Hydration shell of the TS-Kappa protein: higher density than bulk water

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Abstract

The density of the water molecules in the presence of hydrophobic and hydrophilic amino acids was studied. Molecular dynamic simulation were employed to analyze the behavior of hydrated TS Kappa protein in SPC/E and TIP4P-2005 water models. The simulations were performed in the NPT ensemble with the Nosé-Hoover thermostat and the Parrinello-Rahmann barostat. The density profile of these systems were obtained for different temperatures at constant pressure. Two complementary phenomena were observed. The protein-water system exhibits a temperature of maximum density lower than the temperature observed in the pure water system. The densities of the water in vicinity of the hydrophobic and hydrophilic site are higher than the density of the water in the bulk. Our results suggest that interactions between protein and water and the water-water Hydrogen bonds are essential to the understanding of these phenomena.

Keywords:
hydrated proteins, density anomaly, hydrophobic behavior
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1. Introduction

Water is one of the most important substance on the planet. Despite its abundance, liquid water exhibits a number of anomalous thermodynamic, dynamic and structure behaviors when we compared with other liquids [1]. The correlation of the fluctuations of the volume and the fluctuations of the entropy have a minimum for liquid water, while for other liquids these properties decrease when the temperature is decreased [2]. Consequently, at ambient pressure the isothermal compressibility, κ_T, and the fixed pressure heat capacity, C_P, have a minimum at, respectively, 46°C [3] and 35°C [4]. The water molecule can form Hydrogen bond with other water molecules. The competition between this interaction and the van der Waals interaction has been considered the major ingredient that give rise to the water anomalies [2]. From this competition tetramers of water molecules form two structures: an lower density open structure in which two tetramers are H-bonded, and a more dense closed structure in which the tetramers are not H- bonded. Since the H-bond requires more distance to be formed, the bonded tetramers exhibits a larger structure when compared with non bonded tetramers. These two structures competes to create the Hydrogen bond network leading to the thermodynamic, dynamic and structural anomalous behavior.

The most known of these anomalies is the increase of the water density with increasing temperature reaching a maximum at $T = 4°C$ and 1atm. This anomaly arises from the breaking of Hydrogen bonds as temperature is increased what compacts the system up to a certain temperature where the balance of open and closed clusters implies an increase in the number of neighbors [5].

Water anomalies are also important when water is interacting with other substances. The competition between bond and non bonded structures formed in water plays an important role also in biomolecules. For instance, the hydration of the proteins is fundamental to define its three dimensional structure [6, 7, 8]. Dehydrated proteins are generally inactive, and they become functional only after reaching a critical level of hydration [9]. In addition, water influences the motion of protein as seen in experimental [10, 11, 12] and theoretical [13, 14, 15] studies, and determines the biological activity of these biomolecules.

Experimental results indicate that the structure of the proteins behave quite differently when immersed in water [16, 17]. The mechanism suggested is that the water-protein interaction is so strong that water molecules pene-

trate in the cavities of the protein winning against the lost of entropy [18].

Experiments with lysozyme, Escherichia coli thioredoxin reductase, and protein R1 of E. coli ribonucleotide reductase, show that the solvation shell exhibits a higher density than the water in the bulk [16] within a region that covers approximately $7 - 10\mathring{A}$ from the protein surface [19, 20]. In addition X-ray diffraction with Rat mannose-binding protein shows that water is more structured around polar sites [21] than around hydrophobic sites.

In order to understand the mechanism of this increase in density protein-water simulations have been performed [22, 23, 24, 25]. Merzel et al [23] by computing the average density and the dipole orientations around the lysozyme suggested that dense water is found in depression on the surface of the protein where the dipoles aligned. Kuffer et al [24, 25] by comparing the solvation water of the motor head of kinesin and a pure hydrophobic surface suggested that the deformation of the hydrogen bond network in solvation shell is responsible for the average density increase in the solvation shell.

In the protein studies it is difficult to separate the topological [23] from the electrostatic effects [24, 25]. In order to circumvent this difficulties, simulations of water confined between smooth structures such as plates, porous or obstacles has been performed [13, 26, 27, 28, 29]. Even though these studies focus in the change of the anomalous properties and of the melting temperature under hydrophobic [13, 26, 27, 28, 29], hydrophilic [26, 27] and heterogeneous confinement [27], they also show a high density of water in the contact layer for both pure hydrophobic and pure hydrophilic confinements. In addition in the case of heterogeneous confinement [27] the density at the hydrophobic regions is higher than it would be in confinement by pure hydrophobic case. The mechanism that leads to this increase of density in hydrophobic sites in the heterogeneous case is still not clear.

