed adjacent to each other. Hence, in order to reduce the repulsion the center-to-center optimum spacing between the molecules remains slightly larger for the anti-parallel orientation. However, even for the anti-parallel orientation the closeness of these charged groups is not much of a problem for the peptides, since high dielectric constant of water and

1 nm center-to-center spacing among peptides leads to decreased repulsion.

As highlighted in Figure 4.6 the anti-parallel orientation leads to close contacts among aromatic sidechains in vacuum phase, which partially accounts for the lower association free energy of anti-parallel orientation. Also note that the association free energy is not only a function of the peptides, but also surface tension plays a role in determining its magnitude. The anti-parallel orientation leads to a slightly higher surface coverage ($10\text{-}12\text{ nm}^2$ per dimer) and therefore shields more water molecules from the interface. Hence, this increased shielding effect also contributes to the lower association energy among the peptides in the anti-parallel orientation.

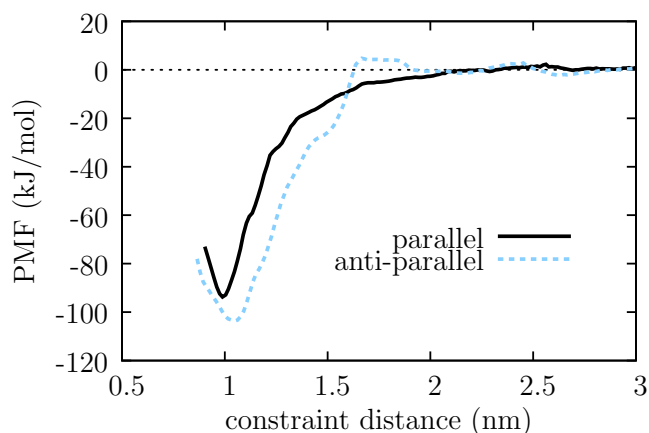


Figure 4.7: PMFs obtained from umbrella sampling simulations of parallel and anti-parallel packed molecules at the air/water interface.

One might argue that the optimum spacing among peptides might be influenced by the presence of other peptides surrounding them. In order to test this, we performed simulations with four peptides at the air/water interface for both parallel and anti-parallel orientations, and monitored the spacing between the two central peptides among the four. The average spacing obtained is identical to the results from the pair of peptide molecules, hence we conclude that the intermolecule spacing is not significantly influenced by the presence of neighboring peptides.

Note that the PMF curves also provide justification for the initial assumption

that the neighboring peptide molecules should be aligned with respect to each other. During the calculation, the peptide molecules are simulated at increasing constraint distances for the center-to-center separation. The PMF minimum is observed when the peptides are perfectly aligned with respect to each other.

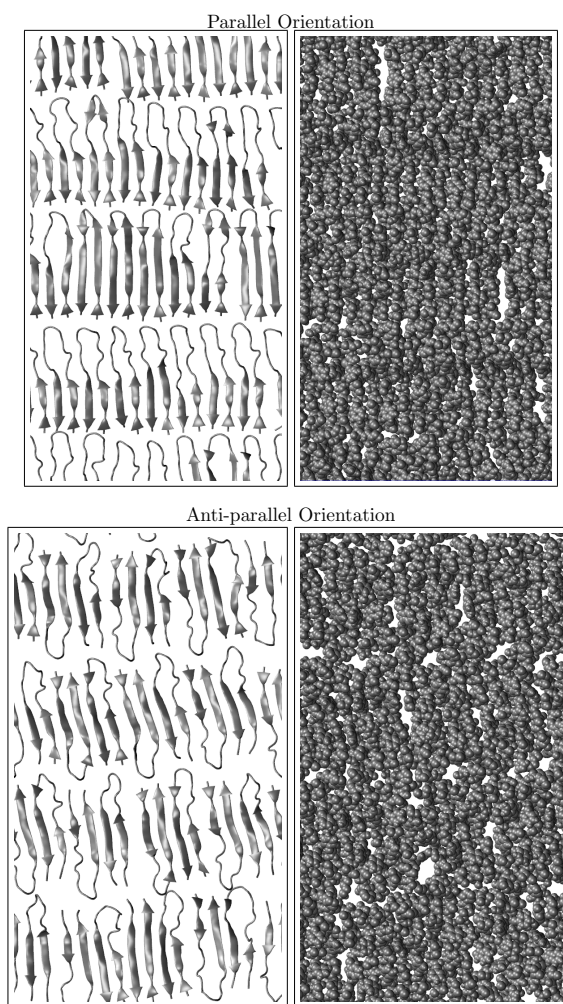


Figure 4.8: Monolayer of peptides in parallel (top) and anti-parallel (bottom) orientation at the air/water interface, where both systems are equilibrated with a lateral pressure of -60 bar. Only the molecules at the top layer of the water slabs are shown from the air-side of the interface.

In order to test the optimum packing distances obtained from PMF curves, we also performed simulations of a slab of water where the interfaces are covered with 32 peptides each. The molecules are initially aligned with respect to each other

into stripes, so that we have 8 molecules in each one of the four stripes. In order to allow fluctuations in box size, the simulations are performed with anisotropic pressure coupling. Starting from the anti-parallel system, we performed simulations of the system under different lateral pressure values. Note that, due to the anisotropic nature of these films pressure (and spacing) along the stripes of peptides and perpendicular to them should be independent from each other. However, for simplicity we applied the same pressure in both directions.

At a lateral pressure of $p_{xx} = p_{yy} = -60$ bar, the anti-parallel packed surface film displays a center-to-center spacing of 1.05 nm for the molecules, consistent with the PMF result. The average stripe thickness is obtained as 3.67 nm from this simulation. Next we applied the same lateral pressure (-60 bar) to the parallel system and this gave us a center-to-center spacing of 1.00 nm and stripe thickness of 3.55 nm. In Figure 4.8 equilibrium snapshots from these systems are shown for parallel (top) and anti-parallel (bottom) orientation. The box-sizes are shown to scale with the actual equilibrium dimensions. Only the upper interface is shown looking down from the air-side of the interface. The cartoon representation shows that in both cases, individual peptides preserve the β -hairpin structure. The space-filling representations, on the right, show that both systems have a similar surface coverage. Hence, we conclude that in agreement with the PMF analyses, the anti-parallel packing is less dense compared to parallel one. In general, the parallel orientation displays a much more ordered structure. Small patches of water are exposed to the interface for both orientations. For the lateral pressure of -60 bar, the partitioning of the sidechains is mostly preserved with only a few side chains flipping to the unfavorable (hydrophobic/hydrophilic) side of the interface.

4.3 Concluding Remarks

In this study, we analyzed folding of an amphiphilic peptide in the presence of the air/water interface. Molecular dynamics simulations of a single peptide molecule in a water slab show that, adsorption to the interface and adoption of the β -hairpin

secondary structure takes place simultaneously. Our earlier results [160] on conformational preference of these molecules in bulk water suggested that these molecules adopt a β -hairpin structure even without the presence of an interface. However, in this study, we have shown that the presence of the interface significantly accelerates adoption of β -hairpin structure and stabilizes it, by eliminating alternative conformations observed in bulk water.

Analysis of the adsorption free energy for these molecules suggests that the stiffness of the molecule determines the decomposition of the free energy into enthalpic and entropic terms. The molecule has a fairly rigid structure in the adopted β -hairpin conformation, due to the perfect alignment of the two halves of the molecule and continuous network of hydrogen bonds in between. This rigid nature and the alternating hydrophobic/hydrophilic residue sequence lead to a fluctuating internal energy and a SAS change during adsorption to the interface.

We also determined key design features required for adsorption and formation of the β -hairpin conformation. *In-silico* mutations showed that turn residues are essential to adopt the targeted β -hairpin conformation, whereas hydrophobic residues are required for adsorption and formation of the β -hairpin conformation at the air/water interface.

Next, we analyzed the possible arrangement of these peptides within a surface monolayer. The molecules lie flat at the interface to accomplish an optimal partitioning of the hydrophilic/phobic residues. PMF calculations among two peptide molecules at the interface suggest that these molecules prefer to be aligned with respect to each other, in order to maximize the inter-peptide hydrogen bonds. Comparison of the parallel and anti-parallel orientations reveals a slightly larger spacing among the molecules for the anti-parallel orientation. Hence, in the anti-parallel orientation more water molecules would be shielded from the interface. Anti-parallel orientation also displays a lower association free energy compared to parallel one, due to closer contact among aromatic residues of neighboring peptides.

These analyses based on a pair of peptide molecules at the interface and monolayer

simulations give us an insight regarding the actual packing of these molecules into continuous surface films. In order to determine the actual reduction in surface tension, further experimental input is necessary, which we hope to accomplish in a future study.

Chapter 5

STRUCTURE AND STABILITY OF NANOFIBERS FORMED BY AMPHIPHILIC PEPTIDES : MOLECULAR DYNAMICS COMPLEMENTS EXPERIMENTS

An increase in the number of individuals subjected to amyloid-related diseases, and progresses in nanotechnology, especially fabrication of nanomaterials, have drawn attention to the elucidation of the triggering mechanism for spontaneous self-assembly of peptide molecules. In terms of treatment, understanding the mechanism will help prevent protein aggregation that triggers many crucial diseases such as Alzheimer's [93, 94], Huntington's [95], diabetes [96, 97]. It will also help improve design strategies for synthesis of nanomaterials with desired properties, which have been used in a wide range of areas including tissue engineering [19], regenerative medicine [21], gene and drug delivery [22, 23], biosensors [24, 25] and hydrogels. [98, 99, 100]

Molecular frustration can be used to control dimensions of the self-assembled structure. In this approach, one can make use of opposing forces between two or more components of the assembling molecules such as attraction versus repulsion, opposing preferences for solvation environment, and/or ordered packing versus disordered one. [162, 163, 164, 165] Recently, nanofibers with controlled-dimensions have been synthesized by Dong *et.al* [4], in which "molecular frustration" is utilized to control the organization and the extent of the assembly. Fibers are composed of multi-domain peptides (MDP), each of which is organized into an ABA motif. In this motif, the central B block is composed of alternating hydrophilic and hydrophobic amino acids, glutamine (Q) and leucine (L), respectively [4] as shown in Figure 5.1, whereas A block is composed of positively charged lysine (K) residues. Alternation of hydrophobic and hydrophilic residues within the B block gives peptides their amphiphilic character.

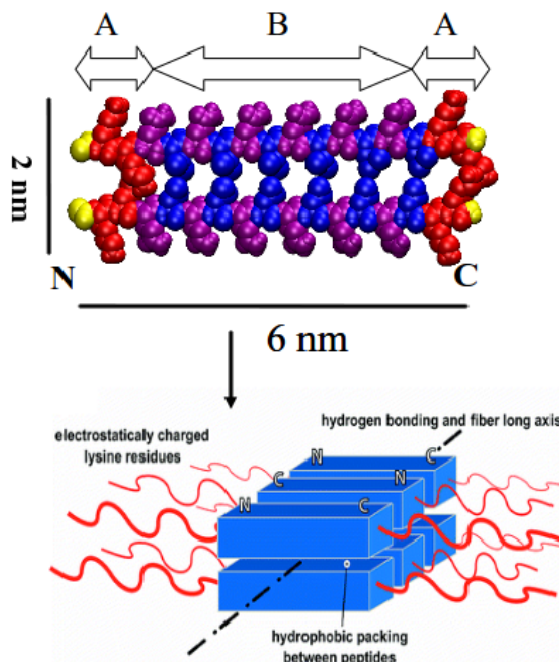


Figure 5.1: Organization of hydrophobic (shown in blue), hydrophilic (shown in purple), charged (shown in red) and capped residues (shown in yellow) is shown for the (QL)₆ dimer (top). Approximate experimental values for dimer length and width are also shown. Proposed model for organization of these dimers into nanofibers [4] along with hydrophobic packing region, and hydrogen bonding axis are also shown (below).

Such an organization allows hydrophobic and hydrophilic side chains to segregate on opposite sides of the peptide backbone when the peptide is in a fully extended β -sheet conformation. Extended conformation is further stabilized in an aqueous environment as a result of strong packing interactions among hydrophobic side chains. Moreover, it provides formation of intermolecular hydrogen bonds among neighboring MDP dimers which eventually grow into high aspect ratio fibers.

Experimental results suggest that there is a critical ratio between the number of QL units located in the B block, and the number of positively charged lysine residues located in the A block. When this ratio is equal to or bigger than 3, soluble fibers with controlled dimensions are formed.[4] Therefore, the smallest MDP that can self-assemble into soluble fibers with controlled-dimension is KK(QL)₆KK, which

is shown as (QL)₆ through the text. In the absence of positively charged lysines, insoluble fibers with infinite dimensions are formed. Therefore, repulsive electrostatic forces among lysine residues act against attractive forces formed among hydrophobic side chains, which eventually leads to formation of fibers with controlled-dimensions. Soluble nature of these fibers is rare among other systems forming β -sheet assemblies. In this respect, they can be used to treat diseases caused by protein aggregation, and they can also be used as scaffolds for bioengineering applications.

