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What Are the Preferred Horizontal Displacements of Aromatic-Aromatic Interactions in Proteins? Comparison with Calculated Benzene-Benzene Potential Energy Surface

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The data from protein structures from the Protein Data Bank and quantum chemical calculations indicate the importance of aromatic-aromatic interactions at large horizontal displacements (offsets). In the proteins stacking interactions of phenylalanine residue show preference for large offsets (3.5-5.0 Å), while the calculations show substantially strong interactions, about -2.0 kcal/mol.

Introduction

The aromatic-aromatic interactions have been intensively studied because of their importance in numerous molecular systems from biomolecules to materials.[1,2] These interactions are of great importance in proteins, since they play a role in protein folding, stability, protein-protein and protein-ligand recognition.[3-12]

A great deal of experimental and theoretical work has focused on studying aromatic interactions.[13] The calculations on benzene dimer show that there are two minima: parallel stacking and T-shaped (edge-to-face) orientations. The calculated interaction energies for these two orientations are -2.73 and -2.84 kcal/mol respectively.[14]

Although aromatic-aromatic interactions have been extensively studied, parallel interactions at large horizontal displacements (offsets) have been reported recently.[15]

Statistical analysis of the data from the Cambridge Structural Database has showed that in crystal structures preferred parallel benzene-benzene and pyridine-pyridine interactions are at large offsets (3.5-5.0 Å). By calculations substantial interaction energies around -2.0 kcal/mol have been obtained for large offsets of 3.5-5.0 Å.[15] For benzene-benzene dimer this is 71% of the stacking interaction energy at the minimum, which is stronger than the interaction at face-to-face orientation.

More than three decades ago aromatic-aromatic interactions were recognized to stabilize protein structures.[3] Studies on aromatic-aromatic interactions in proteins[4-9] show that T-shaped orientations are preferred[3-7] in all classes of proteins.[7] However, parallel stacking orientations also appear in proteins.[5,9,11] Aromatic residues show a high tendency towards forming clusters beyond the dimer which has a significant influence on protein folding, structure, and stability.[7,10]

Herein we present the results on the interactions between aromatic rings of phenylalanine in protein structures including interactions at large horizontal displacements (offsets). We have also calculated benzene-benzene potential energy surface in order to understand data from protein structures. To the best of our knowledge this is the first study comparing the interactions of phenylalanine aromatic rings in proteins with accurate quantum chemical calculations of benzene-benzene potential energy surface, including interactions at large horizontal displacements (offsets).

Results and discussion

Interactions in protein structures

For the purposes of this study, we used the Protein Data Bank`s (PDB`s), release from December 2013. The full protein set is filtered to reduce the redundancy, using PDBSELECT

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(Novembar, 2012)[16] list of nonredundant protein chains, with the threshold 25% and a resolution of 3.0 Å or better.

In the interaction of two phenyl rings of phenylalanine residues one can recognize an acceptor and a donor ring. The acceptor ring has R_1 shorter than R_2 (Fig. 1), while it is opposite in case of the donor ring.

The PDB search yielded 6 919 contacts where centers of donor phenyl rings were found within the area that corresponds to the ellipsoid (r = 7.0 Å and R = 6.0 Å) around an acceptor phenyl ring (Fig. S1).

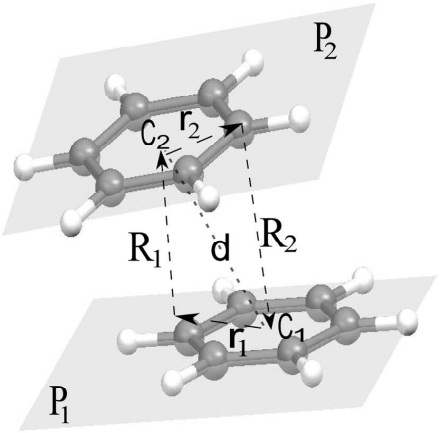

Fig. 1 Geometrical parameters used for describing interactions between benzene rings. The distance between the centers of the interacting pyridine rings is *d*. The R_1 is the normal distance between center of the ring C_2 and the plane P_1. The R_2 is the normal distance between center of the ring C_1 and the P_2. The r_1 and r_2 (offsets) are the distances from the projection of one ring center on the average plane of another ring and the center of that ring. The P_1/P_2 is tilt (dihedral) angle.

