

## Towards an understanding of the molecular basis of hydrophobicity\*

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### Summary

The modern view is stressed that the structuring of water around nonpolar solutes, a process called hydrophobic hydration, actually favors the solubility of nonpolar solutes in water, its associated positive free energy of transfer arising from the enthalpic input required to create a cavity in water to accommodate the solute. The results of a series of molecular dynamics simulations of methane in SPC/E water at different temperatures are reported. These results show the existence of a larger fraction of broken hydrogen bonds in the hydration-shell water of the nonpolar solutes with respect to the bulk water, the difference increasing with a rise in temperature. This supports Muller's modified hydration-shell hydrogen-bond model predictions, where hydration-shell water molecules have lower free energies of hydrogen-bond breaking than those in the bulk.

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### Introduction

Hydrophobic effects make an important contribution to many fundamental processes in biology and chemistry, like the folding and stability of proteins, the conformation and association equilibria of small molecules in water, the formation of micelles and biomembranes, the low solubility of hydrocarbons and inert gases in water, and the recognition processes involved in the binding of substrates or effectors to the active sites or receptors on the surface of proteins.

The simplest case of hydrophobicity lies in the low solubility of nonpolar molecules, where the associated large increase in the standard Gibbs free energy of transfer ( $\Delta G_{tr}^\circ$ ) to water results from the small decrease in the standard enthalpy of transfer ( $\Delta H_{tr}^\circ$ ) and the large decrease in the standard entropy of transfer ( $\Delta S_{tr}^\circ$ ) near room temperature, along with a negative volume of solution and an increase in the heat capacity of solution ( $\Delta C_p^\circ$ ) [1–18]. For many nonpolar solutes, the solubility *decreases* when the temperature is raised near 25 °C, often passing through a minimum not far above room temperature [1–18] where  $\Delta H_{tr}^\circ$  goes to zero and gradually takes increasing positive values as the temperature continues to rise.

The structural theories [1–3,8,17,19] proposed to explain these observations suggest that water molecules in the hydration shell of a nonpolar solute must be capable of rearranging themselves in such a way as to maintain the maximum possible number of hydrogen bonds. This results in a restriction of their orientational freedom [19] because the solute is not able to hydrogen-bond with the water molecules, making certain orientations of the hydration-shell water molecules energetically unfavorable. This results in a negative contribution to  $\Delta S_{tr}^\circ$ . The ordering has also been associated with slowed-down dynamics [20–24].

Hydrophobic interactions [1–3] come from solvent-induced forces that drive nonpolar solutes or groups together to a greater extent when placed in water than if they were dissolved in a nonpolar solvent. The direct attraction between nonpolar solutes has only a small contribution to the hydrophobic interaction, which has been traditionally viewed as arising primarily from the negative  $\Delta S_{tr}^\circ$  of the solutes. The entropy change on bringing two or more nonpolar solutes together should be positive, and can be pictured qualitatively as a process that decreases the  $\Delta G_{tr}^\circ$  of the separate solutes and thus favors aggregation, because the total ordering of the water molecules is

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greatest when the solutes are apart [1–3]. This interaction is expected to increase with the rise of temperature near room temperature, as observed recently in some simulations [25,26]. In a similar way, the stability of proteins [12,13] has been traditionally explained in terms of the ordering of water by nonpolar residues in the protein backbone, so that a compact state of a protein should be favored so as to minimize the unfavorable entropy decrease resulting from the hydration of these groups.

