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Toward True DNA Base-Stacking Energies: MP2, CCSD(T), and Complete Basis Set Calculations

Pavel Hobza* and Jiří Šponer

Contribution from the J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic and Center for Complex Molecular Systems and Biomolecules, Dolejškova 3, 182 23 Prague 8, Czech Republic

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Abstract: Stacking energies in low-energy geometries of pyrimidine, uracil, cytosine, and guanine homodimers were determined by the MP2 and CCSD(T) calculations utilizing a wide range of split-valence, correlation-consistent, and bond-functions basis sets. Complete basis set MP2 (CBS MP2) stacking energies extrapolated using aug-cc-pVXZ (X = D, T, and for pyrimidine dimer Q) basis sets equal to −5.3, −12.3, and −11.2 kcal/mol for the first three dimers, respectively. Higher-order correlation corrections estimated as the difference between MP2 and CCSD(T) stacking energies amount to 2.0, 0.7, and 0.9 kcal/mol and lead to final estimates of the genuine stacking energies for the three dimers of −3.4, −11.6, and −10.4 kcal/mol. The CBS MP2 stacking-energy estimate for guanine dimer (−14.8 kcal/mol) was based on the 6-31G*(0.25) and aug-cc-pVDZ calculations. This simplified extrapolation can be routinely used with a meaningful accuracy around 1 kcal/mol for large aromatic stacking clusters. The final estimate of the guanine stacking energy after the CCSD(T) correction amounts to −12.9 kcal/mol. The MP2/6-31G*(0.25) method previously used as the standard level to calculate aromatic stacking in hundreds of geometries of nucleobase dimers systematically underestimates the base stacking by ca. 1.0−2.5 kcal/mol per stacked dimer, covering 75−90% of the intermolecular correlation stabilization. We suggest that this correction is to be considered in calibration of force fields and other cheaper computational methods. The quality of the MP2/6-31G*-(0.25) predictions is nevertheless considerably better than suggested on the basis of monomer polarizability calculations. Fast and very accurate estimates of the MP2 aromatic stacking energies can be achieved using the RI-MP2 method. The CBS MP2 calculations and the CCSD(T) correction, when taken together, bring only marginal changes to the relative stability of H-bonded and stacked base pairs, with a slight shift of ca. 1 kcal/mol in favor of H-bonding. We suggest that the present values are very close to ultimate predictions of the strength of aromatic base stacking of DNA and RNA bases.

Introduction

Stacking of aromatic systems plays an important role in nature and is responsible for the structure and dynamics of many complexes. The best-known example represents stacking of nucleic acid (NA) bases which fundamentally contributes to the stability and conformational variability of nucleic acids.[1] The physicochemical origin of stacking differs considerably from nucleobase H-bonding. Whereas the H-bonding is mainly of electrostatic origin, the stacking interaction is due to the London dispersion energy.[2−4] The electrostatic interactions are correctly described already at the Hartree−Fock (HF) level of quantum chemical description with rather small basis sets and that is why H-bonding was studied earlier and more extensively. The very popular density functional theory (DFT), including some portions of electron correlation energy, yields accurate data on structure, stabilization energy, and vibration frequencies of H-bonded complexes while the semiempirical methods are less satisfactory. In contrast, for base stacking HF, DFT and semiempirical methods fail completely.[2] We nevertheless wish to underline that there have been considerable recent efforts to improve the DFT predictions for stacked molecular clusters though, to our opinion, ultimate success for stacking (quality comparable to conventional electron correlation methods and available to all stacked NA base pairs) has not yet been achieved.[4b−g] The only exception represents to our best knowledge our recent attempt[4h] where we combined the self-consistent-charge, density-functional tight-binding method with

* Corresponding author. E-mail: hobza@indy.jh-inst.cas.cz.

(1) (a) Mathews, D. H.; Sabina, J.; Zuker, M.; Turner, D. H. *J. Mol. Biol.* **1999**, *288*, 911−940. (b) Bommarito, S.; Peyret, N.; SantaLucia, J. *Nucleic Acids Res.* **2000**, *28*, 1929−1934. (c) Suzuki, M.; Amano, N.; Kakinuma, J.; Tateno, M. *J. Mol. Biol.* **1997**, *274*, 421−435.
(2) (a) Hobza, P.; Šponer, J. *Chem. Rev.* **1999**, *99*, 3247−3276. (b) Šponer, J.; Leszczynski, J.; Hobza, P. *Biopolymers* **2001**, *61*, 3−31.
(3) (a) Šponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem.* **1996**, *100*, 5590−5596. (b) Šponer, J.; Gabb, H. A.; Leszczynski, J.; Hobza, P. *Biophys. J.* **1997**, *73*, 76−87. (c) Šponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. A* **1997**, *101*, 9489−9495.
(4) (a) Šponer, J.; Leszczynski, J.; Hobza, P. *J. Comput. Chem.* **1996**, *17*, 841−850. (b) Adamo, C.; Barone, V. *J. Chem. Phys.* **1998**, *108*, 664−675. (c) Wesolowski, T. A.; Parisel, O.; Ellinger, Y.; Weber, J. *J. Phys. Chem. A* **1997**, *101*, 7818−7825. (d) Williams, H. L.; Chabalowski, C. F. *J. Phys. Chem. A* **2001**, *105*, 646−659. (e) Kurita, N.; Kobayashi, K. *Comput. Chem.* **2000**, *24*, 351−357. (f) Kurita, N.; Araki, M.; Nakao, K.; Kobayashi, K. *Chem. Phys. Lett.* **1999**, *313*, 693−700. (g) Wesolowski, T. A.; Morgantini, P. Y.; Weber, J. *J. Chem. Phys.* **2002**, *116*, 6411−6421. (h) Elstner, M.; Hobza, P.; Frauenheim, T.; Suhai, S.; Kaxiras, E. *J. Chem. Phys.* **2001**, *114*, 5149−5155.