The aim of this paper is to analyze the density of the water molecules within the solvation shell around a system that exhibits a mixture of hydrophobic and hydrophilic sites: the TS-Kappa protein [30]- a specific toxin of brazilian's yellow scorpion. We compare the behavior of the density and H-bonds of water molecules far from the surface of the protein with the water on the hydrophobic and hydrophilic groups on the surface of the protein. In addition we also observe how the presence of the protein influence in the temperature of maximum density. Our results comparing the hydration water close to hydrophobic and hydrophilic sites might shade some light in the influence of the water on the protein and on the protein on water.

2. The Model and the Method

We study the TS-Kappa protein immersed in two types of water model: SPC/E [31] and TIP4P-2005 [32].

The molecular dynamics (MD) simulations for the SPC/E were carried out using OPLS-AA [33] while for the TIP4P-2005 were performed with the Amber03W [34] force fields. Both models were implemented in the GROMACS package [35] version 4.5.5.

The protein was extracted from the Protein Data Bank, with the 1TSK id. The TS-Kappa protein is a globular protein, composed of thirty-five residues, where eight of those have a hydrophobic behavior and twenty-seven have a hydrophilic behavior. It has residues, 20 % of helical structures and 22 % beta sheets and has three disulphide bonds, and it is an alpha toxin acting on potassium channels [36].

The water is modeled by two atomistic potentials. The protein plus water systems were simulated in the NPT ensemble. 3946 SPC/E water molecules were used in one set of simulation while 3916 TIP4P-2005 water molecules were employed in another set of simulations. In both cases the same simulation box size was employed. Periodic boundary conditions were used and the temperature was fixed with the Nose-Hoover thermostat [37, 38]. The pressure was controlled by the Parrinello-Rahman barostat [39].

After the system was solvated, the output file contains a charged protein, with a net charge of $+5e$, four lysine (+), three arginine (+) and two aspartic acid(−). The protein plus water system occupy a cubic box with 5.5 nm of length. The distance of the protein surface and the edge of box is 1.2 nm. In order to neutralize the system was inserted seven ions Cl^- to balance the positive charges, and two Na^+ to balance the negative charges. The electrostatic interactions were calculated by particle mesh Ewald (PME) summation and the non-bonded interactions were truncated at 10 $\mathring{A}$. The systems were subjected to the steepest descent energy minimization process with tolerance of 1000 kJ/mol. The time step for the simulations was set to 2 fs. During the system equilibration process the protein was kept fixed, whilst the solvent molecules and the counter ions were allowed to move during 500 ps by NPT conditions. After the system equilibrate the structure was the used for the following 10 ns production runs. During the production phase, the coordinate data were written to the file every picoseconds.

3. The Results

First, we investigate the total density of the system water plus protein and compare it with the bulk water system for both models for water.

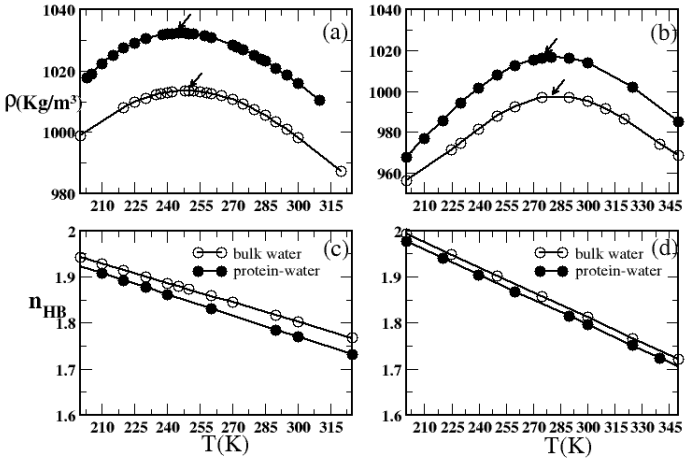

Figure 1: Density *versus* temperature for bulk water system (open circles) and the hydrated protein system (filled circles) for SPC/E (a) and TIP4P-2005 water model (b). Number of water-water H-bonds per water molecule *versus* temperature for bulk SPC/E water model (open circles) and protein hydrated system (filled circles) (c) and bulk for TIP4P-2005 water model (open circles) and protein hydrated system (filled circles) (d). The arrows indicate the TMD.

Figure 1(a) illustrates the density *versus* temperature at $P = 1atm$ for the SPC/E water model. The open circles represent the behavior of the bulk water for this model while the filled circles show the hydrated protein system of the same model. The model SPC/E as in the experimental water exhibits a region in which the density increases with the increase of temperature. The SPC/E bulk water has a maximum in the density at 250K at 1atm. Since SPC/E is under structured this temperature is lower than the TMD (temperature of maximum density) for experimental water at 1atm, namely 277K [40]. Figure 1(a) also illustrates the TMD at 1atm for the water protein system. In this case the TMD is at 245K, 5K bellow the pure water system. This effects of shifting of TMD was also observed in studies of water confined by hydrophobic plates [28, 29, 41, 42].

