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DNA Base Trimers: Empirical and Quantum Chemical Ab Initio Calculations versus Experiment in Vacuo

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Abstract: A complete scan of the potential-energy surfaces for selected DNA base trimers has been performed by a molecular dynamics/quenching technique using the force field of Cornell et al. implemented in the AMBER7 program. The resulting most stable/populated structures were then reoptimized at a correlated ab initio level by employing resolution of the identity, Møller–Plesset second-order perturbation theory (RI-MP2). A systematic study of these trimers at such a complete level of electronic structure theory is presented for the first time. We show that prior experimental and theoretical interpretations were incorrect in assuming that the most stable structures of the methylated trimers corresponded to planar systems characterized by cyclic intermolecular hydrogen bonding. We found that stacked structures of two bases with the third base in a T-shape arrangement are the global minima in all of the methylated systems: they are more stable than the cyclic planar structures by about 10 kcal mol^{-1}. The different behaviors of nonmethylated and methylated trimers is also discussed. The high-level geometries and interaction energies computed for the trimers serve also as a reference for the testing of recently developed density functional theory (DFT) functionals with respect to their ability to correctly describe the balance between the electrostatic and dispersion contributions that bind these trimers together. The recently reported M052X functional with a polarized triple-zeta basis set predicts 11 uracil trimer interaction energies with a root-mean-square error of 2.3 kcal mol^{-1} relative to highly correlated ab initio theoretical calculations.

Keywords: ab initio calculations · DNA structures · nucleic acids · potential-energy surface

Introduction

Noncovalent interactions play an important role in biological systems such as DNA and proteins. The structures of these biopolymers are determined by: 1) hydrogen bonds, which are primarily electrostatic in nature, but the covalent term (covering charge transfer and induction energy) is almost equally important, as shown by Guerra et al.;[1a,b] and 2) van der Waals interactions, including London dispersion, as an attractive force. Specific hydrogen bonds are crucial for correct base pairing in DNA, fidelity of replication, and transcription processes, and for the formation of secondary structures in both DNA and proteins. Nonspecific dispersion forces, by contrast, are responsible for base-stacking interactions in the DNA polymer, as well as for the interaction of aromatic side chains of amino acids with peptide bonds.

Hydrogen-bonded systems can in general be described accurately at both the Hartree–Fock (HF) and density functional (DFT) levels of theory. Theoretical work on hydrogen-bonded DNA base pairs has been reviewed extensively.[1c,2] Stacked clusters of aromatic systems, on the other hand, have attracted less attention. Except for the thoroughly studied benzene dimer[3] and stacked DNA base pairs,[2] relatively few papers have been dedicated to the study of oligomers of aromatic molecules.

The benzene aromatic trimer is the only one for which a complete scan of the potential-energy surface has been carried out. Using a combination of molecular dynamics and molecular mechanics (a molecular dynamics/quenching technique), Gonzalez and Lim[4] explored the potential-energy surface of this cluster. After MP2 reoptimization of the minima found on the empirical potential-energy surface, they predicted a symmetric cyclic T-shaped structure as the

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global minimum[5], which agrees with other theoretical works[3,6] as well as with experiment.[7] Trimers of naphthalene and anthracene have also been studied theoretically;[4,8,9] in each case a combination of a stacked pair with a third molecule in a T-shaped orientation was determined to be the most stable arrangement. This finding was recently verified in a spectroscopic experiment by Piuzzi et al.[10] The stability of stacked pyridine trimers has also been recently reported.[11,12]

Since dispersion arises from nonlocal electron correlation, many commonly used DFT functionals fail badly for the description of systems in which the dispersion energy is dominant.[11,13–16] A new generation of meta-hybrid density functionals[17–20] or functionals with an empirical (C_6/R^6) treatment of the dispersion energy[21,22] appear promising, although these methods still need some intensive testing. The explicitly correlated MP2 level is the first acceptable level for the correct description of the dispersion term within the context of wave-function theory, although it has a tendency to slightly overestimate interaction energies and underestimate intermolecular distances.[4,8,9,23,24]

Besides aromatic amino acids and short polypeptides, which have been intensively examined both theoretically and experimentally over the last decade,[25–32] the DNA base trimer is one of the simplest biologically relevant systems that may include both hydrogen-bonding and stacking interactions. To the best of our knowledge, there is only one experimental study of such trimers in the gas phase.[33] In particular, in the late 1970s Yanson et al.[33] used mass spectrometry to determine gas-phase interaction enthalpies from the temperature dependence of equilibrium constants for dimerization and trimerization of particular combinations of bases. Based on the observation that the equilibrium constants were insensitive to N-9 purine methylation and N-1 pyrimidine methylation, Yanson et al.[33] suggested that the trimers adopted planar, cyclic structures characterized by intermolecular hydrogen bonding. Other experimental works have focused primarily on triplexes of DNA oligonucleotides in solution by using NMR or fluorescent techniques.[34–40] The importance of these triplexes in the inhibition of transcription[41] or as therapies for diseases caused by genetic mutation[42] has led to a number of theoretical molecular dynamics studies of such systems[36,37,43,44] as well as quantum chemical calculations of isolated DNA base trimers.[45–51] In the vast majority of these studies, only planar, hydrogen-bonded trimers were considered. This structural motif was selected either based on solution experiments or from chemical intuition.

Recently, however, Cramer, York, and co-workers[51,52] carried out DFT and semiempirical studies that identified a significant discrepancy between theoretical predictions and experiment[33] for the interaction enthalpies of the cyclic, hydrogen-bonded base trimers. Their computational protocol was highly accurate for base pairs, charged trimers, and the guanine tetrad, but predicted much smaller interaction enthalpies for the base trimers than those reported by Yanson et al. On that basis they suggested that some aspect of the experimental situation must be in error; however, as their method was designed to be accurate only for hydrogen-bonded species, and *not* for stacked species, they were unable to assess whether other minima on the trimer potential-energy surface might have interaction enthalpies corresponding to those reported by Yanson et al.

The aim of this paper is to scan thoroughly the potential-energy surface of the DNA base trimers, representing one of the simplest biological systems in which hydrogen-bonded and stacked interactions can appear together. The size of the system is still acceptable for calculating the interaction energies at the highest accessible level of theory. Further, there are also experimental thermodynamics data available, but without satisfactory reliable assignment of structures. The accurate high-level ab initio data that we obtain are used not only to verify/refute the experimental values, but also to test some new DFT functionals, since these functionals have not yet been widely applied to such complicated systems.