In this study, we investigated structure and stability of amphiphilic peptide nanofibers with all-atom MD simulations. Systems studied here differ from those studied in Chapters 3 and 4 in terms of environment (air/water interface vs. bulk water) and the system size (single peptides vs. pre-formed nanofibers). First, we tested whether the force-field is capable of representing the balance between repulsive electrostatic and attractive hydrophobic forces. Second, we mutated all the aliphatic leucine residues in the original MDP, (QL)₆, to aromatic ones such as phenylalanine (QF)₆, tryptophan (QW)₆, and tyrosine (QY)₆ to investigate how the fiber stability changes with respect to the aromaticity of the residue located within the B block. We also investigated the structural properties and the fiber strength of (QFQL)₃. We used simulated annealing (SA) algorithm to determine equilibrium structural properties of fibers such as inter- and intra-dimer spacing, and orientation of hydrophobic groups within hydrophobic core. Steered MD (S-MD) simulations were performed to address orientational preferences of MDP dimers within fibers. Finally, we investigated possible nucleation mechanisms for self-assembly of these MDPs into fibers via S-MD simulations.

5.1 Methods

MD simulations are performed with Gromacs 4.0 simulation package.[148] Temperature is kept constant at 298 K via Berendsen thermostat with a coupling time of $\tau_T = 0.1$ ps. For constant pressure simulations Berendsen barostat [79] is used with a coupling time of $\tau_P = 0.5$ ps.

GROMOS G53A6 force field,[149] which is parametrized to reproduce hydration

free energies of amino acid analogs, is used for peptides. Water molecules are modeled with SPC/E,[73] which is known to provide accurate hydration free energies. Bond lengths for water molecules and peptides are constrained via SETTLE [80] and P-LINCS [150] algorithms, respectively.

The particle mesh Ewald method (PME) [151] is used for electrostatic interactions with a real space cut-off of 1.0 nm and a grid spacing of 0.12 nm. Lennard-Jones interactions are calculated with a twin-range cut-off scheme of 1.0 and 1.4 nm with neighbor list updated every 10 steps. Dispersion correction is used for both energy and pressure beyond the long-range cut-off. A time step of 2 fs is used for the integration. Prior to production MD runs, systems are energy minimized via steepest descent algorithm to remove all possible steric clashes.

Ionization state of each residue is assigned according to pH 7.0 resulting in a total charge of +4 per peptide. N-termini and C-termini of individual peptides are acetylated, and amidated in accordance with experimental conditions. One counter-ion is added for each charged residue of the peptide to maintain the neutrality.

DSSP algorithm [152] implemented in Gromacs 4.0 package is used for secondary structure analysis. For hydrogen bond (H-bond) analysis angle and distance criteria of 30° and 0.35 nm are used, respectively. Visual molecular dynamics program [154] is used in preparation of all molecular graphics.

Due to limited structural data coming from experiments we start with determining some equilibrium structural properties of fibers such intra-dimer, inter-dimer spacings, and packing of hydrophobic side chains. To do that, we first put a dimer of MDPs on top of each other such that their hydrophobic sidechains are facing each other. Next, eight dimers are stacked along the fiber axis (z axis), where neighboring dimers form H-bonds. We use periodic boundary conditions along the z axis as shown in Figure 5.2 to eliminate end effects.

Semiisotropic pressure coupling is used, where we set the reference pressure values to 1, and 0 atm in x/y , and z directions, respectively to mimic infinite-length fibers. SA algorithm is applied to overcome local energy barriers. We perform a periodic

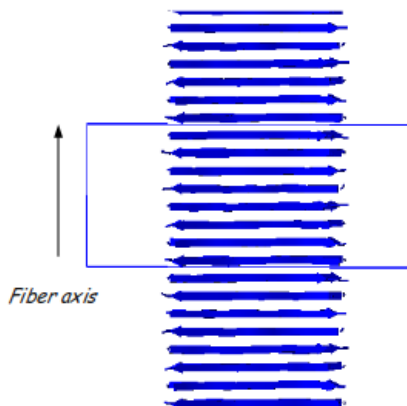


Figure 5.2: Schematic representation of the system with a periodicity along the fiber axis. Each arrow corresponds to a dimer. Boundaries of the simulation box is shown with blue lines. Periodic images are also shown.

annealing cycle, each of which is 2.7 ns. During each cycle, the system temperature is increased from 300 K to 550 K in 1000 ps, and kept constant there for 700 ps. Finally, the system is cooled down to 300 K in 1000 ps. z-dimension of the simulation box is initially set to 0.48 nm which is very close to the experimental value. Infinite-length fiber simulations are performed for both parallel and anti-parallel organization of dimers, and equilibrium structures obtained from these simulations are further used as initial structures for S-MD simulations, and simulations where we test the force field.

Preference of MDP dimers for parallel vs. anti-parallel arrangements are analyzed via S-MD simulations. Initial configurations (6 dimers, 12 MDPs in total) are taken from infinite-length fiber simulations as mentioned above. Pull direction is set along the fiber axis. Reference and pulled groups, which correspond to 3rd and the 4th dimers in the fiber, are pulled away over 400 ps until the center of mass of the two groups do not touch each other. A spring constant of 1000 kJ mol⁻¹ nm⁻², and a pull rate of 0.01 nm/ps are used. Changing the spring constant to 800 or 1200 kJ mol⁻¹ nm⁻² do not change the dissociation profiles of fibers. S-MD simulations are repeated ten times for both parallel and anti-parallel orientations.

In order to investigate possible nucleation mechanisms of MDPs into nanofibers we perform four sets of simulations, each of which is performed in the NPT ensemble. In the first set, we use a single dimer (2 MDPs in total) that packed on top of each other shielding hydrophobic side chains from aqueous environment. In the second set, we use one dimer and an additional MDP, whose backbone is aligned along the peptide that is part of the dimer. In the third and the fourth sets, we perform S-MD simulations with single dimers. Last two simulations differ from each other in terms of the initial organization of the two MDPs within the dimer. In one of the sets, the two MDPs are packed on top of each other such that hydrophobic side chains interact, in the other set, the two MDPs are aligned along their backbones.

5.2 Results and Discussion

5.2.1 Fiber Simulations with Changing QL and Lysine Block Size

Dong *et.al* have shown that fibers with controlled-dimensions are formed when m/n is ≥ 3 , where m corresponds to number of QL repeats in the B block, and n corresponds to number of positively charged lysine residues in the A block.[4] This is due to balancing of repulsive and attractive forces among the residues located in the A and the B blocks, respectively. When m/n is < 3 , which means that the total number of lysine residues is bigger than the total number of QL units, repulsive forces become dominant, and can not be balanced with attractive forces. Here, we test the stability of pre-formed (QL)₂ and (QL)₆ fibers via MD simulations. Simulation results show that strength of attractive forces among hydrophobic residues in the (QL)₂ peptide is not strong enough to balance repulsive forces among lysine residues. Therefore, the integrity of the fiber can not be maintained as shown in Figure 5.3. Note that, disruption of the fiber stability also causes a decrease in the β -sheet content as shown in Figure 5.3 (changing of arrows to rods).

However, strength of attractive forces increases in correspondance with an increase in the number of QL repeats (2-6). Repulsive forces among lysine residues can be balanced with attractive forces, and the integrity of the fiber is maintained as shown

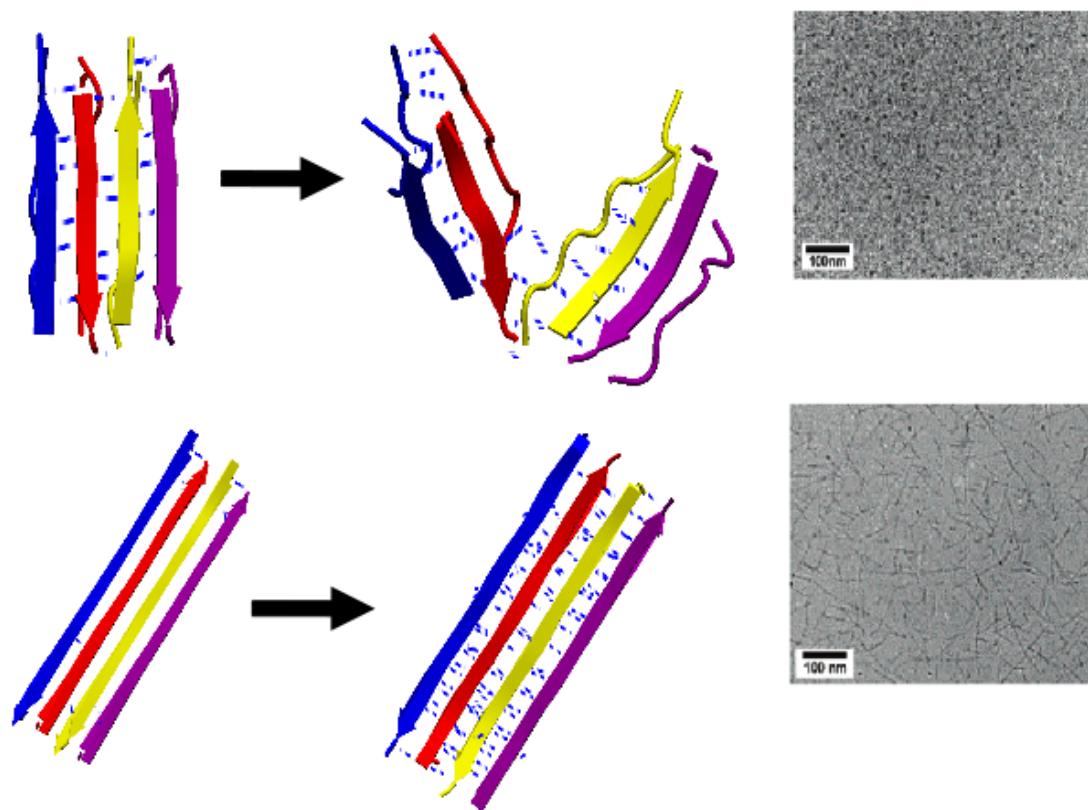


Figure 5.3: Top views of initial and last snapshots taken from MD simulations of fibers are shown for $(QL)_2$ and $(QL)_6$ peptides (left) together with their corresponding cryo-images [4](right). Each dimer is shown with a different color. H-bonds are also shown with blue dashed lines. Water molecules are omitted for clarity.

in Figure 2.3. These MD simulation results confirm experimental data, in which it has been shown that $(QL)_2$ show only random coil conformation, and form amorphous aggregates, and $(QL)_6$ show a strong β -hairpin conformation, and form a population of nanofibers with uniform diameter, and controlled-dimension as shown in Figure 5.3.

We also tested the effect of number of lysine residues on the fiber stability by changing the number of lysines from two ($KK(QL)_6KK$) to four ($KKKK(OL)_6KKKK$) at both termini. The fiber stability is lost as shown in Figure 5.4 (top) due to not balancing of repulsive forces among the positively charged lysines. On the other hand, the stability can still be maintained for fibers with four terminal lysines at

both termini by making these residues neutral as shown in Figure 5.4 (bottom).

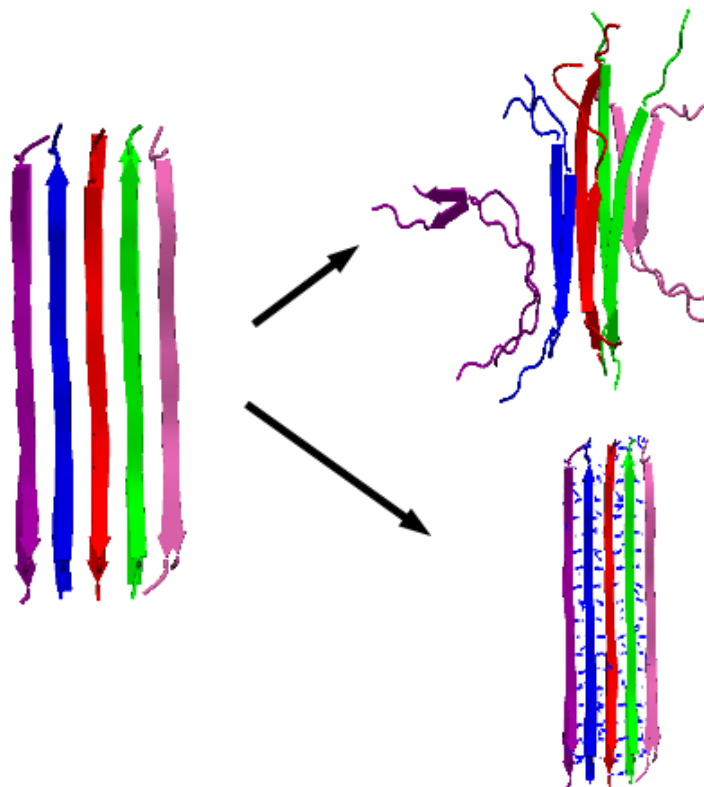


Figure 5.4: Top views of initial and final snapshots taken from MD simulations of fibers are shown for $(QL)_6$ when lysines are positively charged (top) and neutral (bottom). Each dimer is shown with a different color. H-bonds are also shown with blue dashed lines. Water molecules are omitted for clarity.

