Published in final edited form as:

J Phys Chem B. 2009 July 16; 113(28): 9472–9478. doi:10.1021/jp902537f.

Salt Effects on Surface Tethered Peptides in Solution

Jun Feng^a, Ka-Yiu Wong^b, Gillian C. Lynch^b, Xiaolian Gao^{a,b}, and B. Montgomery Pettitt^{a,b,*}

^aDepartment of Biology and Biochemistry, University of Houston, Houston, TX 77204-5001, USA

^bDepartment of Chemistry and Institute for Molecular Design, University of Houston, Houston, TX 77204-5003, USA

Abstract

The capability to manipulate proteins/peptide fragments at liquid-solid interfaces has led to tremendous applications in detectors and biotechnology. Therefore, understanding the detailed molecular behavior of proteins and peptides tethered on a hard material surface is an interesting and important topic. The inhomogeneity presented by surfaces as well as ions in the solution plays an important role in the thermodynamics and kinetics of the tethered proteins. In this study, we perform a series of molecular dynamics simulations of a pentapeptide RHSVV, a p53 epitope, tethered on a prepared microarray surface in various salt concentrations (0 M, 0.14 M, 0.5 M, and 1M NaCl), as well as free in ionic solution (0M, 0.5M, and 1M). The conformational space the tethered peptide visits largely overlaps with the free peptide in solution. However, surface tethering as well as the salt concentration changes both the thermodynamics and kinetics of the peptide. Frequent conformational changes are observed during the simulations, and tend to be slowed down by both increasing the salt concentration and surface tethering. The local composition of ions at different salt concentrations is also compared between the tethered and free peptide.

Introduction

The capability to manipulate proteins/peptide fragments near solid surfaces has led to tremendous applications in synthesis and detection (1-4). Assemblies of proteins with nanomaterials have been successfully utilized in the field of sensing, imaging, implantation, and tissue engineering (1). Protein/peptide microarrays, which require tethering proteins at the microarray surfaces while maintaining their biological functions, enable detection of proteins of interest in trace amounts and have become powerful tools for molecular studies, drug discovery, and clinical diagnostics (5). These applications demand the retention of protein native conformation and biological functionality at the desired material surface. Therefore, understanding the behavior of proteins near the surface is of relevance to furthering the applications in these fields.

The properties of proteins/peptides at the solid-liquid interface have gained much attention in recent years (6). Both experimental and theoretical approaches have been applied to study the effects of surface tethering on protein structure, stability, and folding mechanism. Spectroscopic methods revealed the structure and dynamics of proteins tethered on various surfaces (7,8). Molecular dynamics simulations proved that the presence of surface alters the folding mechanism of the tethered proteins (9). Modeling studies of various tethered proteins/peptides concluded that surface immobilization can either stabilize or destabilize the protein depending on the tethering point (10,11). Stability upon tethering is protein and orientation specific. The absorption of proteins on heterogeneous surfaces has been studied extensively in

* Corresponding author. Fax: +1-713-743-2709; e-mail: pettitt@uh.edu.

colloidal and material science as well (12,13). Many studies on model proteins, such as lysozyme, bovine serum albumin, and fibrinogen, have shown that the behavior of protein absorption on nanomaterial surfaces also exhibit a strong dependence on the individual protein itself and the nature of the surface chemistry (14-17). In many cases, proteins showed a varied degree of partial unfolding or even denaturation upon absorption (18-20). Control of the orientation, stability and therefore the functionality of proteins at the solid interface is still an open issue.

Proteins carry out biological functionality under the influence of salt. In many cases, small organic ions are essential to the catalytic activity of enzymes. Different kinds of salts also exert different influences on the solubility of proteins, which is known as ‘salting in’ and ‘salting out’ effects associated with the Hoffmeister series. Cellular events such as protein association/dissociation can be driven by salt concentration changes.

Thus, biotechnology applications can seek to partially control protein stability/functionality by manipulation of salt type and concentrations. A well-known and common technique is the purification of proteins by ‘salting out’. In recent designs of protein microarrays, reversible immobilization of proteins to a microarray surface was achieved by monitoring calcium mediated calmodulin-target binding (21,22). In our present work, we studied both the surface and salt impacts on structural properties of a model peptide.

