Adsorption of peptides on solid surfaces: An interaction site model study

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Abstract
Computer simulations can be of great help in detailed studies of the adsorption of peptides on solid surfaces. Unfortunately, detailed simulations are limited to infinite dilution situations. Therefore, important characteristics of the adsorption phenomenon such as the morphology of adsorbed layer and the aggregation of the peptide at the solid surface remain unreachable. This prompted us to use the Extended Reference Interaction Site Model (XRISM) model. The adsorption of a model 8-residues peptide, (N)ASP^{1}-ASP^{2}-ILE^{3}-ILE^{4}-ASP^{5}-ASP^{6}-ILE^{7}-ILE^{8}(C), on a charged surface consisting of CH_2 atoms with a fixed lattice arrangement was studied. We covered densities of practical interest. To overcome the inability of the XRISM method to handle a large number of interaction sites (which would arise by treating the peptide atoms explicitly) we used a Monte Carlo (MC) algorithm to determine the united residue potential of the peptide, where each amino acid is represented as a single site. A careful analysis of the correlation functions between the peptide and the surface allowed us to determine the morphology of the adsorbed layer. Inspection of the pair correlation functions between adsorbed peptides shows the presence of clusters of different sizes.

Introduction
A fundamental understanding of protein behavior at solid-liquid interfaces is important to improve our knowledge of numerous processes. The heterogeneity of the protein surface and its shape brings to a multiplicity of possible interactions between the protein and the surface, making protein adsorption studies challenging. The use of customized short model peptides (Mungikar and Forciniti, 2004) simplifies the studies considerable but still finite concentrations of practical interest remain unreachable. Because peptide adsorption is important in the physical and medical sciences (Chandler et. al, 1982; Lue and Blankschtein, 1995), we decided to extend our studies to the adsorption of peptides at finite concentration by using a combination of Monte Carlo simulations and liquid state theories. Monte Carlo simulations were used to determine the force field between amino acid residues and between the residues and the surface whereas a liquid state theory approach was used to study the adsorption of a short peptide at finite concentrations at solid/fluid interfaces.

Liquid state theories such as integral equations are powerful techniques for studying properties of molecular fluids. The Reference Interaction Site Model (RISM) coupled with an additional closure relation such as Hypernetted Chain Approximation (HNC) is one of them. In RISM, pair correlation functions, pcf, between the atoms (sites) that form the molecules are calculated; these pcf yield a “picture” of the structure of the solution from which thermodynamic properties may be calculated. RISM has been used as an economic alternative to molecular simulations methods for the adsorption of polymeric fluids (Yethiraj and Hall, 1991; Striolo and Prausnitz, 2001; Yethiraj, 2000; Janssen et al. 1997). Yethiraj (Yethiraj, 2002) has reviewed the use of computer simulations and liquid state theories for the computation of the structure of continuous-space or off-lattice models of polymers near surfaces. We have extended this theory to the study of the adsorption of peptides.