Note that, disruption of the fiber stability also causes a decrease in the β -sheet content as shown in Figure 5.4 (changing of arrows to rods) similar to the case of $(QL)_2$.

5.2.2 Fiber Simulations with Periodic Boundary Conditions

Due to mildness of temperature (300 K) and pressure (1 atm) values used and big system size there is always a danger of sticking in one of minima. We used SA algorithm to enhance sampling of the equilibrium state of the fibers. It is not guaranteed that the structural properties obtained correspond to equilibrium values, but anyway

we could perturb the initial values for the target structural properties as shown in Figure 5.5. In order to determine maximum temperature values in SA cycles we perform additional sets of simulations, where we increase the system temperature until the stability of fibers is disrupted. Around 600 K the fiber stability start to disappear due to an increase in the intra-dimer spacing values rather than inter-spacing ones, which are stronger due to H-bonding network. Therefore, we set the maximum temperature to 550 K in the SA cycles. We analyze inter-dimer spacing values, orientation of hydrophobic side chains, and number of H-bonds during SA cycles. Time-line inter-dimer spacing values and number of backbone H-bonds together with SA temperature cycles are shown in Figure 5.5.

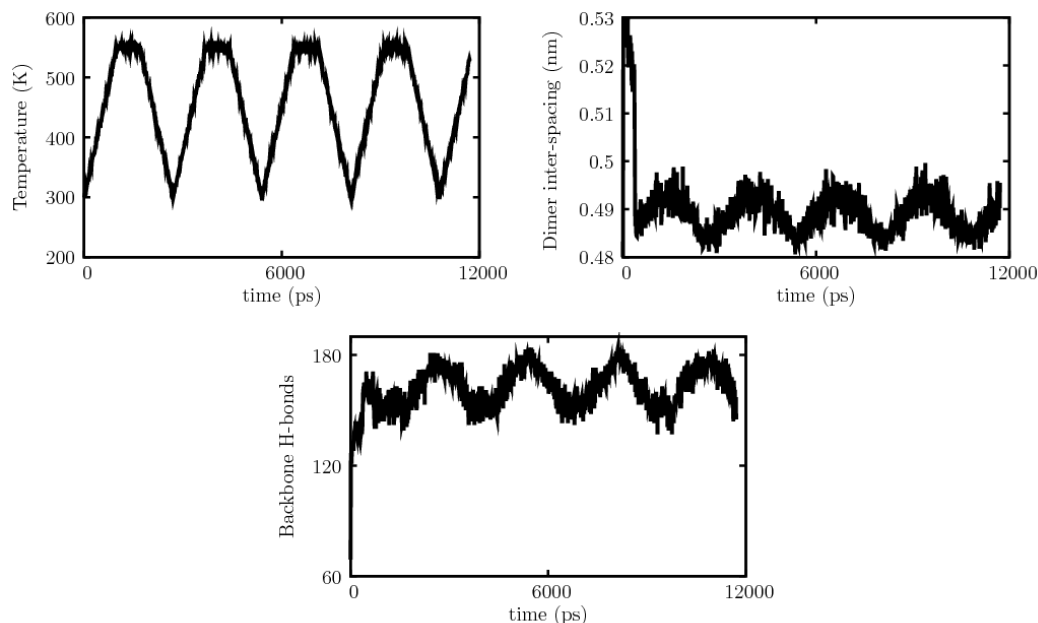


Figure 5.5: Simulated annealing cycles are shown for $(QL)_6$ (top,left) along with corresponding time-line data for inter-dimer spacing values (top,right), and number of backbone H-bonds (bottom).

First thing to notice in Figure 5.5 is that inter-dimer spacing and number of H-bonds change correspondingly with respect to temperature changes in the SA cycles. As the temperature increases the number of backbone H-bonds decreases as a result

of an increase in inter-dimer spacings. However, after each SA cycle inter-dimer spacing values return back to an average value of 0.48 nm, independent of the peptide sequence, which is consistent with the experimental value (0.475 nm). However, the intra-dimer spacing value fluctuates much more (data not shown) compared to the inter-dimer spacing during SA cycles, and it is dependent to the peptide sequence. We find the average inter-dimer spacing values as for 1.25, 1.45, 1.46, 1.53, and 1.71 for (QL)₆, (QF)₆, (QFQL)₃, (QY)₆, and (QW)₆, respectively. This shows that the inter-dimer spacing value is more rigid compared to intra-dimer spacing, and so it is conserved among all peptides due to H-bond network. On the other hand, the intra-dimer spacing is more flexible, and it is dependent on the type of the side chain of the hydrophobic residue within the B block. As the bulkiness of side chain increases the intra-spacing increases, correspondingly.

5.2.3 Determination of Inter-Dimer Packing

There are two possible orientations, parallel and antiparallel, for a dimer pair to pack against each other in the peptide fiber. In parallel orientation, peptide backbones are aligned such that N terminus of one dimer interacts with the N terminus of the neighboring dimer, whereas in the antiparallel orientation, backbones are aligned such that the N terminus of one dimer interacts with the C terminus of the neighboring dimer. In both organizations, backbone H-bonding is maximized, and hydrophobic side chains are packed against each other to be shielded from aqueous environment.

To analyze relative stabilities of parallel and antiparallel orientations we perform S-MD simulations for all types of peptide fibers. Representative initial and final conformations of the fiber obtained from S-MD are shown for (QL)₆ in Figure 5.6.

When the two groups are in contact (before pulling starts), the fiber stability is maintained via backbone H-bonds (shown with red, blue, and green dashed lines) and attractive hydrophobic forces among the residues in the central B block. Upon pulling we disrupt the H-bonding network formed within the fiber partially. H-bonds formed between the two groups are broken, whereas those formed within the two groups

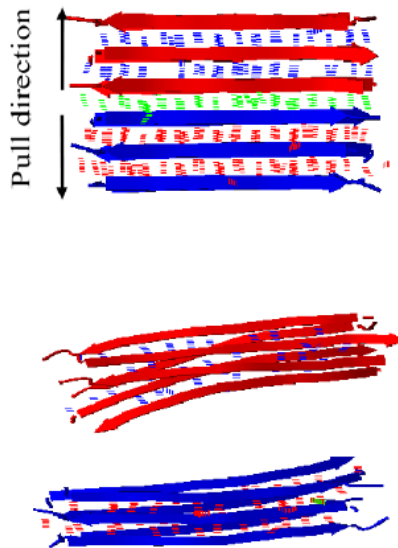


Figure 5.6: Pulling direction used in continuous pulling simulations is depicted for antiparallel (QY)₆ (top). Reference and pulled peptide groups are shown in blue and red colors, respectively. H-bonds formed between peptide backbones are shown in red, blue, and green dashed lines.

(reference and pull groups) are still maintained at the end of the pulling as shown in Figure 5.6. In addition, hydrophobic side chains of the 3rd and the 4th dimers are exposed to water at the end of pulling. Therefore, we measure contributions coming from H-bonding and hydrophobic forces to the fiber stability with these pulling simulations.

Work obtained from a S-MD simulation is a non-equilibrium work, in which individual force values are strongly dependent on the initial conditions. Therefore, we perform 20 independent pulling simulations for each peptide fiber (10x2 (parallel vs. antiparallel orientation)), each of which starts with a different velocity. We calculate average forces and associated standard errors for all peptide sequences and orientations as shown Figure 5.7. We find out that antiparallel orientation is preferred by (QF)₆, whereas parallel orientation is preferred by (QY)₆ and (QW)₆, which is consistent with the experimental data. For the (QL)₆, we show that the average maximum forces between the parallel and the antiparallel orientations are the same within the

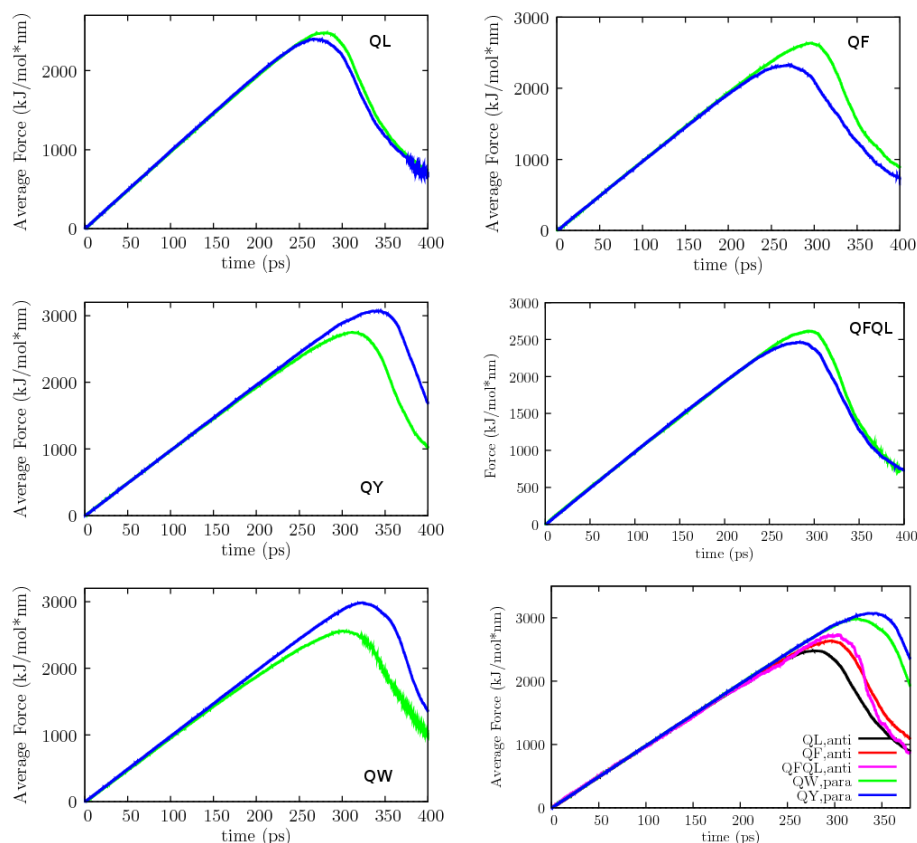


Figure 5.7: Average force curves for the parallel and antiparallel orientations are given for each peptide sequence (top, middle, bottom left). Average force curves for the preferred orientations of peptides are given (bottom right).

error bars (2544.4 ± 32.97 and 2466.3 ± 48.92 , respectively) showing that orientational preference of residues with aromatic side chains is much more pronounced than those having aliphatic side chains.

We also give average forces for the preferred orientations of fibers as shown in Figure 5.6 (bottom). These results suggest that the aromaticity and the bulkiness of the residue increases the strength of the hydrophobic interactions in the B block, which, in turn, leads an increase in the fiber stability.

Orientalional preferences of the peptide fibers are calculated from continuous pulling simulations, so these trajectories are not in equilibrium. In order to check whether the trend seen in the force values is also seen in the energy values of systems

that are in equilibrium we compare the total energies (LJ+Coulomb) of the systems (both in the parallel and the antiparallel orientations), where we use periodicity along the fiber axis, as shown in Table 5.1.

Table 5.1: Energy Differences between the Parallel and the Antiparallel Orientations of Peptide Fibers

Peptide	$(\Delta E_{para} - \Delta E_{anti})_{total}$ (kJ/mol)
(QL) ₆	2759
(QF) ₆	2741
(QFQL) ₃	523
(QY) ₆	-3404
(QW) ₆	-2358

The fibers composed of (QL)₆, (QF)₆, and (QFQL)₃ peptides are more stable in the antiparallel orientation, whereas (QY)₆ and (QW)₆ peptides are more stable in the parallel orientation as it is shown in the energy differences in Table 5.1. This is in agreement with the trend seen in the force values.

We further analyze the trajectories to understand the orientational preferences of peptides in terms of the packing of the side chains buried in the middle of the fiber. More prominent difference in the side chain packing is observed for the QY peptide as shown in Figure 5.8. This is more likely due to having a -OH group of TYR residues in their side chains, which makes the dipole orientation crucial. We show that the orientation of the successive dipoles within the fiber displays a more ordered head-to-tail orientation in the parallel orientation, whereas this orientation is less ordered in the antiparallel one, which may explain the orientational reference of the QY peptide to the parallel orientation. For the (QF)₆ and (QW)₆ peptides, side chains display a more ordered organization (edge-to-face) in the antiparallel and parallel orientation, respectively (data not shown).

