Interplay between adsorbed peptide structure, trapped water, and surface hydrophobicity

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Atomistic molecular dynamics simulations were used to study the influence of interfacial water on the orientation and conformation of a facewise amphipathic α -helical peptide adsorbed to hydrophilic and hydrophobic substrates. Water behavior beneath the peptide adsorbed to a hydrophilic surface was observed to vary with the height of the peptide above the surface. In general, the orientation of water close to the peptide (with the oxygen atom pointing up toward the peptide) was complementary to that observed near the hydrophilic surface in the absence of peptide. That is, no change in orientation of water trapped between the peptide and a hydrophilic surface is required as the peptide approaches the surface. The adsorption of the peptide to the hydrophilic surface was observed to be mediated by a layer of ordered water. Water was found to be largely excluded on adsorption to the hydrophobic surface. However, the small amount of water present was observed to be highly ordered. At the closest point of contact to the hydrophobic surface, the peptide was observed to make direct contact. These findings shed light on the fundamental driving forces of peptide adsorption to hydrophobic and hydrophilic surfaces in aqueous environments. © 2017 American Vacuum Society. [<http://dx.doi.org/10.1116/1.4983408>]

I. INTRODUCTION

It is well recognized that the hydrophobicity of a surface in contact with a solution of proteins is a major factor in determining the amount of protein adsorbed, as well as the orientation and conformation of the adsorbed state.^{1–3} This has important consequences in the biocompatibility of implant materials whether permanent (stents, artificial joints) or temporary (sutures, catheters).^{4–8} Some general principles have been observed: for example, globular proteins often stick and denature on hydrophobic surfaces, but reversibly adsorb to hydrophilic surfaces while retaining their native conformation. However, there are many exceptions to this, illustrating that a macroscopic description of the hydrophobicity, such as contact angle measurement, is in general not a good predictor for adsorbed protein behavior. In addition to such *in vivo* applications, the design and optimization of biosensors often requires precise control of the enzyme orientation and conformation as it is immobilized on a substrate surface.^{9,10} If the surface conformation deviates substantially from the native state, the active site function may be compromised. However, concomitant control of the orientation is of significant importance here, as the active site should be directed toward the solution phase, rather than being sterically blocked by the surface. Finally, the subtlety of protein–material surface interactions is the basis of screening for the selection of molecules with specific binding to a variety of organic and inorganic materials for use in specialty coatings and the self-assembly of designed architectures.¹¹

In addition to the paucity of experimental techniques that offer sufficient surface specificity and structural sensitivity, one of the challenges that remains in characterizing adsorbed

protein structure is the global complexity of the problem. This invariably involves a mixture of proteins, and surface and bulk solution conditions, including the number, type, and distribution of ionic species. For this reason, well-defined model systems have provided valuable information that can be applied to more complex systems. One approach is to use *de novo* peptides that have been engineered to create a specific structural motif, such as a thermodynamically stable α -helix or β -sheet.^{12,13} DeGrado and Lear have proposed and synthesized a class of such molecules, including members consisting of leucine and lysine residues. Of interest here is LK α 14, with the formula Ac-LKKLLKLLKLLKL-COO[–].¹⁴ This molecule forms a facewise amphipathic α -helix in solution, with all of the hydrophobic leucine residues on one side of the helix, and the hydrophilic lysine residues on the other side. LK α 14 therefore is an ideal candidate for studying adhesion on hydrophobic and hydrophilic surfaces. This peptide has been the subject of numerous experimental and computational studies specifically focused on characterizing its surface-adsorbed state.^{12,15–25}

Despite evidence that the dry and hydrated states of adsorbed peptides may differ substantially,^{26,27} there are considerably fewer studies that focus on the role of interfacial water in the adsorption process, and its contribution to the adsorbed state. Water adjacent to surfaces of varying hydrophobicity and charge differs from bulk water in its orientation and the extent and strength of its hydrogen-bonding network.^{28–34} At an ideal surface, if water were to retain its isotropic structure, it would be missing 50% of its hydrogen bonding partners due to the abrupt discontinuation of the water density. As a result, water near the surface reorients in order to maximize hydrogen bonding opportunities with the surface and the bulk water phase, and minimize unfavorable dipole–dipole interactions. This is in addition to any specific

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interactions with the surface that may result from polarizable or charged surface functional groups.