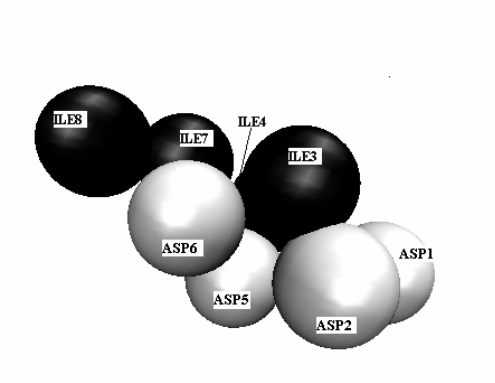

Figure 1. 3-D cartoon of the peptide

Two problems associated with the use of RISM for polymeric-ionic fluids are 1) divergences in the solution of the equations caused by long-ranged potentials and 2) lack of convergence as a result of a very large number of non-equivalent sites (atoms). Some of the formulation of the theory must be changed to avoid the problems associated with long-ranged potentials. The extension of RISM to polar or Columbic fluids is known as Extended Reference Interaction Site Model (XRISM) (Hirata and Rossky, 1981; Hirata et al. 1983). The computational problems associated with a large number of correlation functions, which is the case for an all-explicit atomic model of a peptide, can be avoided by collapsing atoms into larger entities (united atoms). For example, all the atoms in one amino acid can be collapsed into a single site having an equivalent size and interacting via a potential field generated by all the atoms that make the amino acid. For example, Zhou *et. al*. (Zhoue et al., 2003) developed a new united residue peptide-surface interaction model to studying the adsorption of IgG_1 and IgG_2. They showed that the model is consistent with experimental results and can explain them reasonably. We followed a similar methodology to obtain amino acid-surface united residue force fields from the united atom representation of each amino acid.

The sequence of amino acids in the 8-residues peptide used here was (N)ASP^1-ASP^2-ILE^3-ILE^4-ASP^5-ASP^6-ILE^7-ILE^8(C). The superscripts are used to identify these residues later on (a cartoon of the peptide is shown in Fig. 1). The molecular weight and volume of the peptide is 908.964 and 797.4 $Å^3$ respectively. In our earlier adsorption studies of this peptide (Mungikar and Forciniti, 2004), we have shown that it undergoes a total loss of helical structure upon adsorption at a solid/vacuum interface. This extended structure was used here for the 8-residue peptide for consistency with our earlier findings. For the atomistic model used in Monte Carlo simulations to determine the force field between amino acids, all peptide atoms were treated explicitly except for the methyl groups, CH_X (X=1-3), which were treated as united atoms. In all the studies using XRISM/HNC theory presented here a full united atom model of the peptide was used, by doing so, the number of interaction sites was reduced from 72 to 8.

The model surface consisted of CH_2 groups arranged in a single plane with a face centered cubic (FCC) arrangement. An assembly of two surfaces with opposite charges was used. Each surface consisted of an infinite periodic array of identical sites arranged in a hexagonal lattice pattern. The surfaces can be viewed as a single site (denoted by X) surrounded by Γ layers (where Γ = 1,2,3,4 …… N). The distance between adjacent sites of the surface was Δ X= 3.27 Å. One of the assumptions made to solve the peptide-surface XRISM equations is that the surface must be infinite in two-dimensions. Thus, the number of layers (N) used was very large (100) to avoid end effects and make all sites of the wall identical. The charge on the surface site was 0.05e that corresponds to a charge density of 0.00556 e/$Å^2$.

Methods

Each amino acid of the united-residue representation of the peptide is represented by a single sphere centered at the center-of-mass of the amino acid, and of radius equal to the van der Waals diameter of the amino acid. The dispersion energy parameters for amino acid-amino acid and amino acid-surface interactions were estimated by Monte Carlo simulations of a pair of amino acids (or one amino acid and the wall) using an explicit-atom model. The potential used for the interaction between any pair of atoms was the spherically symmetric Lennard-Jones (LJ) plus a Coulomb term,

$$u_{\alpha\beta} = \left\{\frac{A_{\alpha\beta}}{r}\right\}^{12} - \left\{\frac{C_{\alpha\beta}}{r}\right\}^{6} + \frac{e^2 q_\alpha q_\beta}{4\pi\varepsilon_0}\left\{\frac{1}{r}\right\} \qquad (1)$$

where $A_{\alpha\beta}$ and $C_{\alpha\beta}$ are the repulsive and dispersive coefficients of the LJ potential, r is the distance between atoms α and β, q_α and q_β are the partial charges on atoms α and β, and ε_o is the permittivity. Only the first term of the right hand side of equation (1) was used in the calculation of united residue potentials. Non-bonded potential parameters for the united atom model were obtained from the GROMACS 87 force field (van der Spoel et al. 1999).

