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**Biomolecular Structures: From Isolated Molecules to Living Cells**

Guest Editors: Seong Keun Kim, Jean-Pierre Schermann and Taekjip Ha

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Reference MP2/CBS and CCSD(T) quantum-chemical calculations on stacked adenine dimers. Comparison with DFT-D, MP2.5, SCS(MI)-MP2, M06-2X, CBS(SCS-D) and force field descriptions

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We have performed reference quantum-chemical calculations for about 130 structures of adenine dimers in stacked conformations, with special attention given to dimers that are either vertically compressed (parallel structures) or contain close interatomic contacts (non-parallel structures). Such geometries are sampled during thermal fluctuations of nucleic acids and contribute to the local conformational variability of these systems. Their theoretical characterization requires a good description of interaction energies in the short-range repulsion region. The reference calculations have been performed with the CBS(T) method, i.e., MP2/CBS computations corrected for higher-order electron-correlation effects using the CCSD(T) method. These benchmark data have been used to examine the performance of the DFT-D, SCS(MI)-MP2, MP2.5, M06-2X and CBS(SCS-D) quantum-mechanical methods, and of the AMBER Cornell et al. force field. The present results, as well as those of our previous study on stacked uracil dimers, confirm that the force field severely exaggerates the repulsion at short intermolecular distances. This behavior complicates the use of the force field in scans of the stacking-energy dependence on local conformational parameters in nucleic acids. Compared against the previous results obtained in the uracil dimer study, the performance of DFT-D to describe stacking at short intermolecular distances has worsened, showing for the adenine dimers a larger exaggeration of the repulsion, especially for structures where the monomers are parallel to each other. Despite these deviations, the performance of DFT-D is still reasonably good and this method provides, for example, a relatively inexpensive way to monitor stacking energies along molecular dynamics trajectories. The best performers are the MP2.5, SCS(MI)-MP2, and CBS(SCS-D) methods. In addition, the energy profiles given by the SCS(MI)-MP2 and CBS(SCS-D) methods are the ones that most closely resemble the CBS(T) data. Interestingly, the performance of the SCS(MI)-MP2 method for stacked adenine dimers is better than for stacked uracil dimers, indicating that the quality of the description may vary with the nucleobase composition. Even though the SCS(MI)-MP2 method cannot match the speed of DFT-D, the results so far render it a promising tool to study intrinsic interactions in systems of moderate size. In general, for most applications all the QM methods tested here are of sufficient accuracy.

Introduction

It is well established that intermolecular interactions, like hydrogen bonding and base stacking, contribute substantially to the structure, dynamics and, ultimately, function of nucleic acids. Despite years of studies, there are many aspects of these interactions that are not satisfactorily understood. For example, the sequence-dependence of the thermodynamics, structure and dynamics of even small RNA motifs has not been fully elucidated, a knowledge of which would contribute to the prediction of RNA secondary and three-dimensional structures.\textsuperscript{1} In order to tackle such questions, experiments are starting to be combined with theoretical methods in an effort to better understand the relative magnitude and nature of the inter-molecular interactions that take place in these biological systems.\textsuperscript{2–5} The theoretical methods used for this purpose comprise both calculations in the gas phase as well as in the condensed-phase media.

The study of biologically relevant molecules in isolation is often criticized due to the neglect of a real chemical environment. However, studies of complexes of nucleic-acid bases in the gas phase are important for several reasons. First, it is the only way to unambiguously evaluate the nature and magnitude of

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\textsuperscript{†} Electronic supplementary information (ESI) available: The studied geometries (xyz coordinates); additional technical details for the computational methods; interaction-energy Tables; plots of energy gradients; plots of DFT-SAPT energy contributions. See DOI: 10.1039/b924461a
the interactions which are due to direct (intrinsic) forces. Second, this knowledge is indispensable for a proper parameterization of other theoretical methods such as force fields, which can be used to study larger systems. Finally, the description of intrinsic interactions is important for the discrimination between intrinsic and externally imposed properties. Experiments do not offer any unambiguous ways to separate the intrinsic forces from the environmental effects. The study of complexes of increasing size can then simulate the transition from the isolated molecule to the condensed medium, and the analysis of the differences between the gas- and condensed-phase behavior can help in the identification of the environmental influences on the building blocks of nucleic-acids. 

Also, gas-phase quantum-mechanical (QM) calculations can be compared to gas-phase experiments, which allows for a direct testing and calibration of the theory, thus increasing our confidence on the latter. Along these lines, we have studied the potential-energy surface (PES) of the adenine dimer in stacked conformations with a set of QM and empirical methods. The stacking of adenine bases can be seen in the majority of nucleic-acid architectures, and definitely the largest variability of geometries of adenine stacks can be found in ribosomes.