The protein TIP4P-2005 water model exhibits the same trend as observed in the SPC/E model illustrated in the figure 1(b). The bulk system (open circles) exhibits a maximum in density at about $T = 280K$ and $P = 1atm$, while the TMD for protein plus water system (filled circles) has a TMD at $T = 275K$ and also at 1 atm. The maximum of density of the TIP4P-2005 agrees with the experimental value since this model is parametrized to be valid at TMD. The same temperature difference observed for SPC/E water model is also seen for TIP4P-2005 water model. In both cases, the density values for the hydrated protein system have a shift to higher values when compared with the bulk water system. The SPC/E water model the density values for both, bulk water and hydrated protein, are higher than those. The SPC/E leads to more compact structures because it is less structured than the TIP4P-2005 model.

Figures 1(c) and 1(d) show the number of H-bonds between water molecules per water molecule *versus* temperature for the bulk system and for the SPC/E and TIP4P-2005, respectively. The graphics show that as the temperature is increased the number of H-bonds decreases. In both types of water models, the number of H-bonds between water molecules is lower for the water+protein system when compared with the pure water system. The reason for this difference is that the water around the protein breaks H-bonds with other water molecules to interact with the protein sites.

The higher value of the density for the protein+water system when compared with the pure water system can be explained in terms of the particle interactions. Hydrophobic surfaces repel the water generating a region where no water molecule is found and therefore the average density should decrease. Since our criteria for the density of the contact layer is the distance from the protein this empty region is also computed in the density volume. To balance this decrease in density, the presence the protein breaks the H-bonds what increases the density. The competition between these two effects lead to the average increase in the density close to hydrophobic surfaces. Similar behavior is observed in water confined [26, 27].

In order to fully characterize the water structure on the system, we have also calculated the oxygen-oxygen radial distribution function (RDF) of the water molecules in the SPC/E model. This function can be written as $g_{oo}(r)$ and is illustrated in the figure 2. For all temperatures, $g_{oo}(r)$ has a minimum around r= 0.33nm that is close to the coordination shell used in the experiments [16] and simulations [23]. The decrease of temperature does not shift the location of the maximum and minimum of $g_{oo}(r)$ but change their

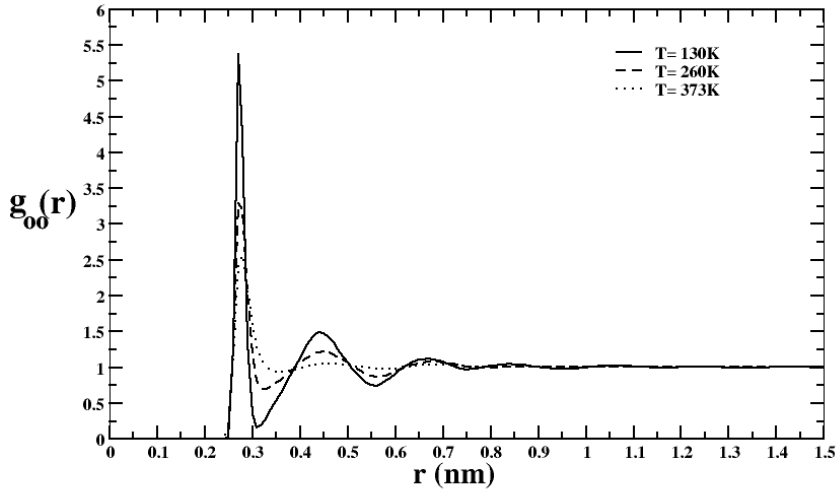

Figure 2: Radial distribution function *versus* r for hydrated protein system for SPC/E water model for temperatures 130K, 260K and 373K.

respective heights. This $g_{oo}(r)$ suggests that the first coordination shell for water comprises the distance until r= 0.33nm. The $g_{oo}(r)$ for the TIP4P-2005 water model, not shown here, gives for this range of temperature approximately the same distance for the first hydration shell and we did not include graph in the paper.

Figure 1 shows that the density of the water-protein is higher than the density of the pure water system. The graph, however, does not provide how the water is distributed in the system and particularly how the water is distributed along the protein. Thus, we focus our attention to the density behavior of water around protein. The protein surface is divided in thirty-five sector. Our approach bares some resemblance with the division in patches employed by Kuffer et al [24, 25] but in our case each sector is an amino acid. Then, since the protein has hydrophobic and hydrophilic patches, the density of water around these different regions is computed. The area of each patch varies from 0.8 to 1.2 nm^2. The height of the patches is taken to be 0.33 nm, the first coordination shell of bulk water.