Results and Discussion

Potential- and free-energy surfaces of base-pair trimers obtained by the molecular-dynamics/quenching (MD/Q) technique: The total number of structures localized by the MD/Q search for each trimer potential-energy surface (PES) and the respective populations of the various structural motifs are presented in Table 1 (for structures of the bases see Figure 1). On each PES we typically found several hundred minima. The schematic side and top views of typical arrangements of trimers are depicted in Figure 2. Thus, the PESs of the trimers are substantially more complicated than those of the various possible base pairs, for each of which

Table 1. A characterization of the PESs of the studied DNA base trimers. The total number of the structures found and the number of each given structural arrangement and its population [in %] are given. All these data were obtained by the molecular dynamics/quenching technique with the Cornell et al. force field employed in the AMBER program package.

Trimer	Str[a]	HB/HB[b]	HB/S[c]	HB/T[d]	T/T[e]	S/T[f]	S/S[g]
UUU	297	46/63.5	1/0.2	17/1.5	154/23.9	74/10.8	5/0.1
mAmUmU	1188	15/0.5	1/0.3	19/0.8	341/73.1	732/9.7	80/15.6
mUmUmU	452	17/0.4	2/0.1	9/0.6	212/71.2	200/21.5	12/5.7
mTmUmU	1234	28/0.3	3/0.1	19/0.3	518/70.5	626/20.7	40/81
mCmCmC	871	8/0.1	7/0.1	15/0.5	398/65.2	389/30.8	54/3.3

[a] Total number of structures found at the PES of DNA base trimer. [b–g] Number of structures/population found for the structural arrangement. [b] All three bases in the planar hydrogen-bonded arrangement. [c] The planar hydrogen-bonded base pair: the third base is stacked above one or both bases. [d] The planar hydrogen-bonded base pair: the third base is oriented T-shaped to the pair. [e] No stacked, no hydrogen-bonded arrangement is observed is such a complex. [f] Stacked base pair: the third base is oriented T-shaped to the pair. [g] All three bases in the stacked arrangement.

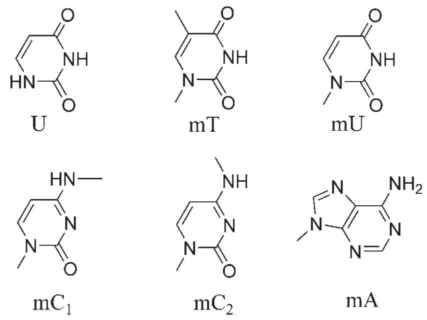

Figure 1. Two-dimensional schematic representations of uracil (U), 1-methylthymine (mT) 1-methyluracil (mU), two rotamers of (N_1, N_4)-dimethylcytosine (mC$_1$, mC$_2$), and 9-methyladenine (mA).

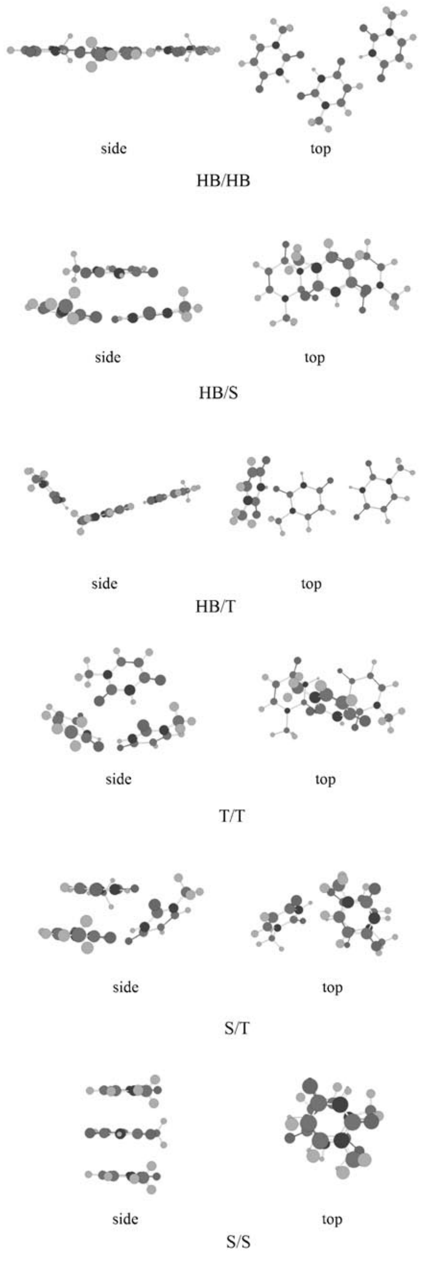

Figure 2. The schematic side and top views of possible arrangements of base trimers.

about 20 structures have been identified previously by the same protocol.[53]

The PES of UUU shows completely different properties from the PESs of the methylated trimers; this will be discussed separately. Although the largest number of structures found (154) corresponded to the T/T (T = T-shaped) arrangement, the total population of this arrangement was only the second largest (24 %), while the planar HB/HB (HB = hydrogen-bonded) arrangement, comprising only 46 structures, was the most populated one (64 %). With the exception of the S/T (S = stacked) arrangement (74 structures, population 10 %), the other structural classes were negligibly represented.

Among the twenty most stable structures, ten were planar HB/HB trimers. However, the global minimum corresponds to the T/T arrangement (cf. structure T/T 1 in Figure 3a) reminiscent of the global minimum for the benzene trimer.[3] The energy difference between the global minimum and the nineteenth higher local minimum is less than 3 kcal mol^{-1}.

We found all of the methylated systems that we studied (mUmUmU, mTmUmU, mAmAmA, mCmCmC) to exhibit very similar behavior with respect to their PESs. S/T and T/T arrangements comprised the largest numbers of structures and also dominated the structural populations (around 70 % for T/T and 20 % for S/T). A significant increase in the population of the S/S structures was observed; for example, 5.7 % for mUmUmU compared to 0.1 % for UUU. Trimers containing a hydrogen-bonded pair (i.e., HB/HB, HB/T, HB/S arrangements) were negligibly populated, which argues against the hypothesis of Yanson et al.[33] that the most stable structures of the methylated base trimers should correspond to cyclic, planar, hydrogen-bonded structures. For all of the methylated trimers, the first hundred of the most stable structures belonged exclusively to S/T or T/T arrangements. The HB/HB mAmUmU structure suggested as the most stable one by Yanson et al.[33] was found as the 274th local minimum, far from the stability of the global minimum. We did not succeed in finding an analogous planar structure for the mCmCmC trimer and attempts to optimize this structure by using the Cornell et al. force field[54] (or ab initio theory) led to a completely different geometry.

Investigating the populations of the individual structures, we found no structure on any PES with a population greater than 8 %. There is little energy separation between the most stable structures and the remaining ones, and interaction energies change in a nearly continuous fashion along the whole family of structures. The experimental stabilization enthalpy thus cannot be taken to correspond to the stabilization enthalpy of a single structure, but rather to an averaged value for an ensemble of structures.