The only experimental data on the stabilization energy of nucleic-acid bases in vacuo can be found in the work of Yanson et al., who employed mass-field spectrometry to measure the stabilization enthalpy of several complexes of nucleic-acid bases. At the experimental conditions the partial pressure of 9-methyladenine was not enough for the formation of its dimer. Had these authors reported a stabilization enthalpy for the 9-methyladenine dimer, the experimental value would have had to be compared against the weighted average of theoretical stabilization enthalpies of all populated structures at the experimental conditions. Such populated structures should be determined theoretically, since those experiments did not reveal any structural information.

On the theoretical side, the stacking interactions between the building blocks of biomacromolecules have been extensively studied with QM methods, and in several studies the authors have reported interaction energies for stacked conformations of the adenine dimer, either as independent dimer or as structural component of a DNA base-pair step. The first electron-correlation characterization of a stacked adenine dimer was reported by Šponer et al., using the MP2/6-31G*(0.25) method. The main conclusion of their study was that the stability of stacking comes from the dispersion attraction, and that the electrostatic interactions determine the orientation between the monomers. The study demonstrated the applicability of the Coulombic term with atom-center point charges to describe the electrostatic component of base stacking. Reference calculations extrapolated to the complete basis set (CBS) limit and including higher-order electron-correlation effects using the CCSD(T) method became available only recently and were reported, e.g., for base stacking in B-DNA base-pair steps. Such calculations are quantitatively more accurate than the MP2/6-31G*(0.25) computations, however both methods reveal the same qualitative picture of stacking. For examples of other relevant work involving stacked adenine dimers, the reader is referred to the references given above.
these systems demands a very high level of accuracy in the computation of interaction energies (mainly the dispersion/repulsion balance), as well as a proper inclusion of solvation effects. Therefore it would be very useful to have a method capable of reaching CCSD(T) accuracy in any nucleic-acid system but at a fraction of its computational cost. This would allow the increase of the sampling and size of the studied systems. Equipped with such a method, meticulous analyses of the PES of nucleic-acid base complexes combined with MD simulations and other computational techniques would help us to shed light on the sequence dependence of nucleic-acid structures.

Local variations are frequently associated with interactions involving non-parallel bases, where close contacts and local unstacking can commonly be observed in the base-pair steps, and such geometries have been deliberately included in the set of structures studied in this work. Such geometries are typically not included in reference QM calculations. It should be noted, when compared to our preceding study on stacked uracil dimers, that stacked adenine dimers show a considerably larger effect of higher-order electron-correlation contributions to base stacking (i.e., the correction when passing from the MP2 to the CCSD(T) level). From this point of view the present study is a significant extension of the earlier stacking analysis of uracil dimers. We have also performed DFT-SAPT\textsuperscript{64} energy decompositions for all of the structures with the intention of gaining some insight into the nature of the interaction.

**Computational details**

**Geometries**

The structures have been obtained as follows. First, an adenine monomer is optimized using the RI-MP2\textsuperscript{65–67} method along with the cc-pVTZ\textsuperscript{68} basis set. Then, it is placed in the \(xy\) plane (\(z = 0\)) with the center of mass coinciding with the origin. The N9–H9 bond is parallel to the \(y\)-axis and is pointing to the direction of negative \(y\) values, and the amino group is oriented towards the direction of positive \(y\) values (Fig. 1). This first monomer is always fixed, and the second monomer is initially superimposed on the first one. Then the relative position of the second monomer is determined via 6 independent parameters. Firstly, three angles are set by applying rotational matrices consecutively and in a counterclockwise manner: rotation around the \(x\) axis (\(\gamma\)), rotation around the \(y\) axis (\(\zeta\)) and rotation around the \(z\) axis (\(\omega\)). Then, the second monomer is shifted along the \(x\) and \(y\) axes by the parameters \(\Delta x\) and \(\Delta y\). Finally, the vertical distance is adjusted by \(\Delta z\). In what follows, we define \(\Delta z \equiv r\) for the PES scans. Following this procedure we obtain structures where \(r\) equals the distance between the center of mass of the second monomer and the \(xy\) plane.

The structures are grouped into two different sets, \(P\) and \(NP\) (Fig. 2). The set \(P\) contains 9 different adenine dimers (\(P_1, P_2, \ldots, P_9\)), in conformations where the planes of the rings are parallel (\(P\)) to each other, whereas the set \(NP\) contains 3 different dimers (\(NP_1, NP_2,\) and \(NP_3\)) in conformations in which the monomers are not parallel (\(NP\)) to each other. The geometry of these dimers can be described in terms of the position of the amino group of the second monomer (in white in Fig. 2) relative to a region in the first monomer (in gray in Fig. 2). In \(NP_1\) the amino group is above the N9–H9 region, in \(NP_2\) the amino group is over the C4–C5 region, and in \(NP_3\) the amino group is above the N3 edge. By varying a free structural parameter while the rest are kept fixed, we have built a potential-energy curve (PEC) for each dimer. This way we have generated a subset of structures for each dimer, and these subsets add up to a total of 131 distinct geometries. With one exception (\(P_9\), for which we have scanned the twist angle in increments of 30°) we have determined the dependence of the stacking energy on \(r\) for a fixed combination of the other five geometrical parameters, scanning the \(r\) parameter in increments of 0.1 Å. For the vertical scans of the parallel structures \(r\) ranges from 2.9 to 4.0 Å, whereas for the non-parallel ones the range goes from 3.6 to 4.5 Å, except for \(NP_1\) for which \(r\) ranges from 3.5 to 4.4 Å.