Modern views of hydrophobicity consider that the real cause of the insolubility of nonpolar solutes at ordinary temperatures is the contribution to the  $\Delta H_{tr}^\circ$  coming out of the cohesive energy of water. Because of enthalpy–entropy compensation, the effects of the superimposed hydrophobic hydration (the ‘structuring’ of the water molecules, which has both entropic *and* enthalpic contributions) have a small but negative contribution to the  $\Delta G_{tr}^\circ$ . Consequently, this ‘structuring’ of water actually *enhances* the room-temperature solubility of nonpolar solutes, and the low solubility of nonpolar solutes in water comes from the large positive *enthalpy* of breaking hydrogen bonds to create a cavity in water in order to accommodate the solute, as has been suggested by several authors [6,7,9–13,18,27]. The positive contribution to the  $\Delta H_{tr}^\circ$  and  $\Delta G_{tr}^\circ$  (arising from differences in the solute–solute, solute–water, and water–water interactions) is the real origin of hydrophobicity, rather than the negative-order-increase thermodynamic contributions that actually stabilize the solutions. In other words, there are two opposing contributions to the  $\Delta G_{tr}^\circ$ : that arising from the structuring of water, consisting of both a negative entropy and a negative enthalpy and resulting in a small negative free energy, and that arising from the large (temperature-independent) energy required to create a cavity in the water to accommodate the solute. This contrasts with the traditional interpretation that, owing to a lack of enthalpy–entropy of solution compensation, the structuring of water leads to a positive  $\Delta G_{tr}^\circ$ . As has just been explained, enthalpy–entropy compensation does in fact occur between the enthalpy and entropy of the structuring of water, producing a relatively small negative contribution to the free energy. The real subjacent cause of hydrophobic effects resides then mainly in the large cohesive forces among water molecules due to their extensive hydrogen-bonding connectivity, with other factors contributing as well, i.e., the differences in van der Waals forces between the solute and water molecules and the small size of water molecules, since a large number of hydrogen bonds have to be broken in order to accommodate the solute [7]. On the other hand, the temperature dependence of the hydrophobic effect remains almost entirely determined by the water-structuring contributions. As the temperature decreases, the increasing importance of the water-structuring process actually enhances the dissolution of larger quantities of solute in water [6,7].

Hydrophobic interaction should then be considered not to arise, as stated before, from the positive entropy of order increase when nonpolar solutes aggregate (since less water molecules solvate the interacting solutes), but from the cohesive energy of water (an enthalpic effect), the process of hydrophobic hydration or water structuring actually favoring the separate solvation of the nonpolar solutes. An implication to protein science is that the stabilization of the compact states of proteins, which traditionally have been explained as a result of hydrophobic interactions, is primarily due to the direct van der Waals attraction between nonpolar residues (and also of intra-protein hydrogen bonds), and the contribution of water structuring of these groups actually destabilizes the compact states and favors the solvation of extended states. This destabilizing effect of water structuring increases as the temperature decreases, and at low temperatures promotes unfolding of the compact structure of the protein, a process called cold denaturation [12,13]. For the area of molecular recognition, this implies that the process of desolvating the hydrophobic groups in the receptor site and the ligand before interaction is not favored in terms of water structuring, although of course the net overall effect after binding is a large increase in entropy and thus a favorable negative contribution to the free energy of interaction.

Muller [4,5] has developed a model to explain the non-zero values of the heat capacity of solution at high temperatures, and the change of nonpolar solutes from structure-makers to structure-breakers as temperature increases, as revealed by proton chemical shifts. In this model, the heat capacity changes can be achieved either by making the hydrogen bonds enthalpically stronger or by increasing the fraction of broken bonds by reducing the bond-breaking free energy. The proton chemical shifts can be explained because the two previous contributions oppose each other with regard to what the experimental probe is measuring (concentration of stronger hydrogen bonds). The calculations by Muller of the fractions of broken hydrogen bonds in the hydration shell around a nonpolar solute and in the bulk showed that both behave nearly linearly below 100 °C with respect to temperature increase, and that the fraction of broken hydrogen bonds is always larger in the hydration shell than in the bulk, the difference increasing steadily with the rise of temperature.

