A molecular dynamics study of interfaces: from pure liquids to biological membranes
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Chapter 4

Surfactin: a “chimeric” peptide*

4.1 Introduction

Surfactin is an amphiphilic lipopeptide produced by various strains of *Bacillus subtilis* [75] and consists of a heptapeptide headgroup with the sequence Glu-Leu-DLeu-Val-Asp-DLeu-Leu linked to a $RC_{14-15} \beta$-hydroxy fatty acid [76] and closed by a lactone ring. Surfactin is a powerful biodegradable surfactant lowering water surface tension from 72 to $\sim 30$ mN/m at concentrations of $\sim 10 \mu$M [77, 78]. At very low concentrations it forms large micelles and the critical micellar concentration of the different analogues is of the order $10^{-5}$ M [77]. Besides its interfacial properties, surfactin exhibits several biological activities: antibacterial [79, 80], hemolytic [81], antiviral [81, 82], and antitumoral [83]. Surfactin interacts with membranes [84], initiates lipid phase transitions [85], and membrane destabilization [86]. Such surface and biological properties have attracted interest in the structure of surfactin and its behaviour at hydrophilic/hydrophobic interfaces.

From $^1$H-NMR studies correlated to distance geometry, energy minimization, and molecular dynamics techniques, a first three-dimensional structure for surfactin in DMSO has been proposed [87]. Two models were presented where in both cases the peptidic moiety adopts a “horse-saddle” conformation with the two hydrophilic residues pointing on one side forming a potentially binding “claw” and the five hydrophobic ones associated to the fatty acid chain pointing on the other side. The two structures differ

mainly with respect to their intramolecular hydrogen bonds, [NH(5)-CO(2)]
and [NH(7)-CO(5), NH(4)-CO(2), NH(6)-C1O] for S1 and S2 structures, re-
spectively. Structure-activity correlation has been extensively studied during
micelle formation [77,88], by FTIR spectroscopy and circular dichroism in
various solvent systems [89,90], and at the air/water interface [91,92] and
hydrophobic/hydrophilic mimicking medium [93]. All those recent results
suggest flexibility of the backbone conformational structure and several sta-
bile configurations are proposed and debated.

The purpose of our work was to explore the conformational flexibility of
surfactin for various interfacial concentrations in a hydrophilic/hydrophobic
medium, similar to a biological system as the lipid/water interface. In order
to avoid perturbations resulting from aliphatic chain order and lipid head-
group interactions, we have mimicked this environment with an amorphous
hexane/water system described at an atomic scale. Furthermore, we have
computed the effect of adding biosurfactant on the interfacial tension at the
oil/water interface and estimated the lateral and rotational diffusion coeffi-
cients.

4.2 Methodology

4.2.1 Molecular dynamics

Simulations

Molecular dynamics computer simulations were carried out using the DL-
POLY package [59]. An all-atom model was employed to describe molecules
at an atomic scale using the potential energy parameter set PARM27 from
the CHARMM package [55]. The TIP3P water model [56] was used in all
simulations. Bonds involving hydrogen were held fixed with the SHAKE
algorithm [18]. Electrostatic interactions were computed using the Smooth
Particle Mesh Ewald method [57]. Our simulations were performed in the
NVT ensemble [60], i.e., with constant temperature, volume, and number of
particles. The equations of motions were solved using the Verlet Leapfrog
integration algorithm [2] and simulations were run with periodic boundary
conditions. All the simulations were performed using a cutoff radius of 12 Å
for the van der Waals terms.

Initially, a single protonated surfactin molecule was equilibrated in vac-
uum. Bonmatin has kindly provided the coordinates of the S1 and S2 hept-
tapeptide conformers of the surfactin molecule which have been completed
with a $R\text{-}C_{14}$ $\beta$-hydroxy fatty acid chain [76]. The analysis of those con-
formers with hydrogen bond criteria [94,95] shows that S1 exhibit a β-turn type II' Asp(5)→Leu(2) with two hydrogen bonds CO(5)-NH(2) and NH(5)-CO(2), whilst S2 contains two reverse γ-turns centred on the D-residues with their respective hydrogen bond, Val(4)→Leu(2) and Leu(7)→Asp(5), and a third hydrogen bond NH(6)-C1O. After this preliminary protonated structure relaxation, we have built our complete models in three steps. First, we have equilibrated a box containing two phases, liquid hexane and vacuum, with interfaces parallel to the xy plane. Subsequently, surfactin molecules have been added to the box, with the fatty-acid chain inserted in the liquid hexane phase and the heptapeptide moiety at the interface. A few runs of equilibration were carried out with a very small time step, which was gradually increased until a final value of 2 fs. Finally, the boxes were filled by adding water molecules. In such a way systems were prepared containing 448 hexane molecules, 2, 4, 8, 18, 24, or 32 molecules of surfactin (corresponding to 1, 2, 4, 9, 12, or 16 molecules per interface, respectively), and about 2000 molecules of water, thus approximatively 17000 atoms. The box dimensions were \(45 \times 45 \times L_z\) Å in the \(x, y, \) and \(z\) directions, respectively, with \(L_z \approx 93\) Å. These systems have been equilibrated for 100,000 steps, with a time step of 2 fs at a temperature of 303 K. During equilibration, density profiles and energy convergence of the system have been monitored. After equilibration, we have recorded the dynamics of the system by accumulating coordinates at an interval of 0.4 ps during two periods of 0.5 ns.