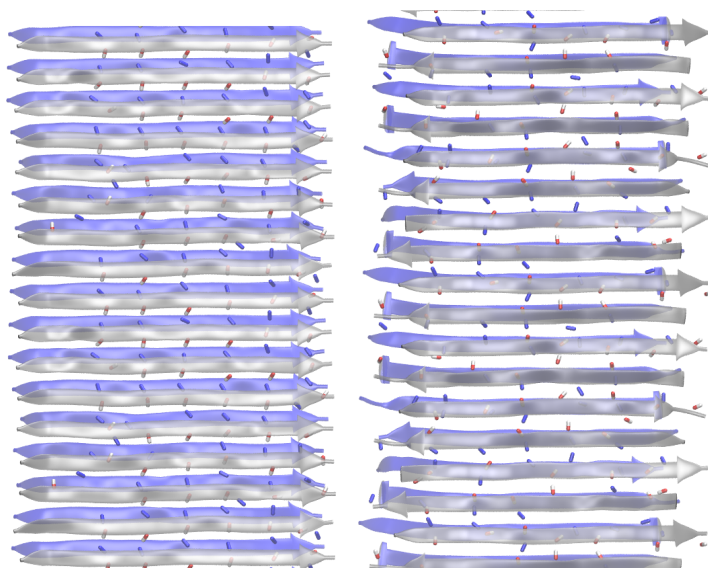


Figure 5.8: Relative orientations of dipoles between the successive -OH groups of TYR side chains in the parallel (left) and antiparallel (right) orientations. Upper layer of the fiber is shown in gray, and lower layer is shown in blue. -OH groups belonging to the lower layer are shown in blue, whereas those belonging to the upper layer are shown in red and white colors.

5.2.4 Fiber Nucleation

There are two possible mechanisms (dimer- and H-bond directed) proposed for the nucleation of individual MDPs into fibers. According to the dimer-directed mechanism individual MDPs are first packed on top of each other shielding hydrophobic groups from aqueous environment. Assembly of individual dimers take place thereafter via H-bonds formed among backbone atoms. On the other hand, according to the H-bond directed mechanism, individual MDPs are first aligned along their backbone atoms, and one of the layers of β -sheet is formed. Exposure of hydrophobic groups in that organization drives assembly of that layer with another pre-formed layer, and arrangement of hydrophobic side chains between these two layers occur thereafter. In order to determine which nucleation mechanism is more likely to occur we perform four simulations. In the first simulation, we perform an MD simulation on the single dimer. The two MDPs are packed against each other such that hydrophobic

side chains are shielded from the aqueous environment. Investigation of trajectories show that one of the MDPs in the dimer turns around to align its backbone along the backbone of the other MDP. In the new organization, the hydrophobic side chains are not interacting with each other and the number of backbone H-bonds is increased as shown in Figure 5.9.

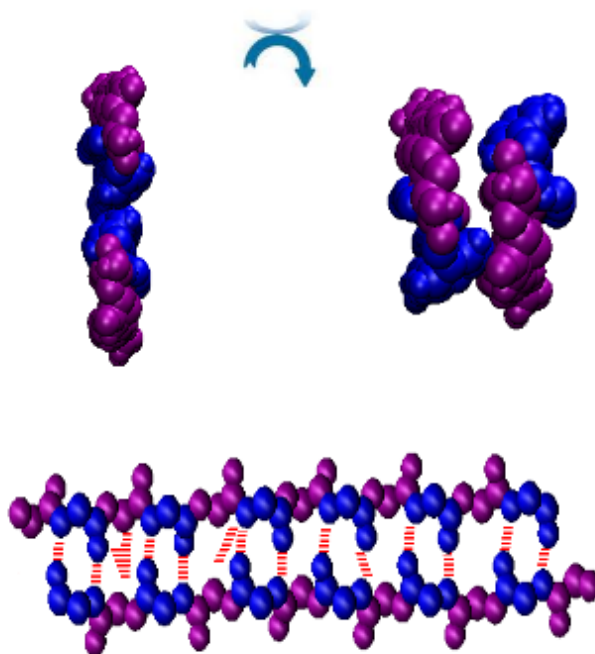


Figure 5.9: Initial and final organization of the two MDPs within the dimer (top). Top view of the final organization of the two MDPs within the dimer (bottom). Only backbone atoms are shown. H-bonds are shown with red dashed lines. Hydrophobic residues are shown in blue, whereas hydrophilic residues are shown in purple color. Water molecules are omitted for clarity.

In the second simulation, we perform simulations with three MDP molecules. We put an extra MDP next to the dimer such that newly added MDP makes backbone H-bonds with one of the MDPs that is part of the dimer as shown in Figure 5.10. We observe a similar behavior for this three-peptide system. The MDP that has no neighbor to make any H-bonding prefers to turn around to align its backbone along the backbone of the other MDP as shown in Figure 5.10.

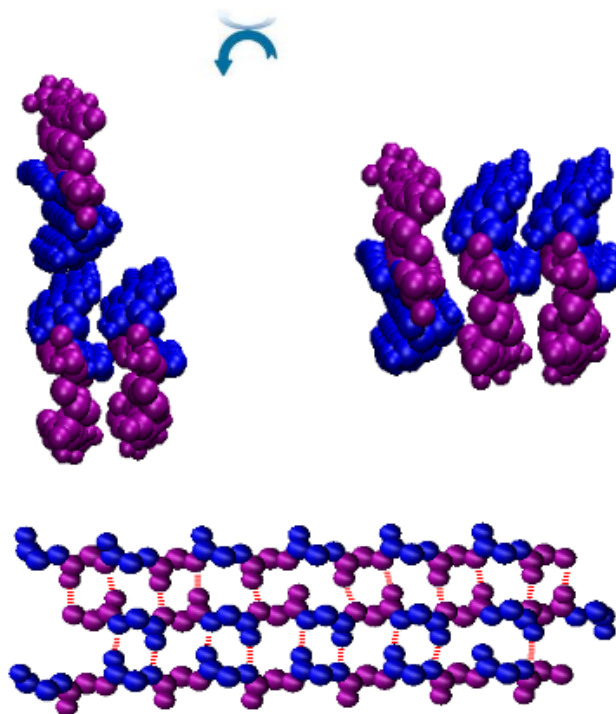


Figure 5.10: Initial and final organization of the three MDPs in the system (top). Top view of the final organization of the three MDPs (bottom). Only backbone atoms are shown. H-bonds are shown with red dashed lines. Hydrophobic residues are shown in blue, whereas hydrophilic residues are shown in purple color. Water molecules are omitted for clarity.

These results show that hydrophobic interactions are not strong enough to hold a lone pair of MDPs together, and an MDP that has no neighbor to make any H-bonds re-orient itself to find a peptide to make backbone H-bonds. In order to further determine the relative stabilities of backbone H-bonding and hydrophobic interactions on the dimer stability we perform two sets of continuous pulling simulations differing in the initial organization of the two MDPs within the dimer as shown in Figure 5.11.

In the first set the two MDPs are packed on top of each other shielding the hydrophobic groups from the aqueous environment, whereas in the second set the two MDPs are aligned along their backbones forming a H-bonding network. Continuous pulling simulations show that H-bonding network made among backbone atoms is

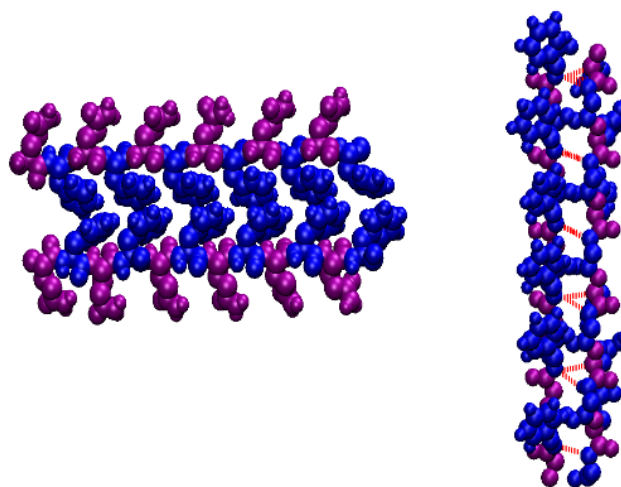


Figure 5.11: Initial and final organization of the three MDPs in the system (top). Top view of the final organization of the three MDPs (bottom). Only backbone atoms are shown. H-bonds are shown with red dashed lines. Hydrophobic residues are shown in blue, whereas hydrophilic residues are shown in purple color. Water molecules are omitted for clarity.

stronger than the hydrophobic interactions for the dimer as shown in Figure 5.12.

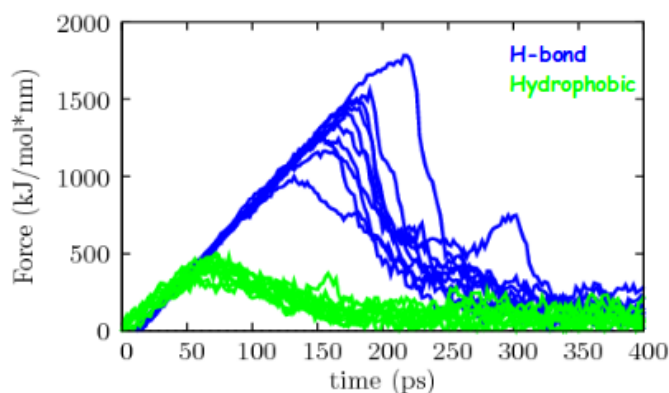


Figure 5.12: Individual force curves for the two sets of continuous pulling simulations. Force curves taken from the simulations, where the two MDPs are initially put on top of each other are shown in blue color, whereas those taken from the simulations, where the two MDPs are aligned along their backbones are shown in green color.

These results give an insight to the nucleation mechanism of individual MDPs

into fibers. If an MDP does not have any neighbors to make H-bonds it re-orientes its backbone to make H-bonds since the strength of hydrophobic interactions in the dimer structure is not strong enough to hold the two MDPs together. Therefore, it is more probable for individual MDPs to self-assemble into fibers via H-bond directed nucleation mechanism.

5.3 Concluding Remarks

We study structure and stability of nanofibers formed by amphiphilic peptides via atomistic MD simulations. We investigate how the ratio between the number of lysine residues and the number of QL repeats in the B block affects the fiber stability. We show that (QL)₂ repeat is not strong enough to balance the repulsive forces among the positively charged lysine residues, which eventually results in the disruption of the fiber stability. On the other hand, (QL)₆ is strong enough to balance the repulsive forces and the fiber stability is maintained in this system. We also analyze the effect of charge status of lysine residues on the fiber stability. We show that if neutral lysine residues are used (mimicking high pH conditions) the number of lysine residues is not crucial on the fiber stability. However, if one uses positively charged lysine residues the length of the lysine block becomes crucial for the fiber stability. These MD results confirm previous experimental data where it is shown that there is a critical value for the ratio between the number of QL repeats in the B block (m) and the number of lysine residues (n). When $m/n \geq 3$ soluble fibers with controlled-dimensions are formed. These results also show that the force field used, Gromos 53A6, can reproduce the balance between the repulsive electrostatic and attractive hydrophobic forces, which are the main forces that control fiber formation with controlled-dimensions.

In order to determine some of the equilibrium structural properties of fibers we use SA algorithm on the system where we use periodicity along the fiber axis. We show that inter-dimer spacing is independent of the peptide sequence; however, intra-dimer spacing is dependent on the peptide sequence. As the bulkiness and the aromaticity of the residues within the B block increase the intra-dimer spacing increases correspond-

ingly. On the other hand, inter-dimer spacing is found to be around 0.482 nm for all peptide sequences, which is in correspondance with the experimental result (0.475 nm). These suggest that organization of dimers within fibers is primarily determined according to the H-bonding distance, and residues with bulky side chains fit to this spacing by rearranging their intra-dimer spacings.

To investigate orientational preferences of dimers within fibers we perform continuous pulling simulations. We show that (QL)₆ and (QF)₆ dimers prefer the antiparallel orientation, whereas (QY)₆ and (QW)₆ dimers prefer the parallel one, which is in agreement with the experimental data. Detailed analyses of trajectories show that orientational preferences are guided primarily by packing of side chains of hydrophobic residues. The orientation of the successive dipoles within the fiber displays a more ordered head-to-tail orientation in the parallel orientation, whereas the orientation becomes less ordered in the antiparallel one, which may explain the orientational reference of the (QY)₆ peptide to the parallel orientation. The side chains of TRP residues of the (QW)₆ peptide, and PHE residues of the (QF)₆ peptide display a more ordered organization (edge-to-face) in the parallel, and antiparallel orientations, respectively.

Lastly, we investigate possible nucleation mechanisms for the fiber formation. We show that hydrophobic interactions within the B block are not strong enough to hold the two MDPs within a dimer, where they are packed on top of each other shielding their hydrophobic side chains. Instead, one of the MDPs in the dimer prefers to turn around to align its backbone along the backbone of the other peptide to maximize H-bonding network. We also perform continuous pulling simulations on dimers to investigate the relative strength of backbone H-bonding and hydrophobic interactions. We show that the force required to take the two MDPs apart is bigger when the backbones of the two peptides are aligned. These results suggest that it is more likely that individual MDPs self-assemble into fibers via H-bond directed nucleation mechanism.

