

NATURE OF THE STACKING OF NUCLEIC ACID BASES IN WATER: A MONTE CARLO STUDY*

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ABSTRACT

The results of a Monte Carlo simulation of the hydration of uracil and thymine molecules, their stacked dimers and hydrogen-bonded base pairs are presented. Simulations have been performed in a cluster approximation. The semiempirical atom-atom potential functions have been used (cluster consisting of 200 water molecules). It has been shown that stacking interactions of uracil and thymine molecules in water arise mainly due to the increase in the water–water interaction during the transition from monomers to dimer. It has been found that stacked base associates are more favourable than base pairs in water. This is mainly due to the energetically more preferable structure of water around stacks.

INTRODUCTION

The use of the thermodynamic and spectroscopic study of the interaction of bases, their derivatives, nucleosides and nucleotides has shown that exclusively stacked associates are formed in aqueous solutions (see data survey [1]). It has been impossible, as yet, to find in-plane hydrogen-bonded (H-bonded) base pairs in water. The data on the Raman scattering spectroscopy [2] indicates the absence of hydrogen bonds (H-bonds) in the mixtures of complementary nucleosides and/or nucleotides, in particular. The results dealing with the detection of the H-bonded nucleotide pairs formed in water [3] by the NMR-spectroscopy were doubted in [4]. Later Raszka [5] showed the data to be erroneous [3].

A further UV-spectroscopy study of the association of the Ade and Ura derivatives dissolved in chloroform has shown [6] that the extent of association does not substantially change in the presence of water. As a result, the authors of the work [6] have drawn the conclusion that the H-bonds in a base pair are stronger than those between the bases and water, and that for

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the dissociation of H-bonded base pairs (H-bonded dimers) a great excess of water is needed. The analysis of the results [6] shows, however, that there is no convincing evidence for base pairing in water.

At the same time, as follows from theoretical investigations [7, 8], H-bonded base pairs in vacuum are energetically more preferable than stacked associates for the majority of the dimers of the same composition. The analysis of the whole complex of the experimental and theoretical data has allowed us to assume [8, 9] an important role of water in the formation of stacked dimers. Despite numerous experiments, the energetic advantage of the base association reaction in water and the factors stabilizing stacks have been studied insufficiently. Besides, it remains unclear why stacked dimers are more preferable than H-bonded base pairs.

METHOD

The study of the water—water interaction, the water—base interaction and the base—base interaction at the molecular level [10] is needed to elucidate the mechanism of stack formation. In this connection we have begun a computer simulation of the behaviour of nucleic acid bases and their dimer associates in water. The entropy has not been considered in simulations which is quite justified taking into account the aim of our work, since it has been shown experimentally that the association of bases and their derivatives in water is determined by enthalpy (see refs. 1, 11). The results of the hydration simulation of Ura, Thy, their stacked and H-bonded dimers by the Monte Carlo—Metropolis method [12] are presented in our work.

Simulations were performed in a cluster approximation [13] at 298 K. The number of water molecules in each of the systems under investigation (a base or its dimer + water) is 200. The system was placed into a sphere of radius 22 Å with impermeable walls, the geometric centre of a base or associate coincided with that of the sphere.

In the calculation we have used semiempirical atom—atom potential functions. In the case of water—water interaction we have applied the model of water developed by D'yakonova and Malenkov [14]. According to the model, a water molecule is represented by five electrostatic point charges at the corners of a tetrahedron and at its centre: the oxygen atom having zero charge is located at the centre of a tetrahedron, two hydrogen atoms with charges +0.1945 and two pseudoatoms representing lone pairs with charges -0.1945 are located at the corners. All the distances between the central oxygen atom and the charges at the corners were taken as 0.98 Å. The interaction energy between two water molecules (*k* and *l*) is the sum of electrostatic interaction

$$U_{kl}^{el} = \sum_{i,j} q_i q_j r_{ij}^{-1} \quad (1)$$

and the van der Waals interaction between the real atoms (oxygens and hydrogens)

$$U_{kl}^{vdW} = \sum_{i,j} [-A_{ij}r_{ij}^{-1} + B_{ij} \exp(-C_{ij}r_{ij})] \quad (2)$$

where, in both cases, i and j belong to molecules k and l , respectively.

