Theor Chem Acc (2011) 130:859–870
DOI 10.1007/s00214-011-1046-1

REGULAR ARTICLE

The nature of base stacking: a Monte Carlo study

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Received: 19 July 2011 / Accepted: 14 September 2011 / Published online: 8 October 2011

Abstract To elucidate the physical origin of the preference of nucleic acid bases for stacking over hydrogen bonding in water, Monte Carlo simulations were performed starting from Watson–Crick structures of the adenine–thymine, adenine–uracil and guanine–cytosine base pairs, as well as from the Hoogsteen adenine–thymine base pair, in clusters comprising 400 and 800 water molecules. The simulations employed a newly implemented Metropolis Monte Carlo algorithm based on the extended cluster approach. All simulations reached stacked structures, confirming that such structures are preferred over the hydrogen-bonded Watson–Crick and Hoogsteen base pairs. The Monte Carlo simulations show the complete transition from hydrogen-bonded base pairs to stacked structures in the Monte Carlo framework. Analysis of the average energies shows that the preference of stacked over hydrogen-bonded structures is due to the increased water–base interaction in these structures. This is corroborated by the increased number of water–base hydrogen bonds in the stacked structures.

Keywords Monte Carlo · Cluster · Base stacking · Hydrophobic interaction

1 Introduction

In the canonical form of DNA, the double helix, nucleic acid bases on opposite strands form base pairs, stabilized by hydrogen bonds (H bonds). Adenine (A) pairs with thymine (T), whereas guanine (G) pairs with cytosine (C). In RNA, uracil (U) takes the place of thymine. The bases pair with characteristic H-bond patterns i.e. Watson–Crick pairing in canonical DNA. Other H-bond patterns may occur in alternate DNA structures. For example, the guanine bases in guanine quartets occurring in four-stranded DNA quadruplex structures are stabilized by Hoogsteen H bonds [1]. Quadruplexes are four-stranded DNA structures occurring in telomeres and may be potential therapeutic targets against cancer. Stacking interactions exist in DNA structures between consecutive bases on one strand, which result from van der Waals forces (electrostatic, exchange-repulsion and dispersion interactions) between the stacked bases. It is generally assumed that both base pairing between bases on complementary strands and stacking interactions are responsible for the stability of the DNA double helix. However, in a recent experimental study, Frank-Kamenetskii et al. have shown that base stacking is the main factor for stabilizing the double helix [2].

In the light of the huge importance of base stacking on DNA stability, a detailed understanding of stacking is of

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Dedicated to Professor Akira Imamura on the occasion of his 77th birthday and published as part of the Imamura Festschrift Issue.

Electronic supplementary material The online version of this article (doi:10.1007/s00214-011-1046-1) contains supplementary material, which is available to authorized users.

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much relevance to increasing our knowledge of the stability of DNA structures. In addition, stacked DNA base associates are in themselves of considerable scientific interest as model systems to study the inherent nature of π stacking. Experimental (thermodynamic and spectroscopic) studies indicate that bases form exclusively stacked structures in aqueous solution [3, 4]. Computational studies have the potential to shed light on the physical origins of the preference for stacking over H bonding in water. In particular, electronic structure methods, which do not rely on parameterization and pre-knowledge of the systems of interest, are well suited for this. However, stacking interactions are innately difficult to study with electronic structure methods, because the large contribution of dispersion to the stacking interaction means that high-level methods are required to obtain accurate results. The benzene dimer has been the prototypical model to study π stacking at high levels of theory such as CCSD(T) (coupled cluster with single, double and perturbative triple excitations) [5–11]; the highly accurate benzene dimer potential energy curves developed in these studies have subsequently been used to assess lower-level methods, such as second-order Møller–Plesset perturbation theory (MP2) and DFT [12–14]. Over the last decade, a large number of electronic structure studies of stacked DNA base pairs have appeared in the literature, see for example [15–20]. The vast majority of studies on DNA stacks are performed in the gas phase, though some studies on microhydrated base pairs are available. Sivanesan et al. studied stacked cytosine–cytosine (C/C) [21] and guanine–cytosine (G/C) [22] structures with up to three and six water molecules, respectively. They found that the stacked base pairs hydrate better than the corresponding H-bonded base pairs. Hobza et al. optimized mono- and dihydrated adenine–thymine and 9-methyladenine–1-methylthymine structures, obtained from molecular dynamics (MD)/quenching calculations, using MP2 [23]. The results indicate that the presence of one water molecule is insufficient to change the H-bonded AT base pairs to stacked structures; however, in the presence of two water molecules, the two types of structure have comparable stability. Dkhissi and Blossey studied mono- and dihydrated AT base pairs using density functional theory (DFT) and MP2 [24]. In agreement with the study by Hobza et al., they found that the presence of two water molecules renders the H-bonded and stacked structures of comparable stability. The preference of stacked over H-bonded structures in a microhydrated environment was found to be due to hydrophilic interactions of the water molecules with the base pairs, which the authors saw as evidence that the stability of stacked structures is not due to hydrophobic interactions. However, to study hydrophobic interactions, bulk hydration needs to be taken into account. Friedman and Honig modelled bulk hydration using continuum solvation models in a study of base stacking in dinucleotides [25]. The results indicate that base-stacking results from both hydrophobicity and the enhancement of Lennard–Jones interactions as a result of close packing. However, the relative contributions are parameter-dependent and therefore difficult to separate. In addition, as the solvent is described by a continuous dielectric constant, continuum solvation models do not describe specific water–base and water–water interactions and may therefore not be sufficiently accurate to reveal the physical origin of base stacking. The alternative to using continuum solvation models is to model bulk water by explicit water molecules. Recently, we showed that the presence of a water cluster consisting of 50 or 100 explicit water molecules reverses the tautomeric preference of 5-bromouracil, rendering the rare (enol) form to be preferred over the canonical (keto) form [26, 27]. This result was not observed with continuum solvation models, indicating that explicit water–water and water–base interactions, which are lacking in continuum solvation models, need to be included to correctly describe these effects. Other examples of the need for explicit water molecules have appeared in the literature. Palafox et al. [28] found that the polarizable continuum model considerably underestimates the deformation of the structure of thymidine nucleosides by water. Other studies have demonstrated the need for explicit water molecules to stabilize the zwitterionic form of L-alanine [29], to reliably reproduce and interpret vibrational absorption, vibrational circular dichroism (VCD), Raman and Raman optical activity spectra [30–38] and to obtain an accurate value of the amino ^{1}H chemical shift in guanine [39]. Explicit waters are particularly essential to model the ‘chirality transfer effect’ in VCD spectra, as shown for methyl lactate in water [35]: Some IR bands of water became VCD active through H bonding with the achiral methyl lactate.