4.2.2 Structure analysis

Peptide shape and orientation

To study the dynamics of surfactin molecules as a function of interfacial concentration, we have computed: the trajectory of the centre of mass of the surfactin's head (thus, all the atoms except those of the fatty-acid chain), its lateral diffusion, and the averaged distance between the centres of mass in order to estimate the molecular area.

Figure 4.1 shows the three-dimensional structure of the surfactin molecule in which the peptide part takes the form of a “horse-saddle”. This structure can be modelled by a tetrahedron, build from four atoms from the cyclopeptide backbone (see legend of figure 4.1). To characterize the shape and orientation of this horse-saddle we have introduced the vectors \(\vec{S}_{\text{top}}, \vec{S}_{\text{base}},\) and \(\vec{S}_{\text{height}}\) and the dihedral angle \(\alpha_{\text{dih}}\). The magnitude of the vectors \(\vec{S}_{\text{top}}, \vec{S}_{\text{base}}\) characterizes the degree of opening of the hydrophilic and hydrophobic side of the horse-saddle, respectively. The rotation of the surfactin molecule
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Figure 4.1: Modelling and parametrization of the “horse-saddle” conformation of surfactin. From four atoms defining a tetrahedral structure, $CO(5)$, $NH(2)$, $CH(4)$, and $C_1H$, are defined three vectors: $\vec{S}_{\text{top}}$: $CO(5) \rightarrow NH(2)$, $\vec{S}_{\text{base}}$: $C_1H \rightarrow \alpha CH(4)$, and $\vec{S}_{\text{height}}$: $[CO(5)-NH(2)] \rightarrow [C_1H-\alpha CH(4)]$. The dihedral angle $\alpha_{\text{dih.}}$ is defined by the angle between two vectors normal to two sides of the tetrahedron, each containing three atoms $(NH(2)-\alpha CH(4)-CO(5))$ and $(\alpha CH(4)-CO(5)-C_1H)$, respectively.

is described by the orientation of the $\vec{S}_{\text{height}}$ vector. In the case of a tetrahedral structure, $\vec{S}_{\text{height}}$ vector is orthogonal to the two orthogonal vectors $\vec{S}_{\text{top}}$ and $\vec{S}_{\text{base}}$. Thus, the orientation of the $\vec{S}_{\text{height}}$ vector can be defined as a sum of contributions from the vectors $\vec{S}_{\text{top}}$ and $\vec{S}_{\text{base}}$. A negative value of $\vec{S}_{\text{height}}$ orientation towards the interface corresponds to a tumbling over of the peptidic part of the surfactin molecule. The dihedral angle $\alpha_{\text{dih.}}$ characterizes the horse-saddle shape which can be modelled by a tetrahedron. It corresponds to the angle between the two vectors normal to two faces of the tetrahedron (see legend of figure 4.6). A symmetrical horse-saddle shape yields an angle $\alpha_{\text{dih.}}$ of $\sim 74-75$ degree. A change in the sign of $\alpha_{\text{dih.}}$ corresponds to an inversion of the horse-saddle conformation, and a value close to 0 corresponds to a flat structure.
Secondary structure

In our simulations we observe that the surfactin structure may fluctuate depending on the molecular orientation and the interfacial concentration. We have computed Ramachandran angles and hydrogen bonds (intramolecular, and intermolecular between surfactins, and with the solvent) in order to describe the secondary structure of the surfactant molecule and its flexibility, and to detect secondary structures as γ- and β-turns.

Hydrogen bonds are described from parameters specific to proteins [94, 95]. These criteria are a maximum distance of 2.5 Å between H (hydrogen atom) and A (hydrogen acceptor) and a minimum angle of 90 degrees for A···H-D (hydrogen donor) when A, H, and D coordinates are available. Such criteria allow a complete screening of the most common hydrogen bonds found in proteins but may underestimate bonds involved in particular secondary structure motifs such as γ- and β-turns, and main-chain lateral-chain interactions. Moreover, we have extended the potential hydrogen bond acceptors to include the main-chain nitrogen atom as described in a previous theoretical study [96].

Rotational and lateral diffusion

The lateral diffusion coefficient ($D_T$) has been obtained from the mean square displacement of the centre of mass of the peptidic moiety. At long times the diffusion coefficient is:

$$D_T = \lim_{t \to \infty} \frac{1}{2d \times t} \langle |\mathbf{r}(t) - \mathbf{r}(0)|^2 \rangle,$$

(4.1)

where $\mathbf{r}(t)$ is the position of the peptide centre of mass at time $t$, and $d$ the spatial dimension of the displacement. In our case, we have studied surfactant molecules remaining in the planar oil/water interface, hence, we have computed the two dimensional (translational) diffusion coefficient.