As to the interaction between the base (b) and water (w), the interaction energy is the sum of the electrostatic interaction U_{wb}^a and the van der Waals interaction of the Lennard—Jones type

$$U_{wb}^{vdW} = \sum_{i,j} [-A'_{ij}r_{ij}^{-6} + B'_{ij}r_{ij}^{-12}] \quad (3)$$

where i and j belong to the atoms of the base and water, respectively [15]. In the water—base interaction a water molecule is represented by three point charges located at oxygen (-0.658) and hydrogen ($+0.329$) atoms. The base—base interaction has been taken into account in the same way [16]. The charges at the base atoms for the evaluation of the electrostatic interaction are calculated by means of the Del Re and Hückel methods.

In the process of the Monte Carlo simulations one of the bases in each of the stacked dimers was moved randomly according to the Metropolis algorithm. As the starting configurations for stacked Ura and Thy dimers the most favourable theoretical configurations for vacuum were chosen: (1) antiparallel; (2) antiparallel with a further rotation of 180° around the glycosidic bond of the upper base. These configurations led to different types of stacks in water. The base pair configuration was being unchanged. It was taken the same as its most preferable theoretical configuration in vacuum [7, 8].

In order to equilibrate each system we have used 700 000 configurations for the systems of Ura + water and stacked Ura dimer + water, 500 000 configurations for the systems of Thy + water and Ura base pair + water and 600 000 configurations for all the systems of Thy dimers + water. The calculations of average properties have been performed on subsequent 500 000 configurations for the systems of Ura + water, Ura dimer + water and stacked Thy dimer + water, 700 000 and 600 000 configurations for the systems of Thy + water and H-bonded Thy dimer + water, respectively.

RESULTS AND DISCUSSION

The computation results of the average values of the potential energy U for the system, the water—water interaction energy U_{ww} , the water—base interaction energy U_{wb} , the hydration energy U_h and the number of water—water H-bonds, in which one water molecule participates, n_{hb} (a water structuring index in the system) are given in Table 1. The results of similar computations [17] for the cluster of 200 water molecules are also presented in this Table.

TABLE 1

Energetic and structural characteristics for the hydration of uracil and thymine. U^a refers to the average potential energy of the system (kcal mol⁻¹ water), U_{ww} refers to the average water-water interaction energy (kcal mol⁻¹ water), U_{wb} refers to the average water-base interaction energy (kcal mol⁻¹ system), U_h refers to the hydration energy (kcal mol⁻¹ system), n_{hb} refers to the water structuring index

System	U	U_{ww}	U_{wb}	U_h	n_{hb}
Ura + water	-7.92 ± 0.02	-7.65 ± 0.02	-54.1 ± 0.1	$-6 (-22^b)$	1.75 ± 0.01
Thy + water	-8.05 ± 0.02	-7.77 ± 0.02	-56.6 ± 0.1	$-32 (-24^b)$	1.78 ± 0.01
Water	-7.89 ± 0.02	-7.89 ± 0.02	—	—	1.95 ± 0.01

^aHere and hereafter estimated energetic values are connected by the relationship $U = U_{ww} + (U_{wb} + U_{bb})/N$. ^bExperimental value of the enthalpy of hydration [18].

It is seen that the calculated magnitudes of base hydration energy are negative. This fact agrees with the experimental data (see Table 1). The absolute value of the energy of hydration for Thy is greater than that for Ura, which also agrees with the experiment.

The values of U_{ww} show that during the transfer of bases from vacuum to water its structure is destroyed. A lower value of U for the system of Thy + water as compared to the system of Ura + water is mainly determined by a lower value of U_{ww} that is associated with the formation of an energetically more preferable structure of water around Thy. The n_{hb} values also testify to this fact.

A detailed analysis of the radial distribution of the water-water interaction energy and water structuring index shows that the methylation of a Ura molecule in the C5 position destroys the structure of water around the base in the region ranging from the centre of the sphere to a distance of 8.3 Å. At the same time, the structuring of water molecules in more distant layers increases. In the proximity of the methyl group located in the region of destruction mentioned above a layer of water molecules (5.8–6.8 Å) where water is more ordered around Thy than around Ura was detected. This is similar to the picture of the structuring of water molecules usually observed near non-polar groups. However, the picture described is not so characteristic of our case because the Thy ring attached to the methyl group and possessing a dipole moment of about 4 D decreases the ordering of water molecules mentioned above.

