

STRUCTURE OF GAS-LIQUID INTERFACE AND HYDROPHOBIC INTERFACE
FOR UREA AQUEOUS SOLUTION SYSTEMS:
A COMPUTER SIMULATION STUDY

A Thesis

by

MENG YU

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2008

Major Subject: Chemistry

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Approved by:

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Robert R Lucchese
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ABSTRACT

Structure of Gas-liquid Interface and Hydrophobic Interface for Urea Aqueous Solution
Systems: A Computer Simulation Study. (May 2008)

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Chair of Advisory Committee: Dr. Yi-Qin Gao

Urea aqueous solution is ubiquitously used to denature protein. Regardless of its extensive use, the mechanism is still unclear and remains an active field of study. There have been two proposed mechanisms, the direct and indirect. The indirect mechanism, which attributes the ability of urea of changing water structure, is susceptible since many research works show that there is little effect of urea on water structure. The current study provided evidence for the indirect mechanism by demonstrating that the introduction of urea slightly changes the water structure in the hydrophobic interfacial areas.

In the current study, the urea aqueous solution systems with either gas-liquid or hydrophobic interface are studied by MD simulations, and the structures of water near the interfacial areas are analyzed in terms of density, orientation and number of hydrogen bonds. For each kind of interface, systems with four different urea concentrations are included, ranging from 0M to 8M. The results show slight change of water structure by the urea solute on the hydrophobic interface in terms of the orientation and number of hydrogen bonds per water molecule.

DEDICATION

To my parents

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I would like to thank my advisor, Dr. Yi-Qin Gao, for the enormous support he provided during my entire master's program. His comments and suggestions have gone a long way in improving this research.

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1. INTRODUCTION

1.1. Urea Molecule and Its Aqueous Solution: An Overview

Urea is an organic compound with the chemical formula $(\text{NH}_2)_2\text{CO}$ and the molecular weight of 60.06. The structure of urea molecule is shown in figure 1a. For each urea molecule, up to six hydrogen bonds may form (see figure 1b). Thus it is highly soluble in water due to extensive possible sites for hydrogen bonding. Also urea molecule is polar with a dipole moment of 4.56 D. Urea and its derivatives have ubiquitous application in many fields, such as the fields of resins, plastics, pharmaceuticals and fertilizers.¹

Urea is not only the first organic molecule to be synthesized,² but also considered the simplest molecule of biological interest. Due to the fact that urea aqueous solutions show a number of biological related properties, such as enhancing solubility of hydrocarbons,³ decreasing micelle formation,⁴ and the ability to denature proteins,⁵ there is persistent interest on the urea/water system. Particularly, urea aqueous solution has been the subject of contrasting opinions on several topics, including the mechanism by which the urea denatures protein and the effect of urea on the structure of water.

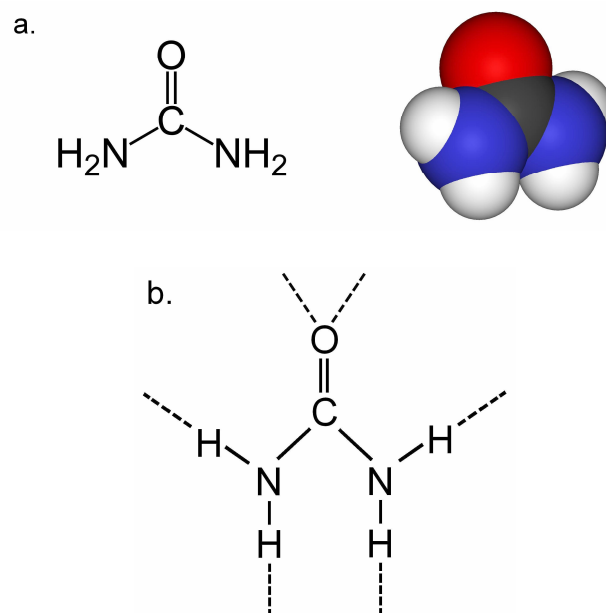


Figure 1. (a) The molecular structure of urea; (b) Possible hydrogen bonding sites of urea molecule.

1.2. Urea Induced Protein Denaturation

Aqueous urea solutions are widely used for protein denaturation.⁵ For example, in 8M urea solution, folded proteins become unstable and tend to have more expanded configurations, leading to the so-called chemical denaturation of proteins.

Due to its extensive use, there has been a persistent interest focusing on the molecular mechanism of protein denaturation induced by urea. In general, protein stability is determined by the balance between the intra-molecular interactions of protein functional groups and their interactions with the solvent environment. For folded proteins in aqueous solution, it is believed that a significant factor contributing to their stability is the hydrophobic interactions among non-polar groups.⁶

Extensive experimental studies have attempted to explain the mechanism of unfolding of proteins in aqueous urea solutions.⁷⁻¹⁷ Since the 1960s, there have been numerous research works focused on urea-induced protein denaturation, and information has been provided by both experiments and computer simulations. For instance, calorimetry,^{7,8} circular dichroism spectroscopy,^{9,10} neutron scattering,¹¹ NMR spectroscopy,¹² fluorescence measurements,¹³ the time resolved optical Kerr effect,¹⁴ IR and Raman spectroscopy,¹⁵⁻¹⁷ and molecular dynamics simulation studies.¹⁸⁻⁴⁷ In particular, there have been two often-used notions about the reason why urea denatures protein, the *direct* mechanism and the *indirect* mechanism.^{6,9,20,30} The former claims that it is the hydrogen bonding interactions with the peptide backbone that causes the protein denaturation, while the latter believes that urea-induced denaturation proceeds by altering the water structure, thereby reducing hydrophobic interactions. Moreover, a

number of recent studies also suggest a combination of direct and indirect effects.^{8,20,27,32}

1.3. The Effect of Urea on Water Structure

Although many recent studies agree on the idea that it is the combination of direct and indirect mechanisms leads to protein denaturation, the indirect mechanism is susceptible regarding to the effect of urea on water structure, and thus is the focus of the current study.