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the empirical expression describing the London dispersion energy. This technique successfully described stacking in all 10 NA base pairs. While stabilization energies of stacked nucleobase dimers are typically around −10 kcal/mol, the HF and most DFT approaches give only repulsive potential.[2,4a]

What makes the theoretical analysis of base-stacking interactions especially difficult is the fact that higher-order electron correlation effects substantially affect the strength of aromatic stacking.[5] The higher-order contributions are not included in the second-order Moeller−Plesset (MP2) perturbational theory conventionally used to study molecular interactions.[5] This is not the case of H-bonding where the higher-order electron correlation terms cancel each other and, therefore, the MP2 method is usually very accurate.[5] Further, evaluation of reasonable stacking energies requires the use of diffuse functions and, especially, diffuse polarization functions. In preceding studies on NA base stacking, we used the MP2 method combined with the 6-31G*(0.25) basis set.[2−4a] Here, the standard d-polarization functions (with exponent of 0.8) were replaced by more diffuse ones (exponent of 0.25)[6] with the aim to improve description of the dispersion attraction.[2−4a] This basis set is obviously not fully balanced and cannot be used, for example, for geometry optimizations. Nevertheless, the utilization of this basis set was crucial in early electron correlation studies of base stacking as the standard 6-31G* basis set would provide a highly distorted picture of stacking.[2]

Stacking energies of NA bases are sensitive to the quality of the basis set and very extended basis sets increase (in absolute value) the calculated stacking energies significantly. The final answer to this problem can be obtained by evaluating the complete basis set (CBS) stacking energies, and the first attempt was made recently when Nielsen et al.[7] studied two gradiently optimized geometries of stacked uracil dimer. The authors obtained CBS MP2 binding energies from basis set extrapolations using cc-pVXZ and aug-cc-pVXZ basis sets. Higher-order correlation contributions were obtained from the difference of MP2 and CCSD(T) stacking energies determined with the 6-31G*(0.25) basis set. CCSD(T) stands for the coupled cluster method with noniterative evaluation of triple excitations. The final estimate of the stacking energy was by 1.2 and 1.7 kcal/mol higher (in absolute values) compared with MP2/6-31G*-(0.25) calculations.[7]

In the present paper, we reevaluate the stacking interaction of smallest NA base pairs (uracil and cytosine homodimer) and a related model system (pyrimidine dimer) using the MP2 procedure combined with extended basis sets. Higher-order correlation contributions are obtained with the CCSD(T) procedure using basis sets larger than the 6-31G*(0.25) one. We are aware of the fact that the higher-level correlation treatment like CCSD(T) requires the use of extended basis sets containing higher polarization functions; such a calculation for the present NA base pairs is, however, clearly impractical. Using a small (symmetrical) model system, we demonstrate that reasonable values of the difference between MP2 and CCSD-(T) interaction energies can be obtained already using smaller basis sets. Further, an efficient procedure for estimation of genuine stacking energies of larger NA dimers (guanine dimer) will be suggested. We will also demonstrate the ability of resolution of identity MP2 (RI-MP2) procedure[8a−c] to correctly describe stacking interactions. It was suggested in the past that the RI-MP2 correlation interaction energy might be smaller than that in the accurate MP2 method. Our recent calculations using the RI-MP2 method for NA base pairs indicated an excellent performance of the RI-MP2 method and it is confirmed here using a more systematic comparison.[8d]

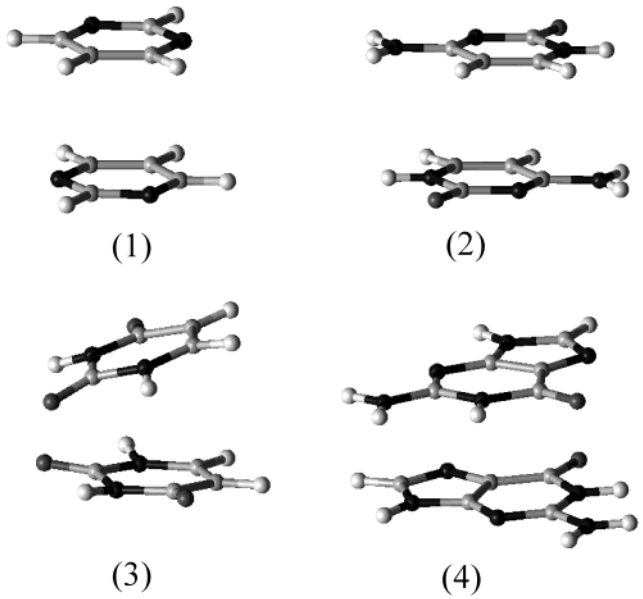

Figure 1. Structures of pyrimidine dimer (1), cytosine dimer (2), uracil dimer (3), and guanine dimer (4).

Accurate characterization of nucleobase stacking allows proper calibration of the balance between stacking and hydrogen bonding of nucleic acid bases. This information is important for studies of a wide variety of systems ranging from advanced gas-phase physicochemical experiments up to the condensed-phase experiments and simulations.[9] Advanced ab initio calculations of base stacking are vital for parametrization of molecular mechanics (empirical) potentials and other computational techniques since gas-phase experiments on the energetics of nucleobase association are presently not available. We suggest that the present values of base stacking are very close to ultimate predictions of the strength of aromatic base stacking of DNA and RNA bases.

Methods

Geometries. Four aromatic stacked structures were investigated (Figure 1). For pyrimidine and cytosine homodimers, we used antiparallel undisplaced face-to-back dimers with a vertical separation between the coplanar monomers of 3.3 Å, assuming rigid monomers.[3a,5]

(5) Šponer, J.; Hobza, P. *Chem. Phys. Lett.* **1997**, *267*, 263−270.
(6) Kroon-Batenburg, L. M. J.; van Duijneveldt, F. B. *J. Mol. Struct. THEOCHEM* **1985**, *121*, 185−199.
(7) Leininger, M. L.; Nielsen, I. M. B.; Colvin, M. E.; Janssen, C. L. *J. Phys. Chem. A* **2002**, *106*, 3850−3854.
(8) (a) Feyereisen, M.; Fitzgerald, G.; Komornicki, A. *Chem. Phys. Lett.* **1993**, *208*, 359−363. (b) Vahtras, O.; Almlöf, J.; Feyereisen, M. W. *Chem. Phys. Lett.* **1993**, *213*, 514−518. (c) Bernholdt, D. E.; Harrison, R. J. *Chem. Phys. Lett.* **1996**, *250*, 477−484. (d) Jurečka, P.; Nachtigall, P.; Hobza, P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 4578−4582.
(9) (a) Nir, E.; Janzen, C.; Imhof, P.; Kleinermanns, K.; de Vries, M. S. *Phys. Chem. Chem. Phys.* **2002**, *4*, 732−739. (b) Nir, E.; Janzen, C.; Imhof, P.; Kleinermanns, K.; de Vries, M. S. *Phys. Chem. Chem. Phys.* **2002**, *4*, 740−750. (c) Desfrancois, C.; Abdoul-Carime, H.; Schultz, L. P.; Schermann, J. P. *Science* **1995**, *269*, 1707−1709. (d) Schnier, P. D.; Klassen, J. S.; Strittmatter, E. E.; Williams, E. R. *J. Am. Chem. Soc.* **1998**, *120*, 9605−9613. (e) Dunger, A.; Limbach, H.-H.; Weisz, K. *J. Am. Chem. Soc.* **2000**, *122*, 10109−10114.