The force field between pairs of amino acids was determined as follows (McCoy and Curro, 1998). The centers-of-mass of the amino acids were fixed at a distance r. Energy functions analogous to equation (1) were used for each pair of atoms. The distance between the centers of mass was kept constant and both molecules were angularly displaced. Each Monte Carlo step consisted of rotating both molecules by a maximum displacement allowed. U(r), the resulting equilibrium interaction potential of the system, was calculated as the average over the angular degrees of freedom of the molecules. The effective united residue potential, w(r), in terms of reversible work (potential of mean force) can be written as,

$$w(r) = -kT \ln < e^{-\beta U(r)} >_o \qquad (2)$$

where,

U(**r**) = Interaction Potential energy for the united atom model of amino acids

$<\ldots>_o$ = Angular average

r = distance between the COM fixed throughout the average

The simplified version of equation (2) is (by taking the logarithm inside the average):

$$w(r) = \left\langle U(r) \right\rangle_o \qquad (3)$$

These calculations were repeated for many separation distances. Therefore, w(r), the effective united residue potential as a function of "r", was obtained. W(r) is a function of temperature; therefore, running the simulations over a temperature range one can calculate both the well depth and the united residue site diameter as a function of temperature.

A similar methodology (Hirata et al., 1983) as described above was used to calculate the force field between the amino acids and the surface. The center-of-mass of the amino acid was kept at a fixed distance from the surface; then, the amino acid was rotated to determine its equilibrium angular position with respect to the surface. Once the optimum orientation of the amino acid was obtained, it was moved away or towards the surface from its initial position. The average interaction energy was obtained at each distance from the peptide to the surface. An energy function analogous to equation (1) was used; i.e,

$$U(d)=4\varepsilon\left[\left(\frac{\sigma}{d}\right)^{12}-\left(\frac{\sigma}{d}\right)^{6}\right]=\left(\frac{A^{12}}{d^{12}}\right)-\left(\frac{C^{6}}{d^{6}}\right) \qquad (4)$$

Where, d (Å) is the distance between interaction sites e.g; center-of-mass of amino acid residue and the surface, σ (Å) is the Van der Waals diameter of each residue, which is calculated using van der Waals volume of each residue (McCoy and Curro, 1998). ε (K) is the interaction energy parameter between the amino acid and the surface and,

$$A^{12}=4\varepsilon\sigma^{12}$$
$$C^{6}=4\varepsilon\sigma^{6} \qquad (5)$$

with A and C in units of Å-$K^{1/12}$ and Å-$K^{1/6}$ respectively.

The set of XRISM equations in the HNC closure for the peptide in bulk was solved for the pcfs by using a standard Piccard algorithm (Watts, 1973; Ortega, and Rheinboldt, 1970). These peptide-peptide pcfs were input into the XRISM/HNC equations for the peptide/surface system, which were solved for the peptide-surface correlation functions again by a Piccard algorithm. (Akiyama and Hirata, 1998) To avoid divergences arising from the long ranged potential, the numerical solution of the equations was renormalized according to Morris and Monson (Morris and Monson, 1983).

Two surfaces of identical structure at infinite dilution, one positively charged and one negatively charged, were introduced into bulk peptide. The force field between each amino acid site in the peptide and the surface was modeled via an equation analogous to equation (1). Note that the surface potential and the geometrical parameters are not chosen to model any type of surface in particular. Rather, they serve as a generic model for any surface in which the atoms have a LJ diameter σ = 3.0 Å and a dispersive constant of ε=70.49 K. Three different volume fractions of the peptide mixture were used in the calculations (3, 4.8, and 6 10^{-5} molecules/$Å^3$).

Results and Discussion

The first step in the construction of our model is the collapsing of the nine atoms that make each of the two amino acids (aspartic acid and isoleucine) of the model peptide into a united residue. By doing so, the peptide can be represented by only eight sites. The L-J parameters for all the possible amino acid pairs (obtained by Monte Carlo Simulations) are listed in Table I. The range of the potential is approximately 15 Å. Following the approach described under Methods, the force field between the amino acids and the surface was also determined. Again, the Monte Carlo results were fitted by a simple L-J expression. The fitted parameters for the residue-surface L-J interaction model are given also in Table I.