The calculation of the rotational diffusion coefficient is based on the Debye theory [97] which assumes a very diluted solution of rigid dipoles with Brownian motion rotating in nonpolar media. Application of the theory has been extended to more complex systems and good results have been obtained for protein/water systems [98]. The rotational diffusion coefficient ($D_R$) can be obtained from the relation:

$$\langle P[\cos\theta(t)]\rangle = e^{-\mu(t+1)D_R t} = e^{-t/\tau},$$

(4.2)
where $\theta(t)$ is the angle between two $\vec{S}_{\text{height}}$ vector orientations spaced in time by $t$, $P_l$ is the $l$th rank Legendre polynomial, and $\tau_l$ the rotational relaxation time associated with each of the Legendre polynomial correlation functions. For molecules undergoing Debye diffusional rotation, a plot of $1/\tau_l$ against $l(l+1)$ should be linear with a slope equal to $D_R$.

4.3 Results and discussion

In this section, we first analyse the behaviour of surfactin molecules at the interface through the study of density profiles and centre of mass motions of the cyclopeptide moiety. Next the secondary structure of the peptidic part is analysed, and finally, we relate our results to interfacial properties.

4.3.1 Behaviour at the interface

Density profiles

From atomic density profiles plotted in figure 4.2 A we observe that surfactin molecules reside at the hexane/water interface. A coordinate analysis of the terminal methyl group of the aliphatic chain (not shown) shows an anchoring of the surfactin molecule in the oil phase. For the three lower concentrations, the surfactin density is increasing with the interfacial concentration, while for the three higher concentrations, the increase of the concentration yields a widening of the surfactin density peak combined with a smoother water interface, as shown in figure 4.2 B. This broadening suggests that the organization of the surfactant layer has changed with surfactin molecules slightly popping out of the surfactant monolayer.

Centre of mass motions

The projection of the centre of mass displacement onto the plane of the interfaces is shown in figure 4.3 for low concentrations and in figure 4.4 at high concentrations. From those snapshots, we observe that molecules exhibit a gas-like behaviour with uncorrelated motions below a concentration of 4 molecules per interface, a solid-like behaviour with collective motions above a concentration of 12 molecules per interface, and a liquid-like behaviour for intermediary concentrations. This behaviour is confirmed by the study of the distance between two surfactin molecules placed at the same interface (not shown). This distance is rather constant at high concentrations but fluctuates at lower concentrations. At the concentration of 4 surfactins per
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Figure 4.2: Density profiles at 303 K, (A) shows hexane (dotted line), water (dashed line), and surfactin headgroup (straight line) at a concentration of 9 surfactins per interface. (B) shows the surfactin (straight line) and water (dashed line) profiles at one interface for the different interfacial concentrations: 16, 12, 9, 4, 2, and 1 surfactin(s) per interface, from left to right, respectively.

interface, figure 4.3 C, where four molecules are located at each interface in the simulation box, few molecules are clustered. Within a cluster, molecules can be surrounded by one or two neighbouring molecules. Molecules are spaced by a minimal distance which decreases from approximatively 15 Å at a concentration of 4 molecules per interface to less than 10 Å at the highest concentration. These intermolecular distance fluctuations suggest a conformational flexibility of the peptide moiety. Intermolecular distances yield an estimation for areas per molecules of surfactin which fluctuate from 177 Å² at a concentration of 4 molecules per interface, where the interface is not completely covered by surfactant but few molecules are already in contact, to 78 Å² at the highest concentration where we observe the onset of a solid phase. Our results are similar to $A_0$ and $A_4$ molecular areas obtained from II-$A$ isotherms [77] at pH 4.2, where the surfactin molecule is fully protonated, equal to 184 and 89 Å², respectively.

As we will demonstrate next, the properties of the surfactin molecules strongly depend on the aggregation state and their orientation.
Figure 4.3: Projection of the centre of mass of each peptidic moiety at various concentrations. Each molecule centre of mass at an interface differs by a color. Each box represents the $xy$ plane and periodic boundary conditions have been applied on coordinates. (A) 1, (B) 2, and (C) 4 surfactins per interface for each interface, left figures and right figures.
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Figure 4.4: Projection of the centre of mass of each peptidic moiety at various concentrations. (A) 9, (B) 12, and (C) 16 surfactins per interface for each interface, left figures and right figures.
Figure 4.5: Radial distribution function of the centre of mass of the peptidic moiety at 4, 9, 12, and 16 surfactins per interface.