In our previous study, we investigated the conformations of a pentapeptide RHSVV, the tumor suppressor protein p53 epitope, tethered on a microarray surface under roughly physiological salt concentration using molecular dynamics techniques (23). In the present work, by performing a series of simulations at different salt concentrations, we continue to investigate the systematic effects of ionic strength of solution and surface tethering on the conformations and kinetics of the peptide RHSVV. Ion distributions along the microarray surface and around the model peptide are also studied in detail.

Methods

Simulation

Two sets of molecular dynamics simulations are carried out in this work: tethered peptide on the microarray surface (labeled as T0, T0.5, and T1) and the free peptide in aqueous solution (labeled as F0, F0.5, and F1), both sets in salt concentrations of 0, 0.5, and 1.0 M. The simulation model and procedure were discussed in detail previously. (23) Briefly, the silica support surface is densely covered with methyl terminated aminosilane and a linker with the peptide affixed near the center of the surface. A summary of our simulation setup is presented in Table 1. In all 6 simulations the peptide RHSVV shared the same starting molecular structure, which was taken from the peptide fragment (residues 213-217) of the NMR structure of p53 (PDB ID: 2FEJ). The structure was then modified by adding an N-terminal amino group. For the tethered peptide simulations, the C-terminus of the peptide was neutral due to the immobilization to the microarray surface. In the free peptide simulations, the C-terminal group was also neutralized and capped, an *N*-methylamide, in order to access the effect of neutralizing the carboxyl terminus tethered to the surface. After the modification, the peptide RHSVV carried two positive charges due to the protonated N-terminal amino group and the arginine guanidine side chain. Therefore, two additional chloride ions were added into each simulation box to achieve charge neutrality. The trajectory of each simulation was run for 160 ns and updated every 0.1 ps. In addition, we also included 3 trajectories of the tethered peptide simulations from our previous work in the analysis (collectively labeled as T0.14), which were performed under physiological salt concentration of 0.14 M for 360 ns. All simulations were carried out in the microcanonical (NVE) ensemble using the ESP (24) program. During the equilibration, the velocities of the atoms were reassigned to achieve a temperature of 300 K. The volumes of the

simulation boxes were adjusted to achieve a pressure close to 1 atm. Each equilibration procedure took about 1 ns.

Analysis

Unless explicitly stated, all atoms of the tethered peptide (the spacer is excluded) are used in the following analysis. For the free peptide, all atoms except the added *N*-methylethylamide group are used in the calculation. To measure the compactness of the peptide, we calculated its radius of gyration (R_g), which has the standard definition as the mass-weighted root-mean-square distance from the center of the mass to every atom in the peptide.

An agglomerative, hierarchical grouping method is also applied in the peptide conformation cluster analysis (25). For each trajectory, to form the initial set of clusters, a snapshot of the peptide conformation is extracted from every 10-ps interval. Then, each step of the iteration reduces the number of clusters by merging the two clusters closest in distance. In the first step, since each cluster contains only one snapshot, the distance between two clusters is simply defined as the mass-weighted, root-mean-square deviation (RMSD) between the pair of snapshots. After the first step, we follow the group-average (average linkage) scheme, which defines the distance between two clusters as the average RMSD of all intercluster pairs of snapshots. Based on *a priori* knowledge, two conformations with an RMSD within 0.30 nm are generally considered structurally similar. Therefore, the clustering procedure is iterated until the distance between two closest clusters is greater than 0.3 nm. For each resulting cluster, an average structure is calculated as the structure with the minimal RMSD from all peptide conformations within the cluster. A representative structure is then chosen as the snapshot with the smallest RMSD to the average structure. In the study of peptide conformational changes, conformation life time τ is defined as the duration time the peptide stays in a conformational cluster before changing its structure into another cluster. The count $n(t)$ is the number of times the conformation life time τ lasts more than t ($= 0.5, 1, 2, 3, \dots$ ns).