The three non-parallel dimers have been selected by visual inspection of a number of \(\alpha, \gamma, \omega, \Delta x,\) and \(\Delta y\) combinations in order to obtain three vertical scans with diverse close interatomic contacts. There are of course many structures that would be worthy of study, but given that the high-level \textit{ab initio} calculations on these systems are expensive, we have limited the study of stacking to the structures described above. The Cartesian coordinates of all structures are given in the ESI.†

**Interaction energies**

The interaction energies have been calculated following the supermolecular approach. According to this approach the interaction energy \(\Delta E^{\text{AB}}\) of a stacked dimer is calculated as the difference in electronic energy between the dimer and the isolated monomers (see equation below).\textsuperscript{69,70} We have used only rigid monomers, and therefore we do not consider the energies associated with the deformation of the monomers. In the case of the SCS(MI)-MP2, MP2.5 and CBS(T) methods, all of the interaction energies have been corrected for the basis set superposition error (BSSE) using the counterpoise\textsuperscript{71,72} (CP) technique. This correction is not applied within the

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**Fig. 1** Orientation of the first adenine monomer in the \(xy\) plane.

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DFT-D formalism as this method was parameterized against BSSE-corrected data.\textsuperscript{45}  

\[
\Delta E_{AB} = \frac{E_{AB}}{C_0} - \frac{E_A}{C_0} - \frac{E_B}{C_0}
\]

The supermolecular approach is applied for all of the methods used in the present work, except for DFT-SAPT, where the interaction energy is expressed as the sum of selected perturbation contributions. In an intermolecular perturbation theory like DFT-SAPT the BSSE occurs only in the $\delta HF$ term.

**Methods**

The theoretical methods used in the present study are described in the following. Additional technical details are provided in the ESI.\textsuperscript{\textsection}
MP2/CBS calculations corrected by the CCSD(T) method with a small basis set (abbreviated as CBS(T)). The MP2/CBS and CCSD(T) calculations have been performed with the Molpro\textsuperscript{23} 2006.1 software package, applying the frozen-core approximation. In the case of MP2, we have also applied the density-fitting\textsuperscript{24,73} approximation. The Helgaker et al.\textsuperscript{16,73} extrapolation scheme has been used to extrapolate the Hartree-Fock (HF) and the correlation energies. Herein we have used the aug-cc-pVZD → aug-cc-pVTZ extrapolation. The correction for higher-order electron-correlation effects (∆CCSD(T)) has been computed with the cc-pVZD basis set.

**AMBER non-bonded empirical potential.** The empirical-potential calculations have been performed with a local code, employing the van der Waals (vdW) and Coulombic terms of the AMBER force field.\textsuperscript{44} The atom-centered point charges were obtained by an electrostatic potential (ESP) fitting at the MP2/aug-cc-pVZD\textsuperscript{28,78} level of theory using the Merz-Kollman\textsuperscript{79,80} approach as implemented in the Gaussian\textsuperscript{81} 03 software package. The reasons why this level of theory has been chosen for the derivation of point charges can be found in the literature.\textsuperscript{50}

**DFT augmented with an empirical dispersion term (DFT-D).** The DFT-D calculations have been performed with the TurboMole\textsuperscript{82} 5.8 software package in combination with a local code that computes the empirical dispersion correction, following the approach developed by Jurečka et al.,\textsuperscript{45} who parameterized the method by a fitting procedure against the CCSD(T) interaction-energy data for the S22\textsuperscript{83} training set. For the DFT part of the calculation we have used the TPSS\textsuperscript{84,85}6-311++G(3df,3pd)\textsuperscript{37} level of theory and the RI approximation,\textsuperscript{85–88} also known as density fitting. This level of theory augmented with the dispersion correction will hereafter be referred to as DFT-D. When discussing the performance of the DFT-D method we refer exclusively to the approach of Jurečka et al.,\textsuperscript{45} without claiming that the results are valid for other methods that also combine DFT with an empirical correction for dispersion interactions,\textsuperscript{12,17,89–97}

**SCS-MP2 for molecular interactions (SCS(MI)-MP2).** The SCS(MI)-MP2\textsuperscript{46} calculations have been performed with the Molpro\textsuperscript{23} 2006.1 software package, applying the frozen-core and density-fitting\textsuperscript{24,73} approximations. This method has also been parameterized by a fitting procedure against the CCSD(T) interaction-energy data for the S22\textsuperscript{83} training set. Here we calculate the SCS(MI)-MP2 interaction energies using the cc-pVZD → cc-pVTZ extrapolation scheme.