Geometrical and energetical properties of the structures—a comparison of ab initio and empirical results: We observed that the accurate RI-MP2 quantum chemical calculations verified the empirical results not only qualitatively (the optimized ab initio geometries are similar to the empirical

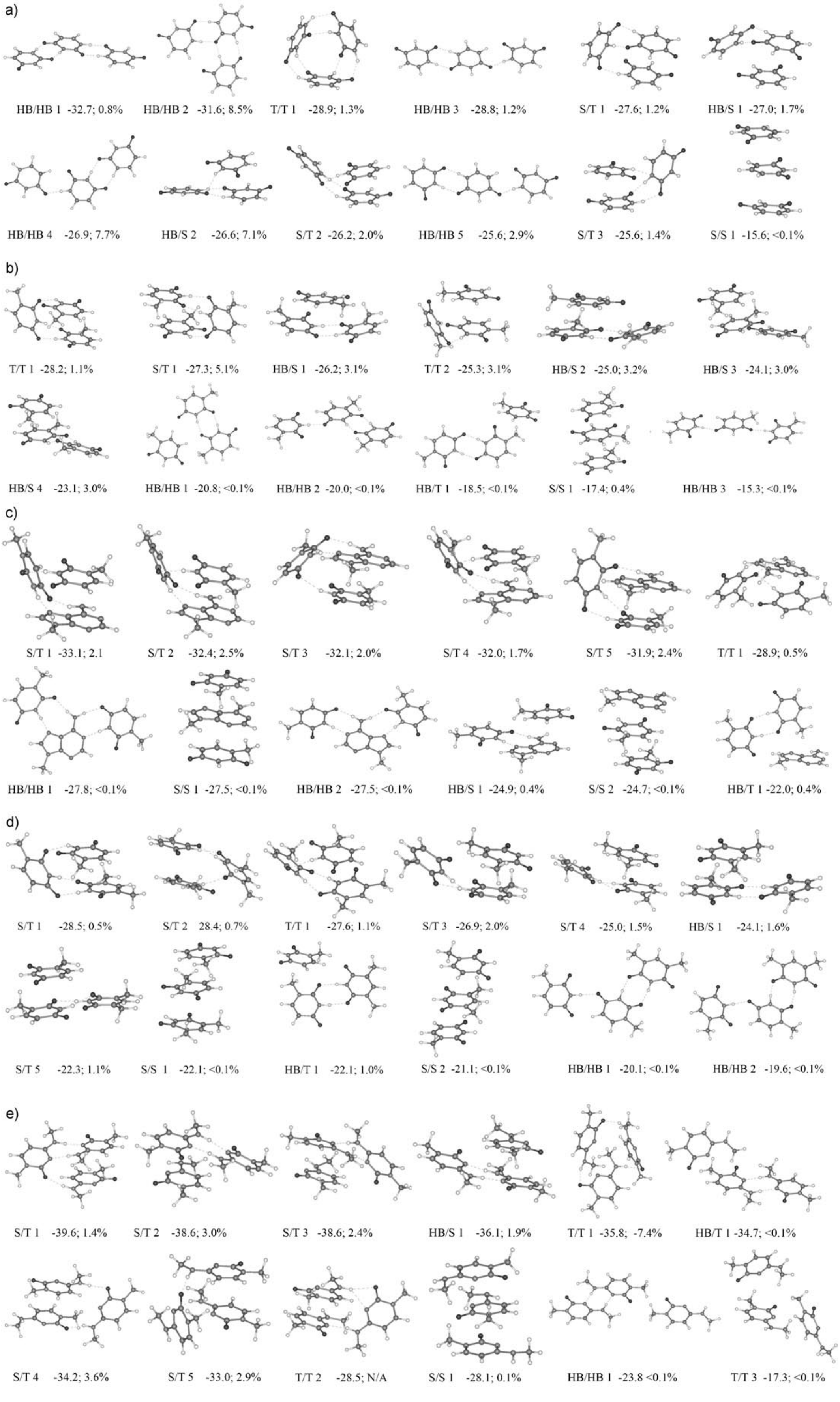
a)
HB/HB 1 -32.7; 0.8%
HB/HB 2 -31.6; 8.5%
T/T 1 -28.9; 1.3%
HB/HB 3 -28.8; 1.2%
S/T 1 -27.6; 1.2%
HB/S 1 -27.0; 1.7%
HB/HB 4 -26.9; 7.7%
HB/S 2 -26.6; 7.1%
S/T 2 -26.2; 2.0%
HB/HB 5 -25.6; 2.9%
S/T 3 -25.6; 1.4%
S/S 1 -15.6; <0.1%
b)
T/T 1 -28.2; 1.1%
S/T 1 -27.3; 5.1%
HB/S 1 -26.2; 3.1%
T/T 2 -25.3; 3.1%
HB/S 2 -25.0; 3.2%
HB/S 3 -24.1; 3.0%
HB/S 4 -23.1; 3.0%
HB/HB 1 -20.8; <0.1%
HB/HB 2 -20.0; <0.1%
HB/T 1 -18.5; <0.1%
S/S 1 -17.4; 0.4%
HB/HB 3 -15.3; <0.1%
c)
S/T 1 -33.1; 2.1
S/T 2 -32.4; 2.5%
S/T 3 -32.1; 2.0%
S/T 4 -32.0; 1.7%
S/T 5 -31.9; 2.4%
T/T 1 -28.9; 0.5%
HB/HB 1 -27.8; <0.1%
S/S 1 -27.5; <0.1%
HB/HB 2 -27.5; <0.1%
HB/S 1 -24.9; 0.4%
S/S 2 -24.7; <0.1%
HB/T 1 -22.0; 0.4%
d)
S/T 1 -28.5; 0.5%
S/T 2 28.4; 0.7%
T/T 1 -27.6; 1.1%
S/T 3 -26.9; 2.0%
S/T 4 -25.0; 1.5%
HB/S 1 -24.1; 1.6%
S/T 5 -22.3; 1.1%
S/S 1 -22.1; <0.1%
HB/T 1 -22.1; 1.0%
S/S 2 -21.1; <0.1%
HB/HB 1 -20.1; <0.1%
HB/HB 2 -19.6; <0.1%
e)
S/T 1 -39.6; 1.4%
S/T 2 -38.6; 3.0%
S/T 3 -38.6; 2.4%
HB/S 1 -36.1; 1.9%
T/T 1 -35.8; -7.4%
HB/T 1 -34.7; <0.1%
S/T 4 -34.2; 3.6%
S/T 5 -33.0; 2.9%
T/T 2 -28.5; N/A
S/S 1 -28.1; 0.1%
HB/HB 1 -23.8 <0.1%
T/T 3 -17.3; <0.1%

Table 2. Energetical characterization of the most stable and populated UUU trimers. All energy values are in kcal mol^{-1}.