The distribution of tilt angle between two planes P_1/P_2 (Fig. 1) for the contacts in ellipsoid is shown in Fig. 2. At low values of tilt angle two aromatic rings form a stacking interaction, while at the values above 60º two aromatic rings are in edge-to-face (T-like) orientation forming CH/π interactions. The data in Fig. 2 show that a large number of phenyl-phenyl contacts in proteins have tilt angles above 60º. This is in agreement with previous data on aromatic interactions in proteins.[3-6,9]

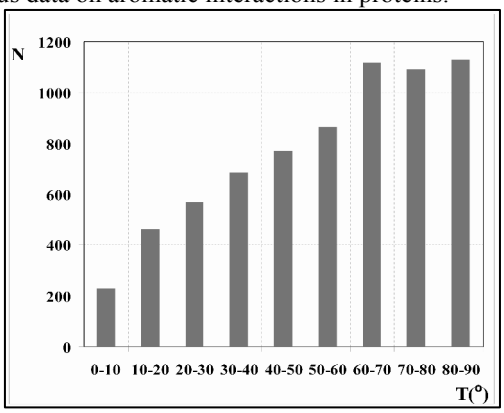

Fig. 2 The distribution of the tilt (dihedral) angle between planes P_1/P_2 of interacting phenylalanine aromatic rings

We have separately analyzed the distribution of offset value r for various values of tilt angle (Fig. 3). For the tilt angles in the range 0-10º, the distribution of offset shows a slight preference for the values above 3.0 Å. For the tilt angles in the range 10-50º (Fig. 3a) the distributions show a large number of interactions with higher offset values, over 50% of the interactions have offsets above 3.5 Å. This is similar to the previous results on Phe-Phe interactions in proteins[5,11] and to the results from crystal structures from the Cambridge Structural Database showing that two stacking benzene rings prefer large offsets.[15] For the tilt angle above 50º (Fig. 3b)the number of structures with smaller offset values increases. However, fraction of the structures with large offset values is still substantial. We have also presented the distribution of mean force (according to Boltzmann law) for different r values in ESI (Fig. S4).