In a previous work, we have used all-atom molecular dynamics simulations to study the detailed water structure adjacent to surfaces of varying hydrophobicity and polarity.³⁵ We later revisited our results on the same substrates to study the LK α 14 adsorbed structure³⁶ and were able to provide further insight into some recent experimental results in the literature.^{20,37} We now return our focus to the interfacial water structure, this time in the presence of the peptide, and use this opportunity to comment on the role of surface water in governing characteristics of the adsorbed state.

II. METHODS

A. Surfaces, peptide, and simulation details

Complete details of how the systems were created and equilibrated, and of how the data were collected are given in Refs. 38 and 36. In brief, for the hydrophobic surface, chains of OPLS/AA methylene united atoms on a hexagonal grid were terminated with OPLS/AA methyl united atoms.³⁹ For the hydrophilic surface, hydroxyl termination was applied to ten-atom methylene chains on the same lattice sites. The CH₂–O–H angles were fixed at 71.5°, but the azimuthal angle about the CH₂–O axis was randomized. A previous study on the effect of the surface OH azimuthal angle found that the same water structure was obtained when the angle was randomized. After assigning these azimuths, we have fixed them throughout the simulation.

LK α 14 was modeled with an OPLS/AA force field. Such nonpolarizable force fields have been shown to be suitable for unraveling subtle features in the solvation and adsorption of peptides on hydrophobic surfaces;⁴⁰ however, the stability and structure of the adsorbed peptide has been shown to depend on the choice of force field parameters in general.^{2,41} The Latour group has demonstrated this specifically for α -helical and β -sheet peptides in the LK family.²³

The peptide started as an α helix in the middle of the simulation box, sufficiently far from the surface, with backbone torsional angles $\phi = -57^\circ$ and $\psi = -47^\circ$, an acetylated N terminus, and a charged C terminus. The system was solvated with 5693 SPC/E water molecules⁴² in the hydrophobic system, and 6627 in the hydrophilic system. A Berendsen thermostat was used to maintain the system at 300 K.⁴³ Particle mesh Ewald summation was set with a real-space equivalent cutoff of 1.2 nm; van der Waals interactions were cut off at 1.2 nm. Simulations were carried out for 50 ns, sampling positions each picosecond for the hydrophilic surface, and each 0.1 ps for the hydrophobic surface. All analyses were performed by averaging over at least eight such trajectories, incorporating a minimum of 400 000 frames over 400 ns.

B. Algorithm for water density analysis

As we are interested in studying water molecules that are between the surface and the peptide, we have developed a method for identifying and quantifying those species. As depicted in the schematic in Fig. 1, the method consisted of

FIG. 1. Schematic representation of the algorithm used to evaluate water structure. A topological map of the peptide [(a) and (b)] was generated with a resolution of 1 Å in x and y and 0.5 Å in z . Trapped water molecules were identified (c) and were converted to density values using a count of the occurrences of each peptide height value (d). Ultimately, we obtain a map of water density as a function of distance from the surface, for each given peptide atom height value (e). Subsequent data (Figs. 5–7) are presented as slices through this map.

three major steps: (1) generating a topological map of the peptide for each frame; (2) identifying the waters trapped between the peptide and the surface using this map; and (3) accumulating and consolidating data from many frames into a usable form.

In the first step, the surface was divided into a virtual (x, y) grid with a resolution of 1×1 Å [Fig. 1(a)]. In each frame, the peptide atom height above the surface was stored in the grid according to its coordinates [Fig. 1(b)]. If the grid already

contained a height datum from a previously evaluated peptide atom [that is, if two or more atoms in the peptide shared an (x, y) coordinate], only the height datum of the peptide atom closest to the surface was retained. By sequentially evaluating each peptide atom, a topological map of the face of the peptide closest to the surface was generated for each frame. Height values were rounded to 0.5 Å precision. To consolidate data independently of the peptide's translation across the surface throughout the simulations, the topological map was regenerated for each frame.

In the second step, the total number of water molecules found beneath the peptide for every possible pair of peptide and water height values over the surface was stored. The grid used to store these data is depicted in Fig. 1(c). The horizontal axis represents the height of the peptide's closest atom to the surface; the vertical axis represents the height of water molecules above the surface. Gray cells indicate pairs of peptide and water heights that were not studied. [For example, if the peptide was 3 Å from the surface for a particular (x, y) coordinate, then data for any water molecules sharing that (x, y) coordinate that were farther than 3 Å from the surface were discarded.] Blue cells show the filling of the grid with the data from water molecules. For each frame, the oxygen coordinates from every water molecule were compared with the peptide topological map. A water molecule was considered trapped if its oxygen atom shared an (x, y) coordinate with any peptide atom and was closer to the surface than that peptide atom. For each such water molecule, the cell in the grid in Fig. 1(c) corresponding to the pair of peptide and water height values above the surface was incremented by one.