Chapter 6

A CHALLENGE FOR PEPTIDE COARSE GRAINING: TRANSFERABILITY OF FRAGMENT-BASED MODELS

Peptide-based materials have gained attention in several fields from medicine to nano-technology. Among many other application areas, they are used in drug delivery,[166, 167] tissue engineering,[19, 20, 168] as biosensors,[169, 170] and nanotubes.[171, 172] Peptides do not only form well-ordered assemblies at the nano-scale, but also serve as excellent building blocks for bio-applications due to the ease of their synthesis, small size, chemical and biological modularity. Understanding physico-chemical principles underlying peptide self-assembly and aggregation is a fundamental step, in view of the rational design of new nano building blocks and drugs for biotechnological applications.

From the computational point of view, investigation of these processes in terms of structure and/or thermodynamics requires long time- and large length-scales, which are beyond atomistic simulations. Therefore, coarse-graining methodology has become an indispensable tool, which led CG models to be used in a wide range of areas such as peptide folding, self-assembly of peptide-based materials and in structure prediction algorithms.[173, 174, 175, 176, 177, 178, 179, 180, 41] In the context of peptide self-assembly it is important that a CG peptide model realistically describes both conformational flexibility (bonded interactions) and intermolecular interactions (non-bonded interactions), and that it reproduces both structure and thermodynamics. In comparison to isotropic homopolymer melts,[181, 182, 183, 184] CG modelling of polypeptides involves additional challenges due to their heteropolymer design with 20 different amino acid residues, with their own specific chemistry and level of conformational flexibility. The heterogeneous nature of peptides results in a vast number

of primary, secondary, and tertiary structures depending on the surrounding environment. This complexity makes the transferability or the generality of CG models extremely difficult for simulation of peptide-like systems. Here, “transferability” will be catalogued in two main groups: “thermodynamic” and “molecular-fragment”. A CG model is considered as “thermodynamically transferable” if it can be used for thermodynamic state points other than those against which it is parameterized for. On the other hand, “molecular-fragment transferability” refers to CG models where a set of molecular building blocks with predefined interactions are used to model any given peptide sequence.

In Chapters 4 and 5, we study structure and stability of preformed peptide monolayers and nanofibers rather than simulating the assembly of these nanostructures from scratch. Modeling the self-assembly itself is beyond all-atom MD simulations due to requirement of big system sizes and long time-scales, so an efficient CG model is required. Villa *et al.* [1] developed a fragment-based CG peptide model that can successfully model the self-assembly of a hydrophobic di-peptide, phenylalanine di-peptide (FF), in water. In this study, we investigated the “transferability” of CG parameters of this CG model to other hydrophobic di-peptides such as valine-phenylalanine (VF) and isoleucine-phenylalanine (IF). We employed the same CG strategy and used previously developed (bonded and nonbonded) potentials to model phenylalanine residue and backbone atoms. With this approach new potentials were only required for new molecular fragments, e.g. valine and isoleucine side-chains. By comparing atomistic and CG intra and intermolecular samplings we analyzed the transferability of the model. The hydrophobic di-peptides chosen for this study are known to self-assemble into microporous materials.[185] Hence, a measure of the success of the CG model would be its ability to capture the association behavior of these di-peptides. The results obtained from such simple peptide systems (di-peptides) should guide CG studies conducted on more complex peptide systems.

6.1 Methods

6.1.1 Mapping Scheme

The original FF CG model consists of seven spherical beads as shown in Figure 6.1 (top), which are located at the center of mass of the atoms that the bead represents. Labels for these seven beads and the chemical groups which they represent for FF can be summarized as follows:

- NH_3^+ : N-terminal bead
- $C\alpha\beta 1$: bead representing C_α and C_β atoms of the first residue of the di-peptide.
- Am : bead representing the amide group and two adjacent C_α atoms
- $C\alpha\beta 2$: bead representing C_α and C_β atoms of the second residue of the di-peptide.
- COO^- : C-terminal bead
- $Phe1$: bead representing the aromatic ring of first phenylalanine residue
- $Phe2$: bead representing the aromatic ring of second phenylalanine residue side-chain

For VF and IF di-peptides, except for interactions that involve the sidechain beads of the first residues, the bonded and nonbonded interaction parameters can be transferred from the FF CG model. Hence, we maintain the seven bead CG model for VF and IF, with the following changes for the sidechains of first residues. For VF the $Phe1$ sidechain bead is replaced by a Met bead to represent C_β and two C_γ atoms of the valine residue (Figure 6.1 middle). For IF the $C\alpha\beta 1$ and $Phe1$ beads of the FF CG model are replaced by $Eta1$ and $Eta2$ beads (Figure 6.1 bottom) where the former represents the C_α , C_β and C_{γ_2} atoms, and the latter represents C_{γ_1} and C_δ .

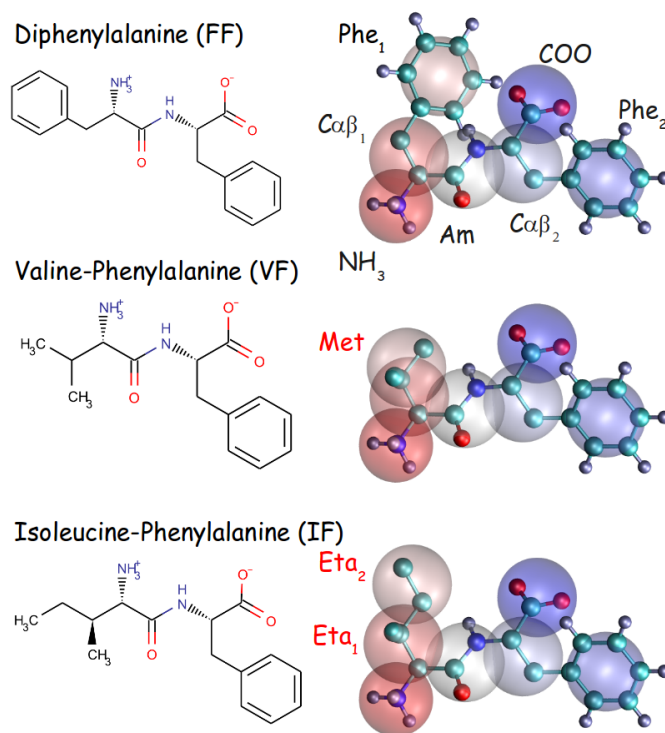


Figure 6.1: Chemical structures (left) and CG mapping schemes (right) for FF, VF and IF di-peptides. Seven CG beads are located at the center of mass of the corresponding united atom groups for each peptide as defined in the text. Interaction parameters for CG beads are transferred from the FF CG model, except for interactions that involve the red labeled beads in VF and IF which have been specifically parameterized.

6.1.2 Bonded Interaction Potentials

In this study, bonded interaction potentials for the CG model are obtained from atomistic simulations of a single di-peptide molecule in water. Here, we briefly summarize the procedure, and we refer the reader to the original FF CG model [1] for details. Using the atomistic trajectory and atom group definitions for each CG bead, first the probability distributions associated with all CG bonded interactions are obtained. Next, the probability distributions are Boltzmann inverted to obtain the corresponding bonded interaction potentials according to Equation 6.1, 6.2, and 6.3.

$$U^{CG}(r, T) = -k_B T \ln (P^{CG}(r, T)/r^2) + C_r \quad (6.1)$$

$$U^{CG}(\theta, T) = -k_B T \ln (P^{CG}(\theta, T)/\sin(\theta)) + C_\theta \quad (6.2)$$

$$U^{CG}(\phi, T) = -k_B T \ln P^{CG}(\phi, T) + C_\phi \quad (6.3)$$

For the CG molecular dynamics simulations, angle and dihedral potentials are given in the tabulated form, whereas harmonic potentials are fitted to the inverted bond length distributions. For VF and IF all bonded interaction parameters, except for the interactions involving valine and isoleucine sidechain beads, are taken from the FF CG model. On the other hand, interactions involving valine and isoleucine sidechain beads are obtained from atomistic simulations of VF and IF, respectively.

It is not guaranteed a priori, that these bonded CG potentials perfectly reproduce the sampling of the target system. We also assume that bonded and non-bonded interactions are decoupled from each other, and therefore they can be parameterized separately. The validity of this assumption has been shown to work for modelling bonded interactions of amorphous polymer systems.[183, 186] With this assumption, bonded CG interactions are developed by excluding non-bonded interactions that are beyond the limit of a CG torsion from the sampling of the (atomistic) reference distributions. Similarly, in the development of nonbonded interactions, to avoid double counting, interactions within the range of the bonded interactions are excluded. This assumption needs to be carefully assessed for biomolecules, especially for peptides, where hydrogen bonding and solvation effects drastically influence the conformational sampling. Indeed, Villa et al.[1] showed that non-bonded interactions are also required for reproducing target bonded interactions. Therefore, as in the original FF model, for VF and IF, we do not exclude long-range non-bonded interactions in the atomistic sampling while deriving CG bonded interactions.

6.1.3 Non-bonded Interaction Potentials

Non-bonded interactions play a two-fold role in defining the conformational space of molecules. Their major contribution is seen in determining the relative orientation and interaction energy of separate molecules. However, they also play a role in defining the conformational space of single molecules, especially for long and flexible molecules. As explained above, for peptides due to the specific role of H-bonds in determining secondary structure, one cannot neglect this coupling.

For the original FF CG model, small-molecule analogues of fragments were used in determining the non-bonded interaction potentials among CG beads. Analogue molecules used for the FF model are: methylammonium for NH_3^+ , methane for $C\alpha\beta1$ and $C\alpha\beta2$, methylacetamide ion for Am , acetate ion for COO^- , and benzene for $Phe1$ and $Phe2$ CG beads. Methane is used for the *Met* bead of VF, whereas ethane is used for the *Eta1* and *Eta2* beads of IF.

CG non-bonded potentials are obtained from potentials of mean force (PMFs) calculated between all possible analogue molecule pairs. We calculate average forces between solute pairs, while constraining the center of mass separation distances between the analogue groups. Next, we obtain PMF curves by integrating the average forces. With this approach we also aim to include the effect of the solvent within the pair potentials.

Since all small-molecule analogues used for VF are already included for FF, non-bonded interaction parameters transferred from FF are sufficient for the VF CG model. However, for the *Eta1* and *Eta2* beads, PMF curves for ethane with all other small-molecule analogues used in the CG model of IF are obtained by performing constraint molecular dynamics simulations. PMFs are directly used as tabulated non-bonded potentials for modelling interactions between CG beads corresponding to hydrophobic and/or polar beads. However, for beads with a net charge the contribution from the electrostatic interactions is subtracted from the PMFs upto the cut-off distance, and they are included via Particle-Mesh-Ewald method during the simulation. [187, 188]

6.2 Results and Discussion

In this section, we evaluate the transferability of the original CG model interaction potentials developed for FF. First, we analyze the bonded interaction distributions and correlations among them. Next, we analyze the inter-peptide PMF curves obtained from constrained simulations of two di-peptides via AA and CG models. We compare both the PMF curves and also the relative orientations of the di-peptides with respect to each other during the constrained simulations.

As our goal in this study is to evaluate the transferability of the CG interaction parameters from FF CG model, in the following analyses we judge the success of the VF and IF CG models by comparing them with the original FF CG model results.

6.2.1 Intra-peptide Distributions

For the VF and IF CG models, a total of six bond, seven angle, and six dihedral angle potentials, including improper torsions, are needed. One can categorize these into two main groups: potentials transferred from FF CG model and those specifically derived for VF/IF di-peptide. In Figure 6.2 the distribution of selected bond angles and dihedrals are given from both of these groups. Remaining angle, dihedral, and bond distributions (which are not shown) display a qualitatively similar behavior. The AA data (solid lines) are obtained from simulations of a single di-peptide in explicit solvent, whereas the CG data (dots) are obtained from simulations of an isolated CG model peptide in implicit water. Transferred potentials are shown in black, whereas specifically derived potentials are shown in red color.

As seen in Figure 6.2, AA and CG angle and dihedral distributions match qualitatively well for VF and IF. Although the relative weights between the multiple peaks and broadness of the distributions are slightly off, positions of maxima and general structure of the distributions are recovered successfully. These potentials were obtained via Boltzmann inversion of the atomistic distributions, and most likely one can improve the matching via iterative Boltzmann inversion.