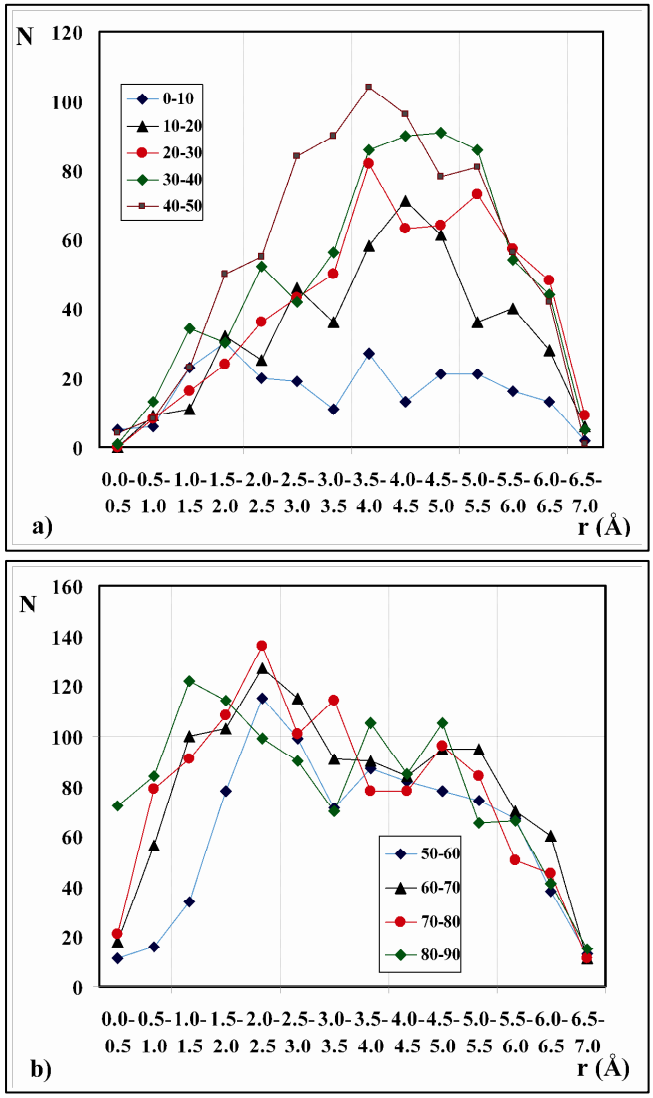

Fig. 3 Distributions of offset value r for various tilt angles: a) for the tilt angles in the range 0-50º, b) for the tilt angles in the range 50-90º

Quantum chemical calculations of benzene-benzene potential energy surface

In order to better understand interactions of phenylalanine aromatic ring we have performed calculations on benzene dimer. All calculations have been done in ORCA (version 2.8) program[17] using B2PLYP-D2[18,19] method and def2-TZVP[20] basis set. This method, without correction for basis-set

superposition error, gives results that are in excellent agreement with the very accurate CCSD(T) data for benzene interactions.[21]

For four benzene-benzene orientations, A, B, C and D (Fig. 4), the interaction energy curves were calculated for tilt angles 0º, 20º, 40º, 60º and 90º. For every tilt angle the monomer geometries were kept rigid while offset r and normal distance R were systematically varied. The calculations were done for the offset values in the range of 0.0 to 6.0 Å. An offset value of 0.0 Å corresponds to the conformation with the centers of the rings above another.

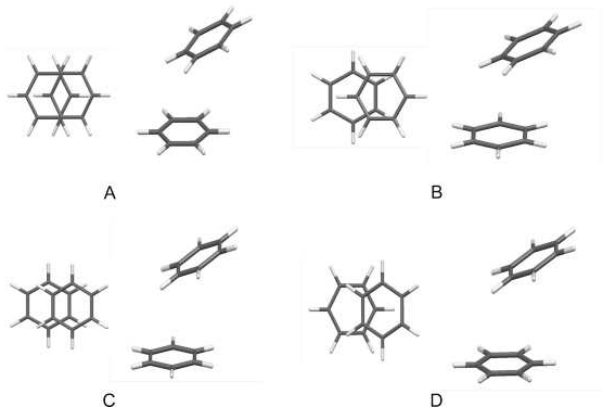

Fig. 4 The top and the side view of A, B, C and D orientations at the offset of 1.5 Å and tilt angle of 20º