After having accumulated the trapped water data from all frames, each cell in the grid depicted in Fig. 1(c) contained a count of the total water molecules observed for each pair of peptide and water height values above the surface. Normalizing the water count value in each cell by the number of times that the particular peptide height value had occurred across all frames [retrieved from the grid of peptide height occurrence frequencies depicted in Fig. 1(d)] yielded a map of water density as a function of the water and the peptide atom distance from the surface as shown in Fig. 1(e). Throughout the remainder of this work, density data are presented as vertical slices of such maps, where each slice shows the water behavior for a particular peptide atom height above the surface.

C. Tilt order parameter

There are several ways of evaluating and quantifying the water structure. One method is to generate histograms of the water tilt and twist angles with respect to distance from the surface.^{28,29,35,44} Here, we consider the water tilt to be the angle θ between the C_2 symmetry axis (H–O–H bisector) and the surface normal. Although such a description in terms of the Euler angles is structurally rich, it does not readily lend itself to combination with other analysis schemes. As a result, it is useful to have a simpler description of the water structure that can serve as an order parameter. We are particularly interested in order parameters that are related to experimental

observables. For this reason, we consider the average value of the cosine of the water tilt with respect to the surface normal, $\langle \cos \theta \rangle$, as this is one of the contributors to the second-order nonlinear response as would be probed in a visible-infrared sum-frequency generation (SFG) experiment. The definitions of this coordinate system and the angle θ are depicted in Fig. 2. Analysis of the tilt order parameter followed the same procedure as that of density: after generating a topological map of the peptide, trapped water molecules were identified. An average of the cosine of the water tilt angle with respect to the surface normal was calculated for every pair of peptide and water height values above the surface.

III. RESULTS AND DISCUSSION

A. Peptide structure in solution and on the two surfaces

In a recent study of LK α 14 on the same two surfaces, the distribution of peptide center of geometry, end-to-end distance, and long axis tilt angle was examined in its adsorbed state.³⁶ Significant differences were found, including a wider distribution of center of geometry and end-to-end distance values on the hydrophilic surface and more tilted orientation on the hydrophobic surface. In addition, Ramachandran plots enabled a detailed examination of the torsional angles, revealing some significant distortions from an ideal α -helix structure when adsorbed on either surface. As a result of this structural variability, end-to-end distance is not an ideal marker for conformation. This also means that the long axis tilt angle, defined based on the endpoint vectors, is most meaningful only when the peptide is in a more-or-less extended conformation. Here, we use a complementary and alternative measure of the peptide's change in secondary structure in its adsorbed state, its radius of gyration R_g . Radii of gyration histograms are displayed in Fig. 3. The results shown in blue were obtained for the peptide solvated in bulk water, in the absence of any surfaces.

Although we can note that in the bulk solution, there is a strong population at the ideal α -helix value ($R_g = 8.5$ Å,⁴⁵ as indicated by the dashed vertical line in Fig. 3), the predominant structure shows $R_g \approx 7$ Å, which indicates a more compact conformation. There is also a significant population with higher radii of gyration than the ideal α -helix, which is indicative of conformational freedom in the bulk solution. When adsorbed on the hydrophilic surface (green histogram in Fig. 3), the

FIG. 2. Angle θ between the water molecule symmetry axis and the surface normal, used in the calculation of the order parameter $\langle \cos \theta \rangle$.

FIG. 3. Peptide radius of gyration in the bulk solvent (blue) and when adsorbed on the hydrophilic (green) and hydrophobic (red) surface. Histograms have been normalized to unit area.

distribution of R_g significantly narrows toward small values. This indicates that the peptide adopts a more compact structure when it interacts with surface OH groups. A more dramatic change is observed upon contact with the hydrophobic surface (red trace), as a bimodal distribution of R_g is observed, with a more compact population centered near 7.5 Å and a more extended conformation with a mean $R_g \approx 9$ Å.

Further insight into the adsorbed peptide structure can be obtained by examining histograms of the height of its closest atom above the surface $z_{\min}$, center of geometry z_{COG} , and furthest atom from the surface $z_{\max}$. Results in Fig. 4 indicate similar features for both surfaces. This indicates that even though the adsorbed structures are significantly different as revealed by the R_g analysis, LK α 14 tends to occupy the same height on both surfaces (Fig. 5).