The effect of urea on water structure has been one of the most controversial issues about urea aqueous solution. In some studies,^{48,49} urea is believed to disrupt the natural water structure in aqueous solutions and termed as “structure breaker” or “chaotrope”. On the contrary, in some other studies,^{27,33} urea is found to enhance the water structure and thus termed as “structure maker” or “kosmotrope”. Both of the two opinions attribute the capability of urea of changing the water structure. However, there are also other studies which show no or only negligible influence of urea on the water structure.^{15,30,31,36,50}

On the basis of thermodynamic arguments, two different models have been proposed to describe the interaction between urea and water, and they still set the framework for ongoing discussions. Frank and Franks proposed the FF model in 1968,⁵¹ which focuses on the changes in the water network induced by urea. In the FF model it is assumed that the liquid water exists in equilibrium between two states, open or ordered versus dense or disordered, and that the hydrogen bonds between water molecules are destroyed by the introduction of the urea solute. The FF model is in agreement with the

findings that urea could be “structure breaker” which is stated earlier. However, there is another model, SKSS model, proposed earlier in the 1960s by Schellman,⁵² Kreshek and Scheraga,⁵³ and Stokes,⁵⁴ which holds the controversial opinion. The SKSS model attributes the properties of aqueous urea solutions to dimerized or oligomerized urea, and suggests that the water structure remains essentially unchanged. The SKSS model is consistent with the findings that urea has no or negligible effect on water structure. The results concerning the validity of the two models, obtained by application of different techniques, are controversial.

However, one must admit that the parameters that describe the water structure in urea aqueous solutions, for example, hydrogen bonding interactions, cannot be measured directly by experiments, but indirectly using some observable properties sensitive to these interactions. Thus the difficulty of interpreting experimental data is that the exact relation linking the observable properties to the actual structures is obtained with the use of given theoretical models based on various approximations.

1.4. Computer Simulation of Urea-Induced Protein Denaturation

According to the available information about the urea aqueous solution, it could be seen that indirect mechanism of urea-induced protein denaturation is challenged by the research works that find no or negligible influence of urea on water structure. Indeed, suggested by experimental data on urea bulk solutions, there is evidence that urea molecule has little-to-no effect on bulk water structure or the effect is not related to protein denaturation.^{55,56}

However, on the other hand, it has been well established that urea accumulates at the protein/solution interface.⁵⁷ Therefore, it is crucial to investigate the structure of interfaces of urea aqueous solutions.

Although there has been an increasing number of experiments dedicated to understanding urea denaturation,⁷⁻¹⁸ more theoretical analyses by computer simulations are needed to address this problem at the molecular level. So far, most computer simulation studies of urea solutions have focused on very small systems to study the radial distribution functions as a means to analyze the influence of urea on water structure. These simulations are helpful in solving the problems at hand; however, in light of the molecular complexity involved, they are probably not able to capture the whole picture. Moreover, it is the interfacial water structure rather than the bulk water structure in urea solution that is crucial towards understanding the urea-induced protein denaturation. As a result, the structure of interfaces in urea aqueous solution needs to be investigated in more detail.

In the current study, large systems of urea solutions with two kinds of interfaces are simulated, and several critical properties of interfacial urea molecules are analyzed. The purpose of the current study is to find out whether the introduction of urea changes the water structure at the interfaces by MD simulations.

2. MODEL AND METHOD

2.1 System Setup

A series of cubic slabs of urea-water solution are constructed and each slab is placed into a rectangular box extended in the z -direction (Figure 2) with three dimensional periodic boundary conditions. To study the interfacial activity of urea and water molecules in the solutions, two kinds of interfaces are constructed: one is gas (vacuum)-liquid interface (Figure 2a) and the other is hydrophobic interface (Figure 2b).

For the systems with air-urea solution interfaces, the empty spaces are vacuum, which serve as the gas phase. The urea solution is in contact with the gas phase on the z -direction, resulting in the formation of infinite slabs with the bulk region residing between two surfaces in the xy -plane. For the systems with hydrophobic interfaces, the alkane layer is composed of one hundred (10×10) $C_{24}H_{50}$ molecules, which are parallel to each other and are parallel to the z -axis. By adjusting the numbers of the urea and water molecules, urea concentration can be controlled. In the current study, the urea concentration ranges from zero, which means no urea molecule is included in the system other than water molecules, to a high concentration of 8M, with two intermediate concentrations being 2M and 4M. The systems simulated in the current study are much larger compared to the systems studied in previous works which contain several hundreds of molecules at most,¹⁸⁻⁴⁷ for example, the system of 8M urea solution with gas-liquid interface contains 900 urea molecules and 4629 water molecules. The sizes of the systems and the number of molecules they contain are listed in Table 1.

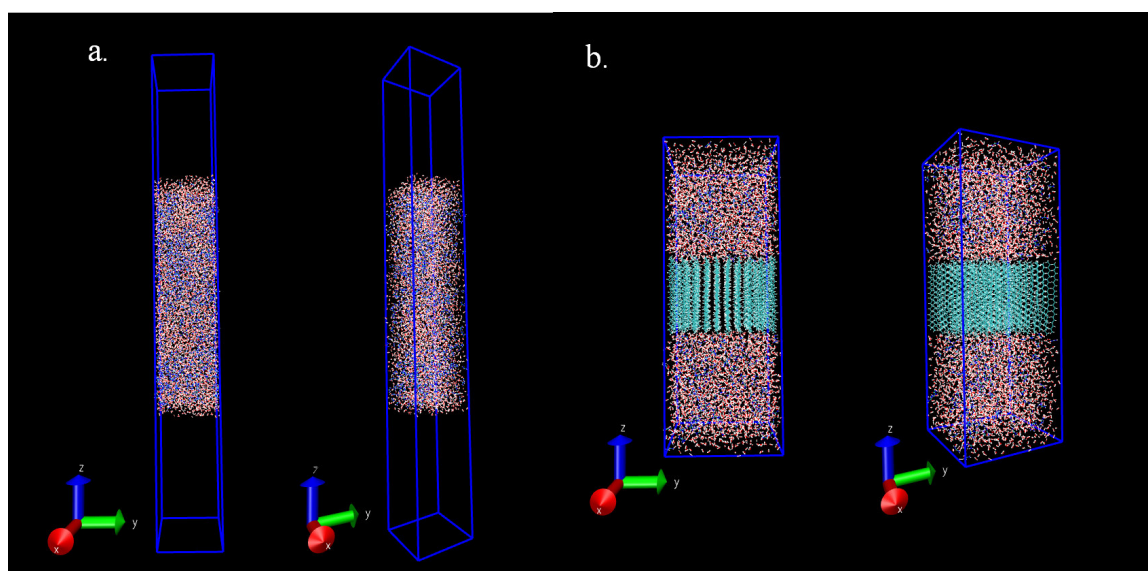


Figure 2. Typical systems constructed in the current study. (a) A system with gas (vacuum)-liquid interface. Oxygen atoms are depicted in red, nitrogen atoms in blue and hydrogen atoms in white. The empty spaces in the box are vacuum; (b) A system with hydrophobic interface. $C_{24}H_{50}$ molecules are depicted in cyan, and the hydrophobic layer formed by 100 $C_{24}H_{50}$ molecules lies in the middle of the water box.