In previous work [28], a large series of molecular dynamics simulations were performed to contrast the behavior of hydrogen bonds in aqueous solutions of nonpolar solutes with the predictions of Muller’s model, and good agreement was found with the temperature trends predicted. In the present paper, similar results using a different potential for water are reported, showing that the intermolecular potential for water has little effect on the trends previously found. It can be concluded that Muller’s model of reduced bond-breaking free energies in the hy-

TABLE 1  
SUMMARY OF SIMULATIONS

T (K)	$T_E/T_R$ (ps)	$\rho$ (g cm <sup>-3</sup> )	U (kJ mol <sup>-1</sup> )	P (MPa)	$D_s$ (10 <sup>-9</sup> m <sup>2</sup> s <sup>-1</sup> )	$D_w$ (10 <sup>-9</sup> m <sup>2</sup> s <sup>-1</sup> )	$D_{whs}$ (10 <sup>-9</sup> m <sup>2</sup> s <sup>-1</sup> )	$D_{wb}$ (10 <sup>-9</sup> m <sup>2</sup> s <sup>-1</sup> )
280.9 ± 0.4	80/100	1.00	-11409 ± 3	-2 ± 4	1.64 ± 0.5	1.72 ± 0.1	1.67 ± 0.2	1.72 ± 0.1
296.3 ± 0.4	70/100	0.99	-11154 ± 2	-12 ± 3	1.23 ± 0.5	2.77 ± 0.1	2.77 ± 0.2	2.77 ± 0.1
322.2 ± 0.4	60/100	0.98	-10750 ± 3	3 ± 3	3.17 ± 0.5	3.61 ± 0.1	3.27 ± 0.2	3.64 ± 0.1
359.4 ± 0.3	50/100	0.97	-10238 ± 2	43 ± 3	5.15 ± 0.5	6.19 ± 0.1	6.05 ± 0.2	6.20 ± 0.1

T = temperature,  $T_E$  = equilibration time,  $T_R$  = run time,  $\rho$  = density, U = average potential energy, P = internal pressure,  $D_s$  = methane diffusion coefficient,  $D_w$  = water diffusion coefficient,  $D_{whs}$  = hydration-shell water diffusion coefficient, and  $D_{wb}$  = bulk water diffusion coefficient.

dration-shell water molecules around nonpolar solutes is confirmed in molecular dynamics simulations, which is a step forward to understanding the molecular basis of hydrophobicity.

### Computational Method

A series of constant-volume (N,V,E) molecular dynamics simulations were done using the program *Moldy* [29], with the same methods as described before [21,28]. Cubic periodic boundary conditions were used throughout, and a time step of 0.5 fs was employed, except for the simulation done at around 360 K, where a time step of 0.25 fs was used. A real-space cutoff of 10 Å was applied to short-range interactions, with long-range corrections for longer distances. Long-range electrostatic interactions were treated using the Ewald sum method. The intermolecular potentials chosen were the three-site SPC/E model of water [30] and the OPLS model of methane [31]. The data generated during the simulations were stored every 10 fs. A different density was used for each temperature, after an approximate estimate of its value was obtained

from (N,P,E) simulations. The simulations consisted of one methane molecule in a box with 256 water molecules. Temperature scaling was done for each simulation during 10 ps. Further periods of between 50 and 80 ps were allowed for equilibration, after which data collection was done for an additional period of 100 ps in all simulations.

### Results and Discussion

A summary of the simulations and thermodynamic data can be found in Table 1. Self-diffusion coefficients were calculated as described before from plots of the mean square displacement against time [21,25,28]. As the temperature increases, the solute and water molecules become more mobile; however, we find that at 296 K the diffusion coefficient of the solute actually drops. This sort of behavior has also been found in the past [21,25,26,28], where the diffusion of the solute(s) decreased at those temperatures where a higher hydrophobic interaction was observed. Further work is being done to investigate this phenomenon. The diffusion coefficients of water in the