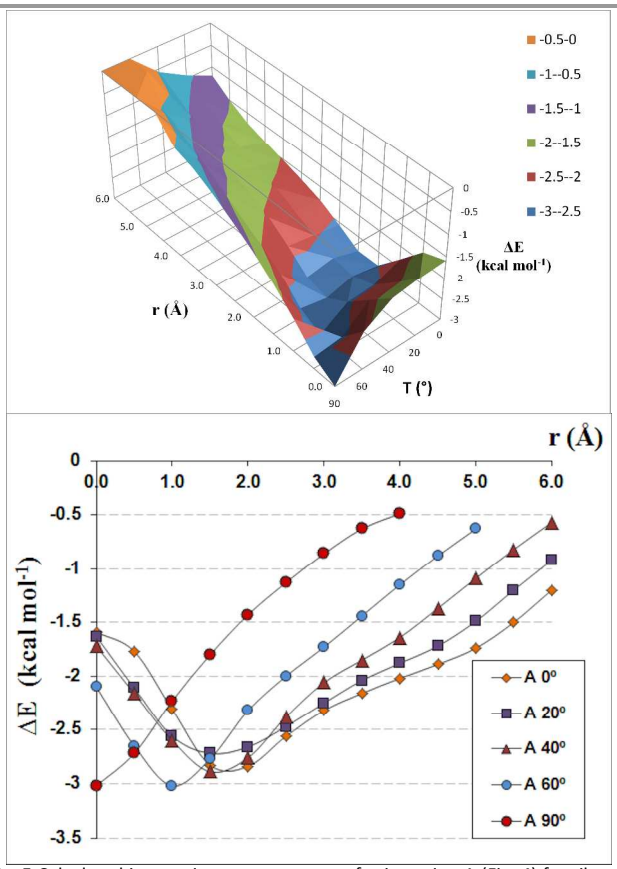

Fig. 5 Calculated interaction energy curves of orientation A (Fig. 4) for tilt angles of 0º, 20º, 40º, 60º and 90º presented on 2-D (down) and 3-D (up) diagrams. The curve for angle 0º was calculated in previous work.[15]

Table 1. Calculated interaction energies at minima of potential curves (Fig. 5, 6, S8, S12) in kcal/mol

Tilt angle [a]	0	20	40	60	90
A	-2.84	-2.72	-2.89	-3.02	-3.02
B	-2.85	-2.73	-2.90	-3.02	-3.02
C	-2.84	-2.64	-2.45	-2.48	-2.56
D	-2.85	-2.64	-2.45	-2.49	-2.57

[a] The calculated minima for tilt angle 0° is at the 1.5 Å in all studied orientations. For orientations A and B for tilt angles 20°, 40°, 60° and 90° minima are at offset 1.5, 1.5, 1.0 and 0.0Å respectively. For C and D for tilt angles 20°, 40°, 60°, and 90°, minima are at offset 1.5, 1.0, 1.0 and 0.0 Å respectively

For orientation A the calculations show that energy curves (Fig. 5) for tilt angles of 0°, 20°, and 40° have minima with energies of -2.84, -2.72 and -2.89, respectively (Table 1). Interaction energies are quite strong even at large offsets of 3.5 Å; they are about -2 kcal/mol (Table 2). The interactions for larger tilt angles (60° and 90°) are somewhat stronger at the minima of potential curves (-3.02 kcal/mol), however, they are much weaker at the large offsets. The results of the calculations on orientation B are very similar to the results for the orientation A. (Tables 1 and 2, Fig. S8).

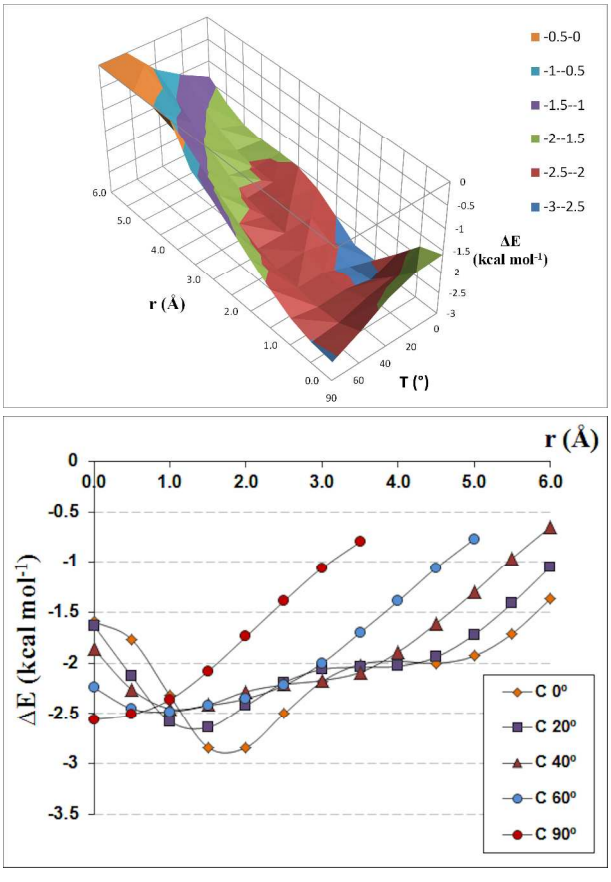

Fig. 6 Calculated interaction energy curves of orientation C (Fig. 4) for tilt angles of 0º, 20º, 40º, 60º and 90º presented on 2-D (down) and 3-D (up) diagrams. The curve for angle 0º was calculated in previous work.[15]