Figures 3 and 4 illustrate the density *versus* temperature at 1 atm at the vicinity of hydrophilic and hydrophobic patches, respectively. The amino

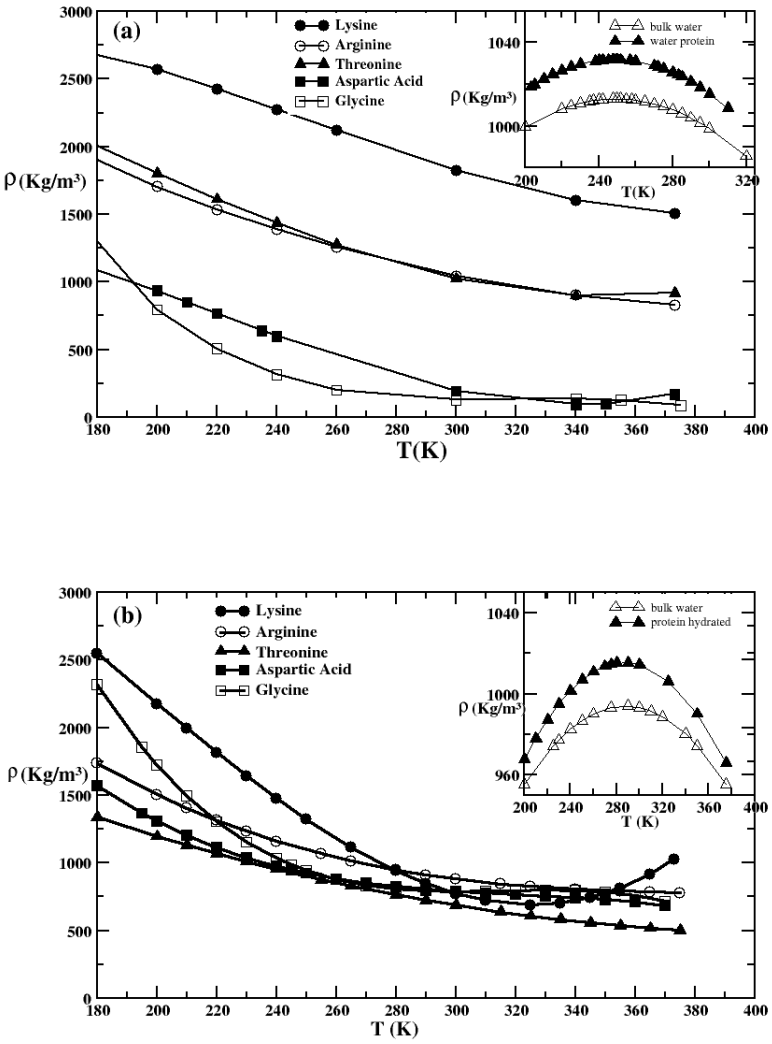

Figure 3: (a) Density *versus* temperature for SPC/E water model around hydrophilic amino acids and (b) density *versus* temperature for TIP4P-2005 water model around hydrophilic amino acids. The inset represents the total density of the water molecules *versus* temperature the for bulk system (open triangles) and for the protein hydrated system (filled triangles).