Motif[a]	AMB Ord[b]	Pop[c]	AMB Ene[d]	MP2/OP[e]	W91/SP[f]	W91/OP[g]	PWB/SP[h]	M05/SP[h]
HB/HB 1	2.	0.8	−30.3	−32.7	−26.3	−30.0	−32.3	−32.3
HB/HB 2	3.	8.5	−30.3	−31.6	−22.9	−26.0	−31.3	−31.1
T/T 1	1.	1.3	−30.8	−28.9	−14.1	−16.9	−22.0	−25.6
HB/HB 3	37.	1.2	−26.4	−28.8	−21.9	−25.2	−28.7	−28.8
S/T 1	7.	1.2	−28.4	−27.6	−7.7	−15.8	−21.4	−25.1
HB/S 1	16.	1.7	−27.6	−27.0			−22.3	−23.5
HB/HB 4	96.	7.7	−24.5	−26.9			−26.5	−26.8
HB/S 2	13.	7.1	−27.8	−26.6	−6.5	[i]	−21.6	−23.9
S/T 2	14.	2.0	−27.6	−26.2			−22.4	−23.6
HB/HB 5	116.	2.9	−23.9	−25.6			−25.1	−25.4
S/T 3	19.	1.4	−27.5	−25.6			−19.0	−21.9
S/S 1	285.	< 0.1	−17.3	−15.9	10.3	[i]	−9.5	−11.8

[a] Structural arrangement of the complex; the number after the arrangement means the stability order of the complex in given class calculated at RI−MP2/TZVPP level. [b] Stability order of the structure at the whole empirical PES. [c] Percentage population of the structure at the whole empirical PES. [d] Interaction energy calculated with the Cornell et al. force field. [e] Total interaction energy (BSSE corrected, deformation energy included) obtained at the RI−MP2/TZVPP//RI−MP2/cc−pVDZ level. [f] Total BSSE-corrected interaction energy obtained at the DFT level, MPWPW91 functional, MIDI! basis set. A single-point calculation using optimized RI−MP2/cc−pVDZ geometry. MIDI! basis set. [g] Total BSSE-corrected interaction energy obtained at the DFT level, MPWPW91 functional. Fully relaxed system, optimization started from the RI−MP2/cc−pVDZ geometry. MIDI! basis set. [h] A single-point calculation using optimized PWB6K/MIDI! or M052X/MIDI! geometry with 6−311+G(2df,2p) basis set. [i] Not stationary.

ones), but also quantitatively (there exists a close correlation between both stabilization energies).

All the optimized structures are shown in Figures 3a–e. The structural motif in most cases remained unchanged during the ab initio optimization: only two structures changed their arrangement, from T/T to S/T. The root-mean-square deviations (RMSD) between the empirical and ab initio geometries were about 0.1 Å for the planar hydrogen-bonded structures, while for the other arrangements the RMSD values were slightly higher (averaging about 0.3 Å). The differences between the lengths of ab initio and empirical hydrogen bonds in the HB motifs did not exceed 0.1 Å in most cases. A difference not exceeding 10° was found for most deviations of the planes of the bases in T/T and S/T arrangements and only in few cases was this difference greater than 20°. The higher RMSDs for the structures containing one or more T-shaped pairs can be explained by the almost barrierless rotations of the bases about the intermolecular axis in this motif.[55]

The energies of the structures studied at the ab initio level can be found in Tables 2–6. The empirical interaction energies are in excellent agreement with RI-MP2/TZVPP interaction energies; the energy difference is usually smaller than 2 kcal mol^{-1} (averaged energy differences and standard deviations for all trimers can be found in Table 7). A significant discrepancy between ab initio and empirical energies was found for only two structures out of sixty: S/S 1 and HB/HB 3 of the mUmUmU trimer had energy differences in excess of 5 kcal mol^{-1}. (see Table 3). It must be stressed here that while the empirical calculations take only seconds of CPU time, the single-point RI-MP2/TZVPP calculations require approximately one day of CPU time.

Table 3. Energetical characterization of the most stable and populated mUmUmU trimers. The column descriptions are the same as for Table 2.

Motif	AMB Ord	Pop	AMB Ene	MP2/OP
T/T 1	1.	1.1	−28.6	−28.2
S/T 1	4.	5.1	−28.4	−27.3
HB/S 1	6.	3.1	−27.7	−26.2
T/T 2	26.	3.1	−25.0	−25.3
HB/S 2	11.	3.2	−27.5	−25.0
HB/S 3	41.	3.0	−26.1	−24.1
HB/S 4	48.	3.0	−26.7	−23.1
HB/HB 1	240.	< 0.1	−26.3	−20.8
HB/HB 2	356.	< 0.1	−23.0	−20.0
HB/T 1	375.	< 0.1	−25.7	−18.5
S/S 1	336.	0.4	−25.9	−17.4
HB/HB 3	427.	< 0.1	−25.5	−15.4

Figure 3. a) The uracil trimer complex; ball and stick representation of twelve structures optimized at the ab initio RI-MP2/cc-pVDZ level. b) The 1-methyluracil trimer complexes. c) The complexes combined from one molecule of 1-methyladenine and two molecules of 1-methyluracil. d) The complexes combined from one molecule of 1-methylthymine and two molecules of 1-methyluracil. e) The (N_1, N_4)-dimethylcytosine complexes. The descriptions below the structures show the structural arrangement (see comments to Table 2), the interaction energies in kcal mol^{-1} (BSSE and deformation energy included) obtained at the RI-MP2/TZVPP//RI-MP2/cc-pVDZ level, and the percentage population of the structure at the empirical PES. Oxygen atoms are colored black, nitrogen atoms are light grey, carbon atoms are dark grey, and hydrogen atoms are white. For an easier comparison of the different structures the position of one of the uracils is kept in the same orientation, except those structures for which such an orientation did not allow a clear view of the spatial arrangement of the bases.

We now characterize the PESs of each trimer in more detail. On the PES of UUU, the global minimum corresponds to the partially open HB/HB structure. The cyclic HB/HB structure, which is the most populated one, follows, and is only 1 kcal mol^{-1} less stable than the global minimum. This observation contrasts with the generally held opinion that cyclic HB/HB structures are more stable than open ones.[46] The next local minimum (found as a global minimum on the empirical PES) corresponds to the cyclic T-shaped structure. Other structural arrangements of these trimers are 5–7 kcal mol^{-1} less stable than the global minimum. The S/S 1 structure is far less stable, suggesting a strong energetic bias against UUU stacked trimers compared to hydrogen-bonded ones in the gas phase.

Table 4. Energetical characterization of the most stable and populated mAmUmU trimers. The column descriptions are the same as for Table 2.