Although we have not investigated the energetics of adsorption in the present work, a recent computational study by Sprenger *et al.*¹⁵ has examined the interaction of LK α 14 with a charged hydrophilic (carboxylic acid/carboxylate self-assembled monolayer) surface and found the adsorption to be both entropically and enthalpically favored. However, changes in entropy on adsorption were found to vary considerably with the bulk solution ionic strength. The same study also examined adsorption of LK α 14 on a hydrophobic surface, and found the adsorption to be enthalpically driven. A net loss in system entropy resulted from a loss in peptide conformational freedom.

B. Water structure between the peptide and surface

1. Adsorbed on hydrophilic surface

Using the algorithm described above, the density and average cosine of the tilt angle of water under the peptide, as functions of distance from the hydrophilic surface are shown in Figs. 6(a) and 6(b). Three distinct regions of water behavior are observed when the peptide is more than 10 Å from the surface. Closest to the surface, water behavior is dictated by the surface, as is suggested by the identical water density profile compared to what is observed when the peptide is not present (blue line). The similarity in order parameter close to the surface compared

FIG. 4. Peptide atom minimum distance of approach ($z_{\min}$, dotted line), center of geometry (z_{COG} , solid line), and furthest atom from the surface ($z_{\max}$, dashed line) when adsorbed on the (a) hydrophilic and (b) hydrophobic surface. Colored regions indicate distances selected for subsequent analysis.

to when the peptide is not present (both with $\langle \cos \theta \rangle \approx 0.8$) further corroborate these findings. We point out that, in our definition of the tilt angle θ (Fig. 2), $\langle \cos \theta \rangle > 0$ indicate trapped water molecules with their hydrogen atoms pointing down toward the surface, and their oxygen atoms directed up toward the peptide. This creates a favorable geometry for hydrogen-bonding with surface hydroxyl groups. In the second region, water attains bulklike structure, with nearly constant water density values (albeit with lower density than that observed in the absence of peptide) and order parameter values tending toward 0 (approaching an isotropic distribution as found in the bulk). In the third region, the water structure is dictated by the peptide: an identical density profile near the peptide is observed when the peptide more than 10 Å from the surface. There is a 1 Å gap between the bottom surface of the peptide and the first water detected, followed by a 1 Å region of low water density. Since density was calculated based on the position of each water oxygen atom, this suggests that the first water layer closest to the peptide is of low density and has its oxygen pointed toward the peptide. This is corroborated by the high, positive values ($\langle \cos \theta \rangle \approx 0.8$) of the order parameter near the surface, which indicate that the water is strongly oriented with its oxygen upward.

Taking the 1-Å-wide shoulder near the peptide (as observed when the peptide is more than 10 Å from the surface) to be characteristic of water structure induced by the

FIG. 5. Representative snapshots of selected water molecules and the peptide adsorbed to the (a) hydrophilic and (b) hydrophobic surfaces. Hydrophilic peptide atoms (nitrogen atoms, oxygen atoms, and hydrogen atoms bonded to either nitrogen or oxygen atoms) are depicted in blue. Hydrophobic peptide atoms (carbon atoms and hydrogen atoms bonded to carbon atoms) are depicted in green. Only water molecules within 10 Å of the peptide are illustrated.

peptide, we see that when the peptide is closer to the surface (6 Å), the water has retained its surface-induced structure. However, it has lost the peak in water density approximately 4 Å from the surface, characteristic of water structure induced by a hydrophilic surface. The concurrent retention of peptide-induced structure and loss of surface-induced structure suggests that the water structure when the peptide is 6 Å from the surface is predominantly influenced by the peptide.

On the other hand, when the peptide is within 5 Å of the surface, the water structure is concurrently affected by both the surface and the peptide, as evidenced by the lack of surface- and density-specific water structure at these distances. The large positive values of order parameter indicate that the water is oriented with oxygen atoms directed toward the peptide. Strong orientation is likely indicative of strong hydrogen-bonding between the interfacial water and the peptide.

Close inspection of the order parameter values of water close to the surface and close to the peptide surface is also

revealing. The same preferred orientation of water molecules—with oxygen atoms pointing away from the surface and toward the peptide, as indicated by the positive tilt order parameter values—is observed both at the surface and close to the bottom face of the peptide. Therefore, as the peptide approaches the surface, water immediately below the peptide does not need to undergo a change in orientation in order to be in an orientation favored by proximity to the surface. This suggests that the presence of water is favorable in mediating the peptide–surface interaction.