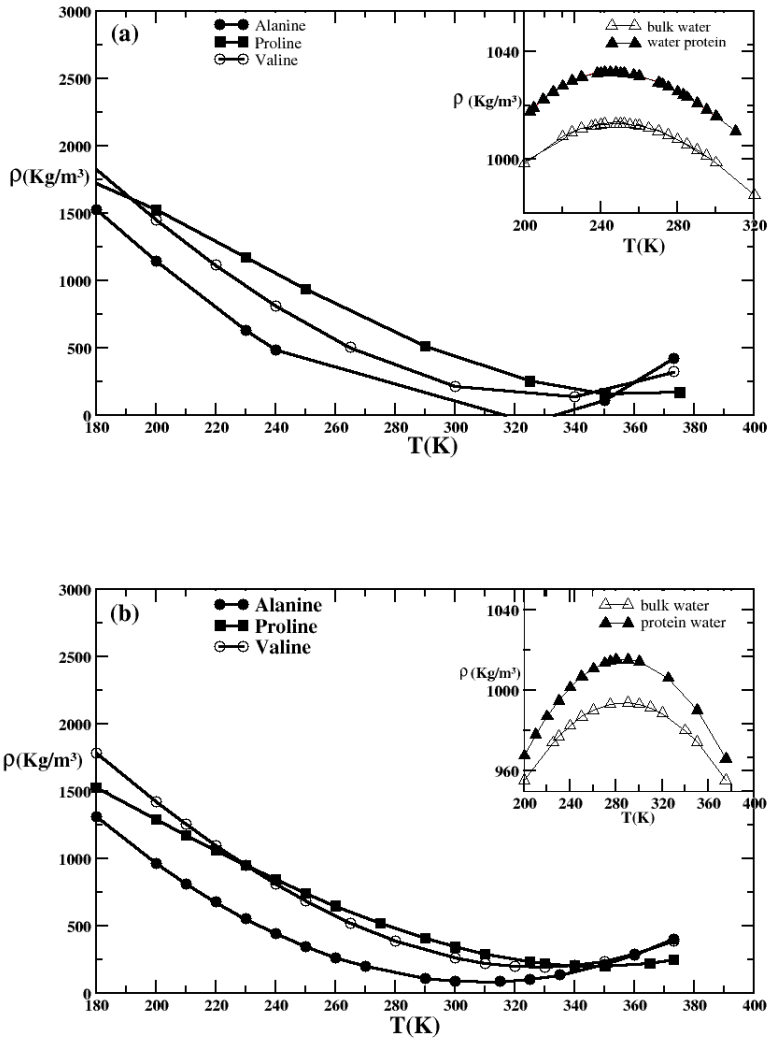

Figure 4: (a) Density *versus* temperature for SPC/E water model around hydrophobic amino acids and (b) Density *versus* temperature for TIP4P-2005 water model around hydrophobic amino acids. The inset represents the total density of the water molecules *versus* temperature for the bulk system (open triangles) and for the protein hydrated system (filled triangles).

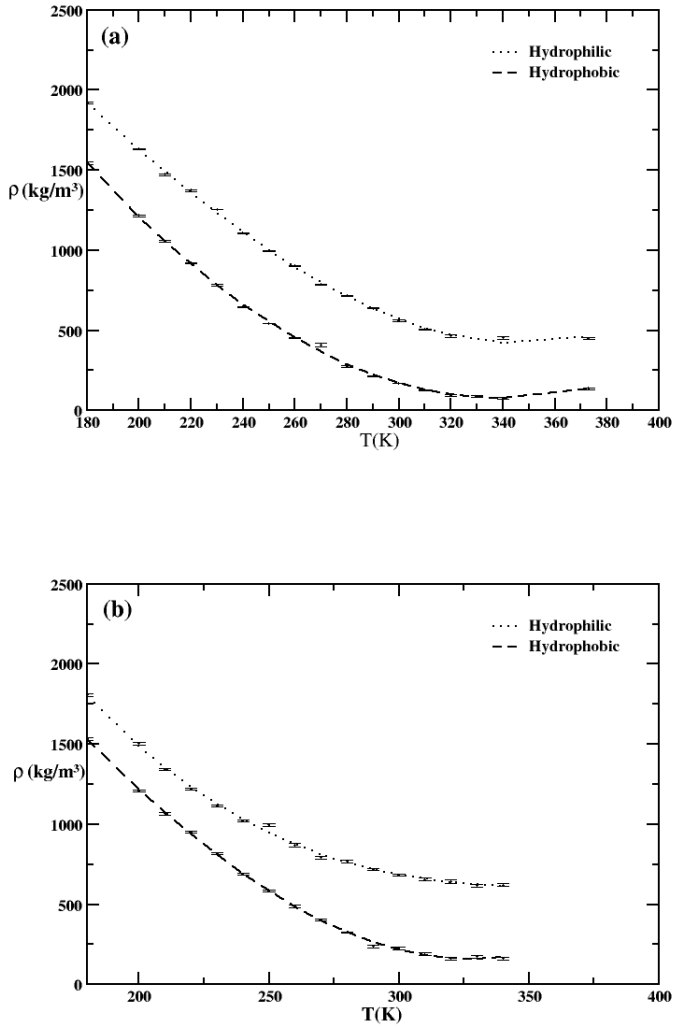

Figure 5: (a) Average density *versus* temperature of the SPC/E water model in the hydration shell and (b) average density *versus* temperature of the TIP4P-2005 water model. The averages are taken for the hydrophilic (dotted line) and the hydrophobic (dashed line) amino acids.

acids present in the protein and its interaction with water can be seen in table 1. For clarity only selected amino acids are illustrated in the figures.