Motif	AMB Ord	Pop	AMB Ene	MP2/OP
S/T 1	2.	2.1	−32.5	−33.1
S/T 2	4.	2.5	−32.0	−32.4
S/T 3	6.	2.0	−32.0	−32.1
S/T 4	8.	1.7	−31.6	−32.0
S/T 5	1.	2.4	−32.5	−31.9
T/T 1	10.	0.5	−31.3	−28.9
HB/HB 1	285.	<0.1	−24.0	−27.8
S/S 1	173.	<0.1	−25.0	−27.5
HB/HB 2	275.	<0.1	−24.1	−27.5
HB/S 1	295.	0.3	−23.8	−24.9
S/S 2	338.	<0.1	−23.5	−24.7
HB/T 1	412.	0.4	−21.9	−22.0

Table 5. Energetical characterization of the most stable and populated mTmUmU trimers. The column descriptions are the same as for Table 2.

Motif	AMB Ord	Pop	AMB Ene	MP2/OP
S/T 1	4.	0.5	−29.0	−28.5
S/T 2	1.	0.7	−29.1	−28.4
T/T 1	10.	1.1	−28.6	−27.6
S/T 3	42.	2.0	−26.6	−26.9
S/T 4	61.	1.5	−26.2	−25.0
HB/S 1	122.	1.6	−25.4	−24.1
S/T 5	169.	1.1	−24.8	−22.3
S/S 1	533.	<0.1	−22.3	−22.1
HB/T 1	380.	1.0	−23.0	−22.1
S/S 2	514.	<0.1	−22.4	−21.1
HB/HB 1	994.	<0.1	−20.3	−20.1
HB/HB 2	1023.	<0.1	−19.5	−19.6

Table 6. Energetical characterization of the most stable and populated mCmCmC trimers. The column descriptions are the same as for Table 2. All possible combinations of two different rotamers of mC were considered.

Motif	AMB Ord	Pop	AMB Ene	MP2/OP
S/T 1[d]	1.	1.4	−39.0	−39.6
S/T 2[b]	2.	3.0	−37.9	−38.6
S/T 3[b]	1.	2.4	−38.2	−38.6
HB/S 1[b]	3.	1.9	−35.9	−36.1
T/T 1[a]	1.	7.4	−37.4	−35.8
HB/T 1[b]	80.	<0.1	−31.9	−34.7
S/T 4[c]	1.	3.6	−35.6	−34.2
S/T 5[c]	3.	2.9	−32.3	−33.0
T/T 2[a]	–[d]	–[d]	−26.4	−28.5
S/S 1[a]	103.	<0.1	−29.3	−28.1
HB/HB 1[a]	144.	<0.1	−23.6	−23.8
T/T 3[b]	203.	<0.1	−20.3	−17.3

[a] Complex combined from the three most stable rotamers of mC (3× mC1 cf. Figure 1). [b] Complex combined from the two most stable rotamers of mC and one less stable (2× mC1 , 1× mC2). [c] Complex combined from the most stable rotamer of mC and two less stable (1× mC1 , 2× mC2). [d] Structure suggested as the most stable in Ref. [33], not found by us in the PES. Optimization of such a structure led to a different structure, whose energetical properties are shown.

The PESs of all four methylated systems continue to be completely different from UUU. The global minimum and first few higher local minima correspond to S/T and T/T arrangements, in agreement with the empirical results. The most stable HB/HB structures are 6–8 kcal mol^{-1} less stable than the global minima, and S/S arrangements, poorly represented in the UUU trimer, now show stabilities comparable to the most stable HB/HB structures.

Table 7. Weighted means of the empirical interaction energies over all structures found on the PESs of the trimers. All energetical characteristics are in kcal mol^{-1}.

Base trimer	AMBER mean[a]	Corr. to MP2[b]	Final E[c]	Exp.[d]
UUU	−25	0 ± 2	−25 ± 2	N/A
mUmUmU	−23	−3 ± 3	−20 ± 3	−20–22 ± 4
mAmUmU	−25	+1 ± 2	−26 ± 2	−27–29 ± 4
mTmUmU	−24	−1 ± 1	−23 ± 1	−23–25 ± 4
mCmCmC[e]	−35	−1 ± 2	−34 ± 2	−33–38 ± 4

[a] Weighted means (by populations) of the empirical interaction energies over all structures found on the PESs of the trimers. [b] Averaged energy difference and the standard deviation between twelve calculated empirical and RI−MP2/TZVPP interaction energies (see Tables 2–6). [c] Weighted mean corrected by [b]. [d] Experimental entropy value taken from reference [33]. [e] Averaged over all possible combinations of rotamers of mC.

How may one explain such a significant difference in the behavior of the methylated and non-methylated systems? The most important factor is the replacement of three hydrogen atoms that can be hydrogen-bond donors with non-polar methyl groups. With the loss of sites for hydrogen bonding, as well as an increase in the polarizability of the system, and, comparing UUU to mUmUmU, the loss of most stable structures owing simply to steric interactions introduced with the methyl groups, the situation changes so that dispersion energy becomes dominant in binding the methylated trimers. Similar conclusions have been reached for methylated and non-methylated base pairs.[56,57] The only difference between the behavior of the base pairs and trimers is that in the base pairs the methylation leads directly to a preference for stacked structures, while for the trimers it leads to a preference for S/T and T/T structures, which is consistent with the most stable structures observed for trimers of aromatic compounds similarly lacking in hydrogen-bonding functionality, for example, benzene, naphthalene, and anthracene.[3,9,10] However, since the dispersion interactions in the S/T and T/T structures can be augmented by hydrogen-bonding interactions in certain arrangements, the interaction energies of the methylated base trimers are substantially higher than those for the simple aromatic trimers.[3,9,10]

If we compare the stability of the global minima of all of the systems studied here, we have the following order: mUmUmU (−28.2) ≈ mTmUmU (−28.5) < UUU (−32.7) ≈ mAmUmU (−33.1) < mCmCmC (−39.6). The numbers in parentheses correspond to the interaction energies in kcal mol^{-1}. From this order it is evident that methylation of UUU destabilizes the system by about 4 kcal mol^{-1}. As noted above, the main reason for this seems to be a loss of the most stable hydrogen-bonded systems by replacement of the hydrogen-bond donor positions with methyl groups.

Comparison of theoretical results with experimental values: As noted in the previous sections, no single dominant structure exists on the PESs of any of the systems studied. Hence we cannot assign the experimentally measured stabilization enthalpy to a single structure. To compare the theoretical data to experimental data we must calculate a weighted mean interaction enthalpy over the complete set of structures. The statistical weight of each structure is given by its population determined on the empirical PES. Since we could not practically reoptimize all of the trimers found at the empirical level at the ab initio level, we first estimate an error, which we introduce by considering the empirical, instead of the more exact ab initio, interaction energies. However, as has already been discussed, the differences between the empirical and ab initio energies are quite small (1–2 kcal mol^{-1}). The statistical expression of this error and the comparison of our results with the experimental ones can be found in Table 7.