Table 2. Calculated interaction energies in kcal/mol at large offsets (Fig. 5, 6, S8, S12)

Tilt angle	0		20		40		60		90[a]
Offset	3.5 Å	4.5 Å	3.5 Å	4.5 Å	3.5 Å	4.5 Å	3.5 Å	4.5 Å	3.5 Å
A	-2.16	-1.89	-2.04	-1.72	-1.85	-1.37	-1.44	-0.88	-0.63
B	-2.08	-1.97	-2.05	-1.83	-1.88	-1.50	-1.49	-1.02	-0.78
C	-2.01	-2.00	-2.04	-1.94	-2.09	-1.61	-1.70	-1.06	-0.80
D	-2.08	-1.97	-2.06	-2.01	-2.13	-1.78	-1.84	-1.22	-0.93

[a]The interaction energies at offset 4.5 Å for tilt angle 90° are omitted because the donor and acceptor molecules swap the roles.

For orientation C the energy curves (Fig. 6) for tilt angles 0° and 20° are similar to the curves for orientation A, while the curves for angles 40°, 60° and 90° are quite different. For tilt angle 40° the most interesting feature is a small difference in the energies at the minimum (-2.45 kcal/mol) and at large offsets (-2.09 kcal/mol). For tilt angle 60° and 90° the minima at the curves are -2.48 and -2.56 kcal/mol, which is weaker than in case of orientation A.

The results of calculations on orientation D are very similar to the results for orientation C (Tables 1 and 2, Fig. S11). Interestingly, for orientations A and B, the edge-to-face geometry (tilt angle above 50º) is more stable than the stacking geometry (tilt angle below 50º), whereas for orientations C and D it is the opposite (Table 1).

The calculated data are in agreement with the data on phenyl-phenyl geometries in proteins. Calculated energies at large offsets for stacking interactions (tilt angles below 50°) are stronger than for edge-to-face orientations (tilt angles above 50°) (Fig. 5 and 6, Table 2). It is in agreement with the difference in the distribution of offsets for tilt angles below 50° and above 50° observed in the protein structures (Fig. 3). Also, by calculations we have obtained a small difference in the energies at the minima and at the larger offsets for tilt angles 20° and 40° for orientations C and D which can explain a preference for large offsets for tilt angles 20°-50° observed in proteins.

The relatively strong stacking interactions at large offsets are a consequence of attractive dispersion and electrostatic interactions. We have compared B3LYP[22] (without dispersion interactions) and B3LYP-D[19,22] energies (with dispersion interactions). At a large offset (5.0 Å) dispersion is substantial, although it is half of the dispersion at the offset 1.5 Å. However, the repulsion is even more reduced, resulting in a substantial attraction at a large offset. Electrostatic potential of benzene molecule (Fig. S13) indicates attractive electrostatic interaction at large offsets. Namely, at a large offset (about 3.0 Å) the potential changes from negative to positive, forming a local dipole. When two benzene rings overlap at the large offsets the dipole-dipole electrostatic interaction is very favourable.

