CHAPTER 6

The interfacial structure of self-assembled switchable peptide surfactants.

Summary:

Recently a series of stimuli-responsive surfactant peptides have been identified that can self-assemble to form robust interfacial networks which can be switched between high- and low- elasticity states as a function of pH or metal ion concentration, reversibly controlling the stability of emulsions and foams. Such peptides have promise in applications ranging from cosmetics to oil recovery. A combination of molecular dynamics simulations and neutron reflection studies has been used to investigate the structure of two such stimuli-responsive peptides, AM1 and Lac21E, at an air-water interface. Both peptides are essentially unstructured in solution but fold cooperatively at an air-water interface to form an ordered monolayer of peptides. The separation between the hydrophobic and hydrophilic residues within these amphipathic peptides (0.55 nm and 0.49 nm for AM1 and Lac21E respectively) predicted by the simulations, consistent with that inferred from neutron reflection experiments (0.75 ± 0.2 nm and 0.60±0.2 nm). The volume fraction of peptides normal to the interface calculated from the MD simulations of AM1 peptide either from helical or random conformation reproduces in detail the experimental neutron reflectivity measurements at different contrast variations. While this demonstrates that the simulations can reproduce the experimental data available it suggests that the neutron reflection measurements are not sufficiently sensitive to be used to determine the conformational state of the peptide at an air/water interface. Together the results suggest the peptides form highly ordered domains of the air/water interface and provide an atomistic model that can be used to explain the mechanical properties of the systems observed experimentally.

Ying Xue, Lizhong He, Anton P. J. Middelberg and Alan. E. Mark. To be submitted
6.1 Introduction.

A defining characteristic of a wide range of biomolecules, including many proteins and peptides, is their ability to spontaneously self-assemble into well-ordered large-scale structures such as tapes, ribbons, fibrils, nanofibers or even nanotubes [1, 2]. The remarkable regularity and diverse structural properties of such systems have meant that self-assembled biomolecular structures are increasingly used in applications ranging from coatings and other functional biomaterials to pharmaceutical preparations [2-6]. Not only can peptides be designed that assemble into a wide range of alternative structures but, the nature and structural properties of the aggregates are strongly dependent on the nature of the external conditions such as pH or the presence of interfaces. Interfaces can dramatically influence the structures formed by peptides by promoting the formation of elements of secondary structure or by acting as a template and inducing the alignment of the peptides. For example, amyloid fibril formation in Alzheimer’s disease which is associated with the misfolding and assembly of the Aβ-peptide is strongly promoted by the presence of water/membrane or air/water interfaces.

As the tendency for a peptide to interact with, for example an air/water interface is strongly dependent on its polarity and thus the protonation state of individual amino acids pH can be used to modulate the tendency of peptides to assemble at interfaces. This has recently been exploited by Middelberg and co workers who have identified a series of stimuli-responsive surfactant peptides that can self-assemble to form robust interfacial networks which can be switched between high- and low- elasticity states as a function of pH or metal ion concentration, reversibly controlling the stability of emulsions and foams. To further develop such peptides as technological agents a detailed understanding of the structure of the peptides at an air/water interface is required. From an experimental perspective determining the structural properties of molecules adhered to surfaces in atomic detail is a major challenge. While techniques such as neutron reflectivity measurements can provide information on the distribution of certain chemical groups they have to be interpreted in terms of a structural model. To circumvent the challenges associated with the experimental investigation of molecules at interfaces theoretical approaches such as molecular dynamics simulations can be used. To date such work has focused primarily on the study of low
molecular weight amphiphilic molecules such as Germini surfactants [7], alkyl benzene sulfonate [8], cetyltrimethylammonium bromide [9], hydrophobins SC3 [10, 11], EAS, a class I hydrophobin from Neurospora crassa [12], and aerosol-OT [13].

In this work, MD simulations are used to investigate the interfacial behavior of two surface active peptides, AM1 and Lac21E. These two peptides have been designed to display specific interfacial properties including changes in aggregation state and mechanical properties in response to stimuli such as a change in pH [5]. The dynamic properties and equilibrium structure of AM1 and Lac21E in solution and at an air-water interface have been examined. To validate the simulations, the interfacial volume fraction profiles of peptides obtained from the MD simulations have been used to predict neutron reflectivity data under different contrast variations. Furthermore, the simulations have been used to predict the effect of pH on the interfacial structures of AM1 and Lac21E, in order to understand why a change in pH results in major changes in the mechanical properties of the system.