These structures are in fact very close to the minima on the potential energy surface (PES) for face-to-back structures.[3a,5] Structure of the uracil dimer (face-to-face) is taken from the MP2/6-31G** gradient optimization reported earlier[10a] and corresponds to the global minimum at the PES.[10] Guanine dimer geometry has been taken from our preceding study and corresponds to the minimum on the PES of face-to-back structures, as localized using MP2-adjusted empirical force field, assuming coplanar arrangement of rigid bases with an interplane separation of 3.4 Å.[3a,11] In contrast to the preceding three dimers, the guanine dimer does not have the C_i symmetry.

Methods and Extrapolation Techniques. Complete basis set (CBS) MP2 stacking energies were estimated using various extrapolation schemes. Besides a simple extrapolation of interaction energy versus 1/*N*, where *N* is the number of contracted AO, also the scheme recently suggested by Truhlar[12a] is utilized. In this procedure, the total HF and MP2 energies are extrapolated and CBS interaction energy is obtained as a difference of CBS energies of the dimer and the monomers. In the pyrimidine dimer, the aug-cc-pVXZ series (X = D, T, and Q) will be utilized while in the NA base pairs the calculations will be performed only for the first two basis sets. The CBS extrapolation technique represents a very efficient method which yields accurate values of interaction energies without making extended (and expensive) calculations. We have recently[12b] evaluated interaction energies of Ar2, Kr2, and Xe2 dimers using CCSD(T) calculations with aug-cc-pVXZ (X = D, T, Q, 5, 6) basis sets. Interaction energies obtained by Truhlar extrapolation[12a] from the aug-cc-pVDZ and aug-cc-pVTZ data agreed very well with the most accurate interaction energies evaluated with the largest basis sets.

In purine NA base dimers (and all larger stacked complexes), even the aug-cc-pVTZ calculations are too expensive. Thus, extrapolation to the complete basis set stacking energies should be attempted using two points of which the aug-cc-pVDZ basis set is the more accurate one. This simplified procedure will be tested for all three small complexes and then utilized for the guanine stacked dimer.

The genuine stacking stabilization energies will be predicted using the extrapolated CBS MP2 stacking energy and the difference between the MP2 and CCSD(T) stacking energy, $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$. The convergence of this term will be tested for the pyrimidine dimer and partially also for uracil and cytosine dimers.

Stacking interaction energies will be determined with a variety of atom-centered basis sets. To improve the convergence toward the basis set limit, the bond functions will also be tested. The main problem when using the bond functions is their localization. In our case, we placed them at a dummy atom localized in the center of mass of the dimer. Besides the single dummy atom bearing the bond functions, we also used a set of six dummy atoms each of which was augmented by bond functions and placed at the plain located between the interacting monomers. This plain was at equal vertical distance (the *z*-coordinate) away from both monomers and was coplanar with them. The xy coordinates of the plain coincided with second-row atoms of one of the monomers.

Table 1. Basis Sets Used

Split-Valence		**Correlation Consistent (Dunning)**	
6-31G*	[3s2p1d/2s]	cc-pVDZ	[3s2p1d/2s1p]
6-31G**	[3s2p1d/2s1p]	aug-cc-pVDZ	[4s3p2d/3s2p]
6-31++G**	[4s3p1d/3s1p]	cc-pVTZ	[4s3p2d1f/3s 2p1d]
		aug-cc-pVTZ	[5s4p3d2f/4s 3p2d]
Dunning		cc-pVQZ	[5s4p3d2f1 g/4s3p2d1f]
SVP	[3s2p1d/2s1p]	aug-cc-pVQZ	[6s5p4d3f2 g/5s4p3d2f]
aug-SVP[a]	[4s3p2d/3s2p]		
TZVPP	[5s3p2d1f/3s 2p1d]	**Bond Functions**	
aug-TZVPP[b]	[6s4p3d2f/4s 3p2d]	bf1	(3s3p3d)[c]
		bf2	(3s3p3d3f)[d]

[a] Diffusion s, p, d/s, p functions taken from aug-cc-pVDZ. [b] Diffusion s, p, d, f/s, p, d functions taken from aug-cc-pVTZ. [c] Exponents of s, p, and d functions are 0.9, 0.6, 0.1, respectively. [d] Exponents of s, p, d, and f functions are 0.9, 0.6, 0.1, respectively.

The MP2 and CCSD(T) calculations were done using the GAUSSIAN 98[13] and MOLPRO[14] codes and the RI-MP2 calculations with the TURBOMOLE[15] code. A variety of Pople[13] and Dunning's[16] basis sets were applied (cf. Table 1). The bond functions used are also specified in Table 1. All interaction energies were calculated using the frozen core approximation and corrected a posteriori for the basis set superposition error using the counterpoise procedure.[17]

Results

1. Pyrimidine Dimer. The most complete calculations were carried out for the pyrimidine dimer (Table 2).

Medium Basis Sets. The HF interaction energy shows no basis set dependence and is repulsive, as expected. Substantial attraction is gained when including part of the electron correlation effects via the MP2 method. The standard medium-sized 6-31G* and cc-pVDZ basis sets underestimate binding qualitatively because of the lack of flexibility for the intermolecular electron correlation. The MP2 stacking energy improves considerably when using diffuse polarization functions and very similar results were obtained with the 6-31G*(0.25), 6-31G**-(0.25, 0.15), and cc-pVDZ(0.25, 0.15) basis sets. The improvement is primarily due to the diffuse d-polarization functions of the second-row atoms while diffuse p-polarization functions localized on hydrogen atoms do not bring any substantial change of stacking energy. Additional diffuse s- and p- functions added to the second-row elements in the 6-31++G**(0.25, 0.15) basis set bring non-negligible improvement of the stacking energy.