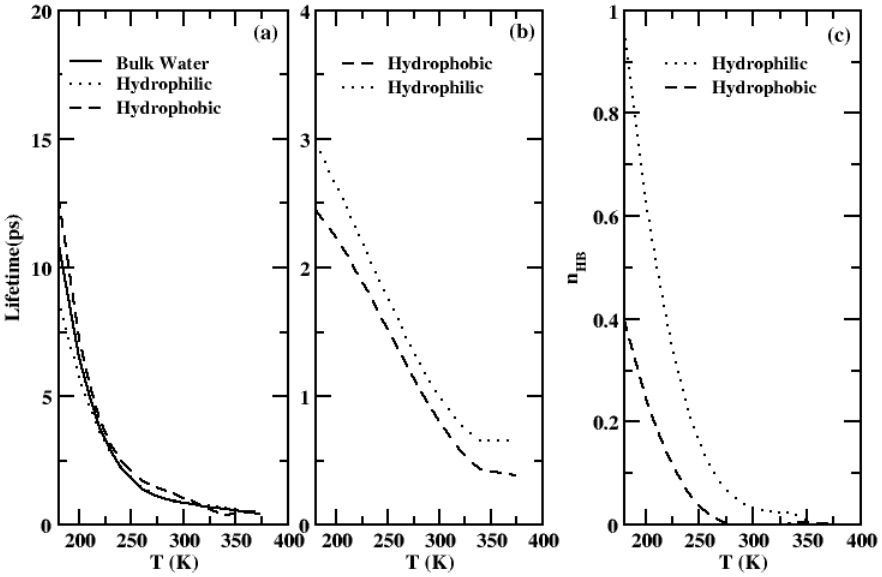

Figure 6: (a) Lifetime of the water-water H-bonds at the bulk (solid line), near the hydrophobic (dashed line) and hydrophilic (dotted line) sites of the protein. (b) Lifetime of the H-bonds of water and hydrophilic and water-hydrophobic sites of the protein (c) Number of the H-bonds between the water and the hydrophobic add the hydrophilic sites of the protein. All the quantities were computed for the SPC/E water model.

Since the number of amino acids of each type is small, an averages over the density of the hydrophobic and the hydrophilic amino acids are illustrated in Figure 5.

As the temperature is decreased the density of water increases. The maximum density observed in the bulk water and water-protein systems are due to the water-water H-bond formation at the liquid phase when the temperature is decreased. The presence of a surface disrupts the H-bond network and either moves the temperature of maximum density to very low temperatures [28, 43] or make it to disappear.

At very low temperatures the values of the densities are in both hydrophilic and hydrophobic sites higher than the bulk density shown in figures 3 and 4. This can be explained since water molecules in lower temperatures

Table 1: Table of amino acids of TS-Kappa protein

Hydrophilic	**Hydrophobic**
Lysine	Valine
Arginine	Alanine
Aspartic Acid	Proline
Glycine	Leucine
Serine	Isoleucine
Threonine	-
Tyrosine	-
Asparagine	-
Glutamine	-

has a decrease in their entropy. As the protein has a majority of surface with hydrophilic behavior and the entropy of water molecules is decreased with the decrease of temperature, the water molecule is more organized and more dense at low temperatures. The same behavior can seen in the water-like molecules confined within the hydrophilic and the hydrophobic parallel plates [27].

The figure 6 (a) illustrates the lifetime of the water-water H-bonds versus temperature for the water at the bulk, near the hydrophobic and hydrophilic sites of the protein. At low temperatures, the H-bonds between water molecules near the hydrophobic sites is larger when compared with the hydrophilic sites. On the other hand figure 6 (b) shows the lifetime of the H-bonds between the water and the hydrophobic and hydrophilic sites of the the protein. The figure indicates that the bonds between the water and the hydrophilic sites exhibit longer lifetimes when compared with the hydrophobic sites. These two effects together with 6 (c) that shows that the number of H-bonds between the water molecules and the hydrophilic sites is higher than the number of H-bonds with the hydrophobic sites suggests that the hydration water is more structured around the hydrophilic sites than around the hydrophobic sites.

The density of the first layer of water around the protein is also obtained for the TIP4P-2005 model. Figures 3 (b) and 4 (b) illustrate the densities for the selected the hydrophilic and hydrophobic amino acids, respectively.

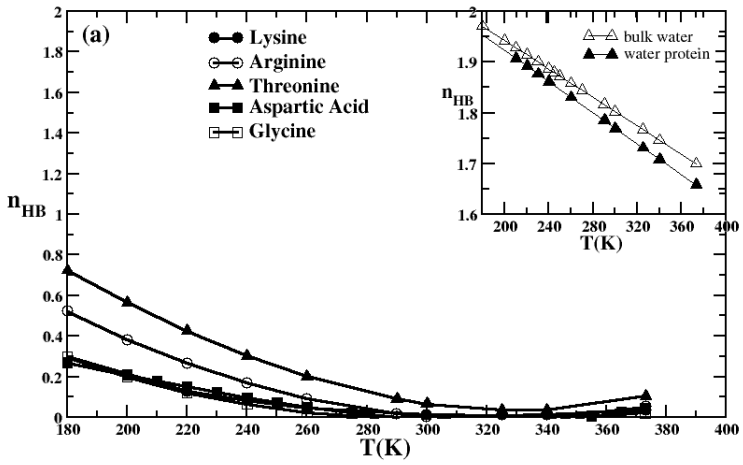

Figure 7: Number of water-water Hydrogen bonds per water molecule *versus* temperature for the SPC/E water model around the hydrophilic amino acids. The inset represents the H-bonds between water molecules for bulk system (open triangles) and protein hydrated system (filled triangles).

The densities averaged over all the hydrophilic and the hydrophobic patches are shown in the figure 5 (b).

The figure 5 (b) show that the average density at hydrophilic sites are higher than at the hydrophobic sites. In both cases at low temperatures the values are higher than the bulk value shown in figure 1 what is consistent with the confinement within heterogeneous plates [27]. It is important to point out that in our approach the distance between the contact layer and the protein is the same for both hydrophobic and hydrophobic sites. In many confined systems this distance is computed by the peak in the density that is different in both cases.