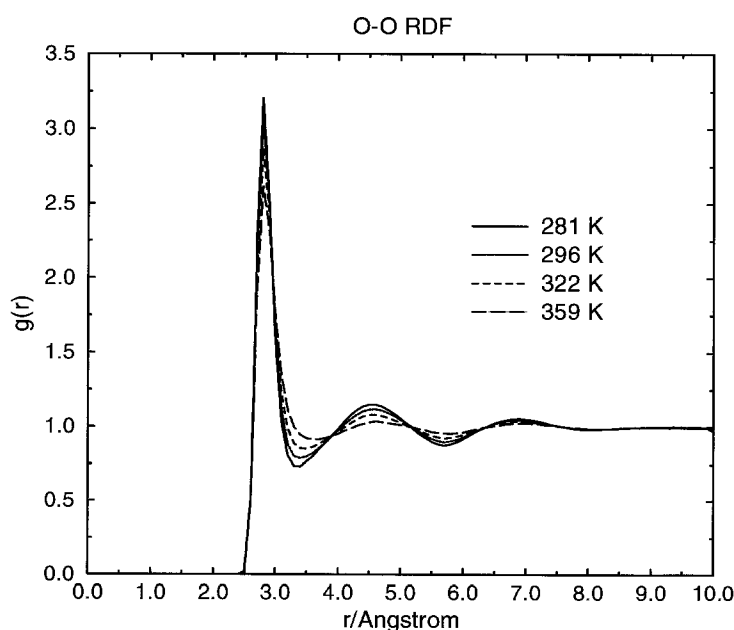


Fig. 1. Oxygen-oxygen radial distribution functions, showing diminishing water structure as the temperature increases.

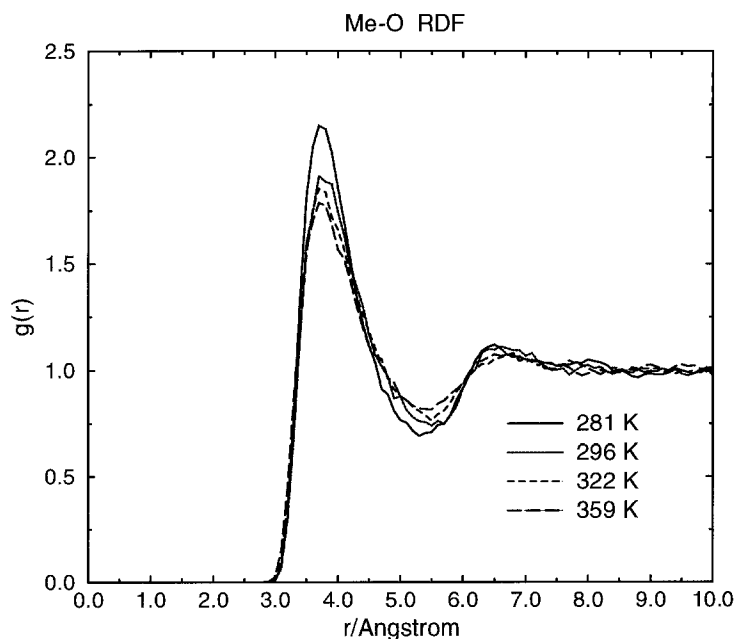


Fig. 2. Methane–oxygen radial distribution functions, showing the hydration structure around the methane solute at different temperatures.

hydration shell of methane are always smaller than those for water in the bulk region, confirming previous findings of slowed-down dynamics in the vicinity of a nonpolar solute [20–24].