In the tethered peptide simulations, the hydrophobic microarray surface is formed by the top layer of the methyl terminated aminosilane derived linkers. The relative density distribution

of ions along the surface is defined as $\rho'(d) = \frac{\rho(d)}{\rho^{\text{bulk}}}$, where $\rho(d)$ is the ion density as a function of perpendicular distance d to the peptide chip surface, and ρ^{bulk} is the bulk (4 nm away from the surface) ion density. Relative density distribution of waters as a function of distance to the surface is defined in a similar way.

In the study of the local distribution of ions in the coordination shells around the peptide, mole

fraction of ions, is defined by $x_i = \frac{n_i}{n_{\text{total}}}$, where n_i is the number of species i (sodium or chloride ions) and n_{total} is the total number of species (sodium and chloride ions, and water) within a coordination shell. Relative mole fraction is simply the local mole fraction normalized by the

bulk, i.e., $x_i' = \frac{x_i}{x_i^{\text{bulk}}}$, where x_i^{bulk} is the mole fraction in the bulk (2 nm away from the peptide).

Results and discussion

In the tethered peptide simulations, the top layer of the surface is formed by methyl groups of the unreacted aminosilane derived linkers, which interacts favorably with the peptide hydrophobic residues. Therefore, the peptide always stays very close to and constantly interacts with the neutral hydrophobic chip surface irrespective of the salt concentrations. This observation is in good agreement with the previous study (23) where the peptide surface interactions and the peptide orientation with respect to the surface were discussed in detail.

1. Peptide Conformations

The radius of gyration (R_g) is calculated for both tethered and free peptide at all salt concentrations. The probability distribution of the radius of gyration is shown in Figure 1. In the simulations of free peptide in solutions, as the salt concentration increases, the distribution of the radius of gyration slightly shifts to the left. The average radius of gyration is 0.263 nm at 0 M, 0.258 nm at 0.5 M, and 0.249 nm at 1 M, which suggests that the peptide is likely to assume a more compact form in higher ionic strength solutions. This agrees with the simulation result of a hydrophobic polymer that addition of salt to the solution drives the conformational equilibrium toward the compact states (26). However, for the tethered peptide, salt impact on the conformational states is complicated by the presence of a nontrivial charge distribution and the surface. The average radius of gyration of the tethered peptide is 0.263 nm at 0 M, 0.269 nm at 0.14 M, 0.252 nm at 0.5 M, and 0.274 nm at 1M. We note the presence of two peaks in the distributions that are tethered. For T1 for example, based on the cluster analysis, conformer a, a compact form with average R_g of 0.24 ± 0.02 (standard deviation) nm, mainly contributes to the first peak in Figure1. Conformer c, an extended form with R_g of 0.30 ± 0.03 nm, corresponds to the second peak. The R_g of conformers b and e are 0.27 ± 0.04 and 0.28 ± 0.03 , respectively, but are broad and contribute over the whole range.

As a precaution, we checked whether the sampling time of our simulations was sufficient. Given the small molecular size of our model peptide, each 160-ns trajectory is long enough to enable frequent conformational changes (discussed later) and thus ensure the convergence of R_g . To understand the changes in the peptide compactness, the effective interactions between the peptide and the surface need to be considered. These can be monitored by calculating the closest distance between the atoms of the peptide and the surface methyl groups. We use 0.35 nm, about the minimum distance between a carbon atom in the peptide and a surface atom, as the criteria for direct surface contact. The percentage of time each residue forms direct contact with the surface is calculated and shown in Table 2. Here, we observe a salt-induced strengthening of hydrophobic interactions (26,27). Two hydrophobic residues Val4 and Val5 interact more often with the surface as the salt concentration increases. Salt, which drives the free peptide toward more compact states, has also induced enhanced hydrophobic interactions between residues of the tethered peptide and the surface. Therefore, the peptide may assume an extended form to enhance the hydrophobic interactions with the surface and thus, the radius of the gyration actually increases at 1 M salt. Hence, surface tethering changes the peptide conformational equilibrium in conjunction with the ions.