In Tables 2 and 3 the values U , U_{ww} and U_{wb} are presented for Ura and Thy dimers, respectively. In addition, the values U_{bb} for the base-base interaction energy are also given here. In case of the stacked dimers these values are listed for the most favourable types of stacks: for Ura dimer it is formed from the first starting configuration, for Thy dimer from the second one (see section entitled METHOD).

The data of these Tables and Table 1 allow one to calculate the changes of the potential energy (ΔU) and water-water (ΔU_{ww}), water-base (ΔU_{wb}),

TABLE 2

Energetic characteristics for the hydration of the stacked dimer and hydrogen-bonded base pair of uracil (U_{bb} refers to the average base—base interaction energy (kcal mol⁻¹ system))

System	<i>N</i>	U^a	U_{ww}^a	U_{wb}^a	U_{bb}
Stacked Ura dimer + water	200	-8.18 ± 0.02	-7.67 ± 0.02	-98.7 ± 0.2	-4.15 ± 0.01
	39	-10.18 ± 0.02	-8.32 ± 0.02	-68.3 ± 0.1	-4.15 ± 0.01
	82	-9.42 ± 0.02	-8.31 ± 0.02	-86.6 ± 0.2	-4.15 ± 0.01
Ura base pair + water	200	-8.10 ± 0.02	-7.60 ± 0.02	-91.6 ± 0.2	-9.29
	39	-9.62 ± 0.02	-7.88 ± 0.02	-58.7 ± 0.1	-9.29
	82	-9.18 ± 0.02	-8.04 ± 0.02	-84.9 ± 0.2	-9.29

^aValue is described in Table 1.

TABLE 3

Energetic characteristics for the hydration of the stacked dimer and hydrogen-bonded base pair of thymine

System	<i>N</i>	U^a	U_{ww}^a	U_{wb}^a	U_{bb}^b
Stacked Thy dimer + water	200	-8.24 ± 0.02	-7.76 ± 0.02	-90.9 ± 0.2	-5.13 ± 0.01
	39	-10.05 ± 0.02	-8.16 ± 0.02	-69.0 ± 0.1	-5.13 ± 0.01
	82	-9.23 ± 0.02	-8.13 ± 0.02	-85.3 ± 0.2	-5.13 ± 0.01
Thy base pair + water	200	-8.11 ± 0.02	-7.64 ± 0.02	-85.5 ± 0.2	-9.38
	39	-9.83 ± 0.02	-8.01 ± 0.02	-61.6 ± 0.1	-9.38
	82	-9.11 ± 0.02	-8.02 ± 0.02	-79.9 ± 0.2	-9.38

^aValue is described in Table 1. ^bValue is described in Table 2.

base—base (ΔU_{bb}) interaction energies during the base association reaction resulting in dimer formation. According to [17, 19] these changes can be written as follows

$$\Delta U = \Delta U_{ww} + \Delta U_{wb} + \Delta U_{bb} \quad (4)$$

$$\Delta U_{ww} = N[(U_{ww}(D) - U(W)) - 2(U_{ww}(M) - U(W))] \quad (5)$$

$$\Delta U_{wb} = U_{wb}(D) - 2 \cdot U_{wb}(M) \quad (6)$$

$$\Delta U_{bb} = U_{bb} \quad (7)$$

In these expressions *N* is a number of water molecules in the system, D refers to the system of dimer + water, M refers to the system of monomer + water, W is the water cluster.

The magnitudes of these changes are given in Table 4. They show that it is the change of the water—water interaction associated with the structural rearrangement of water around monomers during their association that is the main factor promoting stacked dimer stabilization. The stacked associate is

TABLE 4

Energetic characteristics for base stacking reaction in water (ΔU_{ww} refers to the change of the water—water interaction energy, ΔU_{wb} refers to the change of the water—base interaction energy, ΔU_{bb} refers to the change of the base—base interaction energy, ΔU refers to the change of the potential energy of the system (all the values are given in kcal mol⁻¹ system))

Transition	ΔU_{ww}	ΔU_{wb}	ΔU_{bb}	ΔU
Ura + Ura → stacked Ura dimer	-52	9.5	-4.2	-47
Thy + Thy → stacked Thy dimer	-22	22.3	-5.1	-5

considerably less stabilized by the base—base interaction. At the same time, the stack is substantially destabilized by the water—base interaction. This is a direct confirmation of the assumption [8, 9, 20, 21] on the decisive role of the water—water interaction for base stacking.