Radial distribution functions

Figure 4.5 A shows the radial distribution function of the centre of mass of the peptidic part of surfactin molecules at a range of surfactin concentrations. The first peak is observed at about 12, 11.5, 9.5 and 9-12 Å at a concentration of 4, 9, 12, and 16 molecules per interface, respectively, corroborating a compression of surfactin molecules as the interfacial concentration increases. Radial distribution functions have been computed from the projection of the centre of mass coordinates onto the interface. As a consequence, molecules popping out of the interface yield minor peaks placed at distances shorter than 8 Å and contribute to a broadening of the peaks. Moreover, the radial distribution functions plotted in figure 4.5 are an average from the contributions of the two interfaces. As a consequence, at the highest concentration, the contribution from the most ordered interface is counterbalanced by the contribution from the other interface, which is less ordered (see figure 4.4 C), yielding a radial distribution function not representative to an ideal bidimensional solid.
4.3.2 Molecular shape and orientation

Figure 4.6 displays the fluctuations of the $\alpha_{\text{dih}}$ angle related to the tetrahedral shape model of the molecule. At all surface concentrations, the angle distributions exhibit a main peak, sharp and centred on 80-85 degree at high concentrations (figure 4.6 A), and broader with a few other contributions which depend on the orientation of molecules and their environment at low concentrations (figure 4.6 B). At low concentrations, molecules can be described as clustered or free (the latter may tumble over). On the one hand free molecules not tumbled over have a rather flexible tetrahedral shape, on the other hand clustered or tumbled over free molecules exhibit a stable $\alpha_{\text{dih}}$ angle equal to 50-55 and 80-85 degrees, respectively, as shown on figure 4.6 C. However, such an $\alpha_{\text{dih}}$ angle range demonstrates that surfactin molecules at the water/hexane interface adopt a tetrahedral shape, which is similar to the compact “horse-saddle” conformation observed under particular conditions [87] where surfactin was in a DMSO solution. The amplitude of the observed dihedral angle distributions ascertains the flexibility of the secondary structure which never remains flat or adopts a reversed saddle shape.

To characterize the orientation of the molecule, i.e., the saddle up or down, we have computed the angle between $\vec{S}_{\text{height}}$ and the $xy$ plane, parallel to the two hexane/water interfaces. At high concentrations, figure 4.7 A shows angular distributions in a range of 15-90 degree with minor contributions below 15 degree. At 16 molecules per interface, the distribution is rather large and centred around 45 degree while at a concentration of 9 molecules per interface the distribution is made of a main peak with a mean angle value of 70 degree. When the interfacial concentration is increased, the surfactin solid-like molecules popping out of the planar interface may adopt a tilted orientation but have less freedom to tumble. Figures 4.7 B and C illustrate that for concentrations below 4 molecules per interface, molecules may tumble over. At low concentrations, the proportion of tumbled over molecules (corresponding to a negative angle value) increases inversely with the concentration. During our recorded simulations, we have observed one tumbling over motion within a few tens of picoseconds. That means that the other observed tumbled molecules have tilted during the equilibration time. Moreover, at the concentration of 2 molecules per interface, we have observed at an interface, two molecules clustered with opposite orientation, forming a kind of “dimer”.
Figure 4.6: Normalized distributions of the $\alpha_{\text{dih}}$ angle at various interfacial concentrations. (A) 9, 12, and 16 surfactins per interface, (B) 4, 2, and 1 surfactins per interface, and (C) at a concentration of 4 surfactins per interface plotted with the contributions of molecules clustered with two neighbours (straight line), clustered with one neighbour, free and tumbled over (dashed line), and free molecules (dotted line).
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Figure 4.7: Normalized distributions of the $\tilde{S}_{\text{height}}$ angle at various interfacial concentrations. (A) 9, 12, and 16 surfactins per interface, (B) 4, 2, and 1 surfactins per interface and (C) at a concentration of 2 surfactins per interface plotted with contributions from tumbled over molecules (dotted line) or not (straight line).
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The tumbling of molecules is a surprising result as compared to previous models proposed from the molecular structure and experiments suggesting that hydrophilic amino-acids were oriented towards the hydrophilic part and the hydrophobic part was pointing to the hydrophobic medium or laying at the interface [77,78,87]. First, our results cannot be compared with a previous computed simulation study of surfactin conformation [93] where the interface was defined by the position of the hydrophilic and hydrophobic barycentres in a medium of intermediate dielectric constant. Furthermore, at low interfacial concentrations, lateral chains from the two hydrophilic residues of tumbled over molecules point into the core of the peptide avoiding interactions with the hydrophobic oil interface. This phenomenon has been ascertained by the orientation of the lateral chains (not shown) and the existence of hydrogen bonds as shown subsequently. Moreover, the low flexibility of tumbled molecules supports this model of a compact structure.