6.2 Methodology.

6.2.1 Structural properties of the surfactant systems.

The structural properties of two surfactant peptides AM1 (Ac-MKQLADSLHQLARQVSRLHRA-CONH₂) and Lac21E (Ac-MEELADS LEELARQVEELESA-NH₂) were investigated in various environments denoted systems AM1/Lac21E-I to VIII (see Table 6.1).
Table 6.1 Summary of MD simulations performed under various initial conditions.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Peptide</th>
<th>No peptides</th>
<th>Environment</th>
<th>Initial Structure</th>
<th>pH</th>
<th>Pressure coupling</th>
<th>Time (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM1-I</td>
<td>AM1</td>
<td>1</td>
<td>Bulk</td>
<td>Helical</td>
<td>7</td>
<td>Yes (Isotropic)</td>
<td>100</td>
</tr>
<tr>
<td>AM1-II</td>
<td>AM1</td>
<td>1</td>
<td>Bulk</td>
<td>Random</td>
<td>7</td>
<td>Yes (Isotropic)</td>
<td>200</td>
</tr>
<tr>
<td>AM1-III</td>
<td>AM1</td>
<td>1</td>
<td>Interface</td>
<td>Helical</td>
<td>7</td>
<td>No</td>
<td>100</td>
</tr>
<tr>
<td>AM1-IV (a)</td>
<td>AM1</td>
<td>1</td>
<td>Interface</td>
<td>Random</td>
<td>7</td>
<td>No</td>
<td>200</td>
</tr>
<tr>
<td>AM1-IV (b)</td>
<td>AM1</td>
<td>1</td>
<td>Interface</td>
<td>Random</td>
<td>7</td>
<td>No</td>
<td>200</td>
</tr>
<tr>
<td>AM1-V</td>
<td>AM1</td>
<td>4x4</td>
<td>Interface</td>
<td>Helical</td>
<td>7</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>AM1-VI</td>
<td>AM1</td>
<td>4x4</td>
<td>Interface</td>
<td>Random</td>
<td>7</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>AM1-VII</td>
<td>AM1</td>
<td>4x4</td>
<td>Interface</td>
<td>Helical</td>
<td>7</td>
<td>Yes (X-Y plane)</td>
<td>25</td>
</tr>
<tr>
<td>AM1-VIII</td>
<td>AM1</td>
<td>4x4</td>
<td>Interface</td>
<td>Random</td>
<td>7</td>
<td>Yes (X-Y plane)</td>
<td>72</td>
</tr>
<tr>
<td>Lac21E-I</td>
<td>Lac21E</td>
<td>1</td>
<td>Bulk</td>
<td>Helical</td>
<td>3</td>
<td>Yes (Isotropic)</td>
<td>50</td>
</tr>
<tr>
<td>Lac21E-II</td>
<td>Lac21E</td>
<td>1</td>
<td>Bulk</td>
<td>Random</td>
<td>3</td>
<td>Yes (Isotropic)</td>
<td>200</td>
</tr>
<tr>
<td>Lac21E-III</td>
<td>Lac21E</td>
<td>1</td>
<td>Interface</td>
<td>Helical</td>
<td>3</td>
<td>No</td>
<td>50</td>
</tr>
<tr>
<td>Lac21E-IV</td>
<td>Lac21E</td>
<td>1</td>
<td>Interface</td>
<td>Helical</td>
<td>3</td>
<td>No</td>
<td>200</td>
</tr>
<tr>
<td>Lac21E-V</td>
<td>Lac21E</td>
<td>4x4</td>
<td>Interface</td>
<td>Helical</td>
<td>3</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>Lac21E-VI</td>
<td>Lac21E</td>
<td>4x4</td>
<td>Interface</td>
<td>Random</td>
<td>3</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>Lac21E-VII</td>
<td>Lac21E</td>
<td>4x4</td>
<td>Interface</td>
<td>Helical</td>
<td>3</td>
<td>Yes (X-Y plane)</td>
<td>25</td>
</tr>
<tr>
<td>Lac21E-VIII</td>
<td>Lac21E</td>
<td>4x4</td>
<td>Interface</td>
<td>Random</td>
<td>3</td>
<td>Yes (X-Y plane)</td>
<td>50</td>
</tr>
</tbody>
</table>
Systems AM1/Lac21E-I and II consisted of a single copy of the peptide AM1 or Lac21E in aqueous solution. The initial conformations of the individual peptides in systems AM1/Lac21E-I and II, were α-helical and random coil respectively. The random coil conformations were derived from a 20 ns simulation of a single copy of the peptide in water at 500 K. Systems AM1/Lac21E-III and IV were derived from systems AM1/Lac21E-I and II by extending the box in the z direction by 4 nm, creating a vacuum/water interface. Systems AM1/Lac21E-V and VI consisted of a 4 × 4 array of peptides at a vacuum/water interface. The peptides were evenly distributed on a square grid (3.0 nm spacing) parallel with the x-y plane. The spacing of the grid points at which the centers of mass of the peptides were placed was such that the system formed a continuous surface under periodic boundary conditions. The conformations of each of the 16 peptides used to form the array were taken from simulations of the isolated peptides in water. Conformations for the systems AM1/Lac21E-V were selected randomly from the trajectory of system AM1/Lac21E-I (helical) and conformations for system AM1/Lac21E-VI were selected randomly from the trajectory of system AM1/Lac21E-II (random coil). The peptides were placed in a box 12.0 nm × 12.0 nm × 3.0 nm and solvated with water. After equilibration of the solvent, during which time the atoms in the peptides were positionally restrained, the box was extended by 97.0 nm to create a vacuum/water interface. Systems AM1/Lac21E-VII and VIII were identical to systems AM1/Lac21E-V and VI, except that the dimensions of the box along the x and y axes were reduced until the density of the peptides at the air/water interface matched values obtained experimentally.