The overall agreement among the transferred and specifically derived potentials

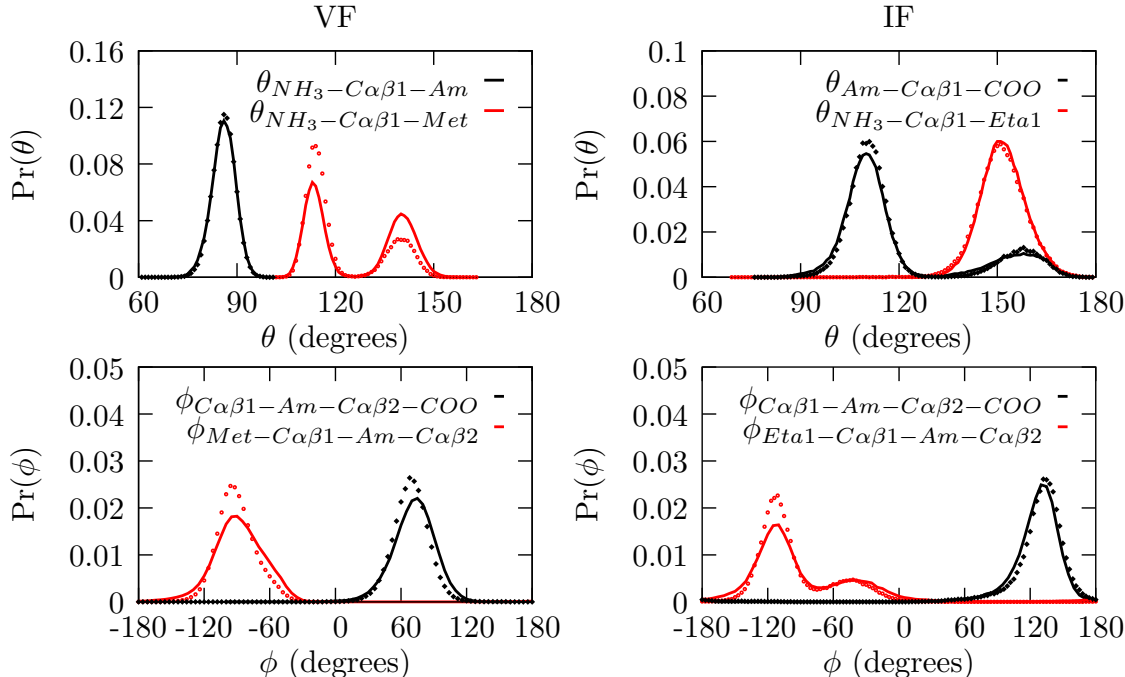


Figure 6.2: AA and CG probability distributions for selected angles and dihedrals of VF (left) and IF (right) di-peptides. AA and CG data are shown with solid lines and dots, respectively. Both the potentials transferred from FF (black lines and dots) and potentials specifically derived for the corresponding di-peptide (red lines and dots) reasonably represent the relevant degree of freedom.

suggests that the original CG model, methodology and assumptions made in the derivation of bonded and non-bonded interactions work successfully for modelling VF and IF, as was observed for the FF CG model. We conclude that transfer of CG potentials among hydrophobic di-peptides is successful, which suggests that similar groups in different molecules display similar conformational preferences.

In both AA and CG simulations the degrees of freedom represented via different potential terms in bonded interactions could be correlated. The characteristic distribution in the Ramachandran map for $\phi-\psi$ angles in peptides is a well known example of this coupling. Since one can not eliminate this coupling, an important test for the CG bonded potentials is to check whether they display similar correlation behavior as in AA system. This was shown to be the case for the original FF CG model. Here,

since we aim to test the transferability of CG model potentials from FF to other hydrophobic di-peptides, we also judge the results by comparing the correlations among transferred and specifically derived potentials.

In Figure 6.3 comparison of AA (left) and CG (right) sampling of the free energy landscape is given for two different angle-dihedral pairs. At the top, the Boltzmann inversion for the joint probability distribution of $\text{NH}_3\text{-C}\alpha\beta 1\text{-Met}$ angle and $\text{NH}_3\text{-C}\alpha\beta 1\text{-Am-C}\alpha\beta 2$ dihedral is shown. The angle $\text{NH}_3\text{-C}\alpha\beta 1\text{-Met}$ is specifically derived for VF CG model, since CG bead Met is not present in FF CG model. The dihedral $\text{NH}_3\text{-C}\alpha\beta 1\text{-Am-C}\alpha\beta 2$ was derived for FF and is one of the transferred potentials for VF. The AA and CG correlation plots for angle $\text{NH}_3\text{-C}\alpha\beta 1\text{-Met}$ and dihedral $\text{Met-C}\alpha\beta 1\text{-Am-C}\alpha\beta 2$ shown at the bottom, display the coupling among two of the specifically derived potentials for VF CG model, since both the angle and dihedral involve the Met CG bead which is only present in VF di-peptide.

As seen in Figure 6.3, CG potentials reproduce the correlations observed in AA system qualitatively well. Here, only two of these correlation plots are shown for VF; however, the results are similar for the remaining pairs of bonded interactions of VF and IF. These results suggest once again that the transferred and specifically derived bonded potentials work in harmony for VF and IF. As in the case of the original FF CG model, for VF and IF the developed CG potentials neither forbid sampling of atomistic conformations nor introduce unphysical correlations.

Finally, we analyze two additional distributions, end-to-end distances and side-chain separations, which are not explicitly represented by the bonded interactions of the CG model. In Figure 6.4 probability distributions of these structural properties are shown for VF and IF. AA and CG data are displayed with straight and dotted lines, respectively. Here, the AA and CG distributions are qualitatively in good agreement. One can note that the smaller peak of AA side-chain separation distribution at large distance and the sharpness of the AA end-to-end distance distribution are not fully captured by the CG model. Both of these quantities provide overall measures for the conformational preference of the peptide and they are not directly

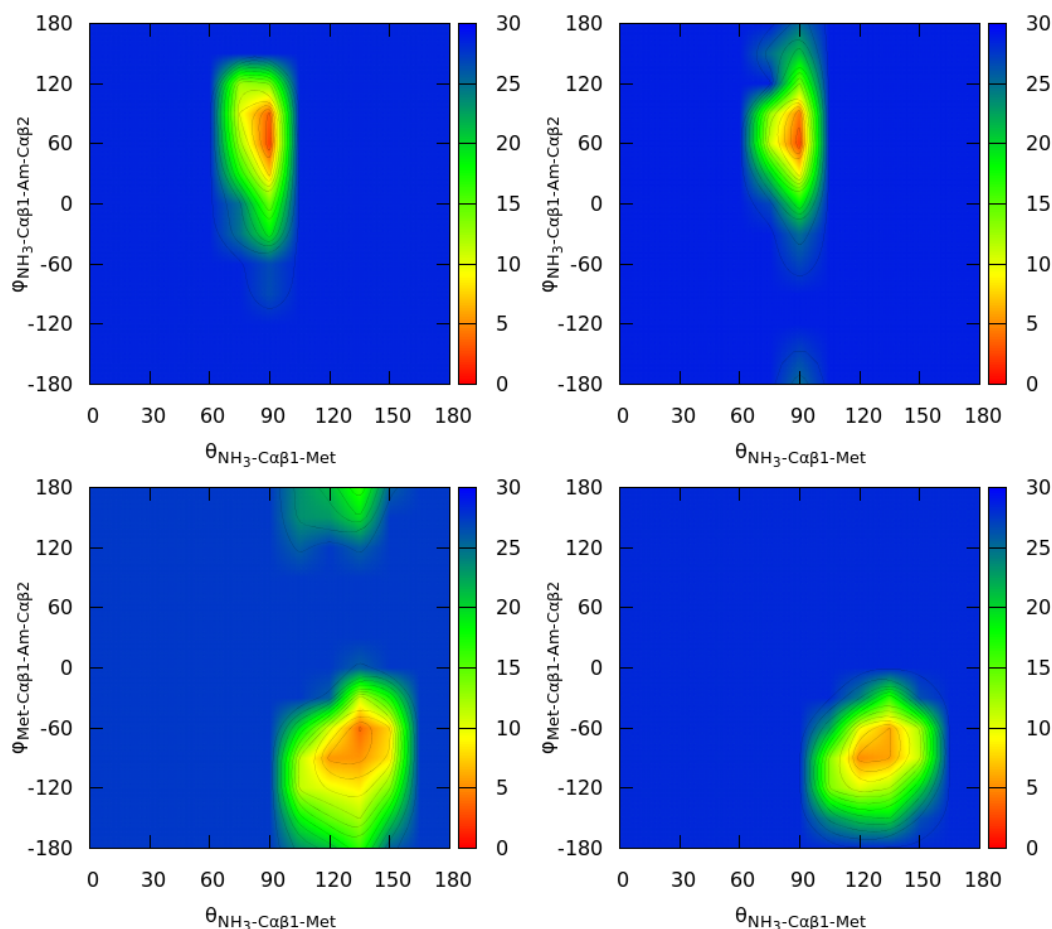


Figure 6.3: AA (left) and CG sampling (right) of the free energy landscape of the $\text{NH}_3\text{-C}\alpha\beta 1\text{-Met}$ angle, and $\text{NH}_3\text{-C}\alpha\beta 1\text{-Am-C}\alpha\beta 2$ dihedral angle (top), and $\text{NH}_3\text{-C}\alpha\beta 1\text{-Met}$ angle and $\text{Met-C}\alpha\beta 1\text{-Am-C}\alpha\beta 2$ dihedral angle correlation plots (bottom). Free energy is given by $-k_B T [\ln P(\theta, \phi) - \ln P_{min}]$ where $P(\theta, \phi)$ corresponds to the probability of finding a conformation with values (θ, ϕ) for two corresponding degrees of freedom, and P_{min} is the population probability of the global minimum. $\sin \theta$ normalization is applied to angles. Angles are given in degrees, and free energy is in kJ/mol.

associated with any particular interaction. Therefore, improving the match requires an overall enhancement of the bonded and non-bonded interaction potentials. As a first attempt, one might consider improving the bonded interactions via iterations, which we hope to accomplish in future studies. Correlations among degrees of freedom along the chain might also be responsible for these deviations. In that case, additional potential terms might be necessary to reproduce chain conformations.

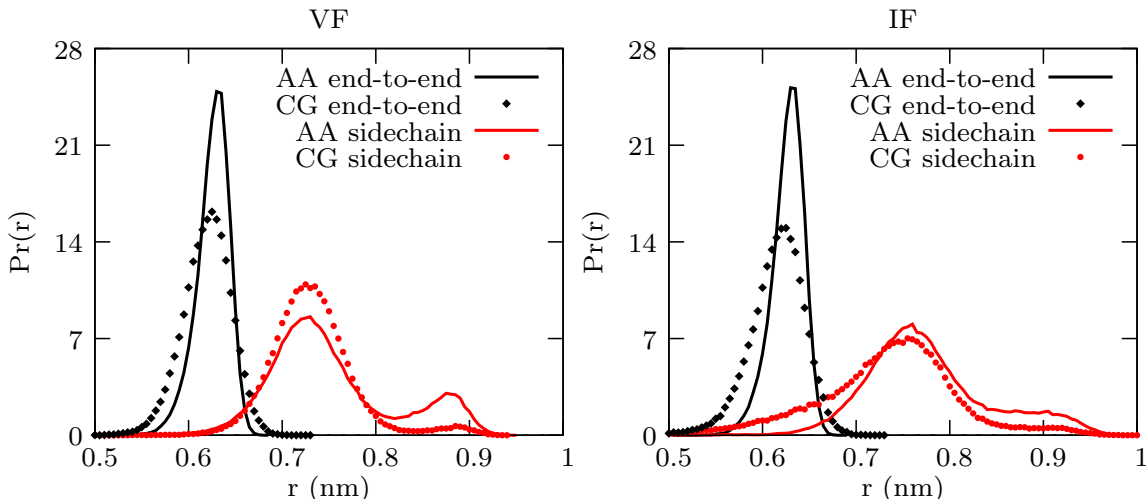


Figure 6.4: Comparison of AA and CG probability distributions for end-to-end distance and side-chain separation for VF (left) and IF di-peptide (right). AA end-to-end and side-chain distance distributions are shown with black and red solid lines, whereas black and red dots are used for CG model data.

6.2.2 Inter-peptide PMFs and Orientational Distributions

In this section we analyze the CG models for VF and IF in terms of their ability to represent intermolecular interactions and distributions in comparison to AA simulations. Here, the non-bonded interactions dominate, this also serves as a means to measure the success of the small-molecule analogue approach used in derivation of these interactions.

First, we compare the PMF curves obtained from AA and CG simulations (Figure 6.5). The data for the following analysis is gathered from simulations of two peptides, where the center of mass distance separation among them is constrained for sampling purposes. AA simulations are performed with SPC/E explicit water, whereas CG simulations are performed at constant relative dielectric constant of 72 (electric permittivity of the SPC/E water model). We measure the average force required to maintain a fixed constraint distance among the peptide molecules and obtain the PMF curves by integration of the average force at each constraint distance.