The global orientation of the molecule given by $\vec{S}_{\text{height}}$ orientation can be explained in terms of the orientation of the two vectors $\vec{S}_{\text{top}}$ and $\vec{S}_{\text{base}}$ placed at the base and the top of the tetrahedral model. At low concentrations, figure 4.8 B (left) shows a broad distribution centred on a mean value of 15 degrees for the $\vec{S}_{\text{base}}$ angle with a contribution in the range of 30-60 degrees for tumbled over molecules, while at higher concentrations A (left) the distribution is broader. At low concentration, the hydrophobic interface is a plane while at higher concentrations molecules are packed and thus create a hydrophobic environment for their neighbours. In figure 4.8 (right) we show fluctuations of the $\vec{S}_{\text{top}}$ vector orientation. The angle distribution is drifting towards low angle values as the interfacial concentration is decreasing. At high concentrations, the angle is about 50 degrees. This behaviour confirms the influence of the aggregation on the orientation of the molecules.

Internal dimensions of the tetrahedral structure

The magnitudes of the three vectors $\vec{S}_{\text{top}}$, $\vec{S}_{\text{base}}$, and $\vec{S}_{\text{height}}$ give a complementary insight into the geometry of the surfactin molecule. The $\vec{S}_{\text{height}}$ vector magnitude varies from 2.9 to 4.9 Å as the secondary structure fluctuates (details not shown). Figure 4.9 shows the $\vec{S}_{\text{base}}$ and $\vec{S}_{\text{top}}$ magnitude distributions versus the surfactin concentration. Above four molecules per interface, the magnitude is about 5.6 Å, and 4.1 Å for the $\vec{S}_{\text{base}}$ and $\vec{S}_{\text{top}}$ vectors, respectively. At low concentrations, the $\vec{S}_{\text{base}}$ magnitude distribution is broad with a mean value of 6.5 Å, and the $\vec{S}_{\text{top}}$ magnitude distribution shows a broad peak around 6 Å and a sharper one around 4.1 Å, almost separated. At the lowest concentrations, where one molecule is upside down
and the other one is tumbling, only the sharp peak is present in figure 4.9 B (right). In conclusion, concerning the free molecules, the hydrophilic peptidic part is compact when the molecule is tumbled over and its opposite side has an opened conformation. In this case, the $\tilde{S}_{\text{base}}$ magnitude distribution reported on figure 4.9 B (left) has only one broad component due to the lactone part. This has a large ability to fluctuate compared to an amino acid residue. At the intermediate concentration of 4 molecules per interface, both distributions have a second component. The separation between the two components in figure 4.9 B (right) demonstrates the existence of two distinguishable conformational states of the hydrophilic side, “opened” or “closed”. Clustered molecules surrounded by two neighbours are in a “closed” conformation while the others, in contact with less than one neighbour, are in an “opened” state. Two phenomena can explain this observation. The tran-

Figure 4.8: Normalized distributions of the angle between $\tilde{S}_{\text{base}}$ (left), $\tilde{S}_{\text{top}}$ (right), and the xy plane parallel to the interface. (A) 9, 12, and 16 surfactins per interface, and (B) 4, 2, and 1 surfactins per interface.
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Figure 4.9: Normalized distributions of the $S_{\text{base}}$ (right) and $S_{\text{top}}$ (left) vector magnitude at various concentrations. (A) 9, 12, and 16 surfactins per interface, and (B) 4, 2, and 1 surfactins per interface.

Transition from one state to the other obeys internal constraints and needs a significant activation energy, or the first transition state during the molecular opening adopts a geometry dependent on the first inserted molecule (as a water molecule in our case). However, the “closed” state of the hydrophilic side is observed in two cases, at a concentration of 4 surfactins per interface when molecules are clustered, and at lower concentrations when molecules are upside down. These occurrences suggest strongly that the “closed” conformation of the hydrophilic side is stabilized by internal hydrogen bonds favoured when interactions of the hydrophilic part with the aqueous phase are concealed or hindered by the environment by tumbling or packing of the peptidic part, respectively.
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Ramachandran angles

To complete the structure analysis and estimate the flexibility of surfactin molecules, we have recorded fluctuations of Ramachandran angles. At concentrations below 12 molecules per interface, upside down molecules have stable Ramachandran angles for all the residues except the terminal ones which are sensitive to the motions of the aliphatic tail and the lactone group. Moreover, those molecules exhibit the Ramachandran angles characteristic of a type II’ β-turn Asp(5)—>Leu(2). At a concentration of 2 and 4 molecules per interface, the free molecules and those clustered with only one neighbour are rather flexible and do not contain a particular structural motif. At a concentration of 9 surfactins per interface, about one third of the molecules are unstable when we consider the Ramachandran angles. They correspond to the molecules which are not yet part of a homogeneous surfactant monolayer.

At a concentration of 12 surfactins per interface, four molecules in total have few unstable angles corresponding to a D-Leu(3)-Val(4) peptide-plane flip with a ($\phi_4; \psi_3$) transition from (-90; -100) to (70; 100), the former state corresponding to the type II’ β-turn and the latter one being metastable. This kind of peptide-plane flip is in agreement with previous work on peptide-plane motions [99], although it does not correspond to a transition between two stable conformations. With the exception of one molecule containing a cis D-Leu(3)-Val(4) conformation, all the other molecules have a type II’ β-turn conformation.