In Fig. 1 the oxygen–oxygen (O–O) radial distribution functions (RDF, or  $g(r)$ ) are reported for all temperatures simulated. It can be seen that as the temperature increases, the structure of water diminishes; this is revealed by the broadening of the peaks. Figures 2 and 3 show the average methane–oxygen (Me–O) and methane–hydrogen (Me–H) structures. The positions of the peaks in the Me–

O and Me–H RDF can be attributed to the existence of a first hydration shell of water molecules with dipole vectors that are nearly tangential to the surface of the solute, as opposed to the situation in the second hydration shell, where a rather radial orientation is observed. This structure has been found consistently in the past [18–26,28], and has been justified as the geometrical disposition allowing for hydrogen bonds to be maintained more easily in the hydration shell, similarly to what occurs in clathrate hydrates. On the other hand, these hydration structures seem to be comparatively more sensi-

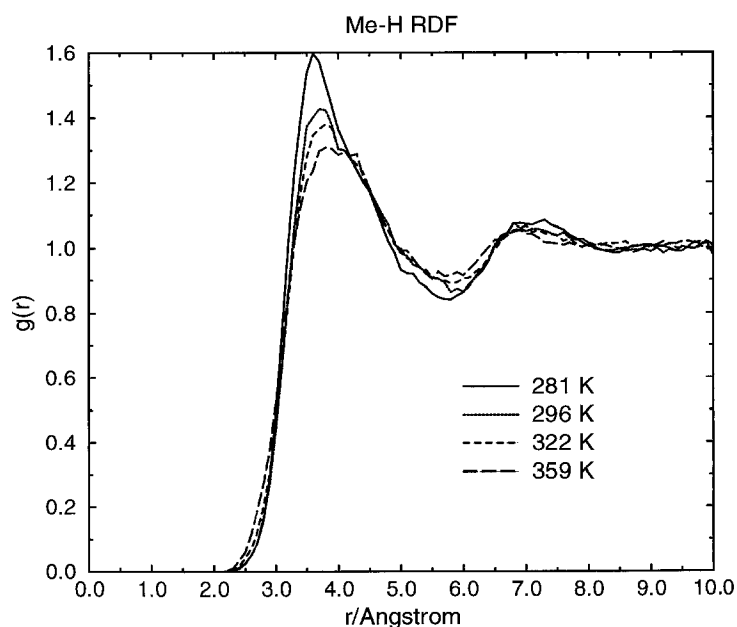


Fig. 3. Methane–hydrogen radial distribution functions, showing the hydration structure around the methane solute at different temperatures.

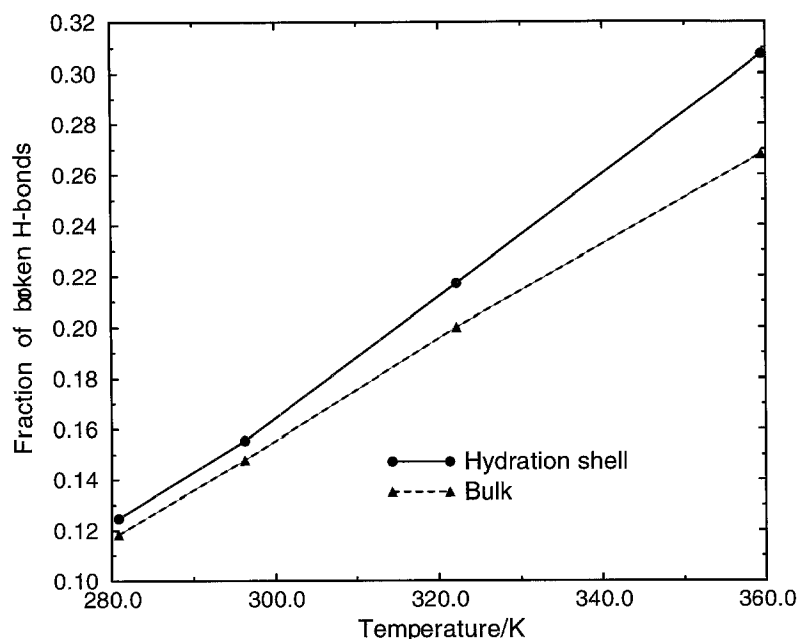


Fig. 4. Fractions of broken hydrogen bonds in the hydration shell and the bulk regions at different temperatures.

tive to temperature in the range studied when compared to previous simulations using the TIP4P potential [21,25, 26].