The simulations of AM1 were performed at pH 7 (with protonated Lys and His), while the simulations of Lac21E were performed at pH 3 (with protonated Asp and Glu), conditions under which the two peptides form robust films.

6.2.2 Mechanical properties of AM1 and Lac21E.

The mechanical properties of the two peptides AM1 and Lac21E were investigated in various pH environments. The simulations were performed under conditions at which the two peptides form robust (“on” state) or weak (“off” state) films, denoted as systems AM1/Lac21E-IX and X respectively (see Table 6.2). The simulations of systems AM1-IX and Lac21E-X were performed at neutral pH (approximately pH 7,
with Lys and His (ND1) protonated), and at low pH (approximately pH 3, with Asp, Lys, Glu, and His (ND1 and NE2) protonated, system AM1-X and Lace1E-IX).

The systems were built as described in 6.2.1 but with one monolayer on either side of an 8.0 nm thick water slab. The length of the z-axis of the box was set to 16.0 nm, to create a vacuum/water interface on either side of the monolayer. Each monolayer consisted of 16 peptides. In all cases except that of system Lac21E-X, the conformations of each of the 16 peptides used to form the aggregates were taken from simulations of the systems AM1-VII or Lac21E-VII. The dimensions of the box along the x and y axes were derived from the systems AM1-III or Lac21E-III in which the total cross sectional area occupied by the peptide was approximately the same as that measured experimentally. However, in the case of Lac21E at neutral pH, aggregation at the interface is believed not to occur, with the interfacial area measured experimentally being only 14±2 nm²/peptide. Using the same packing density as in system Lac21E-IX, the water slab was heavily distorted. As a consequence, Lac21E-X was built with the same dimensions as system Lac21E-IX, but each monolayer contained only 3 peptides so that the total cross sectional area occupied by the peptide (approximately 18 nm²/peptide) was close to that measured experimentally while still retaining a manageable system size. The conformations of each of the 3 peptides were taken from the trajectory of system Lac21E-VII. A 5 ns of NVT simulation was then performed to relax each of the systems.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Peptide</th>
<th>No peptides</th>
<th>Environment</th>
<th>Initial structure</th>
<th>pH</th>
<th>Pressure coupling</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM1-IX</td>
<td>AM1</td>
<td>16x2</td>
<td>interface</td>
<td>Helical</td>
<td>7</td>
<td>Yes (X_Y plane)</td>
</tr>
<tr>
<td>AM1-X</td>
<td>AM1</td>
<td>16x2</td>
<td>interface</td>
<td>Helical</td>
<td>3</td>
<td>Yes (X_Y plane)</td>
</tr>
<tr>
<td>Lac21E-IX</td>
<td>Lac21E</td>
<td>16x2</td>
<td>Interface</td>
<td>Helical</td>
<td>3</td>
<td>Yes (X_Y plane)</td>
</tr>
<tr>
<td>Lac21E-X</td>
<td>Lac21E</td>
<td>3x2</td>
<td>Interface</td>
<td>Helical</td>
<td>7</td>
<td>Yes (X_Y plane)</td>
</tr>
</tbody>
</table>
6.2.3 Simulation parameters.

All simulations were performed using the GROMACS (Groningen Machine for Chemical Simulation) package, version 3.2.1 [14]. The GROMOS 45A3 force field was used to describe the peptides [15] and the simple point charge (SPC) water model was used to describe the solvent water [16].

All simulations were performed under periodic boundary conditions. The conditions under which each system was simulated along with the total time simulated are summarized in Table 6.1 and Table 6.2. In cases where a single peptide was initially placed in a box of water (systems AM1/Lac21E-I and II), the dimensions of the box were chosen such that the minimum distance between any atom of the peptide and the box wall was 1 nm. The non-bonded interactions were evaluated using a twin-range method. Interactions within the short-range cutoff of 0.9 nm were updated every step. Interactions within the long-range cutoff of 1.4 nm were updated every 5 steps together with the pair list. To minimize the effect of truncating the electrostatic interactions beyond the 1.4 nm long range cutoff, a reaction field correction was applied using a relative dielectric constant of $\varepsilon_r = 78$ [17]. The LINCS algorithm [18] was used to constrain the lengths of covalent bonds in the peptide. The SETTLE algorithm [19] was used to constrain the geometry of the water molecules. In order to further extend the timescale that could be simulated, explicit hydrogen atoms in the peptide were replaced with dummy atoms, the positions of which were calculated each step based on the positions of the heavy atoms to which they were attached. This eliminates high frequency degrees of freedom associated with the bond angle vibrations involving hydrogens, allowing a time step of 4 fs to be used to integrate the equations of motion without affecting thermodynamic properties of the system significantly [20]. The temperature was maintained close to the reference value of 298 K by weak coupling to an external temperature bath [21], using a relaxation time constant of 0.1 ps. In systems AM1/Lac21E-VII and VIII semi-isotropic pressure coupling was used to allow the systems to relax in the $x$-$y$ plane to an appropriate surface density. The relaxation time constant was set to 15 ps along $x$ and $y$. The length of the box along the $z$-axis was held fixed. This allowed the dimensions of the box in the $x$-$y$ plane to decrease slowly as the peptides formed a condensed film on the air/water interface during simulation. After the experimental surface density was
achieved, the systems were equilibrated for another 10 ns at constant volume. In systems AM1/Lac21E-IX and X, the length of the box along the z-axis was held fixed. To estimate the Young’s modulus, the dimensions of the box in the x-y plane were either increased or decreased to give the required surface density. A 3 ns NVT simulation was then performed to relax the system. A further 2 ns simulation was then performed for analysis.