In order to study the detailed molecular structure of the peptide, we apply the described hierarchical grouping method to each trajectory. Cluster analysis results in no more than 4 major conformational clusters (RMSD cutoff at 0.3 nm) in each simulation (Table 3). Here we regard any conformational cluster contributing more than 5% of the configuration space as a major cluster. In order to compare these conformational clusters within and between each trajectory, we calculate the average structure of each cluster and the RMSD among the average structures. Again, we apply the hierarchical grouping method to the cluster average structures. Conformationally close clusters (RMSD cutoff at 0.2 nm) are listed on the same row in Table 3 and labeled from a to e. It should be noted that the RMSD cutoff between average structures is smaller than the cutoff between conformational clusters in each trajectory because of the slightly distorted bond length and bond angles resulting from the average structure calculation. Representative snapshots of the peptide conformations are also shown in Figure 2. Either being tethered or free, the peptide visits conformation a, b, and c in all salt concentrations. In the figure, it is easy to see that the peptide in conformation a is stabilized by intramolecular salt bridge interactions. The charged guanidino side chain of the N-terminal Arg residue interacts favorably with the partially charged backbone oxygen atoms of Ser3 or Val4. Therefore the structure is more compact than the peptide in conformation b and c, which assumes an extended

form. The side chains all extend outward from the backbone in conformations b and c, which only differ by the orientation of the peptide backbone of Val4 and Val5.

The peptide in conformation d forms a major cluster in simulations T0, F0.5, and F1 only. The peptide backbone along with the arginine side chain bends like a “U” shape. Again, the structure is partially stabilized by the intramolecular electrostatic interactions between charged/partially charged atoms. Conformation e forms a major cluster only in the tethered peptide in 1 M salt concentration. Unlike other peptide conformations, the Arg and His side chains stay close in conformation e. Conformation a and d, which are partially stabilized by intramolecular interactions, are overall more compact than conformations b and c. In the free peptide simulations, if we simply consider a and d as compact forms, and b and c as extended forms, the ratio of compact to extended form is 1.50, 1.90, and 2.03 in 0 M, 0.5M, and 1M salt, respectively. This observation of an increased ratio of the compact states of the free peptide when increasing salt concentration agrees with the distribution of radius of gyration discussed before. As to the tethered peptide, however, because of the complicated effect from the surface and the ions, no simple trend is observed.

To some extent, the result of our cluster analysis depends on the methodology used. Each clustering algorithm for molecular dynamics trajectories has its strengths and weaknesses. However, compared to other commonly used clustering methods for peptide/protein such as principle component analysis (28-30) and clustering algorithm by Daura (31,32), the agglomerative hierarchical grouping method gives the best result for our model peptide (data not shown). As a test, we also combined the trajectories from all simulations (with one snapshot every 100 ps) and applied the clustering analysis. The result from the combined trajectory (data not shown) is similar to the result from each individual trajectory shown above. A recent evaluation of the performance of many clustering algorithms (33) also recommended the average-linkage hierarchical grouping method used here.

2. Kinetics

From the previous cluster analysis, we know that the peptide exists in a variety of conformations in each simulation. Therefore, we are interested in the following questions: How often do conformational changes happen? Does the surface tethering or the ionic environment have any effect on the kinetics of conformational change? Based on cluster analysis, we are able to calculate the conformation life time τ : how long the peptide stays in a conformation before changing its structure. The majority of conformational clusters appear more than once and usually up to ten times in each simulation. The conformational life time τ can be as long as tens of nanoseconds and can also be as short as a few picoseconds, even for the same peptide conformation. Figure 3 shows $n(t)$, the number of times in the simulation the peptide conformation life time τ is longer than t ($= 0.5, 1, 2, 3, \dots$ ns). Under the same salt concentration, the larger values of τ tend to appear in the tethered peptide simulation. There are more short-life conformers ($\tau < 1$ ns) in the free peptide simulation than the tethered one. We also fit each curve into an exponential decay function $n = n_0 e^{-\lambda t}$, where the values of n_0 , λ , the half life