The preference for phenyl-phenyl interactions at large offsets in protein structures is also caused by additional simultaneous interactions of aromatic rings that can be achieved at large offsets. Namely, in the interactions at large offsets faces of phenyl rings can simultaneously form interactions with other groups in proteins. In our previous work we showed simultaneous additional interactions for aromatic/aromatic interactions at large offsets.[15]

Conclusions

The data from the Protein Data Bank (PDB) and quantum chemical calculations indicate importance of aromatic-aromatic interactions at large horizontal displacements (offsets). The statistical analysis of the data from the PDB on aromatic-aromatic interactions of phenylalanine residues shows a preference for large offsets in stacking interactions. Calculations show that stacking interactions at large offsets are substantially strong, around -2 kcal/mol. Calculations also show that for some benzene-benzene orientations the difference in energy between minimum on the potential curve and large offsets is relatively small (< 0.5 kcal/mol). The preference for large offsets is also caused by additional simultaneous interactions that faces of aromatic rings can form at large offsets.

These results can be very important in recognizing the significance of aromatic-aromatic interactions at large horizontal displacements (offsets) in proteins.

Acknowledgements

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Notes and references

1 L. M. Salonen, M. Ellermann and F. Diederich, *Angew. Chem. Int. Ed.,* 2011, **50**, 4808; C. Bissantz, B. Kuhn and M. Stahl, *J. Med. Chem.*, 2010, **53**, 5061; M. L. Ma, Y. Kuang, Y. Gao, Y. Zhang, P. Gao and B. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 2719; H. Robson Marsden, J. G. E. M. Fraaije and A. Kros, *Angew. Chem. Int. Ed.*, 2010, **49**, 8570; H. J. Schneider, *Angew. Chem. Int. Ed.*, 2009, **48**, 3924; W. B. Motherwell, J. Moise, A. E. Aliev, M. Nič, S. J. Coles, P. N. Horton, M. B. Hursthouse, G. Chessari, C. A. Hunter and J. G. Vinter, Angew. *Chem. Int. Ed.* 2007, **46**, 7823.

2 B. Mészáros, P. Tompa, I. Simon and Z. Dosztányi, *J. Mol. Biol.*, 2007, **372**, 549; A. V. Ruban, A. B. Rozhenko, V. V. Pirozhenko, S. V. Shishkina, O. V. Shishkin, A. M. Sikorsky, S. O. Cherenok and V. I. Kalchenko, *Tetrahedron Letters*, 2013, **54**, 3496; A. Lattanzi, C. De Fusco, A. Russo, A. Poater and L. Cavallo *Chem. Commun.*, 2012, **48**, 1650; J. Liu, J. Li, Y. Jiang, S. Yang, W. Tan and R. Yang *Chem. Commun.*, 2011, **47**, 11321; M. J. M. Muñoz and G. Fernández *Chem. Sci.*, 2012, **3**, 1395.