**DFT-symmetry adapted perturbation theory (DFT-SAPT).** The DFT-SAPT\textsuperscript{98–101} calculations have been performed with the Molpro\textsuperscript{23} 2006.1 software package. The decomposition has been carried out using the LPBE0AC\textsuperscript{101} XC potential with the pure ALDA kernel for both the static and dynamic response, in combination with the aug-cc-pVZD basis set. The density-fitting\textsuperscript{101} approximation has also been used. The ionization potential (IP) of the monomer and the energy of the highest occupied molecular orbital (HOMO) have been calculated at the PBE0\textsuperscript{102} /aug-cc-pVZD level of theory with the Gaussian\textsuperscript{81} 03 software package.

**MP2/CBS plus MP3 corrections (MP2.5).** The MP2/CBS calculations have been performed with the Molpro\textsuperscript{23} 2006.1 software package, applying the frozen-core and the density-fitting\textsuperscript{24,72} approximations, whereas the calculations needed for the third-order correction have been performed with a recently developed module\textsuperscript{103} of the MOLCAS\textsuperscript{104} 7 software package, taking advantage of the Cholesky decomposition of the two-electron integrals.\textsuperscript{105} This correction has been computed with the aug-cc-pVZD basis set, and has been damped by 50%.

**Data analysis**

The data have been analyzed in terms of root-mean-squared (RMS) errors, maximum absolute deviations (MAX), and $xy$ plots. In the case of the plots we have plotted not only the interaction energies but also their gradients. RMS errors are calculated using the standard formula. For the calculation of the MAX values we have defined a deviation as the difference between the value obtained with a given method (AMBER, DFT-D, SCS(MI)-MP2, or MP2.5) and the value obtained with CBS(T), and the MAX value for a given dimer merely corresponds to the absolute value of the largest deviation. We have not included the DFT-SAPT method in this analysis because of the relatively small basis set used in the energy decompositions, which leads to underestimation of the strength of the interaction in all cases. However, the basis set used is enough to obtain qualitatively correct information about the relative magnitude of the energy components. With a larger basis set the DFT-SAPT method was shown to provide very accurate total interaction energies in good agreement with CCSD(T) data.\textsuperscript{101} All of the plots have been generated with the Matplotlib\textsuperscript{106} graphics package in combination with the SciPy\textsuperscript{107} and NumPy\textsuperscript{108} packages. The interaction-energy data and the plots of interaction-energy gradients and of DFT-SAPT energy decompositions are given in the ESI.†

**Results and discussion**

**Interaction energies and interaction-energy gradients for the AMBER, DFT-D, SCS(MI)-MP2 and MP2.5 methods**

The vertical scans of the parallel structures (P1–P8) show that in general the QM methods systematically underestimate the strength of the interaction, except for SCS(MI)-MP2, which slightly overestimates it for the P2, P6 and P8 dimers. For all of the tested QM methods the differences with respect to the reference data increase as the intermolecular distance decreases, and given the resolution used in the scans (0.1 Å) all of the methods predict the same optimal intermonomer distance as CBS(T) does, except for DFT-D that overestimates this parameter for P5 (see Fig. 3). Overall, the performance of the QM methods is very good, as can be seen from Fig. 3 and the RMS errors and MAX values reported in Table 1. The SCS(MI)-MP2 method clearly stands out, producing interaction-energy curves with shapes that closely resemble those of the corresponding CBS(T) ones, making this method the one providing the most reliable energy gradients. Furthermore, this method exhibits RMSD and MAX values of only 0.19 and
0.65 kcal mol$^{-1}$, respectively. MP2.5 also performs very well, with RMSD values somewhat larger than those of SCS(MI)-MP2 and energy profiles that deviate from the CBS(T) ones to some degree. Among the QM methods DFT-D exhibits the largest RMS errors (Table 1) and the PECs generated with this method reveal that the interaction energies at short intermolecular distances are being underestimated to a greater extent. Such underestimation could be the consequence of short-range correlation effects that are neither completely described by the density functional used, nor compensated for by the empirical correction. However, the RMS error of DFT-D for the parallel structures is 0.99 kcal mol$^{-1}$, which is a reasonably good result. Note also that DFT-D, among the tested QM methods, is definitely the most economical one, being the only method that would be applicable to really routine massive scans of potential-energy surfaces of base stacking in B-DNA. Contrary to the good performance shown by the QM methods, the AMBER force field shows substantial deviations from the CBS(T) data, giving PECs that overestimate the strength of the interaction at long distances and underestimate it at short ones, with the exception of P1, for which the method overestimates the strength of the interaction energy in a wide range of intermolecular distances (3.2–4.0 Å). The AMBER curves exhibit an excessively steep slope in the short-range repulsion region, overestimating the optimal intermonomer distance by about 0.1 Å in several cases. The little overestimated stabilization at larger distances does not pose any problem in computations of base stacking. However, the substantially exaggerated repulsion for short interatomic distances is significant and may affect studies of local conformational variations significantly.