TABLE 1: Setup of the simulation systems

Gas-liquid interface			
box size (\AA^3)	# water molecules	# urea molecules	urea concentration (300K, mol/L)
41.1 \times 40.6 \times 300.0	10214	0	0
39.4 \times 40.7 \times 300.0	6325	250	2
38.2 \times 40.3 \times 300.0	4767	400	4
37.1 \times 38.6 \times 300.0	4629	900	8
Hydrophobic interface			
box size (\AA^3)	# water molecules	# urea molecules	urea concentration (300K, mol/L)
40.9 \times 44.4 \times 123.3	5357	0	0
42.3 \times 44.4 \times 117.4	4859	192	2
44.0 \times 43.8 \times 122.4	4714	400	4
44.4 \times 44.7 \times 118.1	4005	800	8

2.2. Simulation Details

Molecular dynamics simulations were performed using the AMBER 9 package with ff99 force field. The TIP3P water model was used and the urea geometry was optimized in advance using B3LYP/cc-pVTZ and PCM in water solution. The particle mesh Ewald method was used to calculate the electrostatic energies and forces. The Van der Waals interactions and the real space part of the Ewald sum were truncated at 10\AA . A time step of 2 fs was employed, and the NH and OH vibrations were frozen using the SHAKE algorithm. All simulations were run at 300 K. In order to obtain statistically meaningful data, relatively long simulation trajectories are needed. For all simulations, the systems were run for 1 ns to be equilibrated and for 30 ns for data collections in which the volumes of the simulation boxes were kept constant.

2.3. System Parameters

The physical behaviors of water molecules at interface can be quantified in different ways. In current study, three parameters are used to determine the water structure, including density, orientation and number of hydrogen bonds (see below). All of the parameters are averaged over the time frames.

2.3.1. Density of Solution

In the current study, the density of the urea solution is characterized as a function of solution depth. The position of the urea molecule is represented by the z coordinate of the central carbon atom, while the position of the water molecule is represented by the z

coordinate of the oxygen atom. Particularly, for systems with hydrophobic interface, the density of carbon atoms in the hydrophobic layer is plotted as a function of depth too.

The position of the interface, at which the depth of the solution is defined as zero, is determined by the densities of the solution. For the systems with gas-liquid interface, the interface is defined as the position where the density of the urea solution is one tenth of its bulk density. For the systems with hydrophobic interface, the interface is defined at the position where the curves of solution density and carbon atom density intersects, that is, at where both the density of carbon atoms of the hydrophobic layer and the density of the urea solution are close to zero.

2.3.2. Orientation of Water Molecules

In order to determine the effects of urea on the change of water structure around the interfacial area, the orientations of water molecules are evaluated by the angles α , which is defined as the angle between one of the OH vectors of the water molecule and the z -axis. In the current study, the probabilities of water molecules are plotted with respect to $\cos(\alpha)$ and the depth of the solution.

2.3.3. Number of Hydrogen Bonds per Water Molecule

On the other hand, according to the assumption of the indirect mechanism of urea-induced protein denaturation, the hydrogen bonds at the interfacial regions are critical to the intra-protein hydrophobic interaction and thus need to be examined. In the current study, the cut off distance between donor and acceptor of a hydrogen bond is 3.5

Å, and the cut off angle of a hydrogen bond is 130° . The average number of hydrogen bonds formed per water molecule is calculated and plotted as a function of solution depth.

3. RESULTS AND DISCUSSION

3.1 Gas-liquid Interface

The densities of urea solutions with different concentrations near the gas-liquid interface are shown in figure 3. It can be seen that the densities of urea solutions, especially for urea solute, decrease slowly from bulk phase to interfacial area. For all the four different concentrations, the range of water density decreasing from the bulk value to 10% bulk value is about 5\AA , while for urea density, the range is about 10\AA - 15\AA .

The probability distributions of the water molecules in urea solutions with four different concentrations with respect to $\cos(\alpha)$ and depth are shown in figure 4. As described before, the angle α is the angle between one of the OH vectors of the water molecule and the z -axis. It can be seen immediately from these figures that, the color representing the probabilities of water molecules appearing in this region is even. This suggests that the orientations of water molecules in the bulk are random. At the interfacial area, however, the color is not even, indicating that the water molecules in this region are oriented. In the region near the interface and where the value of $\cos(\alpha) = 1.0$, the probability of water molecules is much higher than in the bulk solution, which indicates that there are more water molecules with angle α close to zero, that is, more water molecules tend to point one of its two hydrogen atoms towards the gas phase. Nevertheless, as the depth increases to be about 5\AA , there is another relatively high probability region at where $\cos(\alpha) = -1.0$, which means that there are more water molecules tend to point one of its two hydrogen atoms towards the bulk solution.