Unfortunately, the results concerning the experimental study of the association enthalpy of Ura and Thy in water have not been obtained. The data available for the different methylated derivatives of Ura and Thy [11] show that methylation of Ura derivatives in the C5 position leads to the decrease in the absolute value of association enthalpy. The values of ΔU (see Table 4) we have obtained agree with these data.

The changes of the potential energy ($\Delta U^{s,p}$) and water—water ($\Delta U_{ww}^{s,p}$), water—base ($\Delta U_{wb}^{s,p}$), base—base ($\Delta U_{bb}^{s,p}$) interaction energies in the process of transition from the H-bonded dimer to the stacked one are given in Table 5, these changes are calculated from Tables 2 and 3.

From Table 5 it follows that, in water, stacked Ura and Thy associates are energetically more preferable than H-bonded dimers. This preference is mainly related to value $\Delta U_{ww}^{s,p}$ and is due to the energetically more favourable structure of water around stacks. The water—base interaction also stabilizes the stack as compared to the base pair. At the same time the stack is destabilized by the base—base interaction.

The considerably greater energetic preference for stacked dimers in comparison with H-bonded ones obtained by us hampers the detection of the latter in water to a great extent. This difficulty remains, irrespective of whether the base pair formation is favourable or not.

For a more detailed understanding of the nature of the advantage of the stacked dimer as compared to the H-bonded one we have calculated the energetic properties for the transition from the base pair to the stack for the nearest 39 and 82 molecules of water cluster using the data presented in Tables 2 and 3. Table 5 shows that for the subsystems including 39 water molecules there is almost the same energetic advantage for the stacked Ura dimer as compared to the corresponding base pair as for the whole water

TABLE 5

Energetic characteristics for the transition from hydrogen-bonded base pair to stacked dimer $\Delta U^{s,p}$ refers to the change of the potential energy of the system, $\Delta U_{ww}^{s,p}$ refers to the change of the water—water interaction energy, $\Delta U_{wb}^{s,p}$ refers to the change of the water—base interaction energy, $\Delta U_{bb}^{s,p}$ refers to the change of the base—base interaction energy (all the values are given in kcal mol⁻¹ system)

Transition	<i>N</i>	$\Delta U^{s,p}$	$\Delta U_{ww}^{s,p}$	$\Delta U_{wb}^{s,p}$	$\Delta U_{bb}^{s,p}$
Ura base pair →	200	-16	-14	-7.1	5.1
stacked Ura dimer	39	-21.7	-17.2	-9.6	5.1
	82	-18.7	-22.1	-1.7	5.1
Thy base pair →	200	-25	-24	-5.4	4.3
stacked Thy dimer	39	-9.0	-5.9	-7.4	4.3
	82	-10.1	-9.0	-5.4	4.3

cluster (term $\Delta U_{ww}^{s,p}$ contributing mainly to value $\Delta U^{s,p}$). The data for the subsystems of Ura dimer + 82 water molecules lead to similar results. This allows to conclude that the preference in Ura base stacking is due to the nearest water molecules [22].

It is appropriate to note that for the subsystems of Ura dimer + 39 water molecules the water—base interaction makes a tangible contribution to a greater stability of the stack as compared to the base pair. The analysis performed of the radial distribution of the water—base interaction energy shows that there is a layer of water molecules only 1.5 Å thick (5.3–6.8 Å from the centre of the sphere) around the stacked Ura dimer. The interaction of this layer with the bases makes a contribution to U_{wb} as great as -46 kcal mol⁻¹. The existence of this layer around the stack and its absence around the base pair is primarily due to the different character of the distribution of hydrophilic groups for stacked and H-bonded Ura dimers.

The analysis of the data for the analogous subsystems of Thy dimer + water (see Table 5) shows that a greater energetic advantage of the Thy stack as compared to the base pair is also observed for the subsystems including 39 and 82 water molecules. However, for the considered subsystems magnitude $\Delta U^{s,p}$ constitutes but a small part of this value for the system including 200 water molecules. This testifies to the fact that the greater energetic preference of the Thy stack as compared to the base pair is due to the layers of water molecules more distant from the dimer.

Since the magnitudes $\Delta U_{ww}^{s,p}$ for subsystems are small in comparison with value $\Delta U_{ww}^{s,p}$ for the whole cluster (see Table 5), the dominant contribution of the latter is also determined by the more distant layers of water molecules. This fact differentiates the hydration of Thy associates from those of Ura considered above.