$t_{1/2} = \frac{\ln 2}{\lambda}$, and R-squared value of fitting are shown in Table 4. The R-squared values indicate a good fit of $n(t)$ into the single exponential decay. $t_{1/2}$ gives an estimation of the average conformation life time. Three conclusions can be drawn based on the values of $t_{1/2}$ and R^2 . First, the peptide conformational change is a random process. The dynamics has no simple relation to the thermodynamic stability. Second, the ions slow down the conformational changes. It is obvious that as the salt concentration increases, the value of $t_{1/2}$ increases. Whether tethered or not, the peptide tends to stay longer in a conformation in the higher ionic strength environment than the lower one. We also notice that the values of R^2 in the 1 M salt simulations are less satisfactory than other lower salt concentrations, because fewer conformational changes happen in the higher salt environment during the limited simulation

time. Third, the surface tethering also slows down the peptide conformational change. Under the same salt concentration, the half life $t_{1/2}$ of the tethered peptide conformation is always longer than the free one. The hydrophobic interactions between the peptide and the surface are likely the cause of the slow down of the conformational changes. The surface can also act as a steric hindrance for the conformational changes. Careful inspection of the trajectories shows that when the peptide changes its conformation, there is always an accompanying reorientation of the peptide to the surface. As the estimation of the kinetics of the peptide conformational change is based on the cluster analysis, its result is subject to the methodology used here. Nevertheless, the kinetics clearly gives us a qualitative view of the effect of ionic environment and surface tethering on the peptide conformational changes.

3. Ion Distribution Along the Tethering Surface

In the tethered peptide simulations, the silica support for the microarray is grafted with methyl terminated aminosilane derived linkers. The top layer of these somewhat tightly packed linkers formed a neutral and hydrophobic surface. Peptide-surface interaction, peptide orientations, and peptide mobility determined by the surface chemistry was discussed in detail previously (23). Therefore, in this study we focus on the comparisons of ion distributions along the surface, and ion interactions with the tethered and free peptide under different salt concentrations.

Relative density distribution of ions and water as a function of distance to the top atom layer of the surface is shown in Figure 4. The water distribution along the surface (shown in solid green color) is near identical in all simulations of tethered peptide, irrespective of the salt concentrations. Two peaks corresponding to the first and second hydration layers appear at approximately 0.35 and 0.65 nm. Beyond 1.0 nm, the water density quickly reaches its bulk value. Relative density distribution of sodium and chloride ions at 0.14 M, 0.5 M, and 1 M concentration are shown in blue, red, and black, respectively. The exclusion region of ions is much larger than water due to the relatively larger van der Waals radii of ions interacting with the particular surface chemistry.

Because of the positive charge on the peptide, which interacts favorably with the hydrophobic surface through the nonpolar residues, the density of sodium ions close to the surface is lower than the bulk. A small first-coordination peak of sodium ions appears at 0.40 nm and is followed by the second peak at 0.80 nm. The density of sodium gradually reaches its bulk value over 3 nm away from the surface. On the contrary, the negatively charged chloride ions are found to be distributed closer to the surface. The peak of the first-coordination layer appears at 0.6 nm. The second layer of chloride ions cannot be discerned because of the appearance of a broadened peak from 1 to 3 nm due to charge attraction by the peptide. As the bulk concentration of salt increase from 0.14 M to 1 M, the peaks of the favorably attracted chloride ions become smaller. On the other hand, the peaks of the repelled sodium ions become higher. The peak of chloride ions from simulation T0.14 is relatively higher, which also decreases more slowly than T0.5 and T1 to its bulk value. It should also be noted that in the lowest salt concentration simulation T0.14, the statistical noise shown on the tail is still large because of the small number of ions, although the simulation time adds up to 360 ns, which is more than twice of the simulation time of T0.5 and T1.