The most stable structure is P4 (antiparallel undisplaced) at $r = 3.3$ Å, with a CBS(T) stacking energy of $-8.29$ kcal mol$^{-1}$. The highest stability of this structure can easily be explained by the large dispersion overlap combined with favorable electrostatics, which is typical for antiparallel stacks.

The dependence of the stacking energy on twist at $r = 3.3$ Å (P9) is shown in Fig. 4. The DFT-D and MP2.5 methods underestimate the interaction energy (the stabilization) across the entire range of twist angles, whereas SCS(MI)-MP2 slightly underestimates it for angles approximately between 0° and 55°. All of the QM methods show PECs with shapes that more or less match that of the CBS(T) curve. Among these methods SCS(MI)-MP2 is again the best performer, with RMSD and MAX values of 0.10 and 0.20 kcal mol$^{-1}$, respectively. The AMBER force field most underestimates the strength of the interaction, and the shape of its PEC somewhat deviates from the shape of the CBS(T) one. On the contrary, the twist-dependence of the stacking energy in the uracil dimer shows that the force field is more stabilizing than CBS(T), indicating that the force field does not perform even across different nucleic-acid base complexes. However, the effect is not dramatic. For the twist dependencies that do not lead to close atomic contacts, the force field performance is, considering its simplicity, quite amazing. It again justifies the use of the ESP atom-centered point-charge model in the description of base stacking and supports suggestions that base stacking is the best approximated contribution in classical nucleic-acid simulations. For the non-parallel structures the situation is a little bit different from what has been found for the parallel ones (Fig. 5). The SCS(MI)-MP2 and MP2.5 methods underestimate the strength of the interaction energy for all of the dimers in this group, although the underestimation is not very significant. These methods show a similar performance in terms of RMS errors, and both give PECs similar to the reference curve. DFT-D shows deviations from the reference data that in average are larger than the deviations seen for the other two QM methods. For the NP2 and NP3 dimers DFT-D overestimates the strength of the interaction at long intermolecular distances and underestimates it at short ones, and exaggerates the short-range repulsion to some extent. Although not as good as in the case of the parallel dimers, the performance of the SCS(MI)-MP2 method for the non-parallel structures is still remarkable, with RMSD and MAX values of 0.32 and 0.59 kcal mol$^{-1}$, respectively. On the force-field side the AMBER method exhibits the same behavior previously seen for the parallel dimers, that is, the method overestimates the strength of the interaction at long intermolecular distances and underestimates it in the short-range repulsion region, with energy gradients in the latter region whose magnitudes are larger than those produced by the CBS(T) reference method. Both the AMBER and the tested QM methods agree with CBS(T) when predicting the optimal intermolecular separation of the monomers, with only one exception of DFT-D, which overestimates this distance for NP2.

The dashed curves in Fig. 3–5 represent the stacking-energy profiles calculated at the MP2/CBS level of theory. The adenine dimer stacking stabilization energy would be seriously underestimated without the inclusion of the ΔCCSD(T) correction. Evidently, higher-order electron-correlation effects cannot be neglected for aromatic stacking. Note that, contrary to the previously analyzed uracil dimer, the adenine dimer is a system with a very large ΔCCSD(T) correction. Thus, the close match between the tested methods and the CBS(T) data is very satisfactory and demonstrates that even the force field is better suited for the description of stacking than pure MP2/CBS computations. For the most stable antiparallel undisplaced adenine dimer, the ΔCCSD(T) correction amounts to 3.01 kcal mol$^{-1}$, while the correction was 1.14 kcal mol$^{-1}$ for the most stable uracil dimer structure studied in our previous work.