As noted above, the weakly coupling method of Berendsen was used to maintain the temperature of the system at 298 K. Using this approach the velocities of all atoms in the system are scaled in a uniform manner. In general, this scaling factor is less than 1.0 due to excess heat being generated in the system as a consequence of errors in the numerical integration of the equations of motion and other approximations. One consequence of this is that water molecules within the vacuum layer, which are effectively decoupled from the rest of the system and do not experience the same degree of heating, are progressively cooled to temperatures approaching 0 K. To avoid the formation of clusters of frozen water in the vacuum layer, weak stochastic coupling using a friction coefficient 0.01 ps$^{-1}$ was applied to maintain the temperature of the water molecules in the vacuum layer close to 298 K. Note, the friction coefficient used in this case is almost a factor of $10^4$ smaller than the friction coefficient of water (91 ps$^{-1}$) [22]. The effect of this on the thermal fluctuations within the system as a whole was negligible.

6.2.4 Calculation of the structural parameters.

The cross-sectional area of the peptide domains was estimated by superimposing a 0.25 nm grid over the system in the x-y plane and counting the proportion of grid cells that contained a peptide atom. The volume fraction profiles of the solvent, and the hydrophobic and hydrophilic residues within the peptides, along the z-axis, normal to the plane of the membrane, were calculated as follows: First, the location of the surface was identified by averaging the solvent distribution over 21 frames taken from the final 1 ns of the trajectory (1 frame every 50 ps). The box was then divided into 2000 slices along the z-axis, and the density of hydrogens in each slice determined. As the GROMOS force field is a united atom force field the coordinates of the hydrogens were generated based on the coordinates of the heavy atoms to which they
are attached assuming ideal geometries. In order to be able to compare the results of
the simulations with the neutron reflectometry data, all valine and leucine residues in
the peptides were considered to have been selectively deuterated (hydrophobic
residues) in line with the experimental setup. The remaining residues were
undeuterated (hydrophilic residues). Both termini (-NH\_2 and -Ac) were considered to
be hydrophilic. The thickness of the peptide layer was estimated from the width of the
peak at half maximum height of the volume fraction profile.

6.2.5 Neutron Reflectivity.

Neutron reflectivity profiles for AM1 and Lac21E at an air water interface were
collected at the SURF reflectometer, a time-of-flight instrument at Rutherford
Appleton laboratory (ISIS, Oxfordshire, UK). These neutron reflection experiments
have been described in detail previously [5]. Briefly, the peptides were selectively
deuterated and different D\_2O/H\_2O ratios (8.1, 39.1, 70 and 100% (v/v) D\_2O) used to
obtain contrast variation. In previous work, the basic structure of the interface was
inferred by simultaneously fitting the reflectivity data at different contrast conditions
and assuming that the residues of interest had a specific distribution, for example, that
they are Gaussian distributed [5, 23]. In the current work, the volume fraction profiles
of peptides across the interface as obtained from the MD simulations were used to
predict the experimentally measured neutron reflectivity data under different contrast
variations using the method of partial structure factors [5, 23]. This allowed a direct
comparison between the experimental data and the simulations to be made and
avoided the need to assume that a given set of residues (hydrophobic or hydrophilic)
had a particular distribution (i.e. Gaussian). The scattering length of the peptides used
to predict the neutron reflectivity data was based on the chemical composition of the
peptides [24], assuming all labile hydrogens on the peptide had fully exchanged with
solvent.

6.2.6 Young’s modulus

In order to study the mechanical properties of the peptide assemblies at an air/water
interface, a system was constructed with a peptide monolayer on either side of a water
slab (see Figure 6.6). Experimentally, the Young’s modulus is determined from the
slope of a stress-strain curve created by applying tension and compression to a sample of the peptide. In the simulations, the dimensions of the box in the x-y plane were either increased or decreased to give the required surface density. A 3 ns NVT simulation was performed to relax the system. A further 2 ns simulation was then performed for analysis. The elastic properties of the film were estimated from the change in the lateral pressure as a function of the percentage of expansion or compression of the film. This was calculated at neutral and low pH for both peptides.

6.3 Results and Discussion.

The peptide surfactants AM1 (Ac-MKQLADSLHLRQVRQVSRLEHA-CNH₂) [4] and Lac21E (Ac-MEELADSLEELRQVEELESA-NH₂) [25] are both derived from the amphipathic Lac21 peptide (Ac-MKQLADSLMLRQVRVSRLESA-CNH₂) [26]. Specifically, in the case of the AM1 peptide two histidine (H) residues have been incorporated at positions 9 and 20 whereas in Lac21E the residues at positions 2, 3, 9, 10, 16 and 17 have been mutated to glutamate [25]. Both of these peptides are surface active and can be reversibly switched between high and low elasticity states as a function of pH and/or metal ion concentration [4, 5]. Circular dichroism studies suggest that the peptides are essentially unstructured in solution [4, 25-27]. The peptides, which are highly amphipathic, have nevertheless been predicted to form α-helical structures at an air/water interface. The lateral (electrostatic and hydrogen bonding) interactions between the peptides are believed to give rise to the ability of the peptides to stabilize foams or emulsions [6, 28-32].