Table I. Effective L-J Interaction Potential Parameters

Sr. No.	**Type of Interaction**	**A** (Å-K$^{1/12}$)	**C** (Å-K$^{1/6}$)
1	Aspartic Acid – Aspartic Acid	6.5712	8.0102
2	Aspartic Acid - Isoleucine	6.7811	8.4416
3	Isoleucine – Isoleucine	6.9978	8.8962
4	Aspartic Acid – Surface	6.9740	9.3552
5	Isoleucine – Surface	7.1891	9.6912

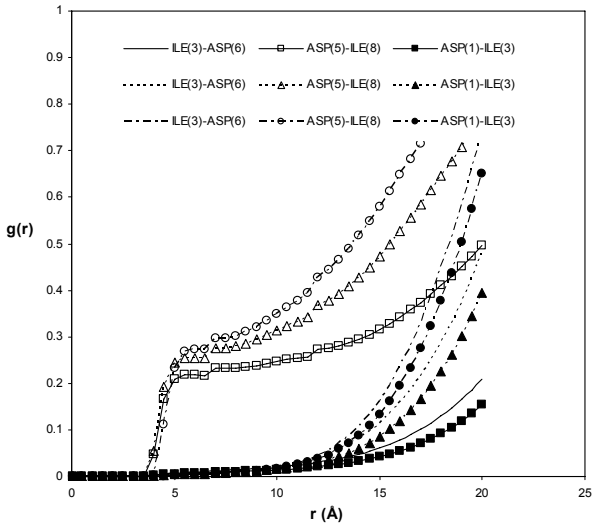

Figure 2 _____ η= 0.02392, …….η= 0.03827, -.-.-.-.-.- η= 0.0478

Using the united atom model of the peptide and the L-J parameters obtained above (plus a charge of -1 on each aspartic acid residue), the XRISM/HNC equations were solved to obtain the bulk correlation functions for the peptide as a function of peptide concentration. A few, representative intermolecular correlation functions between amino acid residues are shown in Fig. 2**.** At all the volume fractions studied here, the potential of mean force remains positive (g(r)<1) as a result of the repulsion between aspartic acid residues. The pair correlation functions between any two aspartic acid residues have the characteristic shape of a pcf between two ions of equal sign. On the contrary, the pcf between any two isoleucine residues exhibits the characteristic structure of a molecule with predominant dispersive forces but somehow masked by the presence of the negative charges in the aspartic acid residues. The "mixed" correlation functions shown in Fig. 2 exhibit an intermediate behavior. The Figure suggests that dispersive forces dominate the interaction between ASP^5 and ILE^8. This seems to be related to the proximity of both sites in the 3-dimensional arrangement of the peptide (see Fig.1). Because isoleucine is a neutral amino acid, dispersion forces did play a role to some extent for isoleucine-isoleucine interaction. Because of chain connectivity the correlation function remains featureless at low volume fractions whereas at higher volume fractions entropic effects are responsible for the presence of features. Still, the electrostatic repulsion between the peptides seems to dominate.

Using the bulk correlation functions for peptide-peptide interactions as the input, the peptide-surface XRISM equations were solved to obtain peptide-surface correlation functions at different peptide concentrations and different distances of separation between the positive and negative surfaces (Fig. 3). Note that the pcfs reported here are based on the radial distance between a peptide site and sites on the surface. Although the exact orientation of the peptide axis with respect to the surface can not be determined because of the complexity of the pcfs, geometrical arguments can be used to determine the most probable structure of the adsorbed layer. At a density of 3 10^{-5} molecules/Å^3 (Fig. 3 a), we observe a well-defined.