Very good performance is obtained with the SCS(MI)-MP2 method when calculating interaction-energy gradients. Looking at the shapes of the PECs in Fig. 3 and 5 one can see that the SCS(MI)-MP2 method is clearly the best performer, when compared with the reference CBS(T) data. Thus, the method should produce reliable interaction-energy differences for studies of stacked conformations of adenine monomers. In addition, this method has also been shown to give interaction-energy gradients in good agreement with CBS(T) data for several uracil dimers in stacked conformations, which makes one believe that a similar performance might be observed if different nucleic-acid base systems are studied with this method. In the case of the AMBER force field the greatest disagreements with the reference data occur at short intermolecular distances, where the force field exaggerates the short-range repulsion. An example of this is given in Fig. 6,
where it can be seen that at $r = 3.1 \text{ Å}$ (only 0.2 Å away from the CBS(T) minimum) the magnitude of the force-field energy gradient is approximately three times larger than the CBS(T) one. Note that for this distance the CBS(T) energy is about $-7.4 \text{ kcal mol}^{-1}$, and the associated structure is indeed accessible on the CBS(T) PEC. As we have explained in our uracil dimer study, such geometries would be penalized when performing a force-field calculation.

The AMBER force field has also been used in the past to study the potential and free energy surfaces of base pairs in the gas phase, in order to estimate the populations of distinct structures in eventual gas-phase experiments. Our study...

Fig. 3 Potential energy curves for **P1** through **P8**, calculated with the AMBER, DFT-D, SCS(MI)-MP2, MP2.5 and CBS(T) methods (the MP2-CBS curve is also given).
indicates that the force field is rather well suited for this purpose, and we would recommend using its variant with gas-phase atom-centered point charges, as used in the present paper. (Note that condensed-phase simulations utilize HF charges which overestimate the polarity of the monomers.) On the other hand we have to exercise caution, because the evaluation of populations of co-existing structures may be very sensitive to even small errors in the energy description and it is unlikely that the force field achieves a true quantitative accuracy, especially in the case of co-existence of H-bonded, T-shaped and stacked structures. It would be thus advisable to combine force-field calculations with highest-quality QM calculations for the key structures in their respective local and global minima. It is usually assumed that the most accurate contemporary gas-phase benchmark calculations may have an accuracy of around 0.5 kcal mol⁻¹ for the individual structures. The force field certainly cannot achieve such accuracy.

Performance of the M06-2X and CBS(SCS-D) methods

Obviously, the above-examined DFT-D, MP2.5 and SCS(MI)-MP2 methods represent only a fraction of the recently introduced techniques capable to describe well base stacking. During the review process we finished testing two additional techniques: M06-2X and CBS(SCS-D). The former method is a hybrid meta exchange–correlation density functional that does not require an additional empirical dispersion term, and the latter method makes use of the SCS-CCSD method to evaluate a ΔSCS-CCSD difference (ΔSCS-CCSD = ΔE_{MP2} - ΔE_{SCS-CCSD}), which is then added to the MP2/CBS energy. The CBS(SCS-D) method therefore aims to offer a viable alternative for accurate calculations of large systems when the CBS(T) method is already inapplicable. In the present work the ΔSCS-CCSD difference has been calculated with the cc-pVDZ basis set. The results of the M06-2X and CBS(SCS-D) calculations are summarized in Fig. S24–S35 in the ESI.

Both methods perform well, but the latter is a much better performer. For the parallel structures the RMS error and MAX value obtained with CBS(SCS-D) are 0.31 and 0.68 kcal mol⁻¹, respectively while for the non-parallel structures these quantities amount to 0.20 and 0.41 kcal mol⁻¹, respectively. In terms of RMS errors, for the parallel structures the performance of SCS(MI)-MP2 is better than that of CBS(SCS-D), but the converse is true in the case of the non-parallel ones. Nevertheless, we would rank both methods as highly accurate and entirely comparable to each other. On the DFT side and also taking as performance parameter the RMS error, DFT-D is better than M06-2X in both cases. Fig. S24–S32 show that for the parallel structures the shapes of the CBS(SCS-D) PECs would ensure very good gradients at all intermolecular distances, whereas M06-2X tends to produce good gradients at short and long intermolecular distances, while showing deviations from the CBS(T) PECs when approaching the minimum. The M06-2X method considerably underestimates the strength of the interaction at long distances, and predicts in most cases a location of the

<table>
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<th>Dimer</th>
<th>AMBER RMSD</th>
<th>AMBER MAX</th>
<th>DFT-D RMSD</th>
<th>DFT-D MAX</th>
<th>SCS(MI)-MP2 RMSD</th>
<th>SCS(MI)-MP2 MAX</th>
<th>MP2.5 RMSD</th>
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Some extreme MAX values are not visualized in the figures because they are out of the range of intermolecular distances in those figures. P9 not included in the calculation of RMSD and MAX values, i.e., the values in this row correspond to the group of dimers for which vertical scans have been performed.

**Table 1** Root-mean-squared deviations (RMSD, kcal mol⁻¹) and maximum absolute deviations (MAX, kcal mol⁻¹) of the AMBER, DFT-D, SCS(MI)-MP2, and MP2.5 interaction energies with respect to the CBS(T) reference data

**Fig. 4** Potential energy curves for P9, calculated with the AMBER, DFT-D, SCS(MI)-MP2, MP2.5 and CBS(T) methods (the MP2/CBS curve is also given). The vertical separation between the monomers is 3.3 Å.
minimum that is 0.1 Å too short when compared to the CBS(T) data.