The same structural definition of a hydrogen bond as used in previous studies was adopted [21,28]. Hydration-shell molecules are defined as those water molecules whose oxygens are less than 5.5 Å away from the methane molecule, according to the positions of the first minima in the Me-O RDF. All the other water molecules in the system are bulk molecules. The average number of hydrogen bonds ( $N_{HB}$ ) that a water molecule can have was calculated for the hydration-shell and bulk molecules at all temperatures. This was done as before [28] by summing every 10 fs the total number of hydrogen bonds of hydration-shell and bulk molecules and dividing it by the number of water molecules in the hydration shell and in the bulk at that moment, respectively. The fraction of broken hydrogen bonds ( $f$ ) was then obtained as  $f = (4 - N_{HB})/2$ , based on an ideal tetrahedral coordination, so that the division by 2 corresponds to considering a water molecule as engaging in two hydrogen bonds with its two hydrogens [4,5].

Table 2 shows the time averages of  $N_{HB}$  and  $f$  over the whole simulations (10 000 configurations), along with the average solute coordination numbers (the average number of water molecules in the hydration shell). The same statistical analysis as described before was done [28]. Figure 4 shows a plot of the temperature-dependent behavior of these fractions of broken hydrogen bonds in the hydration shell and the bulk. It can be seen that as the temperature increases, the fraction of broken hydrogen bonds increases nearly linearly in both the hydration-shell and the bulk molecules, which is to be expected as the average number of nearest neighbors decreases as the temperature increases. It is also observed that the fraction of broken hydrogen bonds in the hydration-shell molecules ( $f_{HS}$ ) is consistently *larger* than that of the bulk molecules ( $f_B$ ), the difference between the two increasing with the rise of temperature. This effect seems to be enhanced near the solute, because of the further reduction in local density as the solute excludes a volume otherwise accessible to water molecules [32]. This work corroborates previous findings with the TIP4P potential for water [28], confirming Muller's predictions of the effect of tempera-

TABLE 2  
HYDROGEN-BONDING STATISTICS

T (K)	$N_{HS}$	$N_B$	$f_{HS}$	$f_B$	$CN_s$
280.9	$3.751 \pm 0.146$	$3.763 \pm 0.045$	0.125	0.118	$21.48 \pm 1.68$
296.3	$3.689 \pm 0.167$	$3.705 \pm 0.048$	0.155	0.148	$21.19 \pm 1.72$
322.2	$3.566 \pm 0.189$	$3.600 \pm 0.052$	0.217	0.200	$20.97 \pm 1.89$
359.4	$3.385 \pm 0.217$	$3.464 \pm 0.058$	0.307	0.268	$20.72 \pm 2.03$

T = temperature,  $N_{HS}$  = average number of hydrogen bonds per molecule in the hydration shell,  $N_B$  = average number of hydrogen bonds per molecule in the bulk,  $f_{HS}$  = fraction of broken bonds in hydration-shell molecules,  $f_B$  = fraction of broken hydrogen bonds in bulk molecules, and  $CN_s$  = solute coordination number.

ture on the fraction of broken hydrogen bonds in aqueous solutions of nonpolar solutes. The larger fraction of broken hydrogen bonds in the hydration-shell water molecules thus can be thermodynamically attributed to smaller bond-breaking free energies.

## Conclusions

A series of (N,V,E) molecular dynamics simulations of aqueous solutions of methane at different temperatures has been performed to try to corroborate some of the predictions of Muller's modified hydration-shell hydrogen-bond model of hydrophobic hydration. It was found that the fraction of broken hydrogen bonds in the hydration shell appears consistently larger than in the bulk, the difference becoming larger with an increase in temperature. This can be related to the existence of hydrogen bonds in the hydration shell with lower bond-breaking free energies or larger bond-breaking enthalpies.

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