For the alanine zwitterion, it was shown that the first and second hydration shells require clusters of 20 and 50 water molecules, respectively [37, 40]. Thus, for a molecule of the size of alanine, a water cluster size of 50 water molecules should be sufficient to approach the bulk water limit. For base pairs, which are larger than alanine, the number of water molecules required to mimic bulk water is, however, expected to be considerably larger. An MD study showed that the observed stability of hydrated RNA base pairs was reproduced when more than 100 water molecules were included in the simulations [41]. It is, however, nearly impossible to include so many water molecules in electronic structure studies. The first quantum-chemical study on base pairs including as many as 200 water molecules used the semiempirical PM6 method [42] to investigate H-bonded and stacked adenine–thymine structures. The results show that, in the water cluster, the base-stacking reaction is favourable, whereas the base-pairing reaction is not. The transition from the H-bonded to the stacked associates is favoured by the water–base interaction. This can be explained by the larger number of exposed polar groups in the stacks compared to the H-bonded structures, which results in more H bonds between the bases and the surrounding water molecules [43–45]. The additional stabilization resulting from this more than compensates the loss of the inter-base H bonds in the stacked structures. Four different stacked structures were studied, which differ in the rotation of the thymine molecule around two twist angles (the first one defining rotation around an axis through thymine's centre of mass and perpendicular to its plane; the second one defining a 180° flip of thymine). The most stable structure found was labelled $A/T_{para/rot}$.

Apart from this PM6 study, most bulk hydration studies on DNA stacks were carried out with classical MD or Monte Carlo (MC) simulations. These methods use force field methods to evaluate the energies of the systems studied, which are much faster (but arguably less accurate) than electronic structure methods. They have the additional advantage that temperature and, in case of MD, dynamical effects are covered. The early MD [46] and MC [47–55] simulations on DNA stacks date back to the 1980s. These show that stacked complexes are more stable than the corresponding H-bonded base pairs in water. However, restrictions on the solvation models used in these early studies may negatively affect the accuracy of the results. For example, Pohorille et al. used fixed gas-phase geometries for the stacked dimers and the position of the base pair in the box was kept fixed throughout the simulation, whereas in the MC simulations by Danilov et al. only one of the bases was moved randomly.

Due to computational advances, presently more accurate simulations can be carried out. Recently, we presented an MC study on the adenine–thymine (AT), adenine–uracil (AU) and guanine–cytosine (GC) base associates in water clusters consisting of 400 water molecules [44]. These MC simulations were based on the physical cluster theory [56–60], which does not employ periodic boundary conditions. As the solubility of the compounds studied is low, their solutions are relatively dilute. When periodic boundary conditions are used, the solute will be close to its corresponding images in neighbouring cells, even if relatively large cells are employed, thus modelling a concentrated solution. To correctly describe a dilute solution with periodic boundary conditions, the 'nearest image' method should be used, which does not take into account interactions between molecules in different cells. However, distribution functions may be distorted when using periodic boundary conditions using relatively small cells. These problems can be avoided using the cluster approach, as done in our MC simulations. The MC simulations in our recent work [44, 45] used a newly implemented algorithm based on the extended cluster approach, which, in contrast to the earlier MC simulations on base stacks, allows all molecules (both bases and all water molecules) to move randomly in the simulations. The forte of the MC method is that it is more likely to find global minima, as the method can easily traverse energy barriers. In addition, the simulations were performed in reduced water density, which allows the individual bases in the dimer to flip around, thereby increasing the chance to reach the global minimum. This work showed that MC simulations starting from the Watson–Crick structures of the AT, AU and GC base pairs converted into stacked structures (A/T, A/U and G/C) for all three base associates. The results therefore showed, for the first time, the transition from H-bonded base pairs to stacked base associates within the Monte Carlo framework.