**DFT-SAPT interaction-energy decompositions**

The plots of the DFT-SAPT interaction-energy terms of all dimers are given in Fig. S11–S23 in the ESI. In all of the structures the dispersion term is the most important stabilizing contribution, followed by the electrostatic term, and in none of them is the magnitude of the electrostatic interaction close to that of the dispersion term, which can be explained in terms of the lower polarity of adenine when compared to other nucleic-acid bases. On the contrary, for dimers of more polar bases like uracil there exist conformations for which the difference between the magnitudes of the dispersion and electrostatic terms is not as large, with the latter term also providing a very significant part of the binding. The contributions of the induction and $\delta HF$ terms are also stabilizing in all cases, but not as significant as the contributions of the dispersion and electrostatic terms. The fact that the induction term is not significant in this case should not be interpreted as polarization effects not being important for nucleic-acid systems. Such effects are relevant for macromolecular simulations in condensed media, and many efforts are currently being devoted to the development of polarizable force fields in order to perform more realistic MD simulations. As is usual for vdW complexes, the dispersion and exchange terms tend to cancel each other in the region around the minimum.

Similar to what has been found for different stacked conformations of the uracil dimer, in the case of stacked adenine dimers the AMBER electrostatic component is in many cases repulsive, and the magnitude of the repulsion increases as the intermolecular distance decreases. This description is physically incorrect and is in disagreement with DFT-SAPT calculations which show that the electrostatic component becomes more stabilizing at short intermolecular separations (see for instance the electrostatic component of P1 in Fig. 7). This penetration effect is not taken into account in the AMBER electrostatic term, but it is implicitly (effectively) included in the vdW part of the force field, at least to some extent. This is sufficient for overall reasonable balance of the force field. For some structures like P6 one can see that the force field is sometimes able to produce electrostatic-energy profiles that show that the contribution is more stabilizing at short intermolecular separations, but the distance dependence of the AMBER electrostatic term is weak when compared against the DFT-SAPT method. This of course does not mean that the force field is able to capture penetration effects in some situations. It is well known that most of the force fields neglect such effects, but several approaches have been explored in order to take them into account.

One interesting feature of P1 is the behavior of its DFT-SAPT electrostatic term, being the most repulsive, except at very short intermolecular distances where this term becomes
more stabilizing than in the case of $P_2$ and $P_3$ (see Fig. S23 in the ESI$^\dagger$). This indicates that electrostatic penetration effects are more favorable for the $P_1$ geometry at very short distances, compared to the other two geometries.

For the angular variations of the electrostatic energy one can see for $P_9$ in Fig. 7 that the AMBER energy profile basically matches the DFT-SAPT one, giving in this case a correct physical description. The horizontal twist scan of $P_9$ shows that the electrostatic and exchange terms clearly depend on the twist angle $\omega$, and that the dispersion term is basically constant across the entire range of twist angles.

Summary and conclusions

We have studied about 130 structures of adenine dimers in stacked conformations in order to assess the performance of the AMBER force field and the DFT-D, SCS(MI)-MP2, and MP2.5 QM methods against CBS(T) data. The PECs reported herein can also serve as reference data for the examination or parameterization of other theoretical methods. Specific attention was paid to structures where short-range repulsion is important, either due to vertical compression of the dimers or the presence of close interatomic contacts due to mutual tilting of the bases. Note that these geometries are not pure artificial constructs, but they appear e.g. in time-averaged nucleic-acid structures (mainly those with tilted bases) or are commonly sampled due to thermal fluctuations. Such structures are not included very often in reference datasets. The present study thus brings a considerable extension of the currently available reference data for base stacking.

Similar to what has been found in the study of stacked uracil dimers, $^{50}$ large differences between the CBS(T) method and the AMBER force field can be seen in the PECs for geometries where the dimers are either vertically compressed or contain close interatomic contacts. The description of such geometries suffers clearly from a sharply exaggerated repulsion by the force field.

The reported differences in the interaction energies are large enough to influence the description of the fine structure of nucleic acids, like local conformational variations in B-DNA. Perhaps, this exaggerated repulsion should not affect MD simulations dramatically, since the sampling of all the other degrees of freedom should effectively alleviate the effects of the excessive repulsion. Nevertheless, thermal fluctuations of stacked systems could be influenced to certain extent. On the other hand, there are likely to be major artifacts in calculations where the empirical force field is used for scanning the dependence of stacking energy on local conformational parameters that can lead to steric clashes, such as propeller twist and base-pair roll. Note that such contacts are supposed to be one of the very driving forces for the sequence dependence of B-DNA geometry. $^{51,53,115}$ The exaggerated repulsion of the

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**Fig. 7** Comparison between DFT-SAPT and AMBER electrostatic energies for $P_1$, $P_3$, $P_6$, and $P_9$. 