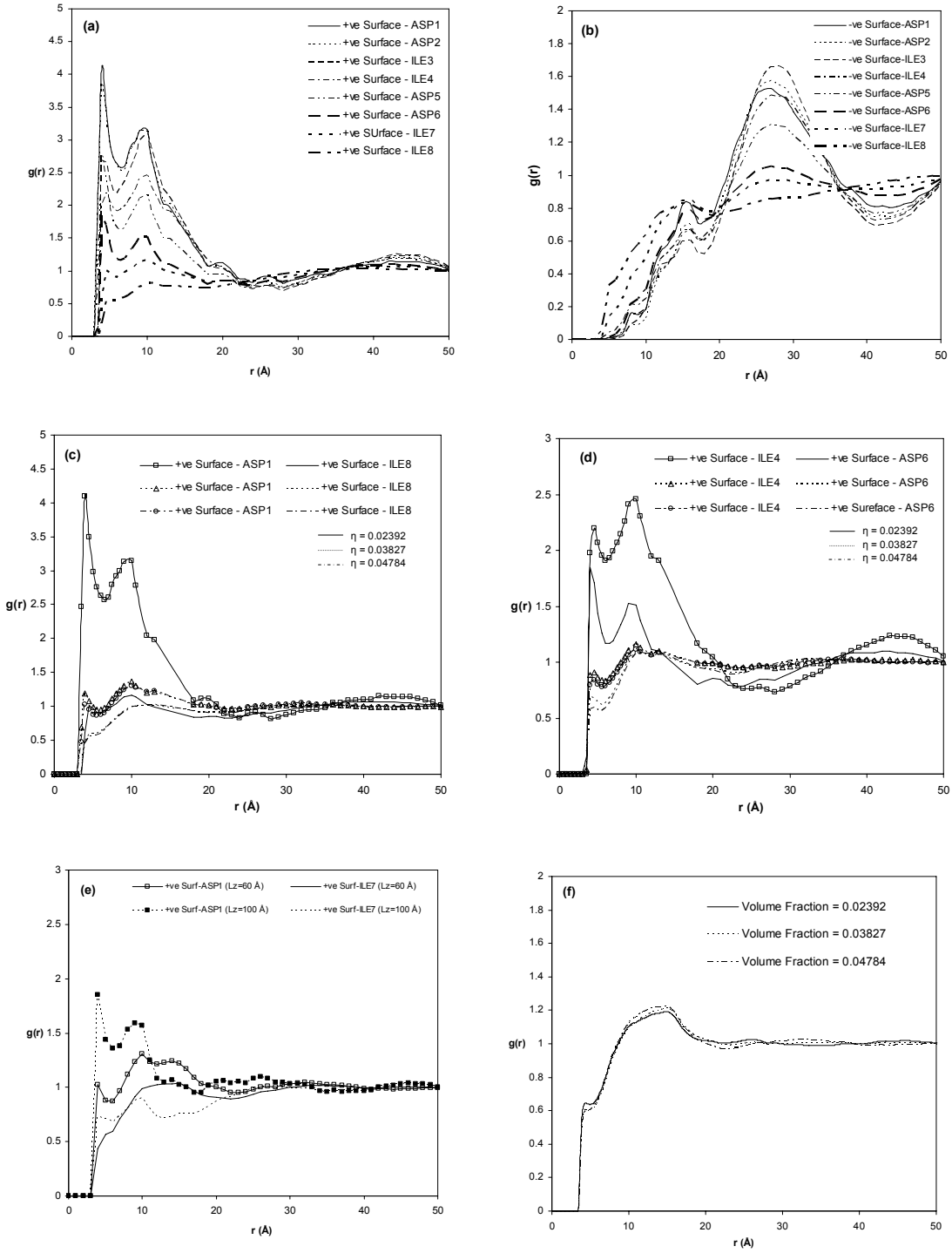

Figure 3. a) Peptide – (+ve) surface correlation function (η= 0.02392); b) Peptide – (-ve) surface correlation fun; c) Peptide–(+ve) surface pair correlation functions; d) Peptide–(+ve) surface pair correlation functions; e) Effect of separation distance between surfaces on the peptide – surface correlations at η= 0.04784; f) Effect of charge density on the peptide – surface correlations

peptide layer (as indicated by the prominent first and second peaks for all the amino acid residues, except ILE^7 and ILE^8) on the surface. Straightforward geometric considerations allow us