At the highest concentration, one third of the molecules have unstable Ramachandran angles. Of the remaining two thirds, three molecules exhibit a peptide-plane flip as described above and four molecules adopt a non-conventional turn with (72±12; -100.5±5.7) and (-137±7; 40.25±9.1) as ($\phi_3; \psi_3$) and ($\phi_4; \psi_4$), respectively, which does not fall into allowed regions of the Ramachandran plot specific to each residue [100]. The remaining molecules contain a type II’ β-turn. This unexpected conformation found at this concentration may result from the large lateral pressure applied on the surfactin molecules, inducing a conformational transition.

Angular fluctuations may explain the “chimeric” character of the molecule [90] observed experimentally. Motions of the peptidic backbone, as the coexistence of different conformers under identical physical conditions, induce a large distribution of the amide and the carboxylic groups orientation, yielding different absorption spectroscopic characteristics. But despite this angular variability, all the molecules at concentrations greater than 4 molecules per interface, have a similar hydrogen bond network.
Intramolecular hydrogen bonds

In figures 4.10 A and B we illustrate the contributions of the most frequent intramolecular hydrogen bonds observed, excluding hydrogen bonds within carboxylic functions, at concentrations of 4 and 2 surfactins per interface, respectively.

Three hydrogen bonds have an occurrence probability longer than half of the simulated time. They are two “weak” bifurcated hydrogen bonds, NH(1)-CO(5) and NH(2)-CO(5), and the hydrogen bond characteristic of the conformer S1, NH(5)-CO(2). Those bonds mainly occur within packed molecules and upside down ones. It is worth noticing that those bonds, as defined by the method outlined above, are also detected from the coordinate set of conformer S1.

When focusing on less frequent hydrogen bonds, we notice that the Glu(1) carboxylic group is more often involved in intramolecular hydrogen bonds than the Asp(5) carboxylic group. This can be explained by the length of the Glu(1) lateral chain being larger than its analogue in the Asp(5) residue, allowing a greater flexibility. The oxygen atoms from Glu(1) interact preferentially with NH(7) and NH(1) while the rare bonds involving Asp(5) concern NH(5). Most of the molecules which are upside down have a hydrogen bond between Glu(1) and CO(7) too. Such interactions confirm the insertion of the carboxylic group in the peptide core when its residue is shielded from the
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hydrophilic medium. Finally, most of the molecules which are not stabilized by the three most abundant hydrogen bonds present various hydrogen bonds such as NH(5)-NH(4), NH(3)-NH(2), NH(2)-NH(1) and NH(3)-CO(1) with various occurrence probabilities (from 10 to 30%).

At concentrations of 9, 12 and 16 surfactins per interface, the two hydrogen bonds NH(2)-CO(5), and NH(5)-CO(2) have an occurrence probability almost equal to the recorded time as NH(7)-COOH(1). Thus, albeit few molecules have conformational transitions as illustrated by their Ramachandran angle analysis, the type II' β-turn hydrogen bonds are preserved. Moreover, we observe few other hydrogen bonds rather stable as NH(5)-NH(4), NH(1)-CO(5), and COOH(1)-CO(7).

Whatever the concentration, we have never noticed hydrogen bonds between the two different carboxylic groups of a single molecule. Moreover, we have detected none of the three hydrogen bonds characteristic of the S2 conformer, NH(7)-CO(5), NH(4)-CO(2) and NH(6)-C\textsuperscript{1}O. This suggests that no transition is allowed from S1 conformer to S2 conformation under the physical conditions used for our simulations. On the other hand, we have also performed simulations starting from the S2 conformer. The characteristic structural parameters of this conformation which contains two γ-turns have not been conserved during the equilibration period. This confirms the S1 conformer as the most stable conformation at a hydrophilic/hydrophobic interface at a wide range of interfacial concentrations.

4.3.3 Interactions between a surfactin and its environment

The peptide is not big enough to have buried hydrogen acceptors or donors and only a few nitrogen and oxygen atoms were part of intramolecular hydrogen bonds. This suggests clearly that most of the remaining oxygen and nitrogen atoms interact with the solvent or other surfactins as hydrogen bond donor or acceptor.

In fact, very few hydrogen bonds between surfactin molecules have been detected. During the simulation, bonds involving the Asp(5) carboxylic group, and CO(1) and CO(2) groups, between two aggregated molecules at an interface, and the Glu(1) carboxylic group, and CO(6) and O(lactone) groups, between two other associated molecules at the other interface, have been identified at the highest surfactin concentration. However, these binding associations have rather different occurrence probabilities, 3.5% and 32.2%, respectively.