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force field upon compression and formation of interatomic contacts is primarily due to the simplicity of the van der Waals Lennard-Jones part of the force field. Its repulsive $r^{-12}$ term may be too hard and could be eventually replaced by some softer term, such as an exponential function.

Compared to previous results on stacked uracil dimers, the agreement between CBS(T) and DFT-D at short intermolecular distances has worsened. For the adenine dimers the amplification of the repulsion shown by DFT-D is larger than in the case of the uracil dimers. The largest deviations of the DFT-D interaction energies occur for the parallel structures at intermolecular distances below the optimum. Despite the deviations of the DFT-D interaction-energy profile in the short-range repulsion region the performance of the method is still satisfactory (considering also the speed of DFT-D), with RMS errors of 0.99 and 0.58 kcal mol$^{-1}$ for the parallel and non-parallel dimers, respectively. Given the reasonably good performance of the method to describe stacking profiles in different conformations of uracil and adenine dimers, we believe that DFT-D could also perform well for other nucleic-acid base complexes, in line with other tests available in the literature. Since DFT-D calculations are not computationally expensive, they could even be used for the monitoring of stacking interactions along MD simulation trajectories, which involves the computation of stacking energies for massive amounts of structures.

The best results, as compared to the reference CBS(T) calculations, were obtained with the MP2.5 and SCS(MI)-MP2 methods. Both methods yield RMS errors basically lower than 0.5 kcal mol$^{-1}$ and the shape of their interaction-energy profiles are more similar to the CBS(T) ones. The performance of SCS(MI)-MP2 is remarkable, with RMS errors of 0.19 and 0.32 kcal mol$^{-1}$ for the parallel and non-parallel dimers, respectively. The MAX values did not exceed 0.65 kcal mol$^{-1}$ and they were below 0.30 kcal mol$^{-1}$ for many scans. In addition, this method is the one providing the profiles that most closely match the CBS(T) ones, which means that the SCS(MI)-MP2 would also outperform the rest of the methods when calculating differences of interaction energies. Interestingly, however, the SCS(MI)-MP2 method was a little less satisfactory for the stacked uracil dimers than for the stacked adenine dimers. This indicates that the performance of the methods for base stacking can modestly vary with the nucleobase composition. Thus, additional studies of some carefully chosen stacked systems would improve our understanding of this phenomenon (work in progress). Nevertheless, even the SCS(MI)-MP2 performance achieved earlier for the uracil dimer would be excellent for the majority of possible applications. It should be mentioned here that the SCS(MI)-MP2 method was parameterized against the interaction-energy data of the S22 dataset, which does not contain adenine dimers. The SCS(MI)-MP2 method is more economical than MP2.5, at least with the method, setup, codes and computers used in our study. However, neither method is sufficiently fast for massive computations and, therefore, they cannot compete with the speed of DFT-D or the force field.

Considering all the results, the CBS(T) method will continue to be the gold standard for QM calculations on nucleic-acid systems. However, having an accurate method that would allow routine calculations on base-pair steps is highly desirable. It seems that the SCS(MI)-MP2 method would perform very well in cases where more geometries and larger systems need to be studied. Regarding SCS(MI)-MP2 and CBS(T) there is still room for accuracy improvements because larger extrapolation schemes can be used. For the former method we have used the cc-pVDZ → cc-pVTZ extrapolation, while the use of the cc-pVTZ → cc-pVQZ extrapolation could still bring some quantitative changes. In the case of CBS(T), the performance of the method can be improved by using larger basis sets for the ΔCCSD(T) correction. For most applications, however, the expected improvement would be insignificant. Concerning the need for massive QM computations on stacking systems, the DFT-D method seems to be suitable for the task. Finally, although having several limitations, the force field remains a choice for the qualitative description of base stacking forces. Nevertheless, when assessing base stacking in nucleic acids all QM methods have one disadvantage, which is not easy to overcome. They include full gas-phase electrostatics and do not allow its exclusion. Due to the presence of solvent screening effects in nucleic acids, the energy contribution of gas-phase electrostatics to the stacking stability is known to be overwhelmingly suppressed. For example, a recent QM investigation of twist-dependence of stacking in B-DNA predicts optimal helical-twist values that are far away from the observed range for most base-pair steps, scattered in a wide range of values between less than 20° and almost 50°. Thus, unless the studied system is appropriately solvated, calculations with the pure vDW term of the force field (retaining the dispersion overlap of the stacked bases and their steric shape while switching off the electrostatics) may achieve better relevance to assess the stability of experimental stacks than calculations with full inclusion of electrostatics.

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