The stereo-drawings of one of the Monte Carlo configurations during the hydration simulation of H-bonded and most strongly preferred stacked Ura and Thy dimers are presented in Figs. 1–4. The figures show that the

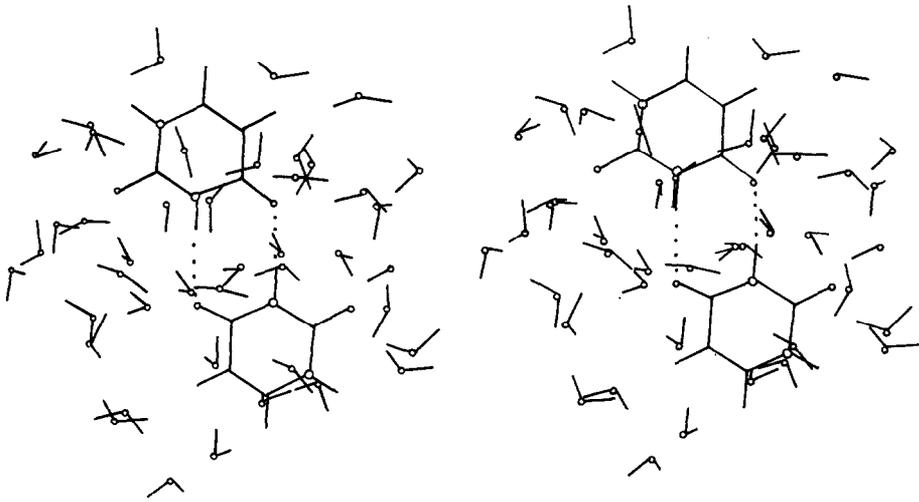


Fig. 1. Stereo-drawing of one of the Monte Carlo configurations during hydration simulation of the hydrogen-bonded base pair of uracil. Only water molecules up to 7 Å from the centre of the base pair are shown.

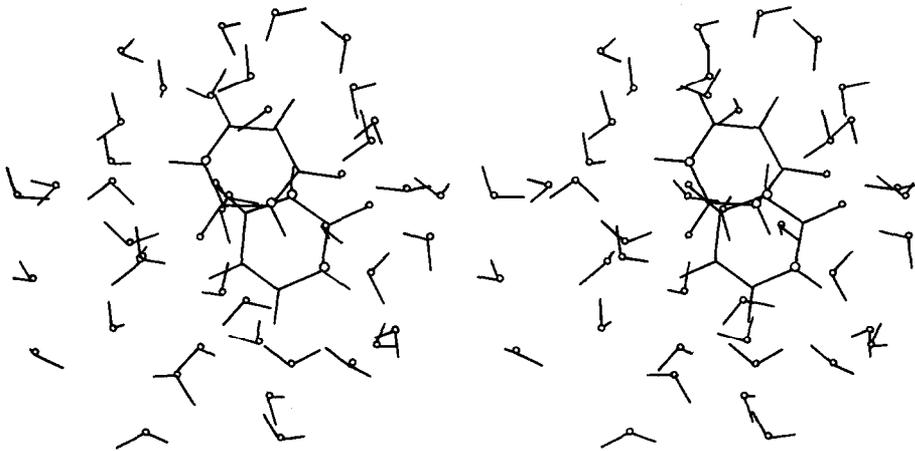


Fig. 2. Stereo-drawing of one of the Monte Carlo configurations during hydration simulation of the stacked uracil dimer. Only water molecules up to 7 Å from the centre of the dimer are shown.

distribution of water molecules around stacks and base pairs is different. The water molecules nearest to base pairs are mainly located above and below the H-bonds between the bases, whereas around the stacks water is distributed more uniformly. The latter leads to a higher structuring of water molecules and a lower energy of the water-water interaction.