Hydrogen bonds between surfactin and water molecules are numerous. We have investigated hydrogen bonds involving a water molecule and two
residues and classified them as type I, II, or III depending on the geometry of the interaction between the water molecule (Hw-Ow-Hw) and the hydrogen bond donor (D) and acceptor (A), D-(Ow)-D, D-(Ow-Hw)-A, A-(Hw-Ow-Hw)-A, respectively. We assume that the donors and acceptors which are not involved in one of the previously described intra- and intermolecular hydrogen bonds interact with a single water molecule as D-Ow, A-Hw.

Most of the intermolecular hydrogen bonds have a probability smaller than 5%. But when we focus on the most stable bonds, we notice that hydrogen bonds of the type I are encountered between two consecutive amino acids NH(n)-NH(n+1) in the less compact surfactin molecules. Their occurrence probabilities are in the range of 30-60%. Hydrogen bonds from type II are the most abundant except for molecules which are upside down. In this case, one of the most stable hydrogen bonds is linking the Glu(1) carboxylic group and CO(7). This bond can have a probability up to 100%. The less compact molecules are stabilized by a large number of hydrogen bonds of this type. The most specific bonds are between NH(1) or NH(2) and CO(5). Their occurrence probabilities are in a range of 10-100%. Their presence is closely related to the increase of the "top" vector magnitude. As a consequence, compact molecules rarely have hydrogen bonds of type II and none of them seems to be stable within this molecular geometry. The last type of hydrogen bond, type III, is rather abundant. In packed molecules, bonds between CO(4)-CO(6) and CO(3)-C1O have a probability of 75 and 60%, respectively, while this value decreases dramatically for the other molecules, except for molecules which are upside down, where CO(3)-C1O is more abundant than CO(4)-CO(6).

In conclusion type I and III hydrogen bonds are mainly linking the peptide with its solvation shell, while type II bonds are characteristic of "opened" conformations of the hydrophilic moiety and take place between residues involved in the intramolecular hydrogen bonds present in packed molecules.

4.3.4 Interfacial properties

Diffusion coefficients

Rotational diffusion coefficient calculations are based on the motions of the \( \vec{S}_{\text{height}} \) vector towards the interfacial plane while translational diffusion coefficients are computed from centres of mass displacements. An average of a vector ensemble motions should give a better description of the rotational behaviour. However, this vector is defined from the \( \vec{S}_{\text{base}} \) and \( \vec{S}_{\text{top}} \) vectors, thus local fluctuations are by definition partially averaged.
4.3 Results and discussion

Figure 4.11: (A) Inverse of the rotational relaxation time $\tau_1$ as a function of $l(l+1)$ corresponding to the five first Legendre polynomials $P_l$. (B) $\ln(P_l[\cos(\theta(t))]$ as a function of time for three Legendre polynomials, $P_1$, $P_3$, and $P_5$, at 16 (a), 12 (b), 9 (c), 4 (d), 2 (e), and 1 (f) surfactins per interface.
In figure 4.11 $A$ $1/\tau$ is plotted versus $l(l+1)$ for all the concentrations. We observe a linear relationship for all concentrations except the lowest density one. This result validates the Debye model to describe the rotational motion despite the insertion of the aliphatic tail and the lateral chains from apolar residues in the hydrophobic medium. This likely tail perturbation might be averaged over all the molecule by interactions between amino acid lateral chains and solvents. Concerning the lowest concentration, two phenomena may explain this nonlinear behaviour. On the one hand, only two molecules contribute to the value, consequently, the statistical accuracy and validity of the results are quite low. Moreover, one of the two molecules has a fast tumbling over motion which brings a "non conventional" contribution to the global rotational motion studied. In figure 4.11 $B$, three logarithms of Legendre polynomial correlation functions are displayed. The short-time part of the curves contains some additional structure which could be related to internal motions of the protein and to rattling of the peptidic moiety within the solvent shell. The variation of the rotational diffusion coefficient with the interfacial concentration is plotted on figure 4.11 $C$.

Table 4.1 contains numerical values of both rotational and lateral diffusion coefficients. The rotational diffusion coefficient decreases as the interfacial concentration increases due to the lack of freedom for molecules at high interfacial concentrations. The lateral diffusion coefficient shows a dependence on the concentration from a concentration of 2-4 molecules per interface. At the lower concentration, our results present a poor statistical value. Moreover, the peptidic part of one of the two molecules has tumbled over on itself during the simulation. This rare motion may have affected the averaged value of the lateral diffusion coefficient.

**Interfacial tension and tangential pressure profile**

Our studies on alkane liquid-vapour systems (chapter 2), hexane-water (chapter 3), and nonane/nonanol/water systems (not shown) show that the interfacial tension is correctly estimated for a large range of temperatures and surfactant concentrations, and differs less than $\sim 10\%$ from experimental results, with the appropriate force field.