(10) (a) Hobza, P.; Šponer; J. *Chem. Phys. Lett.* **1998**, *288*, 7−14. (b) Kratochvíl, M.; Engkvist, O.; Šponer, J.; Jungwirth, P.; Hobza, P. *J. Phys. Chem. A* **1998**, *102*, 6921−6926.

(11) Rigid-monomer and gradiently optimized dimers are equally suitable for the purpose of the present study. The monomers in gradient-optimized structures are deformed upon binding including nonplanarities of the overlapping aromatic rings.[10a] These deformations improve the interaction; however, the stability gain is almost canceled by the deformation energies of the monomers.[10a] Thus, after all, calculations with gradient-optimized dimers provide a rather small improvement of the total binding energies over the rigid-monomer approach. The rigid-monomer approach allows searching over the whole conformational space and is in general preferred in computational studies of bases stacking since stacking in nucleic acids includes a wide range of mutual interbase geometries rarely coinciding with genuine gas-phase stacking minima.[2−4] Further, the pronounced out-of-plane deformations of bases seen in the isolated dimers should be eliminated inside DNA as bases have stacking partners at either side.

(12) (a) Truhlar, D. G. *Chem. Phys. Lett.* **1998**, *294*, 45−48. (b) Slavíček, P.; Kalus, R.; Paška, P.; Odvárková, I.; Hobza, P.; Malijevský, A. *J. Chem. Phys.*, submitted.

(13) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, J. S.; Pople, J. A. *Gaussian 98*, Revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(14) MOLPRO is a package of ab initio programs written by H.-J. Werner and P. J. Knowles, with contributions from J. Almlof, R. D. Amos, A. Berning, P. Celani, D. L. Cooper, M. J. O. Deegan, A. J. Dobbyn, F. Eckert, S. T. Elbert, C. Hampel, G. Hetzer, R. Lindh, A. W. Lloyd, W. Meyer, A. Nicklass, T. Korona, K. Peterson, R. Pitzer, G. Rauhut, A. J. Stone, P. R. Taylor, M. E. Mura, P. Pulay, M. Schutz, H. Stoll, and T. Thorsteinsson.

(15) Ahlrichs, R.; Bär, M.; Häser, M.; Horn, H.; Kö, C. *Chem. Phys. Lett.* **1989**, *162*, 165−169.

(16) (a) Woon, D. E.; Dunning, T. H., Jr. *J. Chem. Phys.* **1993**, *98*, 1358−1371. (b) Kendall, R. A.; Dunning, T. H., Jr.; Harrison, R. J. *J. Chem. Phys.* **1992**, *96*, 6796−6806. (c) Schaefer, A.; Horn, H.; Ahlrichs, R. *J. Chem. Phys.* **1992**, *97*, 2571−2573. Schaefer, A.; Huber, C.; Ahlrichs, R. *J. Chem. Phys.* **1994**, *100*, 5829−5835.

(17) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553−574.

Table 2. Stacking Interaction Energies (kcal/mol) of Antiparallel Undisplaced Pyrimidine Dimer

basis set	HF	MP2	CCSD(T)	Δ|MP2 − CCS D(T)|
6-31G*	5.96	−0.75	0.78	1.53
6-31G*(0.25)	5.74	−3.0	−1.33	1.77
6-31G*(0.25, 0.15)	6.06	−3.04	−1.31	1.73
cc-pVDZ	6.01	−1.46		
cc-pVDZ(0.2 5,0.15)	6.03	−3.17	−1.44	1.73
6-31++G*(0. 25,0.15)	5.70	−3.62	−1.93	1.68
6-31G*(0.25) + bf1	6.16	−3.39	−1.77	1.61
aug-cc-pVDZ	5.82	−4.21	−2.20	2.01
aug-cc-pVDZ + bf1	5.85	−4.45	−2.47	1.99
aug-cc-pVDZ + f/cc-pVTZ[b]	5.78	−4.43	−2.39	2.05
aug-cc-pVDZ + f/cc-pVTZ + bf2	**5.82**	**−4.65**	**−2.61**	**2.04**
cc-pVTZ	5.91	−3.64		
aug-cc-pVTZ	5.82	−4.80		
cc-pVQZ	5.84	−4.53		
aug-cc-pVQZ	**6.20**	**−5.03**		
cc-pVTZ + 6 x bf1[c]	5.85	−4.86		

The most accurate calculated CCSD(T) and MP2 values are highlighted by bold. For extrapolated values, see the text. [b] f-functions taken from the cc-pVTZ basis set. [c] Set of six bf1-type bond function centers placed at a plain localized at equal vertical distance between the monomers (see Methods).

Base-stacking energy is also improved by using one set of bond functions, though not as much as when using diffuse polarization functions.

Large Basis Sets. Further improvement of MP2 binding energies is achieved with extended basis sets provided they contain very diffuse polarization functions. The aug-cc-pVDZ basis set gives already a reliable estimate of the MP2 stacking energy. Further extension of the basis set by adding the bond functions or polarization f-functions did not affect the stacking energy significantly. The cc-pVTZ basis set includes less correlation interaction energy than the smaller aug-cc-pVDZ basis set since energy-optimized f-functions have a rather minor effect on the MP2 stacking.[5] Similarly, the aug-cc-pVTZ basis set gives deeper stacking energy than the cc-pVQZ one. Even a slightly better stacking-energy value is achieved when combining the cc-pVTZ basis set with a set of six bond function centers (bond function plain, see Methods) between the monomers.[18] This basis set is considerably smaller than the aug-cc-pVTZ basis set. Finally, the most attractive MP2 binding energy is obtained with the aug-cc-pVQZ basis set.

Extrapolation to the MP2 Basis Set Limit. Extrapolating the respective MP2/aug-cc-pVXZ (X = D, T, Q) stacking energies with respect to $1/N$ (i.e., extrapolation to zero), where N is the number of basis functions, we found the CBS MP2 stacking energy being about −5.4 kcal/mol. The dependence is perfectly linear which means that using only two points would result exactly in the same extrapolated value. We have also used extrapolation as proposed by Truhlar (Table 3) leading to a slightly reduced binding of −5.3 kcal/mol. Thus, the CBS MP2 value is ca. 2.2−2.3 kcal/mol above (in absolute value) the MP2/6-31G*(0.25) data.