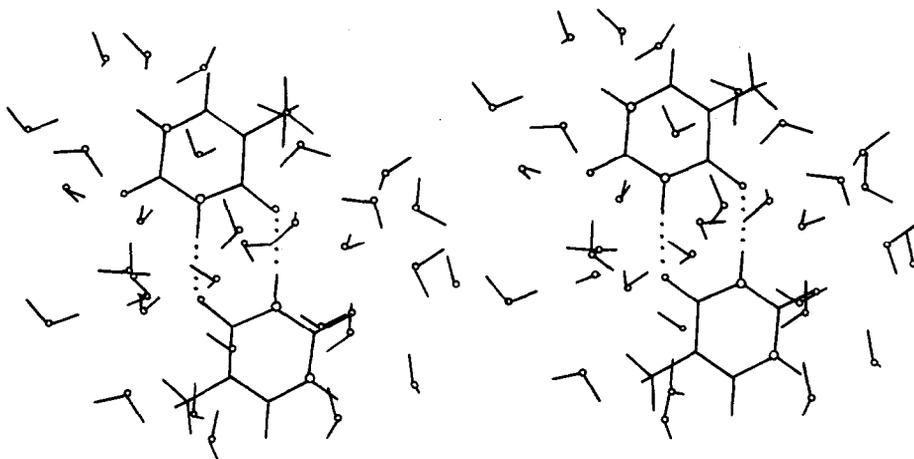


Fig. 3. Stereo-drawing of one of the Monte Carlo configurations during hydration simulation of the hydrogen-bonded base pair of thymine. Only water molecules up to 7 Å from the centre of the base pair are shown.

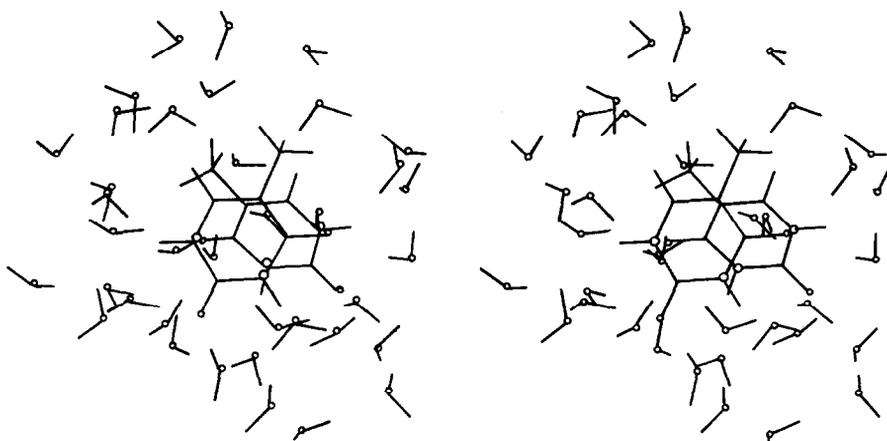


Fig. 4. Stereo-drawing of one of the Monte Carlo configurations during hydration simulation of the stacked thymine dimer. Only water molecules up to 7 Å from the centre of the dimer are shown.

Attention should be drawn to the fact that the rings of the stacked Ura dimer bases do not overlap in practice, and the dihedral angle between the planes is 17° . For the stacked Thy dimer the region of base overlap includes the bonds N3-C4, C4-C5 and C4-O4, a dihedral angle is equal to 39° . The analysis performed for other Monte Carlo configurations has given similar results. A great deviation of the base planes of dimers from parallelism and the overlap of the rings we have found seem to be one of the reasons which

hampers the experimental observation of pyrimidine base stacking by the NMR-spectroscopy.

Thus, simulation of the hydration of stacked Ura and Thy dimers has shown that the obtained configurations of stacks in water differentiate strongly from the most favourable configurations calculated for vacuum (cf., Figs. 2 and 4 with the corresponding figures in [23]). This has led to a tangible decrease in absolute value U_{bb} during the transition from vacuum to water. The comparison of the data we have got for the Ura dimer with the variable and fixed (vacuum) geometry of the stack [24] indicates not only to the change of value U_{bb} but of values U_{ww} and U_{wb} as well. Therefore, one should treat with great care the results of the works devoted to the study of base stacking reaction with the fixed geometry of stacks in water. This should be taken into account under the consideration of the recent work [25].

In conclusion it should be noted that a Monte Carlo simulation of the hydration of uracil and thymine and their different dimers performed by us has allowed to elucidate the mechanisms of base stacking and the preference in the formation of stacked dimers, as compared to H-bonded base pairs.

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ADDENDUM

Whilst this paper was in press, the performed Monte Carlo hydration simulation of m^1 Ura and m^1 Thy confirmed the appearance of a water layer with a higher structuring near to methyl groups, compared with Ura and Thy.

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