6.3.1 The structural characterization of the peptide AM1.

6.3.1.1 The conformation of an isolated AM1 peptide in aqueous solution.

Simulations of a single copy of the peptide AM1 in aqueous solution (AM1-I, AM1-II) showed that the peptide had little propensity to form α-helical structures in solution. As can be seen from Figure 6.1 (a) which shows the secondary structure [33] as a function of time starting from an ideal α-helix, the initial structure was rapidly lost and was not recovered fully during the 100 ns of simulation. Likewise, starting from a random structure no significant formation of α-helical structure was observed within 200 ns (Figure. 6.1 (b)). The results are consistent with CD data suggesting
that AM1 is unstructured in solution [4, 26].

6.3.1.2 The surface adsorption of the peptides.

When a vacuum/water interface was introduced into a system containing a single peptide in water, the peptide readily migrated and bound to the interface irrespective of the conformation of the peptide. The progressive addition of a further 8 peptides into the water layer in trial simulations led to the accumulation of all 9 peptides at the interface. Again this was irrespective of the initial conformation (results not shown). As in all cases the peptides once bound did not leave the interface, all subsequent studies were performed by placing the peptides directly at the interface.

6.3.1.3 The conformation of an isolated peptide AM1 at an air/water interface.

To determine the preferred conformation of the peptide AM1, a single copy of the peptide was placed at an air/water interface in either an ideal α-helical conformation (AM1-III) or a random conformation (AM1-IV). In contrast to the results obtained free in solution, the helical conformation remained stable at the air/water interface on a 100 ns time scale. To examine if an α-helix is the most thermodynamically stable configuration for AM1 at an air/water interface, two 200 ns simulations (systems AM1-VI (a) and (b)) starting from the same random configuration but with different initial velocities were performed. As can be seen from Figure 6.1 (c) and (d), which show the formation of secondary structure as a function of time, the peptide folds spontaneously to form stable regions of α-helical structure in both cases.

6.3.1.4 Interfacial assembly.

Although the peptide spontaneously migrated to the surface when placed in solution, this process was slow on the MD timescale. For this reason simulations of the assembly of the peptides at an interface were performed by placing a 4 × 4 array of peptides at an air/water interface. These peptides were placed either in an ideal α-helical conformation (AM1-V) or the conformations were selected randomly from the simulation of AM1 free in solution (AM1-VI). Figure 6.2 (a) shows snapshots from a 15 ns NVT simulation of system AM1-V at 0, 1, 3, and 15 ns. As can be seen in Figure 6.2 (a), the regular array of randomly orientated helical peptides rapidly
aggregated (within 1 ns) to form several large clusters. These clusters then combined to form a continuous network across the periodic boundary conditions. Once the initial aggregate was formed, the system relaxed slowly. From the electrostatic surface shown in Figure 6.2 (b), it can be seen that the non-polar residues are orientated towards the air layer and the polar or charged residues were orientated preferentially toward the water layer. An analysis of the secondary structure of the peptides using the program DSSP [33] showed that the peptides maintained a predominately helical conformation. The total cross-sectional area occupied by the peptide was approximately 3.79 nm²/molecule. Figure 6.2 (c) shows the initial configuration and the configuration after 72 ns of the surfactant peptide AM1 at an air/water interface (system AM1-VIII). This figure is discussed in more detail in section 6.3.1.6.

6. 3.1.5 Volume fraction profiles of the peptide AM1.