Higher-Order Electron Correlation Contributions. Let us now investigate the role of higher-order correlation energy contributions. The CCSD(T) stacking interaction energy is (in absolute value) always substantially smaller than the MP2 one. The $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ difference is 1.53 kcal/mol for the smallest 6-31G* basis set and increases moderately when improving the basis set quality, with saturation around 2.0−2.1 kcal/mol. Relatively sound values of this difference were found when using the 6-31G*(0.25) or 6-31G**(0.25, 0.15) basis sets, but the respective values were still about 0.3 kcal/mol smaller than the reference value obtained with the aug-cc-pVDZ basis set. The presence of diffuse polarization functions is thus essential to obtain accurate values of the $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ term though the basis set dependence is on the scale of few tenths of kcal/mol.[19] The $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ term shows certainly a better convergence compared to the MP2 and CCSD(T) values themselves.

The genuine stacking interaction energy of the pyrimidine dimer can be determined using the CBS MP2 stacking energy corrected by the $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ term evaluated with the largest basis set (2.04 kcal/mol). The values obtained with the $1/N$ and Truhlar's extrapolations are −3.6 and −3.4 kcal/mol, respectively. These final values are surprisingly close to the MP2/6-31G*(0.25) prediction of −3.0 kcal/mol.

Table 3. HF and MP2 Energies (in a.u.) of the Pyrimidine (p), Uracil (u), and Cytosine (c) Monomers and Dimers Extrapolated Using the Truhlar Scheme to the Infinite Basis Set Limit Using the Aug-cc-pVXZ Basis Sets

X =	D	T	Q	∞
p HF	−262.725471	−262.780610	−262.795526	−262.808152
pp	−525.441662	−525.551938	−525.581179	−525.607021
p MP2	−0.867553	−1.033109	−1.089718	−1.159502
pp	−1.751090	−2.08315	−2.197327	−2.336659
u HF	−412.527226	−412.619564	−412.665687	
uu	−825.053547	−825.238219	−825.330464	
u MP2	−1.255205	−1.506350	−1.698085	
uu	−2.527982	−3.031973	−3.416742	
c HF	−392.676980	−392.763443	−392.806636	
cc	−785.353993	−785.526982	−785.613391	
c MP2	−1.237945	−1.481850	−1.6680578	
cc	−2.492026	−2.980754	−3.3538706	

Table 4. Stacking Interaction Energies (kcal/mol) of Antiparallel Undisplaced Pyrimidine Dimer Calculated Using the RI-MP2 Method

basis set	HF	RI-MP2
SVP	5.85	−1.28
aug-SVP	5.74	−4.17
TZVPP	5.87	−3.87
aug-TZVPP	5.82	−4.86

The RI-MP2 Procedure. Table 4 shows that the RI-MP2 procedure includes a close to identical amount of correlation interaction energy as the accurate MP2 procedure. The extended aug-TZVPP basis set improves the stacking energy by −3.58 kcal/mol compared with the SVP basis set which parallels the MP2/aug-cc-pVTZ and MP2/cc-pVDZ difference of −3.34 kcal/mol. Also, absolute numbers are very similar and stacking interaction energies evaluated at MP2/aug-cc-pVTZ and RI-MP2/aug-TZVPP levels differ by less than 0.1 kcal/mol. This conclusion is very important for the future utilization of the RI-MP2 calculations that are about 1 order of magnitude faster than the exact MP2 procedure. The aug-SVP basis set is essentially equivalent to the aug-cc-pVDZ one.

(18) The calculation illustrates capability of bond functions in stacking calculations, although their common utilization is hampered by lack of general rules regarding where to place the bond function centers for displaced geometries, dimers with irregular shape, dimers with noncoplanar bases, and others.

(19) Calculations lacking any polarization functions (6-31G, 6-311G, 6-311+G basis sets; not shown) yield this difference around 1 kcal/mol.

Table 5. Stacking Interaction Energies (kcal/mol) of Cytosine Dimer, Uracil Dimer, and Guanine Dimer

basis set	dimer	HF	MP2	CCSD(T)	Δ|MP2–CCS D(T)|
6-31G*	cytosine	0.33	−5.27	−4.16	1.11
	uracil	0.99	−6.01	−4.75	1.26
	guanine	−0.64	−8.06		
6-31G*(0.25)	cytosine	−0.04	−8.27	−7.15	1.11
	uracil	0.14	−8.94	−8.01	0.93
	guanine	−0.87	−11.19	−9.34	1.85
6-31G*(0.25, 0.15)	cytosine	0.18	−8.39	−7.39	1.00
	uracil	0.29	−9.01	−8.20	0.81
6-31G*(0.25) + bf1	uracil	0.13	−9.35	−8.58	0.77
6-31G*(0.25) + bf2	cytosine	−0.01	−8.78	−7.95	0.83
cc-pVDZ (0.25,0.15)	cytosine	0.14	−8.51	−7.55	0.96
	uracil	0.48	−8.98	−8.15	0.83
6-31G** (0.25,0.15) + bf2	cytosine	0.05	−8.97	−8.20	0.78
6-31++G** (0.25,0.15)	cytosine	−0.59	−9.75	−8.87	0.88
	uracil	0.19	−9.62	−8.94	0.69
aug-cc-pVDZ	cytosine	−0.02	−10.15		
	uracil	0.57	−10.46		
	guanine	−1.13	−13.22		
aug-cc-pVDZ + bf1	uracil	0.57	−10.87		
aug-cc-pVDZ + bf2	uracil	0.54	−10.99		
aug-cc-pVTZ	cytosine	−0.06	−10.76		
	uracil	0.57	−11.52		
aug-cc-pVTZ + bf2	uracil	0.55	−11.68		

[a] Deformation energies of monomers in gradient-optimized geometry of uracil dimer (+1.47 kcal/mol) is not included in the table.