These findings have implications on the mechanism of peptide adsorption on the hydrophilic surface. We see in Fig. 4(a) that the closest peptide atom to a hydrophilic surface is typically 3–6 Å from the surface. That there is well-ordered water beneath the peptide when the peptide is 4, 5, and 6 Å from the surface [Fig. 6(a)] indicates that the peptide does not always make direct contact with the surface. Rather, it makes contact with a layer of water on the surface, whose structure is influenced by both the surface and by the incoming peptide.

2. Adsorbed on hydrophobic surface

Markedly different structure is observed for water trapped between the peptide and the hydrophobic surface. Because of the depletion of water near the surface (on the order of 4 Å in the absence of peptide), water is nearly excluded, even when the peptide is as far away as 11 Å. Besides the clear decrease in water density beneath the peptide for all heights of the peptide studied, it is also striking that the width of the depletion region increases with the distance of the peptide above the surface. Whereas for any water height above the hydrophilic surface, the density increases the further the peptide is from the surface, on the hydrophobic surface, we see that water density near the surface is higher when the peptide is closer to the surface (11 Å) than when it is far from the surface (20 Å). This suggests that the water has a greater affinity for the peptide than for the hydrophobic surface.

Although water density beneath the peptide is significantly lower than in the absence of peptide, the water that is present is much more ordered. For example, even when a peptide atom is 20 Å from the surface, the trapped water barely attains bulklike behavior (as indicated by a tilt order parameter of zero) before either the surface or the peptide surface induces order on it. It is also noteworthy that although water density is low near the peptide, the strong tendency of water to orient itself with its oxygen pointing toward the peptide (as indicated by large, positive values of the order parameter) is observed close to the peptide adsorbed to both the hydrophilic and hydrophobic surface.

A comparison of water order parameter values close to the peptide (where water is strongly oriented with its oxygen atom pointed toward the peptide) with those close to the surface (where water order parameters are lower) is also revealing. Whereas on the hydrophilic surface, the orientations of water near the peptide and near the surface are complementary, the orientations differ on the hydrophobic surface. That is, as peptide atoms move closer to the surface, the surface

FIG. 6. (a) Density profiles and (b) order parameters for water molecules as a function of distance from the hydrophilic surface. Results obtained in the absence of the peptide ($d = \infty$) are shown in blue. When the peptide is adsorbed, the distance between the surface and the closest peptide atom is indicated according to the colors described in the legend ($d = 4, 5, 6, 11$, and 20 Å). Similar analyses were performed for the hydrophobic surface, with the density profiles in (c) and the order parameters in (d).

and the peptide have competing effects on the orientation of the water. Since the density of water beneath the peptide is as a whole higher on the hydrophilic surface than on the hydrophobic surface, it is conceivable that the exclusion of water beneath the peptide is due at least in part to the unfavorable reordering process required of water as the peptide approaches the surface. This has implications on the mechanism of peptide adsorption to the hydrophobic surface. In Fig. 4(b), it can be seen that the closest approach of the peptide to the surface is typically 6 Å or less. Here we see, based on the exclusion of water beneath peptide atoms located $4, 5$, and 6 Å from the surface [Fig. 6(c)], that the peptide is anchored directly to the surface, rather than to a layer of water above the surface.

Visible-infrared SFG experiments are ideally suited at probing buried surfaces and distinguishing ordered molecules from neighboring isotropic species.⁴⁶ Since vibrational information is obtained, SFG is a unique probe of interfacial water, as the details of the hydrogen-bonding environment are revealed from the distribution of O–H stretching intensity in the $3000\text{--}3800\text{ cm}^{-1}$ region. When adsorbates are introduced, the dominant trend is usually a reduction in the

overall water SFG response. This is due to a decrease in the amount of ordered water near the surface, and a reduction in the degree to which interfacial water is structured. In an SFG study of LK α 14 adsorption onto glass and polystyrene by Mermut *et al.*, the authors observed a nearly identical water response on the hydrophobic substrate before and after peptide adsorption.²⁰ Based on our findings, we can speculate on this anomalous behavior, in that the decrease in water density at the hydrophobic surface in the presence of LK α 14 may be balanced by the strong orientation of the trapped water molecules, resulting in no significant change in the overall SFG response. Such an interpretation is not straightforward, as the frequency of the coupled water OH modes is sensitive to the surface-water-peptide environment, and SFG provides a probe of their collective frequency, amplitude, and phase.