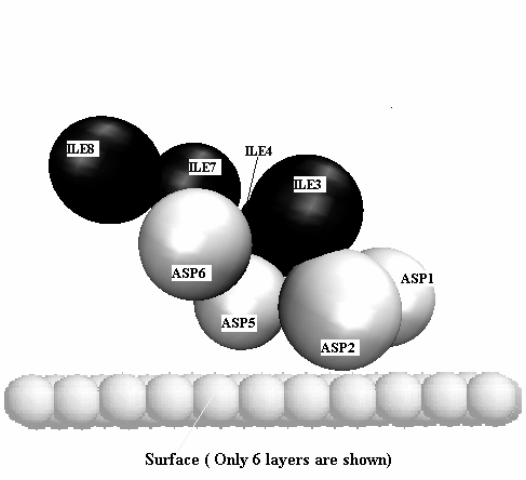

Figure 4 Representation of orientation of peptide on the surface

to immediately discard a surface morphology that would correspond to the peptide laying "head-on" onto the surface. Because of electrostatic complementarily and less sever entropic restrictions (as compared to middle residues), the negatively-charged end terminal residues ASP^1 and ASP^2 stay very close to the surface. The three dimensional shape of the peptide is such that the remaining two aspartic acid residues also lay close to the surface. (Note that the first peak is located at a radial distance of ~4.10 Å from the surface site for ASP^1 and ASP^2 residues). A cartoon representing surface morphology is shown in Fig. 4. For a linear molecule (with similar charge distribution as our non-linear peptide molecule), this kind of arrangement would be energetically favorable only if all the adsorbed molecules attach to the surface with their axis perpendicular to the surface. At higher volume fractions, η= 0.04784, the correlation functions between all of the peptide residues and the surface are almost identical. At higher volume fractions those configurations that maximize the packing efficiency will be favored. This is attainable if the peptide forms layers adjacent to the surface and they are oriented parallel or nearly parallel to it. This is illustrated in Figure 5, which shows preliminary MC results for the same peptide. At distances longer than 30 Å from the positively charged surface the peptide reaches the bulk density (and therefore a random orientation).

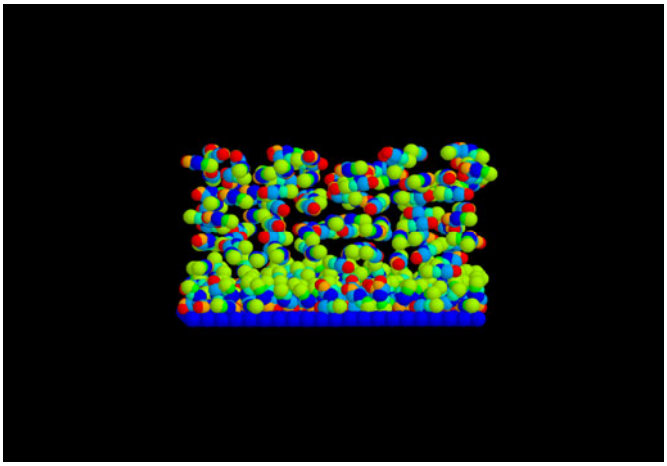

Figure 5. Monte Carlo simulations of the peptide at fine concentrations

Due to electrostatic repulsion, the surface is depleted of peptide up to a distance as high 20 Å from the negatively charged surface (Fig. 3b). Still, some surface enhancement is obvious at a distance of 20 Å. At distances closer than 20 Å (the length of the peptide) the surface is depleted of peptide because of the entropic lost associated with the inability of the peptide to freely rotate