Simulated interfacial tensions are reported in table 4.1. Up to 9 molecules per interface, the interfacial tension is nearly constant. Above this limit, the interfacial tension can decrease dramatically until a minimal value of half the interfacial tension of a pure hexane/water system. This large decline in the interfacial tension illustrates the high interfacial activity of the surfactin molecule and gives an estimation of the "active" range of surfactin interfacial
4.3 Results and discussion

<table>
<thead>
<tr>
<th>Interfacial concentration (molec./interface)</th>
<th>$D_T$ $(10^{-12} \text{m}^2 \text{s}^{-1})$</th>
<th>$D_R$ (ns$^{-1}$)</th>
<th>$\gamma$ (mN.m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>-</td>
<td>-</td>
<td>46.0±1.6</td>
</tr>
<tr>
<td>1</td>
<td>670±120</td>
<td>-</td>
<td>47.4±1.7</td>
</tr>
<tr>
<td>2</td>
<td>950±220</td>
<td>0.280</td>
<td>44.3±1.6</td>
</tr>
<tr>
<td>4</td>
<td>320±140</td>
<td>0.263</td>
<td>49.7±1.7</td>
</tr>
<tr>
<td>9</td>
<td>260±140</td>
<td>0.207</td>
<td>46.2±1.9</td>
</tr>
<tr>
<td>12</td>
<td>230±70</td>
<td>0.124</td>
<td>31±2</td>
</tr>
<tr>
<td>16</td>
<td>180±60</td>
<td>0.055</td>
<td>18±2</td>
</tr>
</tbody>
</table>

Table 4.1: Interfacial tension, rotational and lateral diffusion coefficients as a function of the number of surfactant per interface. * results from previous simulations concerning hexane-water binary system (see chapter 3).

concentrations. The efficiency of surfactin in lowering the interfacial tension of a hexane-water system is comparable to its ability to reduce the water-air interfacial tension [77,78].

Through the plot of the tangential component of the pressure profile, shown on figure 4.12, we can analyse the effect of surfactin molecules at the interface. At low concentrations, up to 4 molecules per interface, pressure profiles show a single structured peak. This peak contains contributions from a sharp peak characteristic of the oil/water interface, and a broader one related to the surfactin layer. While the concentration is increasing, direct contacts between oil and water phases are reduced by the surfactant film. At a concentration of 9 surfactin molecules per interface, the interface is fully covered by the surfactant layer and the lateral pressure profile contains several peaks. While the concentration is increasing, the profile is broader as the interfacial region is becoming thicker with an increasing number of surfactin molecules slightly popping out of the surfactant layer.

The reduction of the interfacial tension at concentrations higher than 9 surfactin molecules per interface (corresponding roughly to the interfacial concentration needed for a total covering of the oil/water interface) results mainly from the contribution of interactions between surfactant molecules which are not fully embedded in the surfactant layer and water or oil phase. They yield a positive contribution to the tangential pressure profile (considering that in the bulk phases, either the oil or water one, the tangential pressure profile is on average null).
Surfactin: a “chimeric” peptide

Figure 4.12: Tangential pressure profiles at various concentrations. The oil interface is located at \( z \approx 25 \, \text{Å} \), with the oil phase on the right side of the graph, and the aqueous one on the left side. This plot is an average of the tangential component of the pressure profile of the two interfaces and over a simulation of 0.5 ns for the different interfacial concentrations: 16, 12, 9, 4, 2, and 1 surfactin(s) per interface, from the bottom to the top, respectively.

4.4 Concluding remarks

These simulations are the first molecular dynamics studies at an atomic level of surfactin in a liquid hydrophilic/hydrophobic interphase. They bring an interesting insight into the structural variability of the surfactin molecule depending on interfacial concentration and the molecular environment, and investigate the interfacial properties of this remarkable molecule. Since very few structure-activity correlation studies at a hydrophilic/hydrophobic interface have been carried out experimentally it is difficult to compare our results with existing data. However, the spectroscopic studies done in a homogeneous medium suggest a structural variability depending on the nature of the solvent and concentration of cations. In our model, which reproduces the native environment of the protonated form of surfactin, besides its salt concentration, we demonstrate that the conformation depends also on the environment of the molecule. Structural variability has already been observed [101,102] when a peptidic segment was placed in a different medium. In this study,
we have demonstrated that placed at the same hydrophilic/hydrophobic interface, the surfactin molecule adopts different conformations depending on its interfacial concentration. Placed in a crowded environment, molecules are associated such that the interactions between hydrophobic residues and the hydrophilic medium are minimized. Such clusters are mainly stabilized by van der Waals interactions and from time to time by intermolecular hydrogen bonds involving carboxylic groups from side chains. Moreover, when hydrophilic residues are shielded from the environment, a complete tumbling over of the peptidic part can occur. This can be related to the ability of a surfactin molecule to pass through a hydrophobic medium as a lipid membrane. We hope that our description of the structural variability at this hydrophobic/hydrophilic interface will bring helpful insights for the interpretation of spectroscopic results.

From these results, we can assume that other environmental factors such as an organized and charged environment (as a zwitterionic lipid bilayer) will strongly affect the conformation of the surfactin molecule and its orientation as suggested by experimental results [85].