In the current article, we present a comprehensive MC study of the AT, AU and GC associates in clusters consisting of 400 and 800 water molecules. Radial distribution functions for water obtained from our previous MC simulations showed that clusters consisting of 400 water molecules accommodated two hydration shells. Clusters with 800 water molecules should even more closely model bulk water. The simulations were started from their Watson–Crick H-bonded base pair structures and, for AT, also from the Hoogsteen structure. All simulations reached stacked structures. Analysis of the average energies confirmed the decisive role of the water–base interaction in favouring the stacked over H-bonded structures.

2 Computational methods

A newly implemented Metropolis Monte Carlo [61] algorithm following the extended cluster approach was used, which allows all molecules to move randomly in the simulations, while still preventing the base molecules from drifting to the edge of the water cluster. This is achieved by forcing the centre of mass of the base pair and the centre of mass of the water cluster to coincide. The algorithm is based on the physical cluster theory of Abraham [56–60]. In a nutshell, the MC algorithm is as follows: (1) the system of interest (in this case: the two bases and water molecules) is placed in a sphere with impenetrable walls; (2) the centre of mass of the base associate and the centre of mass of the water cluster are made to coincide with the centre of the sphere; (3) trial motions of the water and base molecules are carried out so that the overall centre of mass of the system remains fixed. Thus, the centre of mass of the base associate, as well as that of the water cluster, always remains in the centre of the restricting sphere, whereas the individual bases and water molecules can adopt different mutual orientations. In more detail: A water cluster comprising 400 or 800 water molecules, with a density that equals the experimental density of water at room temperature, is placed in the centre of a sphere of radius 24.3 Å (400 water molecules) or 30.6 Å (800 water molecules). These spheres have a volume that is five times larger than the volume of a 400- or 800-water cluster with the same density as liquid water under standard conditions. A base pair, optimized at the MP2/6-311G(2df,pd) level of theory, is placed in this water cluster so that the centre of mass of the solute coincides with the centre of the sphere. Watson–Crick (WC) structures were considered for AU and GC, whereas for AT a WC as well as a Hoogsteen (HS) structure was employed (see Fig. 1). Due to the large volume of the sphere, the density of the water cluster is reduced in the subsequent simulation process. This allows the bases in the stacks to rotate more freely. To remove close contacts between the solute and the water molecules, 10^4 configurations were generated using the MC algorithm. In general, one full MC configuration consists of one move of every molecule. However, at this stage, the geometry of the base pair was kept fixed. The next stage of the simulation consisted of 3×10^6 configurations for the WC starting structures. The simulations starting from the HS AT base pair were propagated for a longer time (400-water cluster: 6×10^6 configurations; 800-water cluster: 5×10^6 configurations). The water molecules as well as the individual bases were now allowed to move. In all simulations, the water molecules were restricted to remain within the spherical constraining volume and the centres of mass of the solute and the water cluster were kept at the centre of the sphere. The last 1×10^6 configurations of the simulations were used to calculate the average properties of the stacked structures. Similar simulations were performed for the corresponding H-bonded base pairs with fixed base pair geometry. These consisted of 2×10^6 configurations, of which the last 0.5×10^6 configurations were used to calculate the average properties. The rigid rotor approximation was applied to the movement of the base and water molecules.

The simulations were carried in the canonical (NVT) ensemble, in the standard state (298 K and 1 atm pressure). Entropy was not considered as it has been shown both experimentally by NMR experiments [4] and theoretically using free-energy perturbation/MD [46] that the association of the bases in water is primarily driven by enthalpy. The potential energy surface was modelled using the refined semiempirical potential functions of Poltev and co-workers [62–65], of which the parameters were chosen for systems containing nucleic acid bases and water. The form of the potential functions is different for interactions between atoms that are not involved in H bonding and for interactions between hydrogen atoms capable of forming H bonds and H-bond acceptor atoms, as shown in Eqs. 1 and 2, respectively:

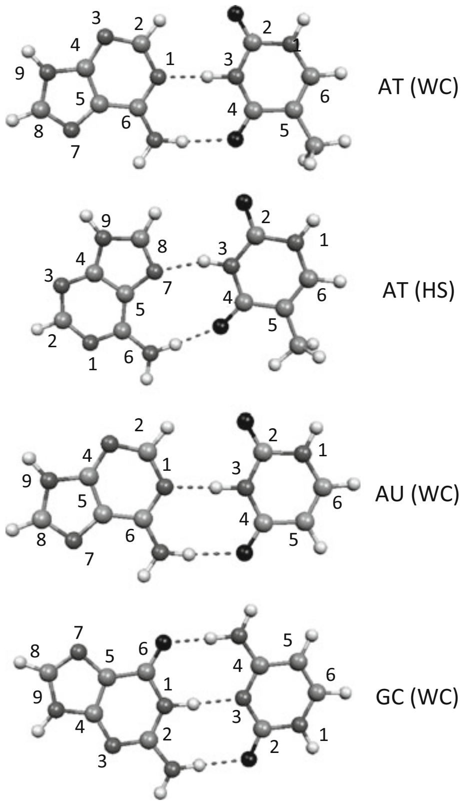


Fig. 1 Starting structures for the MC simulations, optimized with MP2/6-311G(2df,pd)

$$U_{ij} = kq_iq_j/r_{ij} - A_{ij}/r_{ij}^6 + B_{ij}/r_{ij}^{12} \tag{1}$$

$$U_{ij}(r_{ij}) = kq_iq_j/r_{ij} - A_{ij}^{(10)}/r_{ij}^{10} + B_{ij}^{(12)}/r_{ij}^{12}. \tag{2}$$

Here, r_{ij} is the interatomic distance, and k is a numerical constant; q_i and q_j are atomic charges (calculated from semiempirical methods to reproduce experimentally determined dipole moments of the molecules of interest [66, 67]), and A_{ij}, B_{ij}, $A_{ij}^{(10)}$ and $B_{ij}^{(12)}$ are parameters that depend on the atom type (see [68] for their numerical values).