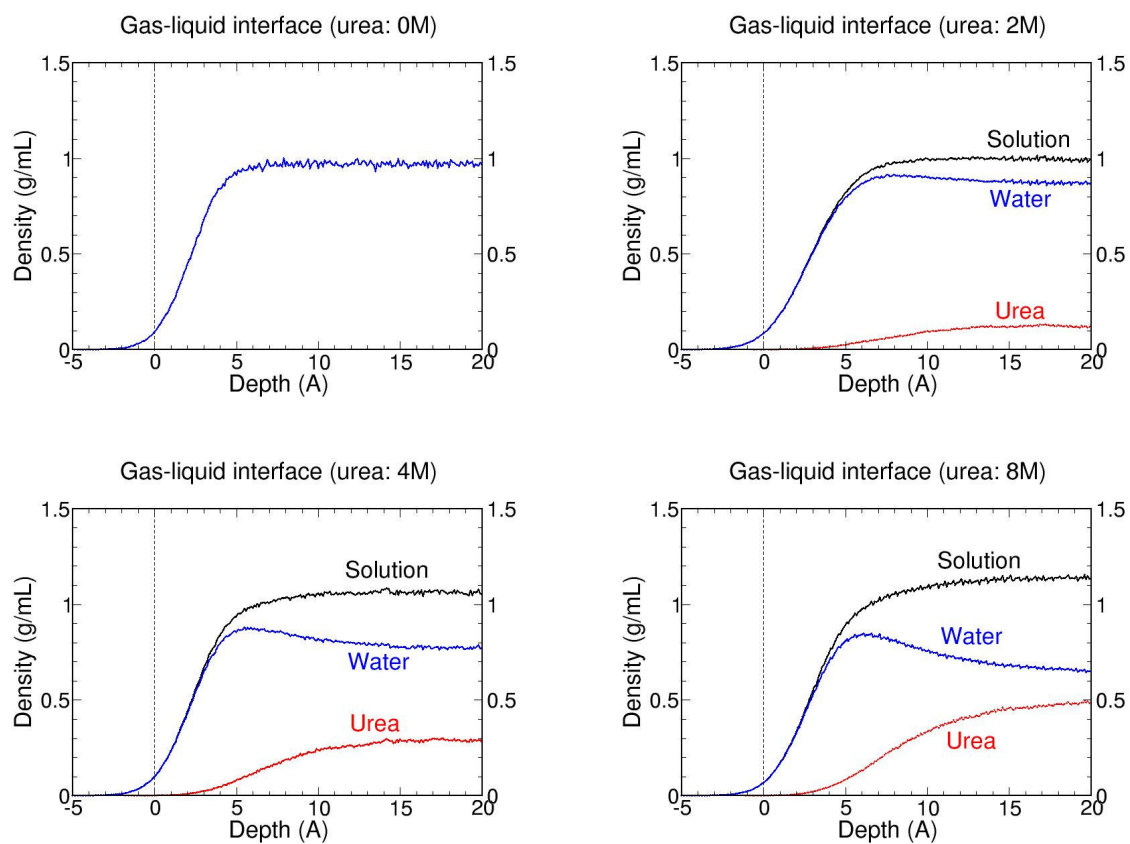


Figure 3. The densities of urea solutions with four different concentrations near the gas-liquid interface. The blue curve represents the density of water, the red curve represents the density of urea and the black curve represents the density of the solution. The dotted lines are drawn as the guide to the eyes.

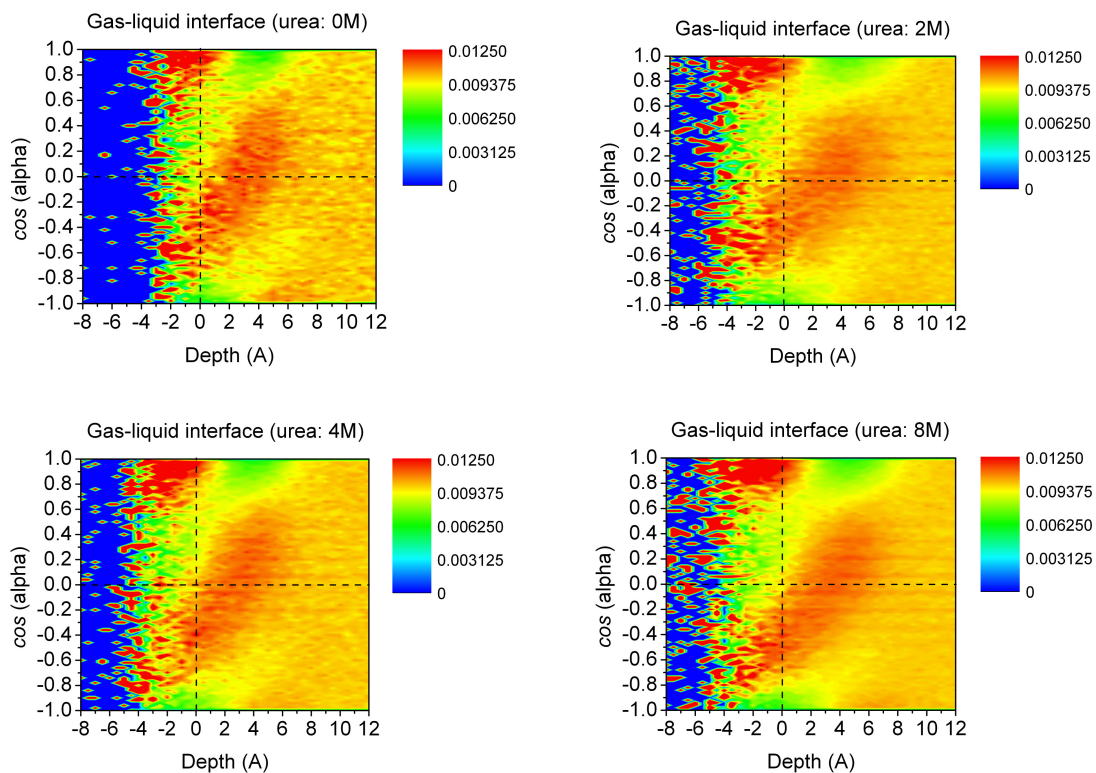


Figure 4. The probability distributions of the water molecules in urea solutions with four different concentrations with respect to $\cos(\alpha)$ and depth near the gas-liquid interface. The dotted lines are drawn as the guide to the eyes.

As a result, from the bulk to the interfacial area of urea solution, the orientation of water molecules change from randomly distributed configurations to relatively ordered configurations. However, judging from the shapes of the four figures, it can be seen that the probability distributions of water molecules are very similar no matter what the concentration of urea is. Thus, it can be concluded that the introduction of urea solute to water has no or negligible influence on the orientation of water molecules.