C. Peptide contribution to the water structure differences

As the radius of gyration histograms (Fig. 3) revealed a wide distribution of peptide conformations on each surface,

it is intuitive to search for an explanation on the basis of interfacial water structure. We have therefore decided to analyze the water density and tilt order parameter on each surface using our algorithm, but now divided according to long and short R_g . For the hydrophobic surface, two main populations were evident, with R_g less than and greater than 8 Å. For the hydrophilic surface, there was no clear separation in the R_g histogram, so the extrema of the population were studied (the bottom 20% with $R_g < 7.3$ Å, and the top 20% with $R_g > 8.4$ Å). In both of these cases, each population produced trapped water density and order parameters that were essentially indistinguishable from each other. This revealed that, even though the overall peptide structures were considerably different, the portion of the peptide closest to the surface likely remained the same.

As LK α 14 contains both hydrophobic and hydrophilic residues, it is worth investigating whether the structure of water trapped beneath the peptide is altered based on the hydrophobicity of the residue with which it is interacting. Once again, this split produced nearly identical results. This makes sense considering that even the hydrophilic lysine residue is composed of mainly hydrophobic atoms. This phenomenon has been described in recent work by Kapcha

et al., who proposed that evaluating hydrophobicity on a per atom, rather than per residue basis is more appropriate.⁴⁷ Using such an approach, the water structure trapped between the surface and a hydrophilic peptide atom and a hydrophobic peptide atom were analyzed separately below. Nitrogen atoms, oxygen atoms, and hydrogen atoms bonded to either nitrogen or oxygen atoms were considered hydrophilic; carbon atoms and hydrogen atoms bonded to carbon atoms were considered hydrophobic.

1. Adsorbed on hydrophilic surface

Of the three regions of water behavior observed previously when the peptide is more than 10 Å from the surface, only that region closest to the peptide is affected by the hydrophobicity of the peptide. The behavior of water is identical for the hydrophilic and hydrophobic peptide atom cases for both the surface-dictated region and the bulklike region [Figs. 7(a) and 7(b)]. However, the change in water structure with changes in peptide atom hydrophobicity in the third region—close to the peptide—is drastic.

In the general case of water behavior between the peptide and hydrophilic surface, without accounting for peptide

FIG. 7. Density profiles for water molecules as a function of distance from the hydrophilic surface, when trapped under a (a) hydrophilic and (b) hydrophobic peptide atom. The distance between the surface and the closest peptide atom is indicated according to the colors described in the legend ($d = 4, 5, 6, 11$, and 20 Å). Similarly, water order parameters were calculated when water was trapped under peptide (c) hydrophilic and (d) hydrophobic atom. For comparison, results obtained in the absence of the peptide ($d = \infty$) are shown in blue.

atom hydrophobicity [Figs. 6(a) and 6(b)], peptide-induced water structure was characterized by a highly ordered layer of water of low density, 1 Å from the bottom side of the peptide. Here, we observe that this structure is due exclusively to water beneath hydrophilic peptide atoms [Fig. 7(c)]. The low values of water order parameter near the hydrophobic peptide atoms are commensurate with those observed near the hydrophobic surface in the absence of peptide, which indicates that the hydrophobic peptide atoms form a surface analogous to the planar hydrophobic surface and induce similar water behavior as does the surface. The contrast in order parameter values near hydrophobic peptide atoms compared to those near hydrophilic atoms suggests that these water molecules are in drastically different hydrogen-bonding environments. This in turn corroborates our previous assertion that the layer of water closest to the peptide is likely involved in strong hydrogen-bonding interactions with the peptide.

When the peptide is close to the surface (4–5 Å), water density is even higher underneath hydrophilic peptide atoms than in the absence of peptide, whereas water is nearly entirely excluded from beneath hydrophobic peptide atoms.

Water between a hydrophilic surface and hydrophilic peptide atoms is the only case in which the water density is higher in the presence of peptide. This hydrophilic peptide atom height above the surface favors high water density, as the hydrogen atoms in each water molecule can form hydrogen bonds with the surface while the oxygen atoms can form hydrogen bonds with the peptide (the large, positive values of the order parameter corroborate this orientation argument). The near-total exclusion of water between the hydrophilic surface and a hydrophobic peptide atom suggests that in order to trap water in appreciable quantities within 5 Å of the surface, it is insufficient for only one of the surface or the peptide face to be hydrophilic; rather, both must be hydrophilic.