4. Ion Distribution Near the Peptide

We also study the distribution of ions near the peptide surface. The ions can be viewed as forming three coordination shells around the peptide. Approximately, the first coordination shell is within a distance of 3 Å from the surface of peptide, and the second shell is from 3 to 6 Å. The third coordination shell from, 6 to 10 Å, is not as distinct as the first two but still discernable. The distribution of ions around the peptide may be measured by the mole fraction and the relative mole fraction of sodium and chloride ions within the 3 coordination shells

(Figure 5 and 6). The error bars in the figures represent the standard deviation of the calculation by cutting each trajectory into 3 batches. As expected, the error bar is relatively larger in the first coordination shell than the other two, because of the smaller number of ions and water in the first shell, which results in larger statistic uncertainties. Nevertheless, the standard deviation is no more than 20% of its mean in the first coordination shell and is always equal to or less than 5% in the second and third shell, which indicates a reliable statistics.

Recall that the peptide carries two positive charges. Because of charge repulsion, the mole fraction of sodium ions in all 3 coordination shells is lower than the bulk, especially in the first shell with over 75% deficit in all simulations of different concentrations. The mole fraction of sodium increases rapidly in the outer coordination shells. In the case of free peptide simulation in 0.5 M and 1 M salt, sodium ion concentration almost reaches its bulk value in the third coordination shell. On the other hand, the chloride ions are attracted to the peptide. The mole fraction of chloride in the first coordination shell is greater than the bulk value. Chloride concentration in the second and third coordination shell is almost the same. We also observe that both the sodium and chloride ion concentration within the second and third coordination shells of the free peptide is a little higher than the tethered one under the same bulk ion concentration. As shown from the relative mole fraction, the extent of sodium deficit and chloride excess is more dramatic as the ion concentration decreases from 1 M to 0.14 M in the tethered peptide simulation, which is in accord with the observation from the density distribution of ions near the tethering surface in Figure 4. However, the trend is not so obvious for the free peptide simulations F0.5 and F1.

We can compare the probability distributions of ions for tethered and free systems directly. The comparison in Figure 7 shows the mole fraction probability distribution of Na^+ (upper) and Cl^- (lower) as a function of distance to the surface of the peptide in F1 and T1. All three peaks for Na^+ are clearly shown. The first and second coordination shells of Cl^- are obvious, with the third one less prominent. All other salt concentrations show similar trend but with different peak intensities.

Conclusions

A series of molecular dynamics simulations of p53 epitope RHSVV gives us a detailed molecular picture of the peptide at various salt concentrations. Either being tethered on the hydrophobic surface or free in the homogeneous solution, the peptide has sampled a similar ensemble of conformations in conformational space. This is an important conclusion in device design as it means the conformations in solution are still available when the peptide is tethered as done here, albeit with different equilibrium constants.

We found the free peptide is likely to assume a more compact form as the ionic strength in the solution increases. However, through constant van der Waals interactions with the hydrophobic surface, the immobilized peptide tends to stay in a more extended state. Multiple conformational changes are also observed during the collective simulation time, which sum up to microseconds. Longer conformation life time is observed at higher salt concentration. Conformational changes are also found to be slowed down by surface tethering. Both surface tethering and salt concentrations have shifted the conformational equilibrium of the peptide as well as the kinetics of conformational changes.

Distributions of water and ions have also been presented in this work. Water distribution along the microarray surface is nearly the same irrespective of the salt concentration. While both sodium and chloride ions are not attracted to the hydrophobic surface, because of the presence of the positively charged peptide close to the surface, chloride ions are preferentially more concentrated near the surface and the sodium ions are somewhat excluded. The distribution of

sodium and chloride ions along the hydrophobic surface follows a concentration series, with chloride at the lowest bulk concentration showing the highest peak. The trend is reversed in the case of sodium ions. The local composition of ions within the first coordination shell of the peptide is similar in either tethered or free state at the same bulk concentration. However, the concentration of both positive and negative ions in the second and third coordination shell of the tethered peptide is relatively lower than the peptide free in the solution as a result of the surface repulsion. This observation gives clues about the nature of the salt concentrations used most effectively in binding versus washing in standard combinatorial uses.

Acknowledgments

Bin Lin is thanked for helpful discussions. We gratefully thank the National Institutes of Health and the Robert A Welch Foundation for partial financial support of this work. This research was supported in part by the National Science Foundation through TeraGrid resources provided by the Pittsburgh Supercomputing Center (Lemieux and Bigben) and the San Diego Supercomputer Center (Datastar). These calculations were performed in part using the Molecular Science Computing Facility (MSCF) in the William R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the U.S. Department of Energy's Office of Biological and Environmental Research and located at the Pacific Northwest National Laboratory, operated for the Department of Energy by Battelle.