The average number of hydrogen bonds per water molecule is also calculated and plotted as a function of depth in figure 5. For all the four concentrations, the total numbers of hydrogen bonds per water molecule in the bulk solution are the same, which is about 3.6, and is very close to the experimental result obtained at 300K and 1atm, which is 3.58.⁵⁸ At the presence of the urea solute, although the contribution from other water molecules decreases, the number of hydrogen bonds per water molecule contributed from the urea increases as the urea concentration increases. Therefore, the net effect is that the total number of hydrogen bonds per water molecule remains the same as in pure water. When approaching the interface, the number of hydrogen bonds per water molecule decreases since the density of molecules decreases.

However, as is shown in figure 6, near the gas-liquid interface, the shapes of the curves of the total number of hydrogen bonds per water molecule for different urea solutions are almost identical, which suggests that the introduction of urea solute in water has no or negligible influence on the averaged number of hydrogen bonds per water molecule.

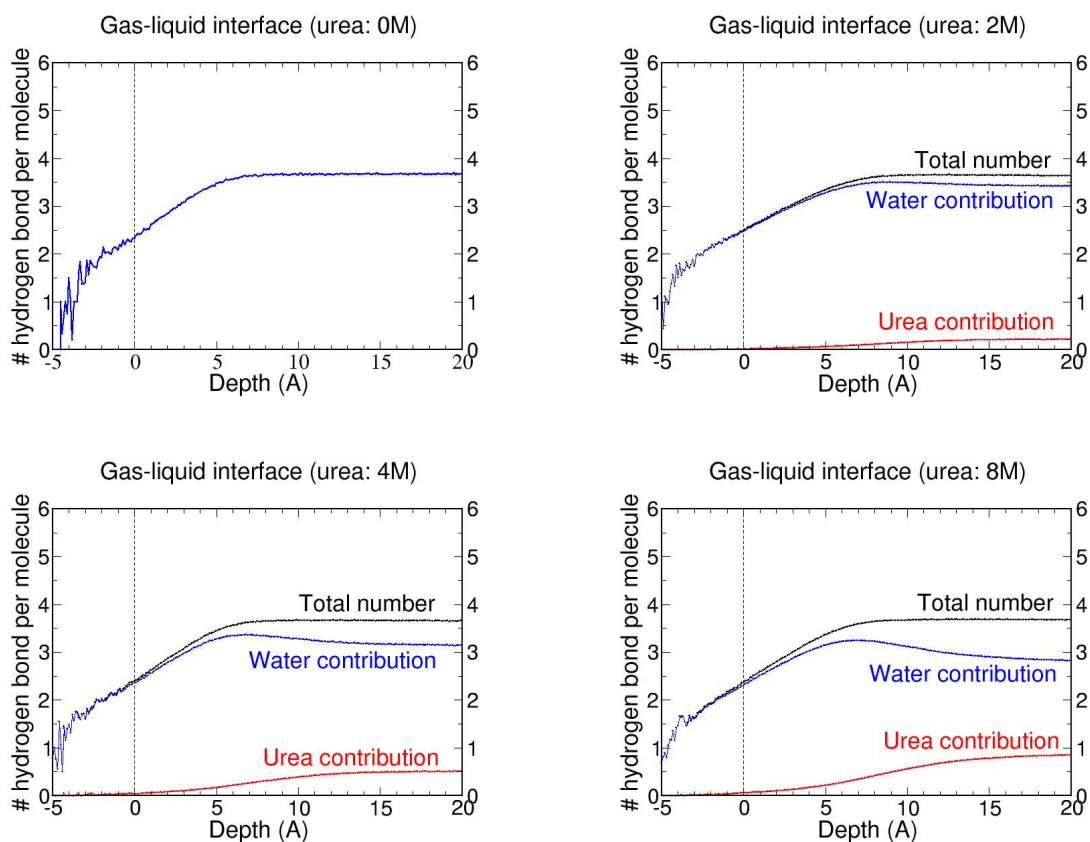


Figure 5. The average number of hydrogen bonds per water molecule as a function of depth in urea solutions with four different concentrations near the gas-liquid interface. The blue curve represent the number of hydrogen bond contributed by other water molecules, the red curve represent the number of hydrogen bond contributed by urea molecules and the black curve represent the total averaged number of hydrogen bond per water molecule. The dotted lines are drawn as the guide to the eyes.

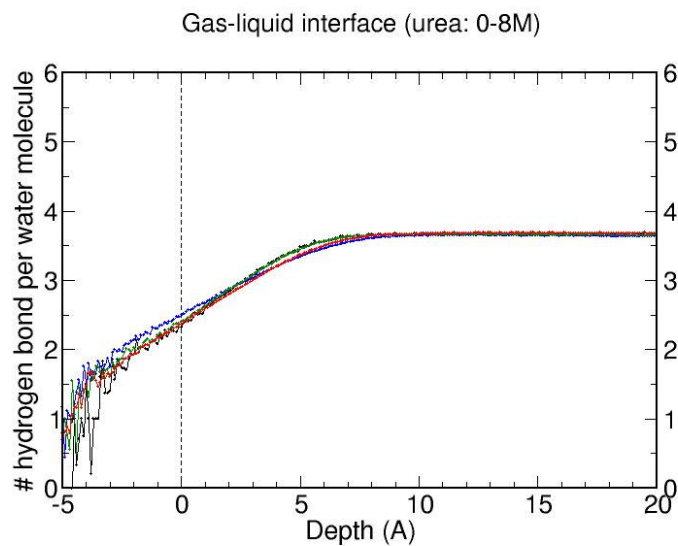


Figure 6. Comparison of the total number of hydrogen bonds per water molecule in urea solutions with four different concentrations near the gas-liquid interface. The black curve represents the total number of hydrogen bonds per water molecule in 0M urea solution, the blue curve represents that in 2M urea solution, the green curve represents that in 4M urea solution and the red curve represents that in 8M urea solution.

In summary, on the gas-liquid interface, the densities of water and urea near the gas-liquid interface decreases very slowly, especially for urea. Additionally, urea has no or negligible influence on the orientation of water molecules, as well as the average number of hydrogen bonds of water molecules.