To obtain volume fraction profiles that could be compared directly to those obtained experimentally semi-isotropic pressure coupling was used to slowly decrease the dimensions of the system in the x-y plane. The systems AM1-VII and AM1-VIII reached a stable surface density of 4 nm² / peptide after approximately 20 ns of simulation. A 5 ns NVT simulation was then performed to further relax the system. The volume fraction distributions of both systems are shown in Figure 6.3 (a) and (b), and given in Table 6.3. In system AM1-VII (helical peptides), the maximum volume fraction of peptide was about 64% and the separation between the hydrophobic and hydrophilic layers was 0.55 nm, slightly less than the maximum expected for an ideal α-helix (~ 0.56 nm). This suggests that the non-polar leucine and valine residues partition almost completely into the air phase, and that the film was essentially a monolayer, the thickness of which was estimated to be 1.3 nm based on the peak width at half maximum height. The maximum of the hydrophobic and hydrophilic layers were at 0.9 nm and 1.1 nm respectively. System AM1-VIII (peptides initially random) gave a volume fraction of protein of about 55% and the two sub-layers were separated by approximately 0.32 nm. In this case the leucine and valine residues are not completely orientated toward the air phase. The film on the interface was more spread, with an estimated thickness of 1.5 nm.
Figure 6.1 A plot of the secondary structure as a function of the simulation time for a single isolated peptide (AM1 or Lac21E) in water. The initial structure (either random coil or ideal α-helix) is shown on the left. The final conformation after 100 ns or 200 ns of simulation is shown on the right. (a) and (b) AM1 in bulk aqueous solution. (c) and (d) duplicate runs of AM1 performed using different initial velocities at an air/water interface. (e) Lac21E in bulk aqueous solution. (f) Lac21E at an air/water interface.
Figure 6.2 (a) A snapshot of the system AM1-V after 15 ns of simulation under periodic boundary conditions. The initial configuration consisted of 16 copies of the surfactant peptide AMI in a helical conformation arranged on a 4×4 grid at an air/water interface. The size of the box was 12 nm ×12 nm ×100 nm. The colors indicate different residue types (basic: yellow; acidic: red; polar: blue; non-polar: white). (b) The system shown is viewed from either the side (left), top (middle) or bottom (right) (red: negative charged; white: neutral; blue: positive charged). (c) The initial configuration (left) and the configuration after 72 ns (right) of the surfactant peptide AM1 at an air/water interface (system AM1-V). The red box indicates the central simulation box surrounded by its periodic images (α-helix: purple; extended-β: yellow; turn: cyan; coil: white).
Figure 6.3 The volume distribution of the peptide film normal to air/water interface. The solvent distribution (dark blue line), total peptide distribution (black line), hydrophobic residues (yellow line) and hydrophilic residues (blue line). (a) Aggregated AM1 starting from an all α-helical conformation (AM1-VII). (b) Aggregated AM1 starting from an all random conformation (AM1-VIII). (c) Aggregated Lac21E starting from an all α-helical conformation (Lac21E-VII).
Figure 6.4 (a) Neutron reflectivity data for a solution of 5 μM protonated AM1 (hAM1) or partially perdeuterated AM1 (dAM1) in 25 mM HEPES buffer at pH 7.4 in the presence of 100 μM ZnSO₄. The volume ratio of D₂O and H₂O was varied to achieve contrast variation. Samples were: hAM1 in 100% D₂O (○), dAM1 in 100% D₂O (●), dAM1 in 70% D₂O (△), dAM1 in 39.1% D₂O (▼), dAM1 in 8.1% D₂O (□), and hAM1 in 8.1% D₂O (■). The red solid lines represent the best fits of the data to a structural model in which the peptide was assumed to adopt an α-helical conformation with the sidechains distributed accordingly by the simultaneous regression of all data as described in [5]. The blue solid lines represent the theoretical curves predicted by using volume fraction profiles of peptides from the simulation AM1-VII. The green solid lines represent the theoretical curves predicted by using volume fraction profiles of peptides from the simulation AM1-VIII. The curves have been scaled to improve clarity of the figure; the top curve in each case is the absolute reflectivity and each subsequent curve has been scaled by a factor of 10. (b) Neutron reflectivity of a 5 μM solution of partially deuterated Lac21E (dLac21E) in 25 mM HEPES buffer at pH 3.0. The volume ratio of D₂O and H₂O was varied to achieve contrast variation. Samples were: dLac21E in 100% D₂O (○), dLac21E in 70% D₂O (●), dLac21E in 39.1% D₂O (△), and dLac21E in 8.1% D₂O (▼). Again the red solid lines represent the best fits of the data to a structural model that assumes the peptides is helical by the simultaneous regression of all data as described previously [5]. The blue solid lines represent the theoretical curves predicted by using volume fraction profiles of peptides from simulation Lac21E-VII. The curves have been scaled as in (a).
6.3.1.6 The conformation of AM1 at an air/water interface.

The simulations of systems AM1-III and AM1-IV showed that a single isolated copy of the peptide AM1 would spontaneously migrate to the interface and fold from random coil to at least a partial helical conformation within 200 ns, suggesting folding was enhanced at an air/water interface. However, the question remained in regard to the preferred conformation in the aggregated state. To address this question, the simulation of system AM1-VIII was extended to 72 ns. Figure 6.2 (c) shows that the peptides gradually fold from a random coil to α-helix. Moreover folding appeared to be cooperative with adjacent peptides preferentially adopting a helical conformation. In simulations performed at a higher packing density (3.80 nm²/molecule) the formation of helical structure was again observed primarily in adjacent peptides.

6.3.2 The structural characterization of the peptide Lac21E.

6.3.2.1 The conformation of an isolated Lac21E peptide in aqueous solution.

The same procedure as used to investigate the conformation properties of the peptide AM1 was used to investigate the conformational properties of the peptide Lac21E. Like AM1 at pH 7, the peptide Lac21E was beta strand in aqueous solution at pH 3 (see Figure 6.1 (e)).

6.3.2.2 The surface adsorption of the peptides.

When a vacuum/water interface was introduced into a system, the peptide Lac21E migrated and bound to the interface irrespective of the initial conformation (results not shown).