References

1. Kane RS, Stroock AD. *Biotechnol Prog* 2007;23(2):316–319. [PubMed: 17335286]
2. Sarikaya M, Tamerler C, Jen AK, Schulten K, Baneyx F. *Nat Mater* 2003;2(9):577–585. [PubMed: 12951599]
3. Jain KK. *Drug Discov Today* 2005;10(21):1435–1442. [PubMed: 16243263]
4. Asuri P, Bale SS, Karajanagi SS, Kane RS. *Curr Opin Biotechnol* 2006;17(6):562–568. [PubMed: 17015011]
5. Tao SC, Chen CS, Zhu H. *Comb Chem High Throughput Screen* 2007;10(8):706–718. [PubMed: 18045082]
6. Raffaini G, Ganazzoli F. *Macromol Biosci* 2007;7(5):552–566. [PubMed: 17477442]
7. Johnson CK. *Biochemistry* 2006;45(48):14233–14246. [PubMed: 17128963]
8. Cheng YY, Chang HC, Hoops G, Su MC. *J Am Chem Soc* 2004;126(35):10828–10829. [PubMed: 15339152]
9. Friedel M, Baumketner A, Shea JE. *Proc Natl Acad Sci U S A* 2006;103(22):8396–8401. [PubMed: 16709672]
10. Knotts Iv TA, Rathore N, De Pablo J. *Biophys J* 2008;94(11):4473–4483. [PubMed: 18326646]
11. Friedel M, Baumketner A, Shea JE. *J Chem Phys* 2007;126(9):095101. [PubMed: 17362129]
12. Gray JJ. *Curr Opin Struct Biol* 2004;14(1):110–115. [PubMed: 15102457]
13. Clarke ML, Chen Z. *Langmuir* 2006;22(21):8627–8630. [PubMed: 17014095]
14. Kim J, Somorjai GA. *J Am Chem Soc* 2003;125(10):3150–3158. [PubMed: 12617683]
15. Agashe M, Raut V, Stuart SJ, Latour RA. *Langmuir* 2005;21(3):1103–1117. [PubMed: 15667197]
16. Vertegel AA, Siegel RW, Dordick JS. *Langmuir* 2004;20(16):6800–6807. [PubMed: 15274588]
17. Roach P, Farrar D, Perry CC. *J Am Chem Soc* 2006;128(12):3939–3945. [PubMed: 16551101]
18. Buijs J, Norde W, Lichtenbelt JWT. *Langmuir* 1996;12(6):1605–1613.
19. Kondo A, Murakami F, Higashitani K. *Biotechnol Bioeng* 1992;40(8):889–894. [PubMed: 18601195]
20. Norde W, Giacomelli CE. *J Biotechnol* 2000;79(3):259–268. [PubMed: 10867186]
21. Daunert S, Bachas LG, Schauer-Vukasinovic V, Gregory KJ, Schrifft G, Deo S. *Colloids Surf B Biointerfaces* 2007;58(1):20–27. [PubMed: 17276043]
22. Jenikova G, Lao UL, Gao D, Mulchandani A, Chen W. *Langmuir* 2007;23(5):2277–2279. [PubMed: 17274635]
23. Feng J, Wong KY, Lynch GC, Gao X, Pettitt BM. *J Phys Chem B* 2007;111(49):13797–13806. [PubMed: 18004834]