3.2 Hydrophobic Interface

The densities of urea solution with different concentrations near the hydrophobic interface, as well as the densities of carbon atoms in the hydrophobic layer, are shown in figure 7. Unlike the gas-liquid interface, the densities of both the urea and water change drastically near the hydrophobic interface. Moreover, the wetting effect is observed for the small head groups ($-\text{CH}_3$ groups) of the hydrophobic layer, since there are peaks of water density beneath the interface (Figure 7), indicating that the water molecules form several relatively ordered layer-like structure near the head groups. The first water density peak appears at the depth of about 2\AA , followed by the second one at about 5\AA . Meanwhile, urea molecules show similar layer-like structure near the hydrophobic interface, and the first and second urea density peaks also appear at the depth of about 2\AA and 5\AA , respectively. Compared with the solution density near the gas-liquid interface, urea molecules tend to accumulate near the hydrophobic surface.

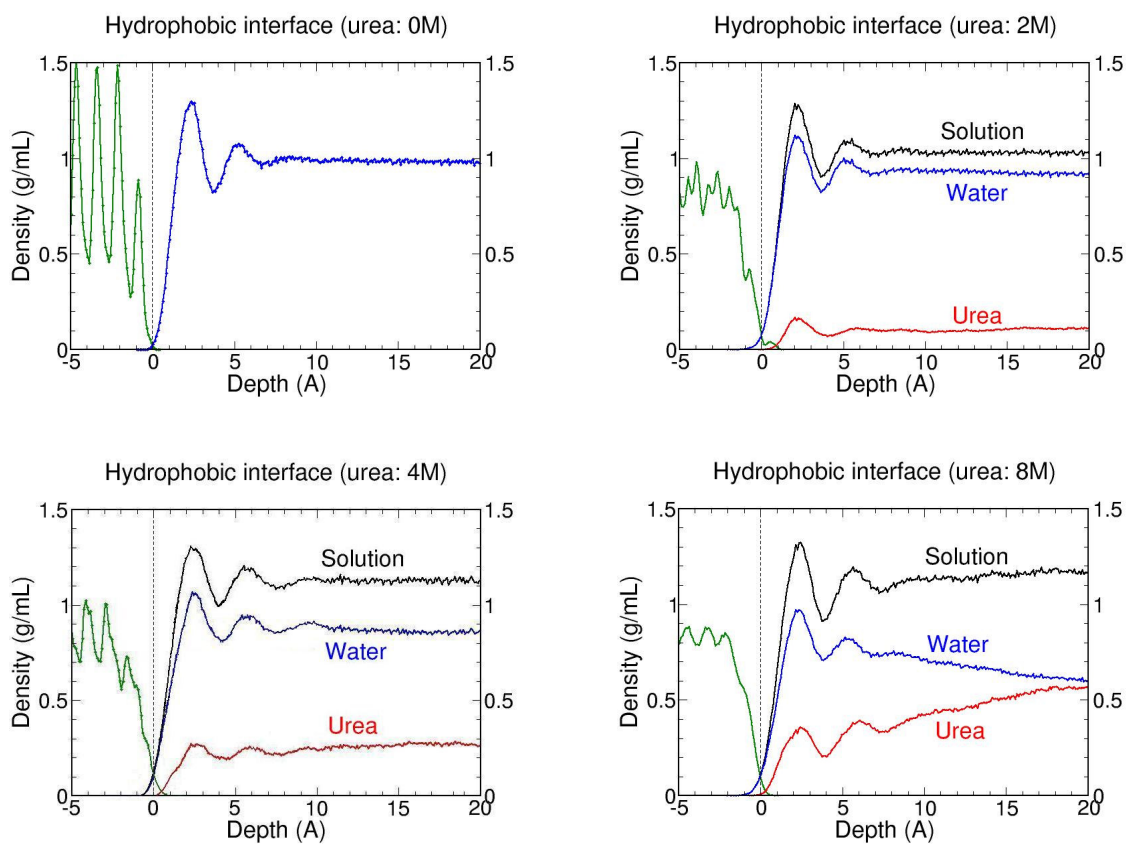


Figure 7. The densities of urea solutions with four different concentrations near the hydrophobic interface. The blue curve represents the density of water, the red curve represents the density of urea, the black curve represents the density of the solution and the green curve represents the density of the carbon atoms in the hydrophobic layer. The dotted lines are drawn as the guide to the eyes.

On the other hand, the densities of carbon atoms in the hydrophobic layer in different urea solutions are obviously different. In the pure water system, the peaks of carbon atom density in the hydrophobic layer are sharp, suggesting that the carbon atoms in the layers are ordered, and those alkane chains are lined up nicely. This ordered structure of the alkane chains can also be seen in the simulation snapshot (Figure 8.a.).

From this simulation snapshot, it can be seen clearly that the hydrophobic layer is compact, and all the alkane chains are parallel to the z -axis. However, the peaks of carbon atom density of the hydrophobic layer become lower in the urea solutions. This is due to the fact that the alkane chains in the hydrophobic layer are tilted (Figure 8.b.) so that the carbon atoms are aligned with less ordered configurations. This tilted configuration of alkane chains leads to a larger area of the hydrophobic surface, as shown in table 2. From table 2, it can be seen clearly that, the surface area of the hydrophobic interface increases with the increase of the urea concentration. Since all hydrophobic layers are consisted of same number of alkane chains, and all the simulations are started with initial configuration with the same hydrophobic surface area and are equilibrated before data collection, it can be assumed that the increase of the hydrophobic surface area is induced by the increase of the urea concentration in the solution. This suggests that urea increases the chance for the hydrophobic layer to contact water, that is, urea weakens the hydrophobic interactions between the alkane chains, which is in agreement with the experimental results.³

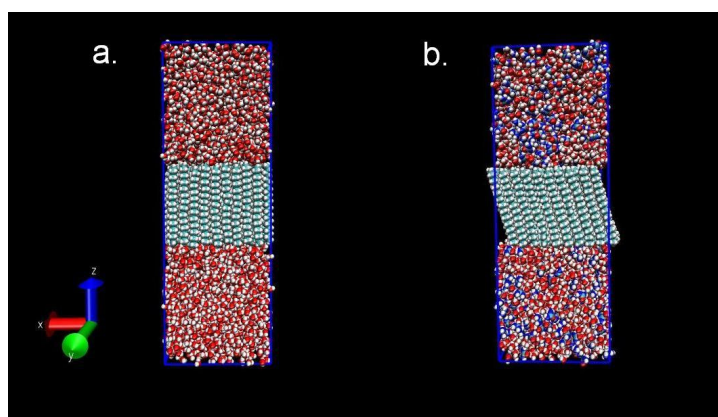


Figure 8. Simulation snapshots: (a) in pure water system; (b) in 8M urea solution system. Oxygen atoms are depicted in red, nitrogen atoms in blue, carbon atoms in cyan and hydrogen atoms in white. The hydrophobic layer formed by 100 C₂₄H₅₀ molecules lies in the middle of the water box.