6.3.2.3 The conformation of an isolated peptide Lac21E at an air/water interface.

Again Lac21E preferentially adopted an α-helical conformation at an air/water interface. Figure 6.1 (f) shows the formation of secondary structure as a function of time for the peptide Lac21E starting from the same random configuration as used to simulate the peptide in aqueous solution. As can be seen the peptide folds spontaneously to form stable regions of α-helical structure within 200 ns.
6.3.2.4 *Interfacial assembly.*

At pH 3, when the glutamate residues are protonated, an array of randomly orientated Lac21E peptides in a helical conformation rapidly aggregated to form several large clusters. These clusters then combined to form a continuous network across the periodic boundary conditions similar to that observed in the case of AM1. Once the initial aggregate was formed, the system relaxed slowly. The nonpolar residues orientated towards the air layer while the polar and charged residues orientated preferentially toward the water layer. The total cross-sectional area occupied by the peptide was approximately 3.38 nm$^2$/molecule.

6.3.2.5 *Volume fraction profiles of peptide Lac21E.*

The volume fraction distributions of the system Lac21E-VII are shown in Figure 6.3 (c), and given in Table 6.3. In the system Lac21E-VII (helical peptides), the volume fraction of the peptide was about 78% and the thickness of the peptide layer estimated to be 1.2 nm. The separation between the hydrophobic and hydrophilic layers was 0.49 nm, the maximum of the hydrophobic and hydrophilic layers were at 0.9 nm and 1.05 nm respectively.

6.3.2.6 *The conformation of Lac21E at an air/water interface.*

In the system Lac21E-VIII in which the peptides were placed initially in a random coil conformation, the peptides gradually folded to adopt $\alpha$-helical conformations when the simulation was extended to 50 ns. Again, folding appeared to be cooperative with adjacent peptides preferentially adopting a helical conformation.

6.3.3 *Direct prediction of neutron reflectivity data based on the volume fraction profiles derived from the simulations.*

The results from a comparison of the structural parameters derived from the MD simulations and neutron reflectivity studies are shown in Table 6.3. As an independent test of the validity of this model, the volume fraction profiles of peptides at an air/water interface derived from the simulations were used to predict neutron reflectivity data at different contrast variations directly.
Table 6.3 Comparison of interfacial structure of peptides from MD simulation and neutron reflection.

<table>
<thead>
<tr>
<th></th>
<th>Area per molecule (nm²)</th>
<th>Volume fraction (%)</th>
<th>Thickness of hydrophobic sub-layer (nm)</th>
<th>Thickness of hydrophilic sub-layer (nm)</th>
<th>Distance between two sub-layers (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM1</td>
<td>Neutron reflection</td>
<td>3.8±0.15</td>
<td>55±5</td>
<td>0.9±0.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>MD simulation</td>
<td>3.79±0.2</td>
<td>64.4</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Lac21E</td>
<td>Neutron reflection</td>
<td>3.3±0.1</td>
<td>70</td>
<td>0.8</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td></td>
<td>MD Simulation</td>
<td>3.38±0.2</td>
<td>78</td>
<td>0.9</td>
<td>1.05</td>
</tr>
</tbody>
</table>

In Figure 6.4, neutron reflectivity measurements are plotted together with values calculated using the volume fraction profiles of the peptides obtained from the simulations. As can be seen the values estimated from the simulations are in very good agreement with the experimental neutron reflectivity data obtained under 6 different contrast variations for the peptide AM1 at pH 7 in both a helical and random conformation (Figure 6.4 (a)) and the peptide Lac21E at pH 3 in a helical conformation (Figure 6.4 (b)). This suggests that the structure of the peptides at the interface in the simulations is able to account for the experimental observation. Most notably, based on the neutron reflectivity data, it is not possible to determine whether the peptide adopts a helical or random conformation at an air/water interface as both simulations yielded similar density profiles. Even if the peptide is unstructured there is a clear layering effect with polar residues partitioning into the water and non-polar residues assembling at the interface. Although the AM1 has a tendency to fold into α-helical conformation at an air/water interface in the simulations and the neutron reflectivity data are consistent with the peptide being helical it does not provide direct evidence that the peptide is helical as previously assumed.

6.3.4 The distribution of water within the peptide layer.

There is considerable interest in the distribution of water in interfacial adsorbed layers. Not only is the distribution of water of interest because hydration plays an important role in determining the structure and thermodynamic properties of adsorbed molecules [34, 35], but also because a specific distribution must be assumed if one is to derive structural properties from reflectivity data. Traditionally, a tanh distribution
has been used to describe solvent distribution [36, 37] in adsorbed monolayers although Penfold and Thomas [35] recently proposed the use of a Gaussian distribution. For a tanh profile, the solvent distribution is described as:

\[ n_s(z) = n_0 \left( \frac{1}{2} + \frac{1}{2} \tanh \left( \frac{z}{\xi} \right) \right) \]  

(6.1)

Where \( \xi \) is the width parameter and \( n_0 \) is the bulk number density. For a Gaussian distribution, Penfold and Thomas assumed that the solvent distribution can be divided into two regions. Below a critical depth \( z_c \), solvent is considered to fill all the space not occupied by the surfactant.

\[ \phi_s(z) = 1 - \phi_{\text{head}}(z) - \phi_{\text{chain}}(z) \]  

(6.2)

Where \( \phi_s \), \( \phi_{\text{head}} \) and \( \phi_{\text{chain}} \) are volume fractions of solvent, surfactant head group and chain group, respectively. Above \( z_c \), the solvent distribution is assumed to decay as a Gaussian of width \( \xi \):

\[ \phi_s(z) = \phi_s(z_c) \exp \left( -\frac{(z - z_c)^2}{\xi^2} \right) \]  

(6.3)

It has been claimed that while a tanh profile is appropriate for an ionic surfactant, a Gaussian distribution brings considerable improvement for non-ionic surfactants [35]. In Figure 6.5, these two alternative methods for fitting the water distribution were compared using data from the simulations of the two peptides. From Figure 6.5 it can be seen that a tanh profile provides a better fit to the water distribution obtained in the simulations of both the AM1 peptide and the Lac21E peptide than the Gaussian distribution in particular for AM1.