2. Adsorbed on hydrophobic surface

While trapped water is apparent between the hydrophobic surface and hydrophilic peptide atoms [Fig. 8(a)], no significant water density is observed between the hydrophobic surface and hydrophobic peptide atoms [Fig. 8(c)]. The density profile of water between the hydrophobic surface and a hydrophobic peptide atom is strikingly similar to the general

FIG. 8. Density profiles for water molecules as a function of distance from the hydrophobic surface, when trapped under a (a) hydrophilic and (b) hydrophobic peptide atom. The distance between the surface and the closest peptide atom is indicated according to the colors described in the legend ($d = 4, 5, 6, 11$, and 20 Å). Similarly, water order parameters were calculated when water was trapped under peptide (c) hydrophilic and (d) hydrophobic atom. For comparison, results obtained in the absence of the peptide ($d = \infty$) are shown in blue.

case of water behavior between the hydrophobic surface and any peptide atom [depicted in Fig. 6(c)], in which water is excluded from beneath the peptide when the peptide is within 6 Å of the surface. The similarity in the water density profiles beneath the peptide in general and beneath hydrophobic peptide atoms indicates that on the hydrophobic surface, hydrophobic atoms are more often the closest peptide atom to the surface. This again illustrates the preferential absorption of the peptide with its hydrophobic face toward the hydrophobic surface.

IV. CONCLUSIONS

Atomistic molecular dynamics simulations of the adsorption of LK α 14 onto two surfaces of different hydrophobicity were used to study the interplay of adsorbed peptide structure, structured interfacial water, and surface hydrophobicity. LK α 14 serves as a model system for such studies as a result of its facewise affinity for both hydrophilic and hydrophobic surfaces. An analysis of water structure beneath a peptide atom greater than 10 Å from a hydrophilic surface allowed us to differentiate surface-induced, bulklike, and peptide-induced water behavior. On the hydrophobic surface, no appreciable bulklike water was observed, and interfacial water was found to be largely excluded, yet highly ordered, beneath the peptide. We found that whereas peptide adsorption to hydrophilic surfaces often occurs through a layer of ordered water, direct interaction is observed on hydrophobic surfaces. We rationalized these findings based on the compatibility of the preferred orientation of water near the peptide and that on the surface in the absence of peptide. These findings were further refined by analyzing differences in the behavior of water beneath hydrophobic and hydrophilic peptide atoms. This study on the structure of interfacial water offers fundamental insight into the role of water in adsorbed peptide orientation and conformation.

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¹R. A. Latour, *Biointerphases* **3**, FC2 (2008).

²R. A. Latour, *Colloids Surf., B* **124**, 25 (2014).

³O. Mermut, R. L. York, D. C. Phillips, K. R. McCrea, R. S. Ward, and G. A. Somorjai, *Biointerphases* **1**, P5 (2006).

⁴B. D. Ratner and S. J. Bryant, *Annu. Rev. Biomed. Eng.* **6**, 41 (2004).

⁵D. G. Castner and B. D. Ratner, *Surf. Sci.* **500**, 28 (2002).

⁶G. Mani, M. D. Feldman, D. Patel, and C. M. Agrawala, *Biomaterials* **28**, 1689 (2007).

⁷D. F. Williams, *Biomaterials* **29**, 2941 (2008).

⁸C. Avery and Z. Chen, *Antimicrobial Polymers* (Wiley, Hoboken, NJ, 2011), Chap. 16.

⁹S. Cosnier, *Biosens. Bioelectron.* **14**, 443 (1999).

¹⁰P. Bartlett and R. Whitaker, *J. Electroanal. Chem. Interfacial Electrochem.* **224**, 27 (1987).

¹¹A. A. Skelton, T. Liang, and T. R. Walsh, *ACS Appl. Mater. Interfaces* **1**, 1482 (2009).

¹²T. Weidner, N. T. Samuel, K. McCrea, L. J. Gamble, R. S. Ward, and D. G. Castner, *Biointerphases* **5**, 9 (2010).

¹³J. S. Apte, L. J. Gamble, D. G. Castner, and C. T. Campbell, *Biointerphases* **5**, 97 (2010).

¹⁴W. F. DeGrado and J. D. Lear, *J. Am. Chem. Soc.* **107**, 7684 (1985).

¹⁵K. G. Sprenger and J. Pfaendtner, *Langmuir* **32**, 5690 (2016).