The accuracy of these potential functions has been verified by comparison of the interaction and dispersion energies of the bare base associates obtained from the final snapshots in the MC simulations conducted in the 400-water clusters to results obtained with the BLYP-D3 method [69] (vide infra). These calculations were done using TURBOMOLE [70] and a dftd3 standalone code (see http://www.toc.uni-muenster.de/DFTD3/getd3.html). The calculations employed the RI (resolution of the identity) approximation and TURBOMOLE's grid4 for the quadrature.

The average potential energy (U) of the different systems in the water cluster (base associates and single bases) was decomposed into base–base (U_{bb}), water–base (U_{wb}) and water–water (U_{ww}) contributions. From these, the potential energy changes (ΔU) and their contributions (ΔU_{ww}, ΔU_{wb} and ΔU_{bb}) for (1) transition from the H-bonded to the stacked associates and (2) the formation of the stacks can be deduced. The potential energy changes for transition from the H-bonded to the stacked associates were computed as the differences in the corresponding average energies of the stacked and H-bonded structures. To obtain the formation energies of the different stacks, the water–water, water–base and base–base contributions to the formation energies were calculated as follows [47]:

$$\begin{aligned}\Delta U_{ww} = U_{ww}(\mathrm{B1/B2}) - U(n\mathrm{H_2O}) \\ - (U_{ww}(\mathrm{B1}) - U(n\mathrm{H_2O})) \\ - (U_{ww}(\mathrm{B2}) - U(n\mathrm{H_2O}))\end{aligned} \qquad (3)$$

$$\Delta U_{wb} = U_{wb}(\mathrm{B1/B2}) - U_{wb}(\mathrm{B1}) - U_{wb}(\mathrm{B2}) \qquad (4)$$

$$\Delta U_{bb} = U_{bb}(\mathrm{B1/B2}), \qquad (5)$$

where B1/B2 is a stack consisting of a B1 and B2 base and $U_{xx}(\mathrm{S})$ (xx = ww, wb or bb) refers to the average energy of the system S (S = B1/B2, B1 or B2) in the water cluster. $U(n\mathrm{H_2O})$ is the average energy of a pure water cluster consisting of n (n = 400 or 800) water molecules: −3,420.5 and −7,019.6 kcal/mol, for the 400- and 800-water clusters, respectively. These values were obtained from MC simulations propagated over 6×10^6 configurations, of which the last 5×10^6 configurations were used to obtain the average energies. The total formation energy of the stacks then follows from summing the water–water, water–base and base–base contributions.

As in our previous paper [44], the statistical error, which occurs because only a finite number of moves can be considered, was estimated using a control function method. The complete series of moves is divided into a finite number of intervals. Mean-square fluctuations are calculated from the values of the functions determined at each interval. The standard deviations of the thermodynamic quantities were obtained from a series of mean values, each representing the average of an interval containing 10^4 configurations. In these calculations, the statistical error (or dispersion value) was calculated to be ±0.5%.

The MC method is implemented in an in-house software program, developed at the National Academy of Sciences of Ukraine [71].

3 Results and discussion

Figure 2 shows the variation of the angle between the plane vectors of the two bases (α_{bb}) during the MC simulations as a function of the MC configuration number. The graphs were obtained by taking the value of every 200th MC configuration and then averaging over every 50 of these. In previous work [44], we enforced this angle to be in the [0–90°] [21] interval (by subtracting α_{bb} values that were larger than 90° from 180°). Here, we did not do this. The α_{bb} values can therefore range from 0 to 180°. An α_{bb} value of 0° indicates a parallel configuration of the two plane vectors, whereas a value of 180° denotes a antiparallel configuration. Both can be achieved by a planar as well as a stacked base pair. At the start of the simulation, α_{bb} is 0° because of the planar arrangement of the WC and HS base pairs (and because the plane vectors point in the same direction, by definition). Note that the simulations starting from the AT (HS) base pair were propagated for a longer time (6×10^6 configurations), as the simulations displayed larger fluctuations in the α_{bb} angle in the 2×10^6–3×10^6 interval as compared to the simulations started from the AT (WC) base pair. All simulations eventually achieved a stacked arrangement of the two bases (α_{bb} close to 0° or 180°). Movies of the MC simulations in the 800-water cluster are provided as Supplementary Materials. The water molecules were removed to more clearly show the geometrical changes in the base associate structure.