In order to understand these differences in the densities, the number of Hydrogen bonds of the molecules inside these patches were also computed. The figures 7 and 8 show the number of Hydrogen bonds between water molecules per SPC/E water molecule in the vicinity of protein. The averages over the bonds over the hydrophobic and the hydrophilic amino acids are illustrated in Figure 9.

In the vicinities of the hydrophobic and the hydrophilic sites the increase

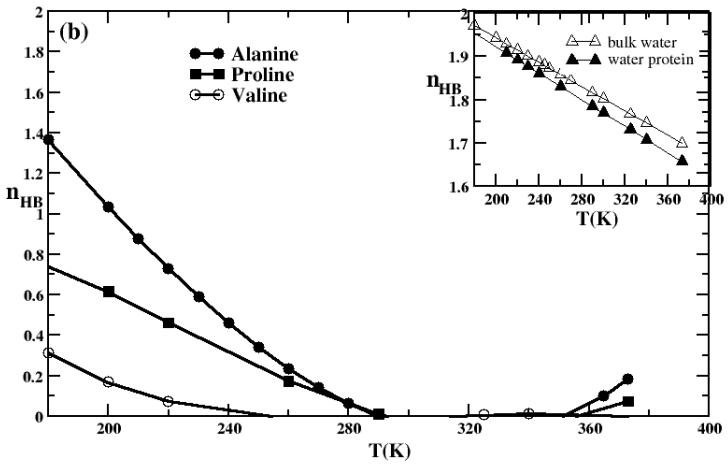

Figure 8: Number of water-water Hydrogen bonds per water molecule *versus* temperature for the SPC/E water model around the hydrophobic amino acids. The inset represents the H-bonds between water molecules for bulk system (open triangles) and protein hydrated system (filled triangles).

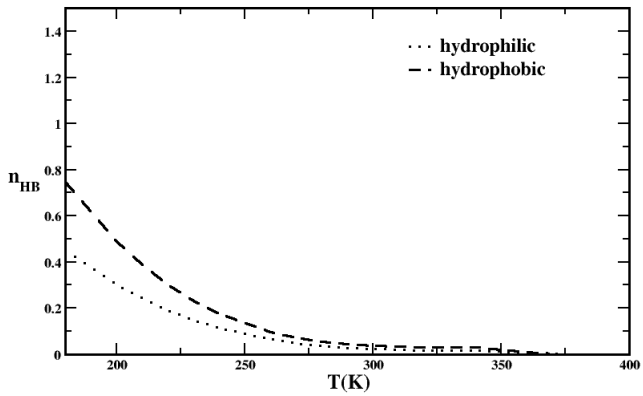

Figure 9: Average number of water-water Hydrogen bonds per water molecule *versus* temperature for the SPC/E water model averaged over the hydrophilic (dotted line) and the hydrophobic (dashed line) amino acids.

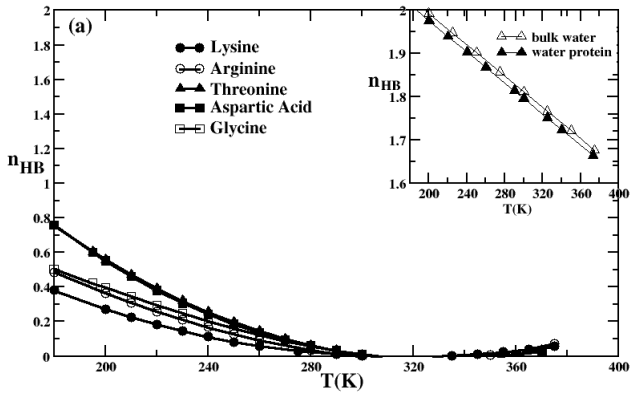

Figure 10: Number of water-water Hydrogen bonds per water molecule *versus* temperature for the TIP4P-2005 water model around the hydrophilic amino acids. The inset represents the H-bonds between water molecules for bulk system (open triangles) and protein hydrated system (filled triangles).