As seen in Figure 6.5 the CG model successfully captures the hydrophobicity

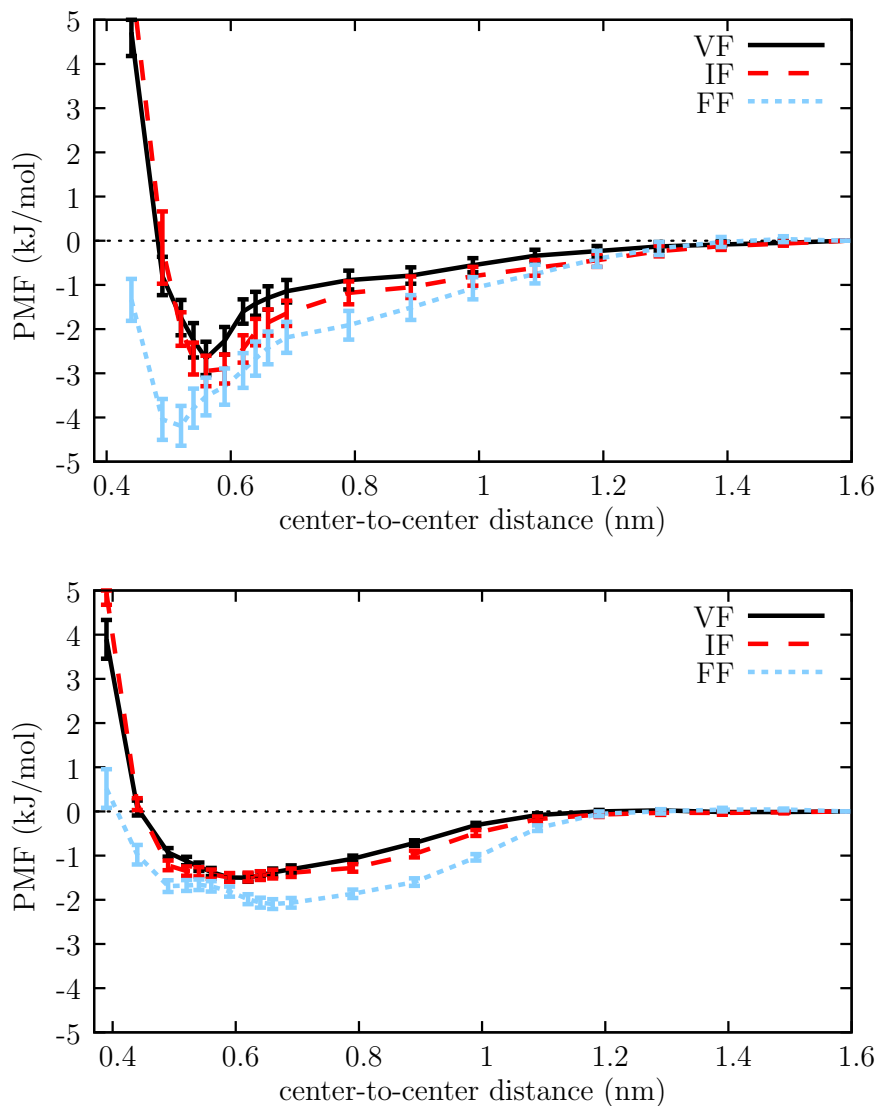


Figure 6.5: Comparison of AA (top) and CG PMFs (bottom) among three hydrophobic di-peptides, VF, IF and FF, as a function of the distance between the center of mass of whole peptides.

scale of the di-peptides FF, IF and VF as observed in AA simulations, and also in experiment. The results are in good agreement for constraint distances down to 0.8 nm. However, specific interactions in atomistic simulations at closer separations can not be reproduced by the CG model. This is not surprising considering the fact that the CG beads are spherically symmetric and so interactions among them

Table 6.1: Atomistic and CG association constants for di-peptides calculated according to Equation 6.4.

Di-peptide	AA (nm ³)	CG (nm ³)
VF	19.22	18.46
IF	20.72	18.97
FF	22.98	20.72

are isotropic. As typically seen in CG simulations, here also the nonbonded CG potentials are softer compared to AA analogues, and this significantly influences the PMF minima obtained. CG PMFs are less attractive than their AA counterparts for distances smaller than 0.8 nm.

In order to quantify the difference between the AA and CG PMFs, we also calculated peptide-peptide association constants using Equation 6.4.

$$K_a = 4\pi \int_0^{R_{max}} r^2 \exp(-V_{PMF}(r)/k_B T) dr \quad (6.4)$$

where $R_{max} = 1.6$ nm. CG association constants are found to be 4-8% lower than those of their AA counterparts as seen in Table. 6.1 for all three di-peptides. Even though the CG model slightly underestimates the affinity between the di-peptides, we observe that the approach used to derive non-bonded bead-bead interaction potentials from corresponding small-molecule analogues successfully captures the trend in association constants.

We further compare two structural properties, distance distribution between phenylalanine side-chains (Figure 6.6) and relative orientations of the two peptides (Figure 6.7) at selected constraint distances of the PMFs. Since these distributions are not controlled via any explicit potential in the CG model, they can be used to assess the success of the CG model in reproducing bonded and non-bonded interactions in the AA target system.

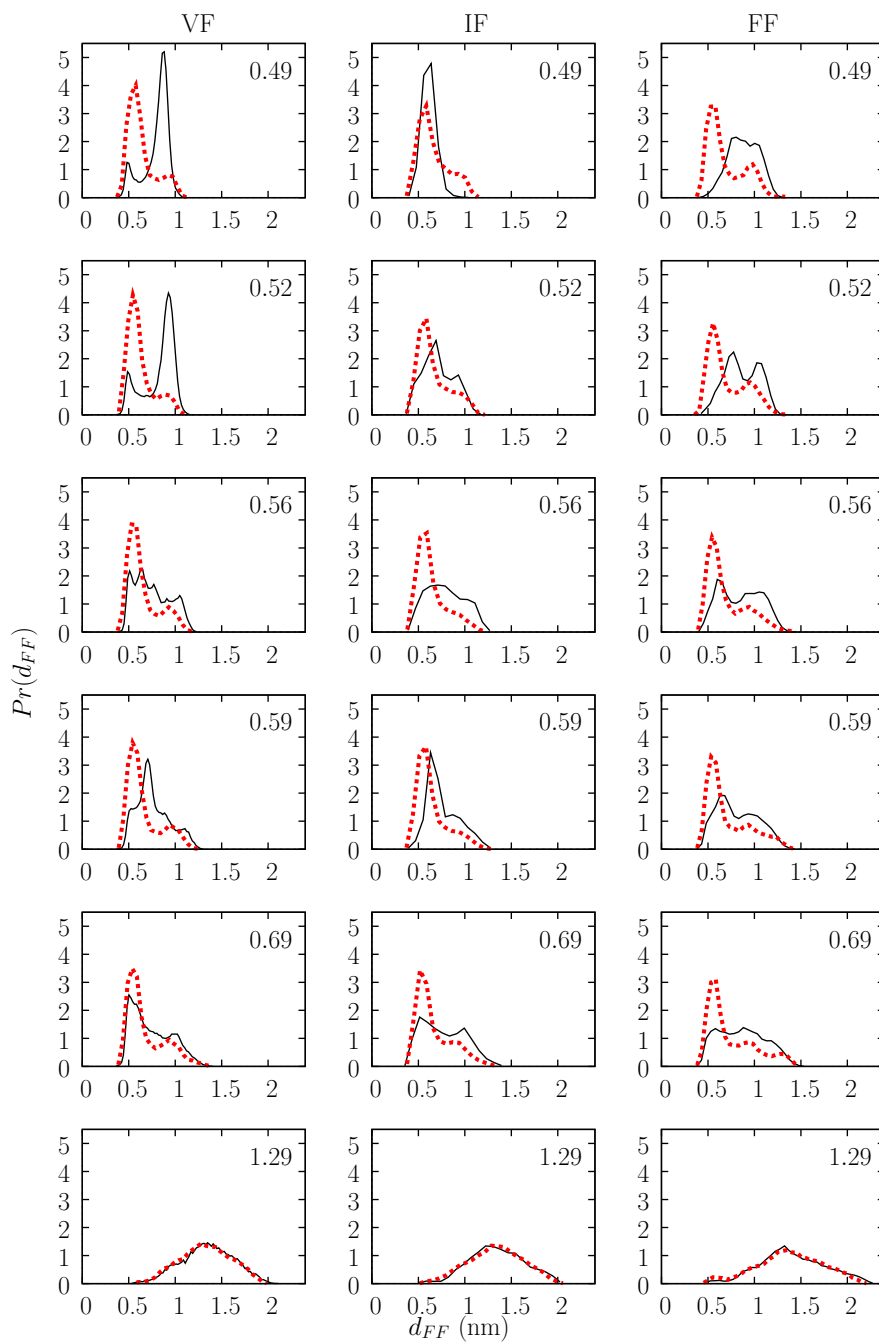


Figure 6.6: Comparison of AA (black) and CG (red) probability distributions for the distance between the phenylalanine side-chains, d_{FF} , at selected peptide center-to-center distances (shown in top-right corner of each subplot). The columns display the probability distributions for VF (left), IF (middle), and FF (right) di-peptides.

AA and CG phenylalanine side-chain distributions for VF, IF, and FF are in good agreement for constraint distances down to 0.59 nm (Figure 6.6). For relative peptide orientations (Figure 6.7) AA and CG distributions are in good agreement for distances down to 0.69 nm. However, at smaller separations CG model fails to capture the AA distributions. Detailed analyses of atomistic trajectories show that the relative orientation of the two peptides at close separations is determined by the charge of the terminal groups (which favor antiparallel orientation) as well as the directionality of the backbone hydrogen bonding. The representation of the amide group with the neutral spherical bead Am, naturally lacks the specificity of the hydrogen bonding present in the AA system, and is most likely the reason for the CG model’s failure at close separation distances. Accurate representation of hydrogen bonding requires a more detailed backbone representation. If one wants to preserve the properties of a donor and an acceptor group in the hydrogen bond, one needs at least one bead representing the CO unit and one bead representing the NH unit (see e.g. Bereau and Deserno).[189]

6.3 Concluding Remarks

Multiscale modelling of biomolecules, especially peptides, is not an easy task due to their complex structural organization and nontrivial dependence on the chemical environment. Reproducing structural and/or thermodynamic properties of such target systems via CG models requires inclusion of conformational flexibility and chemical specificity of the system into some extent, which limits the transferability of CG models for peptides. The majority of current CG models can not reproduce structural and thermodynamic properties of target systems simultaneously. As an attempt in this direction, Villa et al.[1] developed a solvent-free CG peptide model that can capture qualitatively structural and thermodynamic properties, such as the peptide-peptide association constant of the hydrophobic FF di-peptide in water.

Here, we mimic the CG mapping used in the FF CG model and transfer bonded and nonbonded interaction potentials to different hydrophobic di-peptides, namely

VF and IF. These di-peptides differ only in the sidechain group of the first residue. Therefore, we maintain the interaction potentials derived for FF, as long as the beads involved in these interactions are the same. Comparison of atomistic and CG distributions shows that the CG model can qualitatively match structural (such as intra- and inter-peptide conformational distributions) and thermodynamic properties (such as PMF curves and association constants) of atomistic VF and IF di-peptides. Hence, we demonstrate that the original methodology (CG scheme, derivation of bonded and non-bonded potentials) can be used to model similar systems, without the need of an overall re-parameterization. Agreement between atomistic and CG potentials transferred from FF also shows that similar groups in different hydrophobic di-peptides may display similar conformational preferences, which is crucial for the transferability of the CG model.

A general protocol may be developed to transfer the original CG model to other hydrophobic di-peptides, which are in the form of XF. X represents an arbitrary amino acid. The protocol may be summarized as follows. First, an appropriate CG scheme for the new residue, X, is determined. Second, CG bonded potentials specific to the sidechain of residue X are obtained from atomistic simulations of the XF di-peptide in water. Third, an analogue small molecule that mimics the side-chain of the new residue is chosen to model non-bonded interactions via PMF calculations. Fourth, potentials from FF are transferred to model interactions between backbone beads, and the phenylalanine residue.

We tested the transferability of the original CG model for sequences having same length with FF, but the validity of transferability should also be addressed for longer sequences, where “building-block” and “small-molecule analogue” approaches are more likely to be disrupted.

Hydrophobic di-peptides are the smallest molecules forming self-assembled hierarchical structures in aqueous solution. Therefore, developing a transferable CG model for simulation of such systems will help elucidation of driving forces important in the self-assembly of peptide-based materials and peptide aggregation.