The α_{bb} values in Fig. 2 indicate that one of the bases in the AT (WC) base pair flips over in the 400-water simulation (α_{bb} values > 90°), whereas this does not happen in the 800-water simulation. Thus, the two different simulations yield different stacks. The 400-water AT (WC) simulation first goes through a T-shaped orientation (α_{bb} around 90°) and then forms a stacked structure with nearly parallel plane vectors ($\alpha_{bb} \sim 10°$; configuration interval: 0.2×10^6–0.4×10^6). The stacked structure breaks up again, and the AT associate goes once more through a T-stacked structure (configuration interval: 1.0×10^6 – 1.5×10^6) before transforming into a stacked structure with antiparallel plane vectors ($\alpha_{bb} \sim 170°$). In contrast, in the 800-water AT (WC) simulation, a stacked structure (with nearly parallel plane vectors) is formed already within the first 1×10^6 configurations. In both AT (HS) simulations, one of the bases flips over. However, whereas this happens almost immediately in the 400-water simulation, in the 800-water simulation the complex spends a relatively long time in a T-shaped type configuration (α_{bb} around 90°; interval: 1×10^6–3×10^6) and the flip is accomplished only after $\sim 3 \times 10^6$ configurations. A base flip also occurs in the AU complex in the 800-water simulation, and a base flip occurs in the GC complex in the 400-water simulation. These results indicate that the final results of the MC simulations are sensitive to the initial starting structure (WC vs. HS) and the number of water molecules in the water cluster. This indicates that even though MC is more likely to find the global minimum compared to methods such as MD, this cannot be guaranteed. However, it should be emphasized that in all

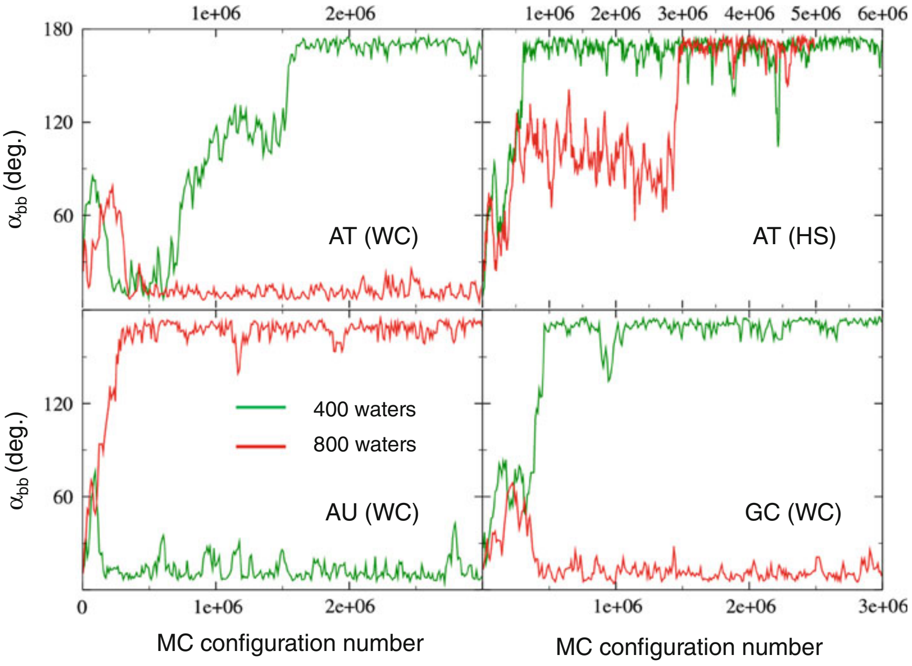


Fig. 2 Angle between the normals to the planes of the two bases (α_{bb}) as a function of the MC configuration

simulations the two bases ultimately form a stacked structure. In the final configurations of all simulations, α_{bb} fluctuates around ~170° or ~10°, indicating that the bases in the stacks are not exactly parallel. It should be noted that the simulations were also done in water clusters with the experimental density of water at room temperature. The obtained results are very similar to those presented in the current work.

Figure 3 shows the variation of the inter-base distance R_{bb}, measured as the distance between the centres of mass of the bases, as a function of the MC configuration number. This distance equals 5.9, 5.7, 5.8 and 5.5 Å in the AT (WC), AT (HS), AU (WC) and GC (WC) base pairs, respectively. The inter-base distances in the final stage of the simulation, estimated by averaging over the last 1×10^6 configurations, range from 3.6 to 3.9 Å. This is slightly larger than the approximate distance between consecutive bases in B-DNA (3.4 Å) [72]. The shorter inter-base distance in the final stack in the AT (WC) simulation in a 400-water cluster as compared to the one formed earlier in this simulation correlates with its greater stability.

The average values of the systems' potential energy (U), water–water interaction energy (U_{ww}), water–base interaction energy (U_{wb}) and base–base interaction energy (U_{bb}) are listed in Table 1. A/T stack1 and A/T stack2 refer to the stacks resulting from the simulations started from the AT (WC) and AT (HS) base pairs, respectively. A comparison of the potential energy values shows that the stacked structures are more stable than their corresponding WC and HS base pairs. This is primarily due to the large water–base interaction energy. The WC and HS base pairs exhibit larger U_{bb} values than the stacked structures. This is as expected because of the presence of inter-base H bonds in these structures.