From Table 7 it is clear that we reproduce the order of stability of the trimers found experimentally: mUmUmU < mTmUmU < mAmUmU ⋘ mCmCmC. Moreover, we obtain excellent quantitative agreement between the theoretical and experimental values. Our theoretical values of the interaction energies (mUmUmU, -20 ± 3 kcal mol^{-1}; mTmUmU, -23 ± 1 kcal mol^{-1}; mAmUmU, -26 ± 2 kcal mol^{-1}; mCmCmC, -34 ± 2 kcal mol^{-1}) lie within the range of reported experimental interaction enthalpies in every case (mUmUmU, −20–22 kcal mol^{-1}; mTmUmU, −23–25 kcal mol^{-1}; mAmUmU, −27–29 kcal mol^{-1}; mCmCmC, −33–38 kcal mol^{-1}). All the experimental data were reported with an error of ±4 kcal mol^{-1}.

How large a difference is there between the interaction energy and enthalpy? If we want to pass from interaction energies to enthalpies, we must first include the zero-point vibrational energy (ZPVE). For the UUU trimer, the ZPVE remains almost constant (±2 kcal mol^{-1}, cf. Table 8) over most of the structures, with the exception of the S/S structure, for which ZPVE is slightly smaller. A scaling factor of 0.965 was applied to the computed vibrational frequencies in all cases, as has been previously discussed for nucleic acid base pairs.[58] The sum of the interaction energy and the ZPVE gives us the enthalpy at 0 K. An inclusion of thermal corrections yields the enthalpy at 298 K. From Table 8 it is evident that the 0 K and 298 K enthalpies are very close (the difference is smaller than 1 kcal mol^{-1}) in all cases.

Table 8. UUU trimer. Sensitivity of the calculated interaction energies on the size of the basis set; thermodynamics data. All values in kcal mol^{-1}.

Motif	aDZ[a]	aTZ[b]	CBS[c]	Cor.[d]	ZPVE[e]	ΔH_0[f]	ΔH_0^{298}[g]
HB/HB 1	−32.4	−35.8	−37.0	−0.8	2.3	−35.5	−35.0
HB/HB 2	−32.6	−35.5	−36.5	−0.9	2.3	−35.2	−34.6
T/T 1	−29.4	−34.8	−36.4	−0.2	2.4	−34.1	−33.5
HB/HB 3	−28.5	−31.9	−32.6	−0.9	2.2	−31.4	−30.6
S/T 1	−25.1	−31.9	−34.1	0.9	1.9	−31.3	−30.2
HB/S 1	−25.8	−31.6	−33.8	1.0	1.9	−30.9	−30.2
HB/T 1	−25.0	−31.3	−33.3	0.7	1.8	−30.7	−29.9
S/S 1	−12.1	−18.7	−22.8	2.7	1.1	−19.1	−18.6

[a] Total interaction energy obtained at the RI−MP2/aug−cc−pVDZ//RI−MP2/cc−pVDZ level. [b] Total interaction energy obtained at the RI−MP2/aug−cc−pVTZ//RI−MP2/cc−pVDZ level. [c] Total interaction energy extrapolated to the infinite basis set. [d] (ΔE CCSD(T)−ΔE MP2) correction term obtained as a difference between the CCSD(T)/6−31G*//RI−MP2/cc−pVDZ and MP2/6−31G*//RI−MP2/cc−pVDZ interaction energies. [e] Zero-point vibration energy, scaled by a factor of 0.965. [f] Enthalpy at 0 K, calculated as a sum of the interaction energy extrapolated to the infinite basis set and the zero-point vibration energy. [g] Enthalpy at 298 K.

Sensitivity of the interaction energy to the size of the basis set: The interaction energies obtained with the cc-pVDZ basis set (results not shown) are systematically much smaller (by 5 kcal mol^{-1} for the structures containing exclusively hydrogen-bonded and T-shaped motifs and by 10–12 kcal mol^{-1} for the structures possessing at least one base pair in the stacked arrangement) than those obtained with the TZVPP basis set. It is thus evident that a DZ basis set is insufficient for a correct and accurate description of the stabilization energies, especially of the S/S and S/T structures of the base trimers.

MP2 calculations for such systems with basis sets larger than TZP are impractical. Nevertheless, for the smallest trimer, UUU, we calculated also the RI-MP2 interaction energies extrapolated to the CBS limit (cf. Table 8). A two-point extrapolation was made from the aug-cc-pVDZ and the aug-cc-pVTZ energies. The differences between the TZVPP interaction energies and values at the CBS limit ranged between 4–6 kcal mol^{-1} independently of the structural arrangement. It must be stressed that the stability order of the structures calculated using the TZVPP basis set remained unchanged when passing to the CBS limit.

The aug-cc-pVDZ basis set provides results almost identical with those from the TZVPP basis set. The interaction energies calculated employing the aug-cc-pVTZ basis set are only 1–2 kcal mol^{-1} smaller than those at the CBS limit.

The ($\Delta E_{\mathrm{CCSD(T)}}-\Delta E_{\mathrm{MP2}}$) correction terms determined with the 6–31G(d) (0.25) basis set are also presented in Table 8. A smaller basis set than for RI-MP2 calculations was used because the ($\Delta E_{\mathrm{CCSD(T)}}-\Delta E_{\mathrm{MP2}}$) correction term is almost independent of the size of the basis set.[59] As expected, the ($\Delta E_{\mathrm{CCSD(T)}}-\Delta E_{\mathrm{MP2}}$) correction term is small (−1 kcal mol^{-1}) for the HB/HB structures, which means that the RI-MP2 stabilization energies are still slightly underestimated in comparison with the CCSD(T) ones. The ($\Delta E_{\mathrm{CCSD(T)}}-\Delta E_{\mathrm{MP2}}$) correction term is positive and rather large (+2.7 kcal mol^{-1}) for the S/S structures, which also agrees with our previous results. For systems containing both hydrogen-bonded and stacked bases (HB/S, S/T) the value of the ($\Delta E_{\mathrm{CCSD(T)}}-\Delta E_{\mathrm{MP2}}$) correction term is slightly positive (+1 kcal mol^{-1}).

Thus, going from the TZVPP basis set to a complete basis systematically increases the interaction energy in each trimer by 4–6 kcal mol^{-1}. The ($\Delta E_{\mathrm{CCSD(T)}}-\Delta E_{\mathrm{MP2}}$) correction term depends on the structural arrangement of the system, but the effect is modest (from −1 to +1 kcal mol^{-1}). Taking the ZPVE term into account leads to a systematic destabilization of each structure by about 2 kcal mol^{-1}. The thermal contribution is negligible. Putting all of these effects together we conclude that the RI-MP2/TZVPP stabilization energies are approximately 3 kcal mol^{-1} smaller than the 298 K enthalpies with a complete basis and the energy order of our structures will remain unchanged.