24. Smith PE, Holder ME, Dang LX, Feig M, Pettitt BM. University of Houston. 1996
25. Everitt, B. Cluster Analysis. Vol. 3rd. New York Halsted Press; London: 1993.
26. Ghosh T, Kalra A, Garde S. J Phys Chem B 2005;109(1):642–651. [PubMed: 16851057]
27. Athawale MV, Sarupria S, Garde S. J Phys Chem B 2008;112(18):5661–5670. [PubMed: 18447346]
28. Balsera MA, Wriggers W, Oono Y, Schulten K. J Chem Phys 1996;100(7):2567–2572.
29. Hess B. Phys Rev E Stat Nonlin Soft Matter Phys 2002;65(3 Pt 1):031910. [PubMed: 11909112]
30. Sugita Y, Okamoto Y. Biophys J 2005;88(5):3180–3190. [PubMed: 15749777]
31. Daura X, van Gunsteren WF, Mark AE. Proteins 1999;34(3):269–280. [PubMed: 10024015]
32. Smith LJ, Daura X, van Gunsteren WF. Proteins 2002;48(3):487–496. [PubMed: 12112673]
33. Shao J, Tanner SW, Thompson N, Cheatham TE. J Chem Theory Comput 2007;3(6):2312–2334.

Figure 1. Probability distribution (p) of radius of gyration (R_g) of the free (upper) and tethered (lower) peptide.

Figure 2.
Representative conformations from cluster analysis.

Figure 3.
Number of the peptide conformations versus duration time.

Figure 4. Relative density distribution (ρ') of sodium ion (solid line), chloride ion (dotted line) as a function of distance (d) to the surface in simulation T0.14 (blue line), T0.5 (red line) and T1 (black line). Relative density distribution of water is shown with the green line.

Figure 5. Mole fraction x and relative mole fraction x' of sodium ion in the first coordination shell (downward shade), second coordination shell (horizontal shade) and third coordination shell (upward shade) of the peptide.

Figure 6.

Mole fraction x and relative mole fraction x' of chloride ion in the first coordination shell (downward shade), second coordination shell (horizontal shade) and third coordination shell (upward shade) of the peptide.

Figure 7.

The mole fraction probability distribution of Na^+ (upper) and Cl^- (lower) as a function of distance to the surface of the peptide in simulations F1 and T1

Table 1
Simulation Setup of RSHVV Tethered on the Surface (T0, T0.14, T0.5 and T1) and Free in Solution (F0, F0.5 and F1)

simulation	peptide	salt concentration (M)	box size (nm ³)	water molecules	sodium	chloride
T0	tethered	0	4.05 × 3.50 × 6.70	2726	0	2
T0.14	tethered	0.14	4.05 × 3.50 × 6.70	2712	7	9
T0.5	tethered	0.5	4.05 × 3.50 × 6.70	2676	25	27
T1	tethered	1	4.05 × 3.50 × 6.70	2628	49	51
F0	free	0	4.02 × 4.02 × 4.02	2164	0	2
F0.5	free	0.5	4.00 × 4.00 × 4.00	2124	20	22
F1	free	1	3.98 × 3.98 × 3.98	2086	39	41

Table 2
Percentage of Time Each Residue is in Contact with the Surface

residue	T0	T0.14	T0.5	T1
Arg1	10.9	16.7	3.0	25.0
His2	20.0	26.2	40.2	24.1
Ser3	22.8	22.0	37.1	20.6
Val4	45.9	55.1	64.6	66.9
Val5	49.1	59.2	61.3	79.0

Table 3
 Percentage of Each Cluster in Each Simulation from Cluster Analysis

cluster	T0	T0.14	T0.5	T1	F0	F0.5	F1
a	25.5	50.5	69.4	29.9	58.3	52.9	58.8
b	28.8	21.1	19.5	25.8	11.6	16.9	12.8
c	22.3	26.2	6.7	28.3	27.2	15.4	20.0
d	20.3					8.4	7.7
e				13.1			
total	97.0	97.9	95.7	97.1	97.1	93.5	99.2

Table 4

Exponential ($n = n_0e^{-\lambda t}$) Fitting Parameters of Peptide Conformation Life Time

	T0	T0.14	T0.5	T1	F0	F0.5	F1
λ (ns ⁻¹)	0.51	0.30	0.19	0.12	0.75	0.51	0.20
n_0	69.91	91.53	27.82	19.90	90.10	63.20	28.66
R^2	0.98	0.93	0.95	0.87	0.95	0.96	0.87
$t_{1/2}$ (ns)	1.36	2.34	3.67	5.73	0.93	1.35	3.45