Figure 4 shows the final snapshot in the simulations. Even though the presence of temperature in the simulations means that the stacks are not rigid, snapshots in the equilibrated part of the simulation will have structural similarities. Analysis of snapshots at the end of the simulations shows that the A/T stack2 (400 waters) and the A/T stack1 (800 waters) are similar to the most stable A/T stack identified in our earlier PM6 study [73], labelled A/T$_{anti/rot}$. To reach this stacked structure, a base flip was required in the A/T stack2 (400 waters) simulation but not in the AT stack1 (800 waters) simulation. This is because the adenine base in the AT (HS) base pair is flipped compared to the AT (WC) base pair (see Fig. 1). The A/U stack resulting from the simulation in 400 waters is more similar to A/T$_{anti/rot}$, whereas the A/U stack resulting from the simulation in 800 waters is comparable to A/T$_{para}$.

Table 2 shows the potential energy changes (ΔU) for transition from the H-bonded to the stacked associates in water. For all base associates, the formation of a stack in an aqueous cluster is favourable. The A/U stack formed in the 800-water simulation, which contains a flipped base, is considerably more stable, compared to the H-bonded base pair, than the one formed in the 400-water cluster. Base flips can occur in our MC simulations due to the reduced density resulting from using constraining spheres with volumes that are five times larger than the volume of a cluster with the same number of water molecules and with the density of liquid water under standard conditions. It is clear that base flips need to be able to happen to find the

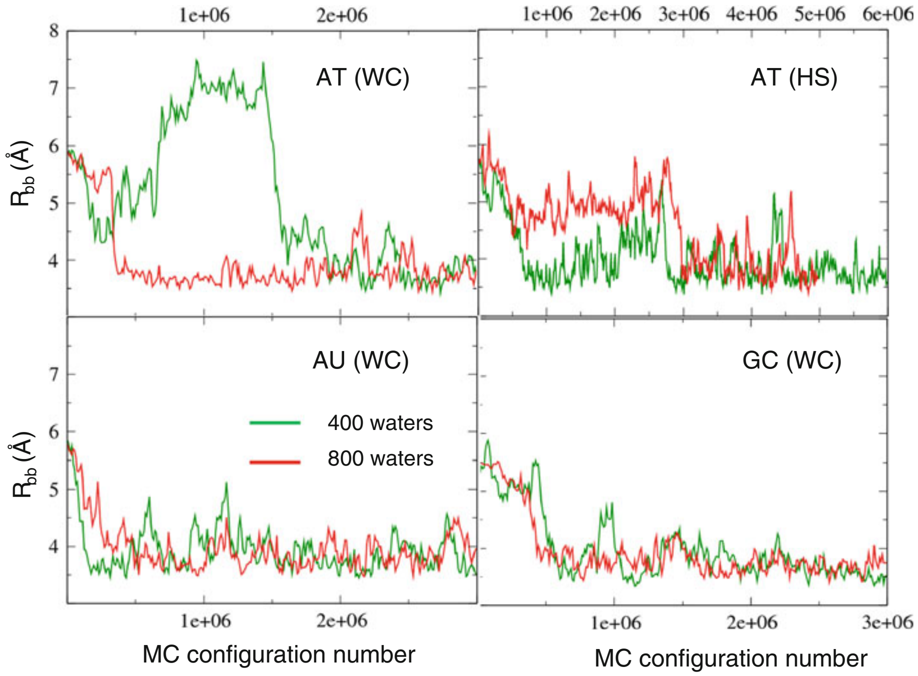


Fig. 3 Distance between the centres of mass of the two bases (R_{bb}) as a function of the MC configuration

most stable stacked configuration. The energy changes evidence the deciding role of the water–base interaction in favouring the stacked structures. In the stacks, all hydrophilic groups (NH; C=O) are available for H bonding with water, whereas the hydrophobic surface area in contact with the water surroundings is smaller than in the H-bonded base pairs. This leads to increased water–base interaction. As mentioned earlier, the base–base interaction favours the H-bonded base pairs. The ΔU_{bb} term is particularly large for the GC associate. This indicates the considerable strength of the GC (WC) base pair, which is stabilized by three H bonds. Also the water–water interaction disfavours the GC stacks. Even so, the water–base interaction in the GC stacks is sufficiently large to compensate the loss of favourable water–water and base–base interactions.

Table 3 shows the average number of water–base H bonds in the H-bonded and stacked structures. A particular base atom was considered to form an H bond with a water molecule if the donor–acceptor distance, R(D···A), was <3.20 Å and the hydrogen–acceptor distance, R(H···A), <2.40 Å. It can be clearly seen that the bases in the stacked structures form more H bonds with nearby water molecules than the H-bonded base pairs. This is in agreement with the larger water–base interaction in the stacks. The results are very similar for the two differently sized water clusters. A more detailed analysis of the water–base H bonds, including breakdown per base atom and average geometrical parameters, is provided in Tables S1 and S2 (Supplementary Materials). These data show that the increased number of H bonds in the stacks compared to the H-bonded structures is primarily due to additional H bonds with the atoms that, in the H-bonded structures, are involved in the formation of the H bonds. The average donor–acceptor H-bond distances range from 2.8 to 3.0 Å, whereas the H-bond angle, ∠(D–H···A), ranges from 146 to 162°.