Fig. 3c and d shows the peptide-surface correlations at three volume fractions η= 0.02392, η= 0.03827 and η= 0.04784 at the positively charged surface for the end-terminal (ASP^1 and ILE^8) and middle residues (ILE^4 and ASP^6) respectively. As the peptide volume fraction increases, the surface concentration seems to decrease (as indicated by a decrease in the size of the peaks) but the location of the peptide at the surface does not change (as indicated by the position of the peaks). The negatively charged terminal end residues ASP^1 and ASP^2 seem to be affected the most. Several reasons can explain this behavior of the peptide at the surface. Figure 2 indicated that an increase in volume fraction weakens the electrostatic repulsion between the peptide. Therefore, it is reasonable to infer that as the volume fraction increases the peptide will have a weaker tendency to deposit at the surface. A more

efficient packing of the peptide in the bulk as a result of a heavier role of dispersion forces at high volume fractions decreases adsorption. Because the adsorbed peptides align parallel to the surface at high volume fractions, lateral repulsion between adsorbed peptide molecules would reduced the abundance of ASP^1 and ASP^2 residues at the surface. The effect of increasing the volume fraction was felt more by the ILE^4 residue than by the ASP^6 one (Fig. 3d). This again confirms our observations that the residues which were close to the surface were mainly affected by the changes in the volume fraction of the peptide.

We investigated the effect of the separation distance between the two surfaces (either 60 Å or 100 Å) on the morphology of the adsorbed layer. Fig. 3e shows peptide-surface correlation functions at these separation distances at a volume fraction of 0.04784. Near the positively charged surface the separation distance between the surfaces showed a significant effect on the density of adsorbed peptides. For the larger separation distance (100 Å) we observed an increased abundance of negatively charged ASP^1 at the positively charged surface. This change in pcfs can be attributed to either changes in peptide-peptide interactions or peptide–negatively charged surface interactions. Although the free volume available to the peptides increases as the separation distance increases, the amount of peptide at the surface increases -- because of the dominating electrostatic repulsion in the bulk. This suggests that peptide-peptide interactions in the bulk are more important than interactions between the peptide and the negatively charged surface.

The correlation function remained largely featureless at neutral surfaces (Fig. 3f), where only the ASP^1 terminal end- surface correlation function is shown at three volume fractions. In the absence of electrostatic interactions between the peptide and the surface, peptide-peptide electrostatic interactions dominate and as a result we see a depletion of the peptide at the surface. This is the case of very weak adsorption or no adsorption. No change in peptide-surface interaction was observed in the correlation functions as the volume fraction of the peptide was increased, confirming the dominant role of electrostatic repulsion between peptides in the absence of strong peptide-surface interactions. Dispersion forces between the peptide and the surface are responsible for the formation of a dispersed layer of peptides which extends 20 Å into the bulk. A similar phenomenon was observed by one of us in a neutron reflectivity study of several proteins at solid/fluid interfaces (Hamilton and Forciniti, 2005).

Conclusions

A 72 atoms peptide was reduced to just eight sites by collapsing the atoms belonging to each amino acid into a united atom. The force field between united atoms and between united atoms an a surface was determined by rigorous Monte Carlo simulations. Our study indicates that only a relatively large volume fractions dispersion forces play a role in determined the structure of the fluid; at low volume fractions the structure is completely dominated by electrostatic effects. We have also shown that XRISM/HNC theory can be successfully used to describe the effect of bulk densities and charge density of the surface on the behavior of a peptide at a solid/fluid interface. The results obtained here indicate that electrostatics as well as entropic effects played an important role in determining the fate of peptide. The model suggests that even in the absence of electrostatic attraction between peptide and the surface a diffuse adsorbed layer would form.

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