We anticipate that this same analysis will hold for the methylated trimers. Adding this difference to the weighted averaged interaction energies of the methylated trimers (cf. Table 7) the theoretical and experimental values would remain in outstanding quantitative agreement.

New DFT functionals: Structures obtained using *m*PWPW91/MIDI!, a method previously noted by Cramer, York, and co-workers to do well for hydrogen-bonded base clusters compared variably to those found on the MD/Q PES and subsequently reminimized at the RI-MP2 level. The agreement between minimized structures was found to be dependent upon the nature of the conformation, with structures characterized by tight hydrogen bonds being much more faithfully reproduced by using the fast DFT methodology than other kinds of structures. This result is expected given the known successes and deficiencies, respectively, of older DFT models in handling hydrogen bonding compared to dispersion. In the case of UUU, seven conformations (see Table 1) were optimized. Five of these structures minimized to give small RMSDs (0.06, 0.09, 0.13, 0.14, and 0.70 Å), while two structures had large geometric deviations from their initial structures (RMSD values of 2.15 and 2.87 Å, respectively). The first highly deviant structure was HB/T1, which minimized to an HB/HB arrangement with *m*PWPW91/MIDI!. The second highly deviant structure was S/S1, which also optimized to an HB/HB configuration, albeit a different one from the first case. All structures for which the motifs were conserved at the *m*PWPW91/MIDI! level had stronger networks of hydrogen bonds relative to the two deviant cases. When the trimer includes stacking, the *m*PWPW91/MIDI! PES appears to be featureless with respect to this interaction, and it is lost in favor of hydrogen bonding when such an interaction can be realized.

Single-point interaction energies computed with BSSE correction do not agree particularly well with the best ab initio energies, with especially large deviations being observed for those structures with S or T motifs. Relaxation of the geometries leads to semiquantitative agreement for the HB/HB structures, but errors remain large for T/T1 and S/T1, and HB/T1 and S/S1 are not stationary.

Given the large number of structures not well characterized as HB/HB in nature, we decided to consider the recently developed PWB6K and M052X functionals, both of which were designed to be accurate not only for hydrogen bonding interactions, but also for dispersion interactions like those that are present in the trimers with T or S interactions. In contrast to the *m*PWPW91 level, the overall character of all structures was maintained when either of these density functionals was used for geometry optimization with the MIDI! basis set. Indeed, the average RMS error between the DFT structures and the MP2 structures for the 12 UUU trimers reported in Table 2 was only 0.13 Å for PWB6K and 0.19 Å for M052X.

When interaction energies are computed for the PWB6K/MIDI! or M052X/MIDI! geometries (minimizations of RI-MP2 input geometries) at the PWB6K and M052X levels with the 6–311+G(2df,2p) basis set, the agreement with RI-MP2/TZVPP energies is extremely good. Since the MIDI! geometries and the RI-MP2 geometries are so similar, we would expect to see equally good energetic agreement between the RI-MP2/TZVPP interaction energies and single points performed on the RI-MP2 geometries using the 6–311+G(2df,2p) set at the PWB6K and M052X levels. The RMS errors between PWB6K and M052X and RI-MP2 for the 11 interaction energies reported in Table 2 are 4.2 and 2.3 kcal mol^{-1}, respectively. The PWB6K and M052X results are very similar for HB/HB structures, but M052X performs better for structures with S or T interactions, although the error still tends to be in the direction of underestimation of the interaction energy in all cases.

For the methylated trimers, we did not do an exhaustive study of geometries and interaction energies with the PWB6K and M052X functionals, but we did examine their ability to predict the nature of S/S structures. For the S/S2 mAmUmU, S/S1 mCmCmC, S/S2 mTmUmU, and S/S1 mUmUmU structures listed in Tables 3–6, the average RMS error between the PWB6K/MIDI! and M052X/MIDI! structures and the RI-MP2 structures was 0.18 and 0.25 Å, respectively. The S/S character was maintained in every case. Interestingly, when the PWB6K functional was used for geometry optimization with the cc-pVDZ basis set instead of the MIDI! basis set, the average RMS error compared to the RI-MP2 structures increased to 0.23 Å. The MIDI! basis set was developed specifically for use in geometry optimization, and it seems to continue to work particularly well for that purpose with these recent functionals.

It appears that the combination of PWB6K/MIDI! geometries with M052X/6–311+G(2df,2p) single-point energies might be a very efficient approach for the computation of interaction energies between nucleic acid bases of arbitrary geometry. Future investigation may wish to examine this point more closely.

Conclusion

Trimer PESs: Several hundreds of energy minima were identified on the various trimer PESs, making the number of minima on these surfaces about an order of magnitude larger than is found for base pair PESs (which typically are characterized by about 20 minima). No structures on the trimer PESs were found to dominate the 298 K equilibrium population significantly, indicating that weighted averages of the interaction enthalpies over all structures should be used when comparing theoretical and experimental results.

Dominant structures: The structural motifs identified as being dominant for the non-methylated trimer (UUU) differed significantly from those identified for the methylated trimers. For UUU the most stable and populated structures were planar and hydrogen bonded, while for the methylated trimers the most stable structures belonged to the S/T and T/T motifs, consistent with the analogous situation observed for the nonpolar aromatic compounds benzene, naphthalene and anthracene.[3,9,10] The replacement of three polar hydrogen atoms by methyl groups in the methylated monomers decreases the polarity and increases the polarizability of these bases, and this decisively affects the preferred trimer structures.

Comparison of empirical and theoretical results: By comparing the empirical and ab initio results we can conclude that the Cornell et al. force field reproduces the ab initio data not only qualitatively (the match between the geometries of the optimized structures), but also quantitatively (the difference between the interaction energies does not exceed 1–2 $\text{kcal}\,\text{mol}^{-1}$ in most cases).

Trimerization enthalpies: We successfully reproduced the experimental trimerization enthalpies reported for all four methylated systems studied by Yanson et al.[33] We verified that the stability order of the trimers increases in the order $\text{mUmUmU} < \text{mTmUmU} < \text{mAmUmU} \ll \text{mCmCmC}$. Furthermore, we found that the calculated interaction energies agreed well with the experimental enthalpies, suggesting that the time-consuming step of computing vibrational frequencies in order to compute enthalpies may be avoided in the interest of efficiency without introducing significant error.

The *m*PWPW91/MIDI! model: Cramer and co-workers were correct that the experimental results of Yanson[33] could not correspond to hydrogen-bonded structures. However, because their *m*PWPW91/MIDI! model was optimized only for hydrogen-bonding interactions, they were unable to identify that stacked geometries were consistent with the reported interaction enthalpies.