Table 4 lists the formation energies of the different stacks in the 400- and 800-water clusters. The formation of all stacks is favourable. The AT stack formed in the simulations starting from the WC base pair (stack1) is slightly more favourable than the stack obtained in the simulations starting from the AT (HS) base pair. The largest stabilizing contribution results from ΔU_{ww}. This confirms the crucial role played by the water–water interaction in base stacking reported in earlier work [44, 47, 54, 55, 73].

The accuracy of the refined potential functions used in the current MC simulations has been examined by comparison of the interaction energies of the last snapshots in the MC simulations of the bare base associates obtained from the simulations in the 400-water clusters to results obtained with the BLYP-D3 method [69] using the def2-TZVP basis set (see Table 5). DFT-D3 is the latest refinement of the DFT-D method, which adds an empirical dispersion correction to standard Kohn–Sham density functional theory. The new parameterization uses atom-pairwise-specific dispersion coefficients and cut-off radii that are computed from first principles. It also includes eighth-order dispersion terms, system/geometry-dependent information and three-body non-additivity terms. DFT-D3 reduces the mean absolute deviations in several test sets by up to 30% compared to the (already accurate) DFT-D2 method [69]. To be able to interpret the D3 term as

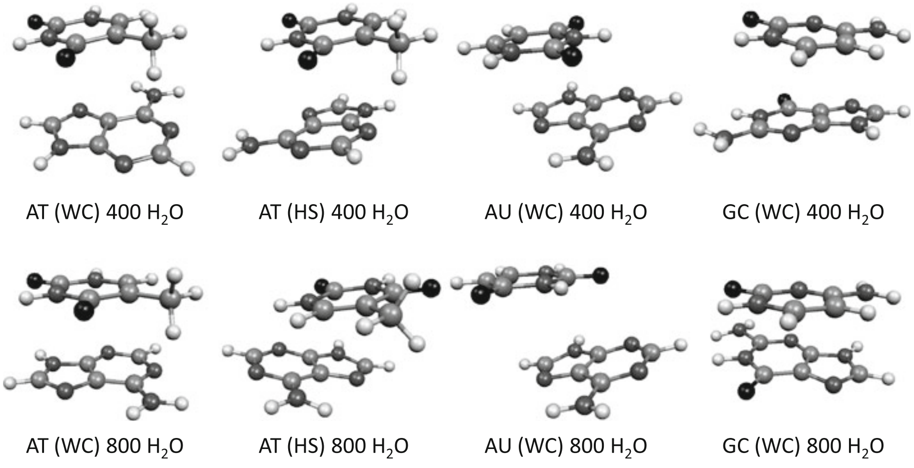
Table 1 Total average interaction energy and its components for the isolated bases and H-bonded and stacked base associates in water clusters consisting of 400 or 800 water molecules (in kcal/mol)

Compound	U	U_{ww}	U_{wb}	U_{bb}
400-water cluster				
Adenine	−3,453	−3,365	−87	–
Thymine	−3,454	−3,397	−57	–
Uracil	−3,452	−3,394	−58	–
Guanine	−3,475	−3,360	−115	–
Cytosine	−3,468	−3,378	−91	–
AT WC	−3,487	−3,351	−126	−11
AT HS	−3,490	−3,350	−128	−13
AU WC	−3,492	−3,354	−127	−11
GC WC	−3,524	−3,344	−159	−22
A/T stack1[b]	−3,503	−3,355	−144	−4
A/T stack2[c]	−3,493	−3,344	−146	−4
A/U stack	−3,494	−3,342	−148	−4
GC stack	−3,535	−3,324	−209	−2
800-water cluster				
Adenine	−7,060	−6,970	−90	–
Thymine	−7,035	−6,978	−57	–
Uracil	−7,047	−6,988	−59	–
Guanine	−7,078	−6,963	−115	–
Cytosine	−7,076	−6,985	−91	–
AT WC	−7,099	−6,962	−127	−11
AT HS	−7,101	−6,956	−132	−13
AU WC	−7,095	−6,954	−129	−11
GC WC	−7,146	−6,960	−164	−22
A/T stack1[b]	−7,109	−6,958	−146	−5
A/T stack2[c]	−7,103	−6,949	−149	−4
A/U stack	−7,114	−6,959	−152	−3
GC stack	−7,152	−6,940	−206	−7

[a] Number of water molecules in the cluster

[b] Stack obtained from the AT (WC) simulation

[c] Stack obtained from the AT (HS) simulation



AT (WC) 400 H_2O AT (HS) 400 H_2O AU (WC) 400 H_2O GC (WC) 400 H_2O

AT (WC) 800 H_2O AT (HS) 800 H_2O AU (WC) 800 H_2O GC (WC) 800 H_2O

Fig. 4 Last snapshot in the different MC simulations. The Cartesian coordinates of these snapshots are given in the Supplementary Materials

dispersion, one needs to use a density functional that does not describe dispersion at all. The BLYP functional fits this requirement. Although the BLYP functional is not suited for the calculation of properties that require a balanced account of opposite- and equal-spin correlation in the core region, such as magnetic properties [74], or for situations where the wave function is not well described as a single determinant (for which multiconfigurational DFT methods are more appropriate [74–76]), BLYP was found to be among the best functionals for DFT-D3, with BLYP-D3

Table 2 Energy changes for transition from the H-bonded base pairs to the stacked associates in water clusters consisting of 400 or 800 water molecules (in kcal/mol)