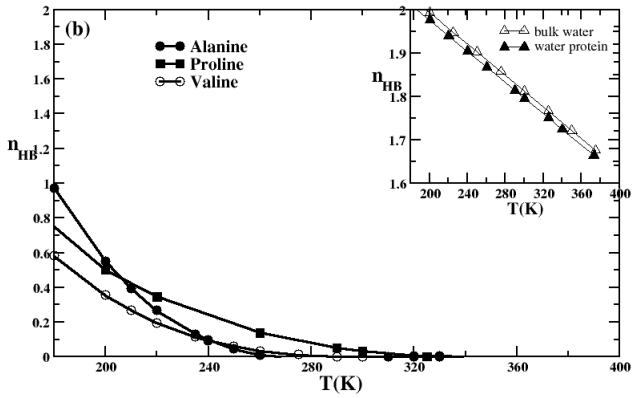

Figure 11: Number of water-water Hydrogen bonds per water molecule *versus* temperature for the TIP4P-2005 water model around the hydrophobic amino acids. The inset represents the H-bonds between water molecules for bulk system (open triangles) and protein hydrated system (filled triangles).

in the number of Hydrogen bonds occurs around $T = 240K$, the temperature

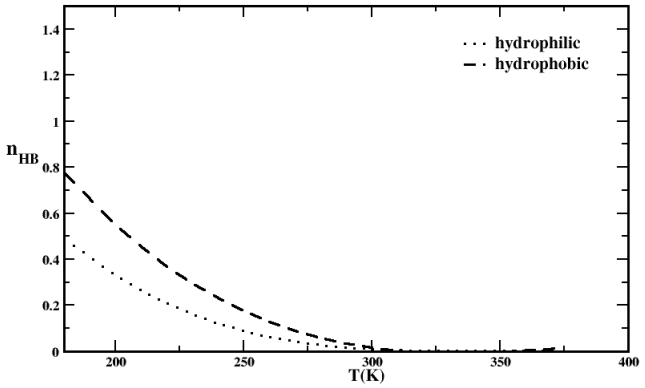

Figure 12: Average number of water-water Hydrogen bonds per water molecule *versus* temperature for the TIP4P-2005 water model averaged over the hydrophilic (dotted line) and the hydrophobic (dashed line) amino acids.

of the global maximum of density. The more pronounced increase in the number of H-bonds as temperature is decreased suggests that the system becomes more structured in the region close to the protein as the temperature is decreased. The very low number of H-bonds for T<300K occurs because at this temperature very few water molecules are at the protein surface as can be seen in figures 3 and 4.

Since the number of amino acids of each type is small, an averages over the number of hydrogen bonds of all the hydrophobic and all of the hydrophilic amino acids are illustrated in Figure 9.

The comparison of the figures 7 and 8 with the figure 1 (c) shows that the number of H-bonds per water molecule close to the protein is lower than this number for the bulk. This indicates that the water close to the protein is less ordered than in the bulk.

Figures 10 and 11 illustrate the number of H-bonds between water molecules per molecule, close to the hydrophilic and hydrophobic amino acids for the TIP4P-2005 water model. The averages of all the hydrophilic and all the hydrophobic amino acids are shown in the figure 12. The number of H-bonds for both cases have a more pronounced increases around $T < 275K$, where this is the TMD for the bulk system. The number of H-bonds per molecule at the protein surface is lower than the H-bonds per molecule for the bulk

system as show in figure 1 (d). The number of H-bonds close to hydrophobic sites is higher than close to hydrophilic segments. This indicates that water is more structured at the hydrophobic patches.

Figure 12 suggests that the water network is distorted locally at the vicinity of hydrophilic sites, breaking water-water H-bonds to form bonds with the protein while close to the the hydrophobic sites the network is more intact. This result is consistent with the high density of water at the protein surface [44].

The difference between the number of H-bonds in the vicinity of the different hydrophobic amino acids is relate to the different degrees of hydrophobicity and topology.

The number of water-water H-bonds close to the Alanine is particularly larger for the SPC/E water than for the TIP4P-2005. Since Alanine is a weakly hydrophobic amino acid, repelling less the water molecules, within the hydration shell, there are more space for water to make bonds. Since SPC/E water forms more packed clusters than the TIP4P-2005 water [45] more water-water H-bonds for the SPC/E water are formed.

4. Conclusions

In this paper we studied the density and the number of H-bond *versus* temperature of water in TS-Kappa protein system. For the bulk system, both SPC/E and TIP4P-2005 water models exhibit a density maximum in T= 250K and T= 280K, respectively. For the protein plus water systems the temperature of maximum density appears at lower values when compared lower with the temperature of the bulk system. The presence of the protein increases the entropy and consequently affects the competition between the bonded and non bonded water clusters. This temperature shift is not universal and might depend on the size and concentration of the protein [46].

The density *versus* temperature for the protein plus water system show higher values of density when compared with the bulk system. The i increase in the density can be explained because the TS Kappa has a majority of hydrophilic sites that attract water molecules.

Analyzing the behavior of water molecules near the protein surface, the anomalous behavior is no longer verified. In this region, as the temperature decreases the density of water increases. This surface water does not have the density anomaly present in the bulk water. At very low temperatures the density of water around the hydrophobic and hydrophilic sites is higher than

the density of bulk water. This suggests that even though the hydrophobic sites repeal water, the neighbor hydrophilic sites attract water. Then in order to preserve the H-bond network the density at the vicinity of hydrophobic sites increases.

5. Acknowledgment

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6. References

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