2. Cytosine and Uracil Homodimer. Stacking interaction energies of both dimers are summarized in Table 5. Comparison of medium-sized 6-31G*, 6-31G*(0.25), and 6-31G**(0.25, 0.15) basis sets shows that the use of diffuse polarization functions is essential and brings systematical improvement of stablization energy by about 3 kcal/mol. Further extension of the basis set by adding the diffuse p-polarization functions on hydrogens does not play (as in the pyrimidine dimer) any important role, and a similar conclusion is obtained for addition of bond functions. Similarly as with the pyrimidine dimer, cc-pVDZ(0.25, 0.15) and 6-31G**(0.25, 0.15) basis sets yield almost identical stacking energies. The flexible diffuse basis set (aug-cc-pVDZ) improves the MP2 stacking interaction energy by more than 1 kcal/mol when compared with the 6-31G**(0.25, 0.15) data. The aug-cc-pVDZ basis set provides already reliable values of MP2 stacking interaction energies as seen by only a moderate improvement when applying the extended aug-cc-pVTZ basis set. Notably, the respective stacking stabilization energy gain (passing from aug-cc-pVDZ to aug-cc-pVTZ basis set) is for the uracil dimer about twice larger than that for the cytosine dimer (−1.12 and −0.61 kcal/mol, respectively). This suggests the necessity to use some kind of an extrapolation technique. 1/N extrapolations using the aug-cc-pVDZ and aug-cc-pVTZ MP2 stacking energies give for uracil and cytosine dimers the following CBS MP2 stacking energies: −12.5 and −11.3 kcal/mol. Results of Truhlar-type extrapolation are very similar (−12.3 and −11.2 kcal/mol, see Table 3).

CCSD(T) Correction. The CCSD(T) stacking stabilization energy is for both dimers systematically smaller than the MP2 one. The reduction is comparable for both dimers but smaller than that found for the pyrimidine dimer. Whereas $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ converged for the pyrimidine dimer to about 2 kcal/mol, for the nucleobase dimers it is less than 1 kcal/mol. The largest basis set used (6-31++G**(0.25, 0.15)) yields this correction for the cytosine dimer slightly larger (0.88 kcal/mol) than for the uracil dimer (0.69). In the pyrimidine dimer, the value of $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ increased with the basis set extension while the opposite is true for the two nucleobase dimers. Apparently, the convergence of the $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ term should be considered case by case and cannot be transferred from one dimer to another. The $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ term varies with dimer composition/configuration and there exists no unique correction.

Estimated Genuine Stacking Energies. The genuine stacking interaction energy of cytosine and uracil homodimers (in given geometries, see Methods and Figure 1) can be determined on the basis of the CBS MP2 stacking energies and $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ values taken from the 6-31++G**(0.25, 0.15) calculations. Considering the Truhlar-type extrapolation, one obtains for the uracil and cytosine dimers −11.6 and −10.4 kcal/mol, respectively. Very similar values of −11.8 and −10.5 kcal/mol are obtained using the simple $1/N$ extrapolation. Thus, the final binding energies are 2.7 and 2.1 kcal/mol deeper than the MP2/6-31G*(0.25) data and 1.1 and 0.2 kcal/mol away from the MP2/aug-cc-pVDZ results.

Comparison with Literature Data. Nielsen et al.[7] determined recently the CBS MP2 stacking energy for the face-to-face and face-to-back structures of the uracil homodimer. Their face-to-face structure corresponds to the uracil dimer studied in the present study. The CBS MP2 stacking energy for this structure was −10.7 kcal/mol using extrapolations up to the aug-cc-pVQZ level. This value nicely agrees with the present estimate based on the Truhlar's extrapolation. To compare these two studies, we need to add the deformation energy of +1.47 kcal/mol to our data, leading to a binding stacking energy of −10.8 kcal/mol. Nielsen et al. include the deformation energies of monomers into all their values. In the present study, we have obtained more accurate $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ term well beyond the 6-31G*(0.25) basis set (see above). Adding our $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ correction to the MP2 CBS data by Nielsen et al. would lead to ca. 2.0 kcal/mol difference between their final and MP2/6-31G*(0.25) stacking data.

For the second uracil dimer geometry (face-to-back) studied by Nielsen et al., they report a notably larger $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ correction of 1.4 kcal/mol and faster convergence of the MP2 stacking energies. This leads to a stacking energy difference of only 1.2 kcal/mol between the MP2/6-31G*(0.25) data and the final prediction, much less than found for the face-to-face arrangement.

3. Guanine Dimer. Stacking interaction energies of the guanine dimer are presented in Table 5. The 6-31G*(0.25) basis set again improves the stacking energy by 3.1 kcal/mol compared with the standard 6-31G* basis set. Passing to the flexible basis set (aug-cc-pVDZ) brings an additional 2 kcal/mol of the stabilization energy, again in agreement with data found for the uracil and cytosine dimers.

Simplified Extrapolation Procedure for Extended Stacked Clusters. The guanine dimer is already too large for making the aug-cc-pVTZ calculations necessary for extrapolation to the CBS limit. Because this is also the case for larger stacked complexes (like stacked NA base pair steps[3b] and NA base pairs with intercalators[20]), it is tempting to consider a cheaper

(20) (a) Řeha, D.; Kabeláč, M.; Ryjáček, F.; Šponer, J.; Šponer, J. E.; Elstner, M.; Suhai, S.; Hobza, P. *J. Am. Chem. Soc.* **2002**, *124*, 3366−3376. (b) Bondarev, D. A.; Skawinski, W. J.; Venanzi, C. A. *J. Phys. Chem. B* **2000**, *104*, 815−822.

extrapolation procedure. Evidently, the extrapolation should consider the aug-cc-pVDZ results because they were in all three above cases already reasonably close to the CBS limit. In the following, we suggest how to calculate the first point for the simple 1/*N* extrapolation. For the pyrimidine stack dimer, we found that among lower quality results it is the 6-31G*(0.25) value which perfectly extends the straight line determined by the aug-cc-pVXZ (X = D, T, Q) values. Extrapolating the CBS MP2 stacking energy from 6-31G*(0.25) and aug-cc-pVDZ data, we obtained an identical result as that based on aug-cc-pVXZ data. Performing this simplified 6-31G*(0.25) − aug-cc-pVDZ extrapolation for the uracil dimer and cytosine dimer, we obtained CBS MP2 stacking energy estimates of −12.2 and −12.3 kcal/mol. These differ from the respective aug-cc-pVDZ − aug-cc-pVTZ estimates (−12.5 and −11.3 kcal/mol using 1/*N*) by 0.3 and −1.0 kcal/mol, respectively. It is thus possible to suggest that extrapolation based on the 6-31G*(0.25) and aug-cc-pVDZ MP2 data provides results quite comparable to those based on aug-cc-pVXZ data; the difference between these two estimates was smaller than (or equal to) 1 kcal/mol. Extrapolating now the MP2/6-31G*(0.25) and MP2/aug-cc-pVDZ guanine stacking energies, we obtained the CBS MP2 stacking energy estimate of −14.8 kcal/mol. The final estimate of the genuine stacking energy of −12.9 kcal/mol is obtained by adding a rather large $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ correction of +1.9 kcal/mol taken from the 6-31G*(0.25) calculations. This final estimate is 1.7 kcal/mol away from the MP2/6-31G*(0.25).