Process	n^{a}_{water}	ΔU	ΔU_{ww}	ΔU_{wb}	ΔU_{bb}
AT WC → A/T stack1[b]	400	−16	−3	−19	6
	800	−10	4	−20	5
AT HS → A/T stack2[c]	400	−3	6	−18	9
	800	−2	7	−17	8
AU WC → A/U stack	400	−1	13	−22	8
	800	−19	−4	−23	8
GC WC → G/C stack	400	−10	20	−50	20
	800	−6	21	−43	16

[a] Number of water molecules in the cluster

[b] Stack obtained from the AT (WC) simulation

[c] Stack obtained from the AT (HS) simulation

Table 3 Average number of water–base H bonds in the H-bonded and stacked associates

n_{HB}	400-water cluster	800-water cluster
AT (WC)	9.7	9.5
AT (HS)	9.9	9.8
AU (WC)	9.7	9.4
GC (WC)	11.7	12.1
AT (stack1)	12.2	12.2
AT (stack2)	12.4	12.6
AU (stack)	12.4	12.5
GC (stack)	16.0	16.0

giving a mean absolute deviation (MAD) of 0.23 for the S22 benchmark set, much better than, for example, MP2/CBS (MAD 0.78) [69]. The BLYP-D3 dispersion terms calculated for the A/T, A/U and W/C stacks (−10.9, −10.5 and −11.4 kcal/mol, respectively) agree very favourably with the R^{-6} van der Waals term in the model potential, which describes the dispersion contribution in the MC method (−10.0, −10.2 and −10.9 kcal/mol, respectively). This indicates the reliability of the potential functions used in the MC simulations. The analysis shows that the dispersion term is paramount in stabilizing the stacks, and thus, a reliable description of the dispersion contribution is essential to model the formation of the stacks.

4 Conclusion

MC simulations started from the WC structures of the AT, AU and GC base pairs, and from the HS structure of the AT base pair, in clusters consisting of 400 and 800 water molecules, show that all base pairs eventually transform into stacked structures. The simulations were done in reduced water density, achieved by restricting the solvent molecules to a sphere with a volume that is five times larger than the volume of a 400- or 800-water cluster with the same density as liquid water under standard conditions. The reduced density permits base flips to occur. Such flips happened in five of the eight simulations. The results show that the structure of the achieved stack is sensitive to the starting structure and to the number of water molecules in the cluster. The averaged energies confirm the deciding role of the water–base interaction energy in favouring the stacks over the H-bonded base pairs. Analysis of the average numbers of water–base H bonds shows that the bases in the stacked structures form more H bonds with nearby water molecules than the H-bonded base pairs. The

Table 4 Energetic characteristics of the base-stacking reaction in water clusters consisting of 400 or 800 water molecules (in kcal/mol)

Process	n^{a}_{water}	ΔU	ΔU_{ww}	ΔU_{wb}	ΔU_{bb}
A + T → A/T stack1	400	−16.9	−13.0	0.4	−4.3
	800	−33.7	−29.9	1.4	−5.2
A + T → A/T stack2	400	−6.9	−2.4	−0.8	−3.7
	800	−27.2	−21.5	−1.4	−4.4
A + U → A/U stack	400	−9.6	−3.2	−2.8	−3.6
	800	−26.3	−20.4	−2.7	−3.2
G + C → G/C stack	400	−12.5	−6.7	−3.5	−2.3
	800	−17.2	−10.4	−0.3	−6.5

[a] Number of water molecules in the cluster

Table 5 Comparison of the interaction energies of the stacks (last snapshots in the MC simulations in the 400-water cluster) calculated with the model potential used in the MC simulations and with the BLYP-D3 method

Stack	Refined model potential				BLYP-D3	
	$E_{\mathrm{el}}^{\mathrm{a}}$	$E_{\mathrm{vdw}}(\mathrm{R}^{-6})^{\mathrm{b}}$	$E_{\mathrm{vdw}}(\mathrm{R}^{-12})^{\mathrm{c}}$	$E_{\mathrm{tot}}^{\mathrm{d}}$	$E_{\mathrm{disp}}^{\mathrm{e}}$	$E_{\mathrm{tot}}^{\mathrm{d}}$
A/T	−0.56	−9.98	4.95	−5.60	−10.89	−7.46
A/U	−0.81	−10.25	6.11	−4.95	−10.47	−6.68
G/C	1.37	−10.87	6.53	−2.96	−11.38	−5.45

[a] Electrostatic contribution

[b] R^{-6}-dependent van der Waals term

[c] R^{-12}-dependent van der Waals term

[d] Total interaction energy

[e] Dispersion contribution

increased number of H bonds in the stacks is predominantly due to additional H bonds with the atoms that, in the H-bonded structures, are involved in the formation of the H bonds, but are available to directly H bond with water molecules in the stacked structures. The formation of the stacks from their individual bases in water is shown to be stabilized by favourable water–water interactions.

Acknowledgments We are grateful to Professor Stefan Grimme for providing us with BLYP-D3 energies for the AT, AU and GC stacks. TvM and HAF thank EaStCHEM for support via the EaStCHEM Research Computing Facility. TvM and VID acknowledge financial support from the Royal Society through an International Joint Project grant.

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