The PWB6K and M052X functionals: The newly developed PWB6K and M052X functionals perform extremely well with respect to the trimers studied here. The PWB6K functional when combined with the MIDI! basis set predicts geometries in very good agreement with RI-MP2; M052X is almost as good. For interaction energies, single-point calculations with these functionals and the 6–311+G(2df, 2p) basis set are also in very good agreement with RI-MP2/TZVPP, indicating that the functionals do a good job of accounting for dispersion interactions in complexes not entirely characterized by hydrogen-bonding interactions. MO52X is more accurate than PWB6K in this regard, although a small systematic underestimation of dispersion interactions remains.

Computational Methods

Molecular dynamics/quenching (MD/Q) technique: Using the MD/Q technique introduced elsewhere[60] and employing the empirical force field of Cornell et al.[54] with standard parameters, we characterized the potential-energy surface (PES) of the uracil trimer (UUU), the smallest trimer of nucleic acid bases, and four trimers for which experimental data are available, namely the 1-methyluracil trimer, (mUmUmU), the 1,4-dimethylcytosine trimer (mCmCmC), a heterotrimer containing one molecule of 9-methyladenine and two molecules of 1-methyluracil (mAmUmU), and a heterotrimer containing one molecule of 1-methylthymine and two molecules of 1-methyluracil (mTmUmU) (see Figure 1). The Cornell et al. force field has been shown to provide reliable results for nucleic acid base pairs in both stacked and hydrogen-bonded motifs.[53,61,62] For mCmCmC all four possible combinations of two rotamers of mC (mC_1 and mC_2) were considered (cf. Figure 1). All simulations were performed in the NVE microcanonical ensemble (N, V, and E refer to the number of particles, the volume, and the total energy, respectively), with the total energy selected to corresponded to a temperature of 298 K. This temperature agrees with that used for the experimental gas-phase measurements,[33] and moreover it was high enough to overcome all relevant energy barriers to allow complete sampling of the conformational PES. The total length of each simulation was 250 ns. Populations of the structures correspond to the number of times a given structure was generated from the minimization of a particular MD/Q starting point.

Ab initio quantum chemical calculations: After locating all the energy minima using the MD/Q method, reoptimizations of the twelve most stable and most populated structures representing all possible structural classes were undertaken at the resolution of identity (RI-) MP2 level by using a DZ+P (cc-pVDZ [3s2p1d/2s1p]) basis set with a standard (default) auxiliary basis set. Interaction energies, being more sensitive to the quality of the calculation than geometries, were determined at the RI-MP2/TZVPP [5s3p2d1f/3s2p1d]//RI-MP2/cc-pVDZ level of theory. All RI-MP2 calculations were carried out using the TURBOMOLE 5.7 package.[63] To test the dependency of the interaction energies on the size of the basis set, the interaction energies for selected structures of UUU were extrapolated to the complete basis set limit (CBS). The CBS energies were determined by Helgaker's two-point extrapolation[64] by using aug-cc-pVXZ basis sets for X=D and T, as described elsewhere.[59] The ($\Delta E_{\text{CCSD(T)}} - \Delta E_{\text{MP2}}$) correction term,[59] which is practically basis set independent, was evaluated with the 6–31G(d) (0.25) basis set. All the CCSD(T) calculations were carried out using the MOLPRO program.[65]

Density functional levels of theory: The geometries of all nucleic acid base trimers (and corresponding base monomers) located on the MD/Q PES and minimized at the RI-MP2 level were re-optimized by using DFT in conjunction with the MIDI! basis set.[66] Optimizations were carried out using *m*PWPW91, previously identified to reproduce gas-phase base-pairing energetics well after correction for the basis-set-superposition error (see below).[51,52] This functional employs the gradient corrected exchange[67] and correlation[68] functionals of Perdew and co-workers, as modified by Adamo and Barone.[69]

Additional optimizations of the base trimers were carried out by using the DFT functionals PWB6K and MO5–2X, described by Truhlar et al.[19,70] The *m*PWPW91 functional did not perform favorably for the S/S orientation of bases (see below), so the Truhlar functionals were employed, since they have been shown to perform well for stacked structures. These optimizations were carried out for the S/S conformations using the MIDI! and cc-pVDZ basis sets.

All stationary points were verified as minima by computation of analytic vibrational frequencies. These frequencies were used to compute zero-point vibrational energies and thermal contributions to the 298 K enthalpies using standard ideal gas statistical mechanics and the rigid rotor harmonic oscillator approximation.[71] Špirko et al.[72] found that harmonic frequencies for base pairs agree well with independently calculated anharmonic frequencies. The frequencies were not scaled,[73] since for pure DFT methods most scale factors fail to differ significantly from 1.00.

When working with relatively small basis sets, the interaction energies are significantly overestimated. We employ the procedure of Xantheas[74] to correct for basis-set-superposition error (BSSE). The performance of the different levels of theory in combination with the two basis sets was judged both from an interaction energy standpoint, and the geometrical deviation from the starting structures. All-atom RMSD calculations were computed for respective pairs using the program MolMol version 2 K.2.[75]

A separation of the structures into the classes based on the orientation of the bases: While for nucleic acid base pairs three basic arrangements exist in the gas phase (hydrogen-bonded, T-shaped, and stacked) the situation is more complicated for the base trimers, and all three possible combinations of the three base pairs defining the trimer should be taken into account. As has been shown previously,[53] a distance between the centers of masses of the bases can be used for a separation of hydrogen-bonded and stacked structures. For hydrogen-bonded structures this distance is about 6 Å (the arrangement of two bases was assigned here as hydrogen bonded when the distance between centers of mass was greater than 5 Å). For stacked structures this distance is significantly smaller, about 3.5 Å (the arrangement of two bases was assigned here as stacked when the distance between centers of mass was less than or equal to 5 Å). In addition to the intermolecular distance, deviation between the planes of the base pairs was also taken into account. The structural arrangement of two bases was consider as T-shaped when the deviation of the planes of the bases was greater than 20°. Thus, six arrangements of the trimers based on the combination of these three structural types were considered: 1) HB/HB: all three bases connected with hydrogen bonds lie in one plane; 2) HB/S: one hydrogen-bonded base pair and the third base stacked above one or both bases; 3) HB/T: one hydrogen-bonded base pair and the third base in the T-shape arrangement; 4) T/T: a complex with neither stacked nor planar hydrogen-bonded arrangements of any two bases; 5) S/T: one stacked base pair and the third base in the T-shape arrangement and 6) S/S: structures in which all three bases are stacked upon one another. The schematic side and top views of each motif are depicted in Figure 2.

For a separation of the localized structures into the classes enumerated above we used a modified script originally developed for a study of hydrated base pairs[76,77] combined with the program for trajectory analysis "carnal" (part of the AMBER program package).

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