4. Comparison of the Reference Stacking Energies with Medium Level Calculations. The final estimates of the CBS MP2 stacking energies of pyrimidine, uracil, cytosine, and guanine homodimers are −5.3, −12.3, −11.2, and −14.8, respectively.[21] After introducing the CCSD(T) correction, the estimated genuine stacking energies drop to ca −3.4, −11.6, −10.4, and −12.9 kcal/mol, respectively. The MP2/6-31G*-(0.25) stacking energies which made up to now the reference values for all stacked NA base pairs, base pair steps, nucleobase−intercalator complexes, and other systems are smaller (in absolute values) by 0.4, 2.7, 2.1, and 1.7 kcal/mol, respectively. At the relative scale, the MP2/6-31G*(0.25) calculations cover 88, 77, 80, and 87% of the genuine stacking energy, respectively. Subtracting the HF term and considering thus only the electron correlation component of stacking (which is in fact more relevant), the MP2/6-31G*(0.25) method includes 75−91% of the genuine electron correlation contribution to the interaction energy. More demanding MP2/aug-cc-pVDZ calculations differ from the final energies by −0.8, +1.1, +0.3, and −0.3 kcal/mol.

Discussion and Conclusions

We have carried advanced ab initio calculations of the stacking interaction energies of pyrimidine, cytosine, uracil, and guanine dimers. In absence of relevant gas-phase experiments, the ab initio calculations represent new reference data regarding the nature and magnitude of aromatic stacking effects. This is one of the two fundamental nucleobase interactions in DNA and RNA molecules.

For the three smaller complexes, the MP2/aug-cc-pVXZ calculations were used to estimate the CBS MP2 stacking interaction energy. The final value of stacking interaction energy was then constructed by combining the CBS MP2 values and what appears to be a converged value of $\Delta|\Delta E^{MP2} - \Delta E^{CCSD(T)}|$ correction. Genuine values of stacking interaction energies of cytosine dimer and uracil dimer were larger (in absolute values) than 10 kcal/mol.

For the guanine dimer, a rigorous extrapolation using the aug-cc-pVXZ basis sets could not be achieved, and we suggested therefore a simplified 1/*N* extrapolation scheme based on MP2/6-31G*(0.25) and MP2/aug-cc-pVDZ calculations. This procedure was tested for the pyrimidine, uracil, and cytosine homodimers and the difference (with respect to the rigorous treatment) was smaller or equal to 1 kcal/mol. Applying the method for the guanine dimer and after correcting for the higher correlation energy terms at the CCSD(T)/6-31G*(0.25) level, we received the genuine stacking energy close to −13 kcal/mol. The present procedure can be recommended for estimates of accurate stacking energies of large clusters including stacking of bases with drugs.[20]

On the basis of the present data and the preceding study by Nielsen et al.,[7] we conclude that the genuine stacking energies of stacked NA base pairs are larger (in absolute value) by about 1.0−2.5 kcal/mol than predicted in the middle of the nineties using the MP2/6-31G*(0.25) method. The new results are still somewhat sensitive to the quality of the CBS MP2 extrapolation and the CCSD(T) correction but, in contrast to the former data, should not contain any systematic error. To guarantee accuracy better than 1 kcal/mol, one needs to carry out the CBS MP2 extrapolation (preferably up to the aug-cc-pVTZ level at least) combined with a CCSD(T) correction with medium-sized basis set for each structure of interest. The difference between the medium-level MP2/6-31G*(0.25) data and the new reference values varies with the base composition and the dimer geometry; thus, no common correction factor can be proposed to extrapolate the MP2/6-31G*(0.25) data to the final values. This is further illustrated by the pyrimidine dimer where the MP2/6-31G*(0.25) procedure agrees within 0.4 kcal/mol with the final prediction! The CCSD(T) correction is significant in all aromatic stacking clusters studied so far, in contrast to hydrogen bonding and nonaromatic stacking.[5,7,22] Nevertheless, for stacked nucleobase dimers, the MP2/6-31G*(0.25) method provides already 75−90% of the intermolecular electron correlation stabilization. This accuracy is entirely sufficient for most purposes. Several hundred structures of nucleobase dimers and larger systems have been evaluated at the MP2/6-31G*(0.25) level so far.[2−5,20a] The actual underestimation of aromatic stacking by the MP2/6-31G*-(0.25) procedure is much smaller than proposed, for example, by Cybulski and co-workers on the basis of monomer polarizability calculations.[23] The MP2/aug-cc-pVDZ calculations would be even closer to the final values except for the pyrimidine dimer.

Reliable values of stacking interaction energies were obtained only if diffuse polarization functions were considered and the aug-cc-pVDZ basis set can be recommended for this purpose. The MP2/aug-cc-pVDZ data appear to be the best balanced and in fact oscillate around the genuine stacking energies as

(21) Truhlar's extrapolation was used, except of guanine dimer.

(22) (a) Hobza, P.; Selzle, H. L.; Schlag, E. W. *J. Phys. Chem.* **1996**, *100*, 18790−18794. (b) Tsuzuki, S.; Honda, K.; Uchimaru, T.; Mikami, M.; Tanabe, K. *J. Am. Chem. Soc.* **2002**, *124*, 104−112.

(23) Johnson, R. C.; Power, T. D.; Holt, J. S.; Immaraporn, B.; Monat, J. E.; Sissoko, A. A.; Yanik, M. M.; Zagorodny, A. V.; Cybulski, S. M. *J. Phys. Chem.* **1996**, *100*, 18875−18881.

predicted on the basis of the MP2 CBS extrapolations and CCSD(T) corrections. When the MP2/aug-cc-pVDZ calculations are impractical, the MP2/6-31G*(0.25) method utilizing one set of momentum-optimized basis functions remains an option to provide sufficiently accurate data.