TABLE 2: Surface areas of hydrophobic layers in urea solutions with different concentrations

urea concentration (mol/L)	X (Å)	Y (Å)	Surface area (Å ²)	Area increase (%)
0	40.97	44.44	1820.85	0
2	42.31	44.39	1878.17	3.05
4	44.00	43.78	1926.43	5.80
8	44.38	44.71	1984.06	8.96

The probability distributions of the water molecules in urea solutions with four different concentrations with respect to $\cos(\alpha)$ and depth are shown in figure 9. Near the hydrophobic interface, the water molecules form relatively ordered structures with similar pattern to the gas-liquid interface. As the same as near the gas-liquid interface, the color representing the probabilities of water molecules appearing in the bulk region is even, suggesting that the orientations of water molecules in the bulk are random. At the interfacial area, the water molecules are oriented. First, there is a layer near the interface where more water molecules tend to point one of its hydrogen atoms towards the gas phase, followed by a layer beneath the interface with more water molecules pointing one of its hydrogen atoms towards the bulk solution. As a result, the orientation of water molecules change from randomly distributed configurations in the bulk solution to relatively ordered configurations near the interface.

Meanwhile, it can be noticed that the width of the two ordered layers are broadened with the increase of urea concentration. Additionally, the space that is available for water molecules to reach near the hydrophobic layer increases as well, which suggests that it is easier for water molecules to get close to the hydrophobic layer. This is consistent with the result that the hydrophobicity of the alkane chain layer is decreased by the introduction of the urea molecules, so that water molecules are able to influx close to the hydrophobic layer.

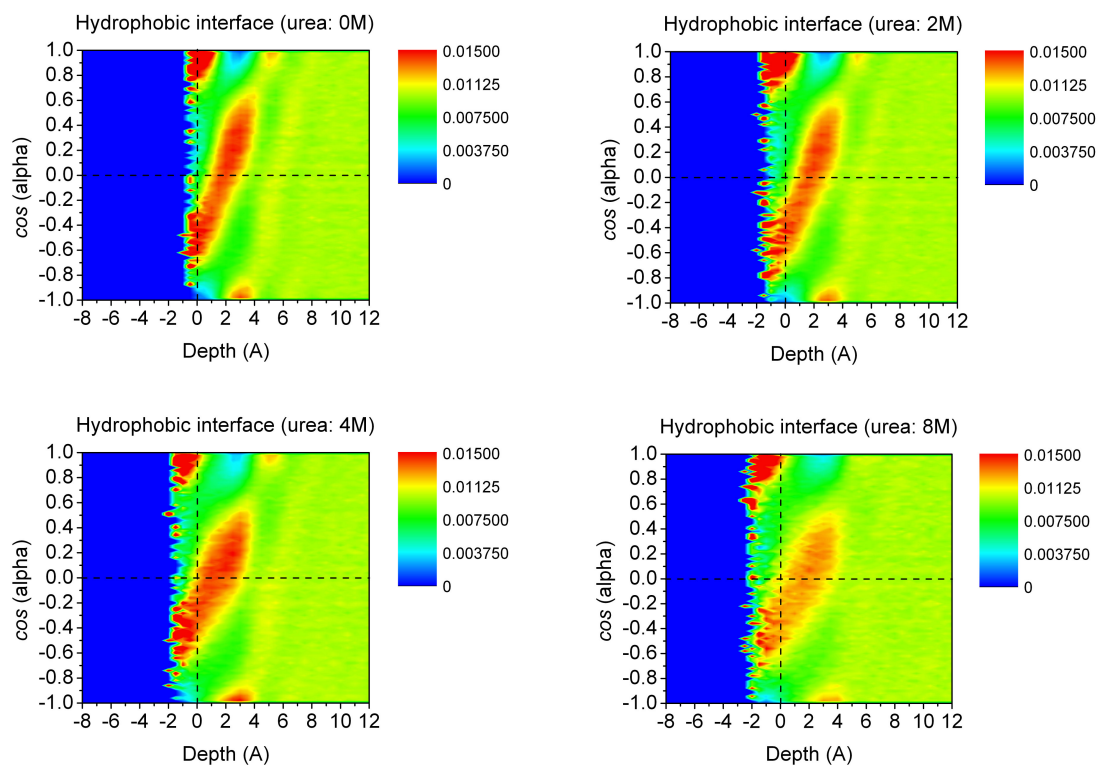


Figure 9. The probability distributions of the water molecules in urea solutions with four different concentrations with respect to $\cos(\alpha)$ and depth near the hydrophobic interface. The dotted lines are drawn as the guide to the eyes.

The average number of hydrogen bonds per water molecule near the hydrophobic interface is plotted versus the depth in figure 10. In the bulk solution, very similar to the gas-liquid interface, the total number of hydrogen bonds per water molecule is about 3.6 and is the same for all the four concentrations; however, near the hydrophobic interface, this number is different for different urea solutions (Figure 11). As the urea concentration increases, the number of hydrogen bonds per water molecule decreases slower at the interfacial area, which indicates that water molecules have more chance to form hydrogen bonds near the hydrophobic interface under high urea concentrations. This suggests that in the solutions with higher urea concentration, there are more water and urea molecules that are able to come closer to the hydrophobic interface, so that they are able to form more hydrogen bonds with others.