¹⁶D. C. Phillips, R. L. York, O. Mermut, K. R. McCrea, R. S. Ward, and G. A. Somorjai, *J. Phys. Chem. C* **111**, 255 (2007).

¹⁷T. Weidner, J. S. Apte, L. J. Gamble, and D. G. Castner, *Langmuir* **26**, 3433 (2010).

¹⁸T. Weidner, N. F. Breen, K. Li, G. P. Drobny, and D. G. Castner, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 13288 (2010).

¹⁹R. L. York, W. K. Browne, P. L. Geissler, and G. A. Somorjai, *Isr. J. Chem.* **47**, 51 (2007).

²⁰O. Mermut, D. C. Phillips, R. L. York, K. R. McCrea, R. S. Ward, and G. A. Somorjai, *J. Am. Chem. Soc.* **128**, 3598 (2006).

²¹T. Weidner and D. G. Castner, *Phys. Chem. Chem. Phys.* **15**, 12516 (2013).

²²T. Weidner, N. F. Breen, G. P. Drobny, and D. G. Castner, *J. Phys. Chem. B* **113**, 15423 (2009).

²³G. Collier, N. A. Vellore, J. A. Yancey, S. J. Stuart, and R. A. Latour, *Biointerphases* **7**, 24 (2012).

²⁴M. Deighan and J. Pfaendtner, *Langmuir* **29**, 7999 (2013).

²⁵C. Dalgicdir and M. Sayar, *J. Phys. Chem. B* **119**, 15164 (2015).

²⁶P.-H. Chen, Y.-H. Tseng, Y. Mou, Y.-L. Tsai, S.-M. Guo, S.-J. Huang, S. S.-F. Yu, and J. C. C. Chan, *J. Am. Chem. Soc.* **130**, 2862 (2008).

²⁷G. Goobes, R. Goobes, O. Schueler-Furman, D. Baker, P. S. Stayton, and G. P. Drobny, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16083 (2016).

²⁸C. Y. Lee, J. A. McCammon, and P. J. Rossky, *J. Chem. Phys.* **80**, 4448 (1984).

²⁹S. H. Lee and P. J. Rossky, *J. Chem. Phys.* **100**, 3334 (1994).

³⁰D. S. Walker, D. K. Hore, and G. L. Richmond, *J. Phys. Chem. B* **110**, 20451 (2006).

³¹D. K. Hore, D. S. Walker, and G. L. Richmond, *J. Phys. Chem. C* **111**, 8832 (2007).

³²G. L. R. D. K. Hore and D. S. Walker, *J. Am. Chem. Soc.* **130**, 1800 (2008).

³³M. J. Stevens and G. S. Grest, *Biointerphases* **3**, FC13 (2008).

³⁴M. Maccarini, *Biointerphases* **2**, MR1 (2007).

³⁵T. G. Trudeau, K. C. Jena, and D. K. Hore, *J. Phys. Chem. C* **113**, 20002 (2009).

³⁶S. Roy, T. L. Naka, and D. K. Hore, *J. Phys. Chem. C* **117**, 24955 (2013).

³⁷R. L. York, O. Mermut, D. C. Phillips, K. R. McCrea, R. S. Ward, and G. A. Somorjai, *J. Phys. Chem. C* **111**, 8866 (2007).

³⁸S. Roy and D. K. Hore, *J. Phys. Chem. C* **116**, 22867 (2012).

³⁹G. A. Kaminski and R. A. Friesner, *J. Phys. Chem. B* **105**, 6474 (2001).

⁴⁰D. Horinek, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2842 (2008).

⁴¹L. Martin, M. M. Bilek, A. S. Weiss, and S. Kuyucak, *Interface Focus* **6**, 45 (2015).

⁴²W. L. Jorgensen and J. Tirado-Rives, *J. Am. Chem. Soc.* **110**, 1657 (1988).

⁴³H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. Dinola, and J. R. Haak, *J. Chem. Phys.* **81**, 3684 (1984).

⁴⁴T. G. Trudeau and D. K. Hore, *Langmuir* **26**, 11095 (2010).

⁴⁵B. Zagrovic, G. Jayachandran, I. Millett, S. Doniach, and V. S. Pande, *J. Mol. Biol.* **353**, 232 (2005).

⁴⁶S. Roy, P. A. Covert, W. R. FitzGerald, and D. K. Hore, *Chem. Rev.* **114**, 8388 (2014).

⁴⁷L. H. Kapcha and P. J. Rossky, *J. Mol. Biol.* **426**, 484 (2014).