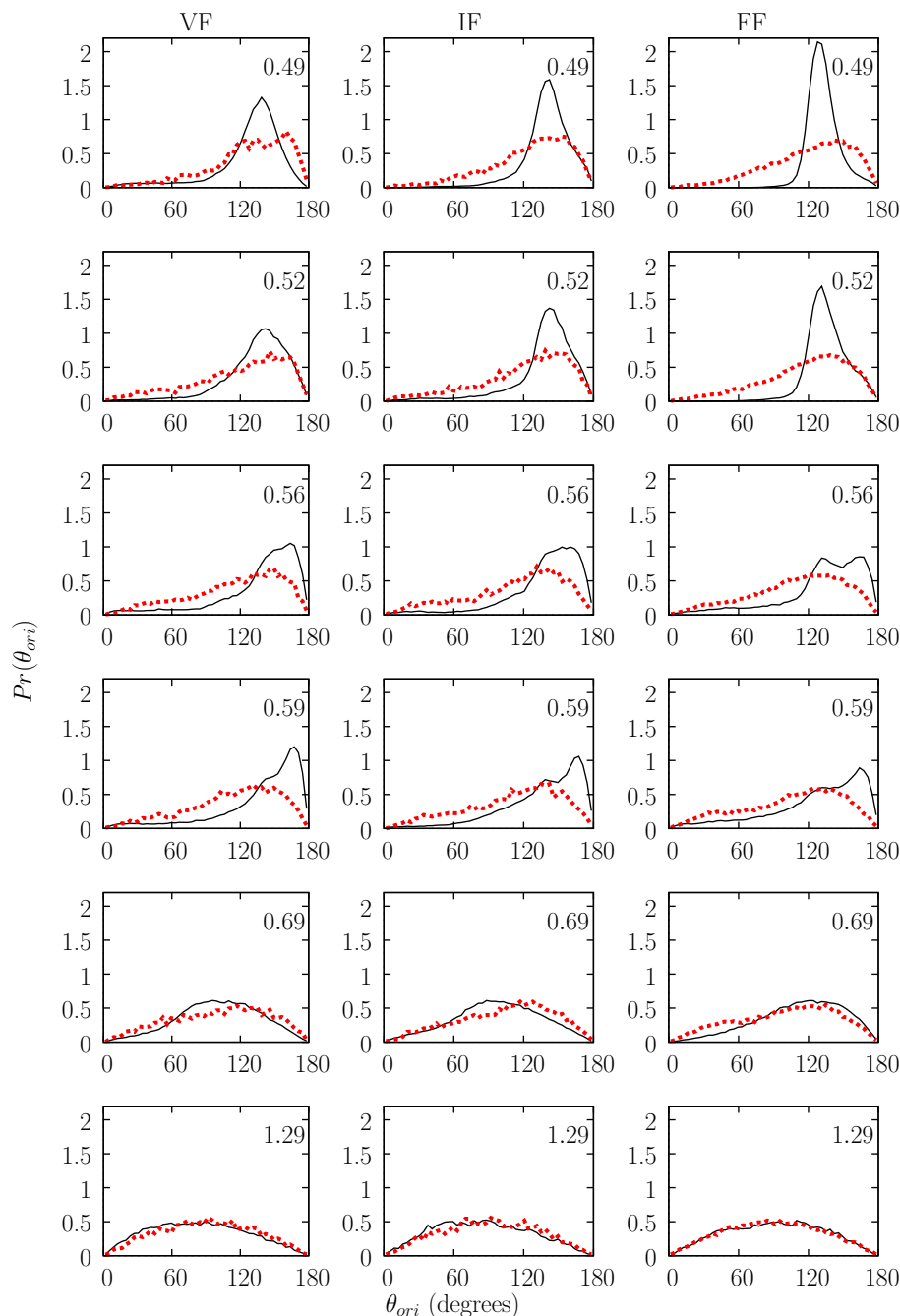


Figure 6.7: Orientation of peptides in PMF calculations for AA (black) and CG (red). Scalar product of the end-to-end vectors of the peptides are used for measuring the relative orientation, θ_{ori} . The probability distribution, $Pr(\theta)$, for VF, IF, and FF are given for selected center-to-center distance (shown in top-right corner of each subplot) separation of the peptides.

Chapter 7

CONCLUSIONS

Discovery of naturally occurring functional peptides has accelerated peptide design studies during the past decade. Besides serving a variety of purposes as drugs, antigens, ligands, and antibiotics, these molecules also self-assemble to form higher-order nanostructures such as micelles, nanotubes, nanofibers, and monolayers that are used in various application areas. In this dissertation, we thoroughly study structure and thermodynamics of amphiphilic peptides, which are shown to be powerful candidates for nanomaterials via MD simulations.

We first analyzed the structure and thermodynamic properties of small amphiphilic peptides at the air/water interface. This will act as a first step for understanding more complex systems as well as improving design strategies for new nanomaterials with desired properties. We accurately determined the free energy of transferring peptides from bulk water to the air/water interface, for the given force field and two water models (SPC and SPC/E). We showed that free energy of adsorption at the air/water interface is negative, whose minima range between 5 to 11 $k_B T$ depending on the peptide length. The shape of the PMF is the same for all peptides and water models with a single deep minimum which is few Ångstroms wide. It was shown that the ΔG of amphiphilic peptides is dominated by one favorable contribution: the de-solvation of hydrophobic side chains. For these peptide lengths two minor terms that also play a role are the partial dehydration of the backbone and the loss of orientational entropy, which is the only significant unfavorable term. The net effect of the three main contributions produces a free energy difference which is linear for the chosen sequence at least up to hexa-peptides. At the interface the peptides cluster through intramolecular hydrogen bonds between backbones. The resulting layer

lowers the surface tension by 30% compared to a pure SPC/E air-water interface.

In the second study, we analyzed folding of a carefully designed 24-residue amphiphilic peptide at the air/water interface in light of information gathered from small amphiphilic peptides. In contrast to small peptides, this molecule adapts to a well-defined β -hairpin conformation. MD simulations show that adsorption to the interface and adaptation of the β -hairpin secondary structure take place simultaneously. However, the molecule does not adapt to the designed β -hairpin conformation in bulk water, where hydrophobic/hydrophilic partitioning can not take place. Adsorption free energy of the molecule is highly negative suggesting that the peptide spontaneously goes to the interface and stays there forming a monolayer by partitioning its hydrophobic/philic groups between the two media. *In-silico* mutations showed that turn residues are essential to adopt the targeted β -hairpin conformation, whereas hydrophobic residues are required both for adsorption and stabilization of the β -hairpin conformation at the air/water interface. We also determine possible packings of individual β -hairpins within monolayers. The results obtained from this study give us an insight on key factors for formation of β -hairpin structure as well as adsorption at the interface which is crucial in design of design peptide monolayers.

In the third study, we tried to understand the link between the sequence and structure of nanofibers formed by amphiphilic peptides in bulk water. The results showed that structural preferences such as inter-dimer and intra-dimer spacings and the stability of peptide nanofibers are determined according to the type of the hydrophobic residue located in the central B block. As the hydrophobicity and the aromaticity of the residue in the B block increase the stability and the intra-dimer spacing increases. However, inter-dimer spacing does not change upon changing the hydrophobic residue, showing that this dimension is primarily determined by H-bonding distance between neighboring dimers. We also showed that individual dimers within nanofibers display an orientational preference (parallel vs. antiparallel) depending on the type of the residue in the B block.

We also investigated possible nucleation mechanisms for fiber formation. We

showed that hydrophobic interactions within a dimer are not strong enough to hold the two MDPs together, but instead they are held together via backbone H-bonding suggesting that individual MDPs self-assemble into nanofibers via H-bond directed nucleation mechanism. These results give some hints for designing stable and functional peptide nanofibers.

In our final study, we investigated the “transferability” of a recently developed solvent-free CG peptide model [1] that was shown to capture quantitatively structural and thermodynamic properties of a hydrophobic di-peptide, phenylalanine di-peptide, by mimicking the CG mapping used in the FF CG model and transferred bonded and nonbonded interaction potentials to different hydrophobic di-peptides, namely VF and IF. These di-peptides differ only in the sidechain group of the first residue. Therefore, we maintain the interaction potentials derived for FF, as long as the beads involved in these interactions are the same. Comparison of atomistic and CG distributions showed that the CG model can qualitatively match structural (such as intra- and inter-peptide conformational distributions) and thermodynamic properties (such as PMF curves and association constants) of atomistic VF and IF di-peptides showing that the original methodology (CG scheme, derivation of bonded and non-bonded potentials) can be used to model similar systems, without the need of an overall re-parameterization. In addition, a general protocol may be developed to transfer the original CG model to other hydrophobic di-peptides, which are in the form of XF. X represents an arbitrary amino acid. Hydrophobic di-peptides are the smallest molecules forming self-assembled hierarchical structures in aqueous solution. Therefore, developing a transferable CG model for simulation of such systems will help elucidation of driving forces important in the self-assembly of peptide-based materials and peptide aggregation.

Chapter 8

APPENDIX

In umbrella sampling one often needs a potential that acts on the centers of mass (COM) of groups of particles. Without periodic boundary conditions or for spatially confined groups this is not an issue; one can simply use the mass-weighted averaged coordinates of the particles in the group. But when a group consists of several molecules in a periodic system problems will occur when a molecule moves one periodic distance with respect to the other molecules in the group. We will derive a general solution to this problem.

Consider a group of N particles with coordinates x_i and masses m_i . These particles are part of a larger system, the size of which is irrelevant for this derivation. For umbrella sampling one would like to add a potential $V_u(X)$ or $V_u(X_1, X_2)$ that acts on the COM X or the distance between COMs. The following derivation will be one dimensional, extension to multiple dimensions is trivial.

We will define $X(\mathbf{x})$ as the coordinate that minimizes the mass weighted sum of a spatial function s as follows:

$$\min_X \sum_{i=1}^N m_i s(x_i - X) \quad (8.1)$$

To minimize this, one needs to solve

$$\sum_{i=1}^N m_i s'(x_i - X) = 0 \quad (8.2)$$

for X and check the sign of the second derivative. The force due to the umbrella on particle i is:

$$f_i = -\frac{dV_u(X(\mathbf{x}))}{dx_i} = -\frac{dV_u(X)}{dX} \frac{dX(\mathbf{x})}{dx_i} \quad (8.3)$$

To evaluate f_i we need the derivative of X with respect to x_i . We can obtain this through a first order expansion of Eq. 8.2 around $x_i - X$. The zeroth order term is

zero by definition, so we are left with:

$$m_i s''(x_i - X) dx_i - \sum_{j=1}^N m_j s''(x_j - X) dX = 0 \quad (8.4)$$

The derivative is then:

$$\frac{dX}{dx_i} = \frac{m_i s''(x_i - X)}{\sum_{j=1}^N m_j s''(x_j - X)} = \frac{m_i w_i}{\sum_{j=1}^N m_j w_j} \quad (8.5)$$

where we have introduced w_i , the weight of particle i .

For $s(x) = x^2$ we recover the standard COM. Equation 8.2 can be solved directly, since s' is linear and the weights given by equation 8.5 are proportional to the masses since s'' is constant. In this framework it is obvious that the standard COM will fail in periodic systems, since x^2 is not a periodic function. The solution is straightforward: use a periodic function for s . The simplest choice is:

$$s(x) = \cos\left(\frac{2\pi x}{\ell}\right) \quad (8.6)$$

where ℓ is the length of the periodic unit cell. Equation 8.2 can be solved directly:

$$X = \frac{\ell}{2\pi} \arctan\left(\frac{\sum_{i=1}^N m_i \sin(2\pi x_i/\ell)}{\sum_{i=1}^N m_i \cos(2\pi x_i/\ell)}\right) \quad (8.7)$$

This choice of s will produce a unique X for all coordinate distributions, except those that have are periodic with a period of half the box length. For the particular case of a slab of particles in contact with a vapour, the relative weights of particles at the edges of the slab will be smaller than in the middle. For a slab thickness of a quarter of the unit cell the difference in weights is up to $\frac{1}{2}\sqrt{2}$, while for a thickness of a third of the unit cell it is $\frac{1}{2}$. In most cases this weight difference will not have a significant effect, since the force on the COM is distributed over a large number of particles. When a particle escapes from the slab and travels through the unit cell its weight will decrease and might become negative, meaning that the force on the escaped particle will have the opposite direction compared to the other particles. The particle can merge back into the slab having crossed through the periodic boundary without causing jumps in the potential or the force as would occur with a standard COM.

Instead of adding an umbrella potential on a COM another option is constraining a COM. Constraints can be added to the Hamiltonian with the principle of least action through a Lagrange multiplier. We won't go into the details of the procedure here and only provide the solution. What is required additionally for a constraint is the effective mass of the COM, this is:

$$m_X = \frac{\left(\sum_{i=1}^N m_i w_i\right)^2}{\sum_{i=1}^N m_i w_i^2} \quad (8.8)$$

The COM constraint can then be solved as a normal constraint. What remains to be done is redistribute the constraint displacement ΔX to the particles. This is done proportional to the weights as follows:

$$\Delta x_i = \frac{\sum_{j=1}^N m_j w_j w_i}{\sum_{j=1}^N m_j w_j^2} \Delta X \quad (8.9)$$

It can be verified easily that the sum of the mass times displacement of the particles equals $m_X \Delta X$.

For constraining a COM with $s(x) = \cos(x)$, 7 sums are required involving masses, cosines and sines, 5 for the reference coordinates and 2 for the unconstrained coordinates. These 7 sums can be accumulated in a single loop and when simulating in parallel can be summed at once over all the processors. The main additional cost compared to a standard COM calculation is the evaluation of one cosine and sine per particle, which is negligible in most cases.

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