In summary, compared with gas-liquid interface, the densities of both urea and water change more rapidly in the hydrophobic interfacial area. Urea molecules tend to accumulate on the hydrophobic interface, which is not observed in the case of gas-liquid interface. The hydrophobicity of the alkane chains in the hydrophobic layer is weakened by the introduction of urea. Moreover, the introduction of urea changes slightly the orientations of water molecules and the average number of hydrogen bonds per water molecule in the hydrophobic interfacial area, which suggests that urea may have slight influences on the water structure near the hydrophobic interfaces.

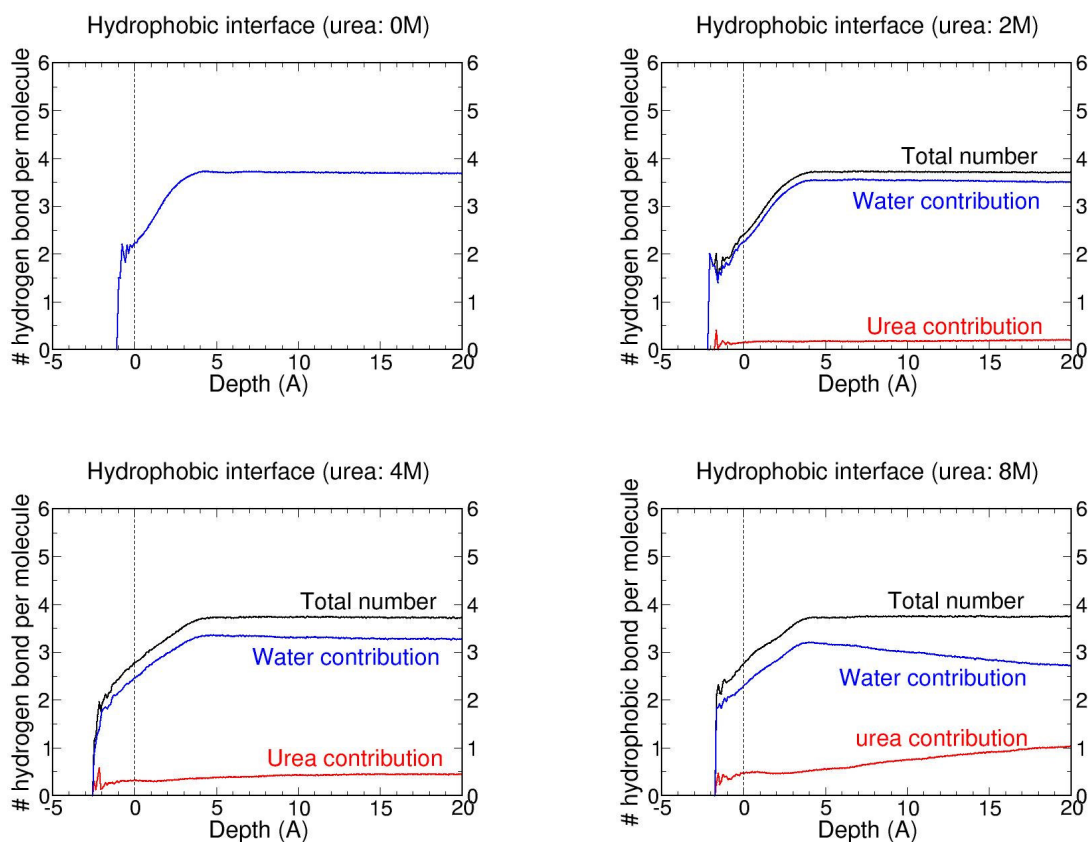


Figure 10. The average number of hydrogen bonds per water molecule as a function of depth in urea solutions with four different concentrations near the hydrophobic interface. The blue curve represent the number of hydrogen bond contributed by other water molecules, the red curve represent the number of hydrogen bond contributed by urea molecules and the black curve represent the total averaged number of hydrogen bond per water molecule. The dotted lines are drawn as the guide to the eyes.

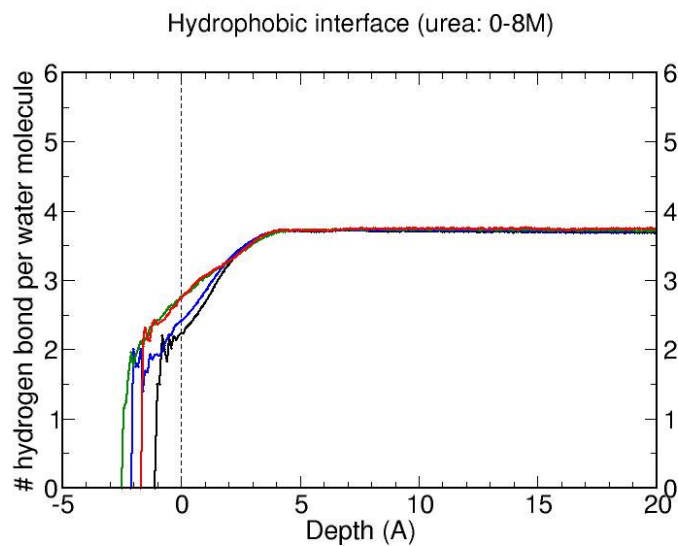


Figure 11. Comparison of the total number of hydrogen bonds per water molecule in urea solutions with four different concentrations near the hydrophobic interface. The black curve represents the total number of hydrogen bonds per water molecule in 0M urea solution, the blue curve represents that in 2M urea solution, the green curve represents that in 4M urea solution and the red curve represents that in 8M urea solution.

4. CONCLUSION

According to the results obtained in the current work, on the gas-liquid interface, the densities of water and urea decrease very slowly, especially urea. While on the hydrophobic interface, the densities of both urea and water change more rapidly, and urea molecules tend to accumulate on the hydrophobic interface, which is not observed in the case of gas-liquid interface. Additionally, the hydrophobicity of the alkane chains in the hydrophobic layer is weakened by the introduction of urea. Urea has no or negligible influence on the orientation of water and number of hydrogen bonds of water on the gas-liquid interface, but on the hydrophobic interface, the introduction of urea slightly changes the orientations of water molecules and the average number of hydrogen bonds per water molecule. The current work provides evidences that support the notion that urea are capable of changing interfacial water structure around the hydrophobic layer, in terms of water molecule orientation and number of hydrogen bonds per water molecule.

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