6.3.5 The mechanical properties of peptide AM1 and Lac21E.

In order to study the mechanical properties of the peptide assemblies at an air/water interface, a system was constructed with a peptide monolayer on either side of a water slab (see Figure 6.6). Experimentally, the Young’s modulus is determined from the slope of a stress-strain curve created by applying tension and compression to a sample of the peptide. In the simulations the elastic properties of the film were estimated from the change in the lateral pressure as a function of the percentage of expansion or compression of the film. This was calculated at neutral and low pH for both peptides. The results are shown in Figure 6.7 and Table 6.4.
Figure 6.5 Volume fraction profile of water at an air/water interface. (a) In the AM1 layer (b) In the Lac21E layer.
Figure 6.6 A snapshot of the system used to study the mechanical properties of peptides with one monolayer on either side of a water slab.

Figure 6.7 Elastic properties of the peptides AM1 and Lac21E. The film was stretched (strain <0) or compressed (strain >0). Each Symbol represents a 5 ns NVT simulation the last 2 ns of which was used for analysis. The dotted and solid lines represent lines of best fits to the data by linear regression using all data as described in methods section. (a) Peptide AM1, at pH 3.6 (○) and at pH 7 (♦). (b) Peptide Lac21E, at pH 7 (○) and at pH 3 (♦).
Table 6.4 Comparison of the Young’s modulus of the peptides obtained from the MD simulations and experiment.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>State</th>
<th>pH</th>
<th>Exp. (MPa)</th>
<th>Sim. (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM1</td>
<td>On</td>
<td>7</td>
<td>80</td>
<td>9.1 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>3</td>
<td>20</td>
<td>1.6 ± 3.0</td>
</tr>
<tr>
<td>Lac21E</td>
<td>On</td>
<td>3</td>
<td>335</td>
<td>10.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>7</td>
<td>&lt;4</td>
<td>0.2 ± 0.7</td>
</tr>
</tbody>
</table>

As can be seen from Figure 6.7 the change in the lateral pressure of the film as a function of the percentage strain is strongly dependent on the pH. In the case of AM1, at low pH, the slope of the stress-strain curve is nearly flat suggesting the film has low mechanical strength. However, at neutral pH, the curve slopes steeply, suggesting the film is highly resistant to mechanical strain. The magnitudes of the Young’s modulus estimated from the simulations are approximately an order of magnitude lower than those obtained experimentally. This could be due to system size effects, the precise nature of the model, differences in the approach used to estimate the Young’s modulus or incomplete equilibration of the system. This said there is a strong correlation between the results from the simulations and the results obtained experimentally for the high and low elasticity states and it is clear that the simulations do reproduce the main differences in the mechanical properties of the systems under different external conditions.

6.3.6 The orderliness of the film.

The film formed by the peptide Lac21E was more ordered at an air/water interface than that of AM1. This suggests that the orderliness of interfacial assembly may contribute to its mechanical properties (Young’s modulus $E = 80$ MPa for AM1, and $E = 335$ MPa for LacE21).
6.4 Conclusions.

A combination of MD simulation techniques and neutron reflection studies has been used to investigate the structural properties of two stimuli-responsive peptides, AM1 and Lac21E, at an air-water interface. While AM1 and Lac21E appear largely unstructured in solution both form primarily α-helical structures at an air/water interface. The simulations accurately reproduced the available experimental data with respect to neutron reflectivity, area/molecule, volume fraction, and the apparent thickness of the assembled monolayer and the distance between hydrophobic and hydrophilic residues. This said, the volume fraction of peptides normal to the interface calculated from the MD simulations of AM1 peptide either from helical or random conformation were very similar and either was capable of accounting for the experimental neutron reflectivity measurements obtained at different contrast demonstrating that neutron reflection data are not sensitive to conformational differences within the peptides at an air/water interface. The elastic properties of the film were estimated at different pH values from the change in the lateral pressure as a function of applied stress and compared to experimental measurements of the Young's modulus. It was shown that the mechanical properties of the systems observed experimentally were correlated with the degree of order in the peptide domains. The two types of files (tanh distribution and Gaussian distribution) that are commonly used to describe solvent distribution in the interfacial adsorbed layers were compared with the results from the simulations. It was found that a tanh profile reproduces better the solvent distribution observed in the simulations than a Gaussian distribution.
References


34. Hines, J.D., A molecular thermodynamic approach to the prediction of adsorbed layer properties of single and mixed surfactant systems. Langmuir, 2000, 16, 7575-7588.