The MP2/aug-cc-pVDZ data can be excellently reproduced using the very efficient RI-MP2 procedure with the aug-SVP basis set. The RI-MP2 method is very economical and can be utilized for highly reliable calculations of extended stacked clusters. More complicated appears to be the application of the local MP2 (LMP2) method for aromatic stacking because of a substantial delocalization of the electronic structure of NA bases. We have attempted such calculations, but so far we did not succeed to get correct numbers. This can also be nicely illustrated by a recent paper reporting application of the LMP2 method to the benzene dimer and other aromatic hydrocarbon dimers.[24] While the authors concluded that the LMP2 method provides excellent results, closer inspection of their data shows the opposite. The LMP2 benzene dimer interaction energies were much smaller than the actual MP2 ones available in the literature[22a] and were comparable to the CCSD(T) results. Such comparison is, however, not adequate since the CCSD(T) interaction energies are smaller than the MP2 ones.[22a] Thus, the LMP2 data converge toward a different value of stacking energy compared with the genuine MP2 method. Further, the authors[24] claim that the LMP2 correctly predicts larger stabilization for the T-shaped structure than for the parallel-displaced one. From the paper, it is apparent that it is true for three smaller basis sets used but not for the largest (and hence the most reliable) one where the latter structure is 0.3 kcal/mol more stable.

The new reference values of stacking are important for parametrization of other computational tools. The presently used biomolecular force fields have been extensively verified using the MP2/6-31G*(0.25) procedure.[2a] It has been shown before that outcomes of explicit solvent simulations of nucleic acids are rather insensitive to adjustments of the magnitude of the dispersion stabilization.[25] Thus, the presently used pair additive biomolecular force field such as Cornell et al.[26] does not appear to need any imminent correction. Nevertheless, we suggest that for parametrization of accurate force fields for nucleic acid bases, including polarization force fields, the reference values for base stacking should be shifted by ca. 2 kcal/mol toward more negative values.

The present results bring an important conclusion regarding the relative stability of H-bonding and stacking of bases. The MP2 method with any medium-sized basis set underestimates binding energies of H-bonded base pairs by about 2−3.5 kcal/mol compared with the latest reevaluations.[27] This is very similar to the inaccuracy of the MP2/6-31G*(0.25) calculations of base stacking. Thus, the MP2/6-31G*(0.25) method provides surprisingly accurate relative values of interaction energies essentially over the whole conformational space of nucleobase dimers. This ultimately validates several preceding studies on nucleobase association and relative importance of stacked and H-bonded structures, as these studies heavily relied, directly or indirectly, on quality of the MP2/6-31G*(0.25) method.[3a,28]

The *intrinsic* stacking energies (interaction of two or more bases, in a given geometry and free of any additional environment) do not always unambiguously determine the effect of base stacking on the stability of nucleic acids (NA).[1,28b,e] The effect of base stacking on nucleic acid stability is a result of a complex interplay of the intrinsic base-stacking energies and additional external (environmental) contributions, such as continuum solvent screening effects, specific hydration, counterions, proximity, and disposition of phosphate groups and others. In the first approximation, we can consider the intrinsic and external contributions to stacking as additive; thus, the external contributions do not affect the intrinsic interactions. The external contributions, however, may reverse the stability order as given by the intrinsic stacking energies.[28b,e] The external contributions primarily respond to the electrostatic component of base stacking. We would like to underline that the actual magnitude of the external contributions to stacking stability is substantially dependent on the DNA or RNA three-dimensional architecture.[29] In other words, a given stacking arrangement (having thus the same intrinsic stacking stability) may have a very different effect on the stability in different NA forms. One well-documented example is stacking of two protonated cytosines. This arrangement is exceptionally stable in four-stranded intercalated DNA[30] while the same interaction sharply destabilizes triplexes.[31] Caution is necessary when making extrapolations from one NA form to another, as a given stacking geometry can play multiple roles in different NA forms. In this situation, the accurate description of intrinsic base stacking is of fundamental importance. On one hand, it is always one of the leading forces. More importantly, it is the only contribution that is invariant to changes of the environment and can be unambiguously associated with a given geometry.[29a] Thus, analysis of the intrinsic stacking terms is the key first step to understand the role of stacking in nucleic acids and to properly separate the intrinsic and external contributions to stability of aromatic stacking in nucleic acids.

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(24) Fomine, S.; Tlenkopatchev, M.; Martinez, S.; Fomina, L. *J. Phys. Chem. A* **2002**, *104*, 4817−4824.
(25) Cheatham, T. E., III; Kollman, P. A. In *Structure, Motion, Interactions and Expression of Biological Macromolecules*; Sarma, R. H., Sarma, M. H., Eds.; Adenine Press: Albany, NY, 1998; pp 99−116.
(26) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179−5197.
(27) Šponer, J.; Hobza, P. *J. Phys. Chem. A* **2000**, *104*, 4592−4597.
(28) (a) Kabeláč, M.; Hobza, P. *J. Phys. Chem. B* **2001**, *105*, 5804−5817. (b) Florián, J.; Šponer, J.; Warshel, A. *J. Phys. Chem. B* **1999**, *103*, 884−892. (c) Kabeláč, M.; Hobza, P. *Chem. Eur. J.* **2001**, *7*, 2064−2974. (d) Kratochvíl, M.; Šponer, J.; Hobza, P. *J. Am. Chem. Soc.* **2000**, *122*, 3495−3499. (e) Luo, R.; Gilson, H. S. R.; Potter, M. J.; Gilson, M. K. *Biophys. J.* **2001**, *80*, 140−148.
(29) (a) Šponer, J.; Berger, I.; Špačková, N.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn.* **2000**, *S2* (11th Conversation Special Issue), 383−407. (b) Lankaš, F.; Cheatham, T. E., III; Špačková, N.; Hobza, P.; Langowski, J.; Šponer, J. *Biophys. J.* **2002**, *82*, 2592−2609. (c) Chou, S. H.; Chin, K. H. *J. Mol. Biol.* **2001**, *314*, 139−152.
(30) (a) Špačková, N.; Berger, I.; Egli, M.; Šponer, J. *J. Am. Chem. Soc.* **1998**, *120*, 6147−6151. (b) Leroy, J. L.; Snoussi, K.; Gueron, M. *Magn. Reson. Chem.* **2001**, *39* (Special Issue), S171−S176. (c) Gallego, J.; Golden, E. B.; Stanley, D. E.; Reid, B. R. *J. Mol. Biol.* **1999**, *285*, 1039−1052. (d) Gehring, K.; Leroy, J. L.; Gueron, M. *Nature* **1993**, *363*, 561−565.
(31) Soliva, R.; Laughton, C. A.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1998**, *120*, 11226−11233 and references therein.