3 S. K. Burley and G. A. Petsko, *Science*, 1985, **229**, 23.

4 J. Singh and J. M. Thornton, *FEBS Lett*, 1985, **191**, 1.

5 C. A. Hunter, J. Singh and J. M. Thornton, *J. Mol. Biol.*, 1991, **218**, 837.
6 A.Thomas, R. Meurisse, B. Charloteaux and R. Brasseur, *Proteins Struct. Funct. Genet.*, 2002, **48**, 628.
7 M. Chourasia and G. M. Sastry, *Int. J. Biol. Macromol.*, 2011, **48**, 540.
8 R. Bhattacharyya, U. Samanta, P. Chakrabarti, *Protein Eng.*, 2002. **15**, 91.
9 G. B. McGaughey, M. Gagné and A. K. Rappé, *J. Biol. Chem.*, 1998 **273**, 15458.
10 N. Kannan and S. Vishveshwara, *Protein Eng.*, 2000, **13**, 753; E. Lanzarotti, R. R. Biekofsky, D. A. Estrin, M. A. Marti and A. G. Turjanski, *J. Chem. Inf. Model*., 2011, **51**, 1623.
11 P. Chakrabarti and R. Bhattacharyya, *Prog. Biohys. Mol. Bio.*, 2007, **95**, 83.
12 K. Berka, R. A. Laskowski, P. Hobza and J. Vondrášek, *J. Chem. Theory Comput.*, 2010, **6**, 2191; I. L. Budyak, A. Zhuravleva and L. M. Gierasch, *J. Mol. Biol.*, 2013, **425**, 3522; I.S. Moreira, J. M. Martins, R. M. Ramos, P. A. Fernandes and M.J. Ramos, Biochimica et Biophysica Acta, 2013, **1834**, 404; K. E. Riley and P. Hobza, *Acc. Chem.Res*, 2013, **46**, 927.
13 E. R. T. Tiekink and J. Z. Schpector, *Chem. Commun.*, 2011, **47**, 6623; J. Z. Schpector, I. Haiduc and E. R.T. Tiekink, *Chem. Commun.*, 2011, **47,** 12682 ; W. B. Schweizer and J. D. Dunitz, *J. Chem. Theory Comput.*, 2006, **2**, 288; J. Řezáč and P. Hobza, *J. Chem. Theory Comput.*, 2008, **4**, 1835; S. Tsuzuki, K. Honda, T. Uchimaru, M. Mikami and K. Tanabe, *J. Am. Chem. Soc.*, 2002, **124**, 104; M. O. Sinnokrot, E. F. Valeev, C. D. Sherrill, *J. Am. Chem. Soc.*, 2002, **124**, 10887; A. Robertazzi, F. Krull, E.W. Knapp and P. Gamez *CrystEngComm*, 2011, **13**, 3293; R. K. Raju, J. W. G. Bloom, Y. An, S. E. Wheeler, *ChemPhysChem*, 2011, **12**, 3116; N. A. Seifert, A. L. Steber, J. L. Neill, C. Pérez, D. P. Zaleski, B. H. Pate and A. Lesarri *Phys. Chem. Chem. Phys.*, 2013, **15**, 11468; H. Li, Y. Lu, Y. Liu, X. Zhu, H. Liu and W. Zhu *Phys. Chem. Chem. Phys.*, 2012, **14**, 9948; C. R. Martinez and B. L. Iverson *Chem. Sci.*, 2012, **3**, 2191; E. G. Hohenstein and C. D. Sherrill, *J. Phys. Chem. A*, 2009, **113**, 878; I. Geronimo, E. C. Lee, N. J. Singh and K. S. Kim, *J. Chem. Theory Comput.*, 2010, **6**, 1931; M. Pitoňák, P. Neogrády, J. Řezáč, P. Jurecka, M. Urban and P. Hobza, *J. Chem. Theory Comput.*, 2008, **4**, 1829.
14 E. C. Lee, D. Kim, P. Jurečka, P. Tarakeshwar, P. Hobza, K. S. Kim, *J. Phys. Chem. A*, 2007, **111**, 3446
15 D. B. Ninković, G. V. Janjić, D. Ž . Veljković, D. N. Sredojević and S. D. Zarić, *ChemPhysChem*, 2011, **12**, 1; D. B. Ninković, J. M. Andrić and S. D. Zarić, *ChemPhysChem*, 2013, **14**, 237.
16 S. Griep and U. Hobohm, *Nucleic. Acids. Res.*, 2010, **38**, D318.
17 F. Neese, ORCA 2.8, University of Bonn, Bonn, Germany, http://www.thch.uni-bonn.de/tc/orca/.
18 S. Grimme, *J. Chem. Phys.*, 2006, **124**, 034108/1.
19 S. Grimme, *J. Comput. Chem.*, 2006, **27**, 1787.
20 F. Weigend and R. Ahlrichs, *Phys. Chem. Chem. Phys.*, 2005, **7**, 3297; A. Schaefer, H. Horn and R. Ahlrichs, *J. Chem. Phys.*, 1992, **97**, 2571
21 J. C. Sancho-Garcia, A. J. Perez-Jimenez, J. Chem. Phys. 2009, 131, 084108/1.
22 A. D. Becke, *J. Chem. Phys*., 1993, **98**, 5648; A. D. Becke, *Phys. Rev. A*, 1988, **38**, 3098; C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785– 789.

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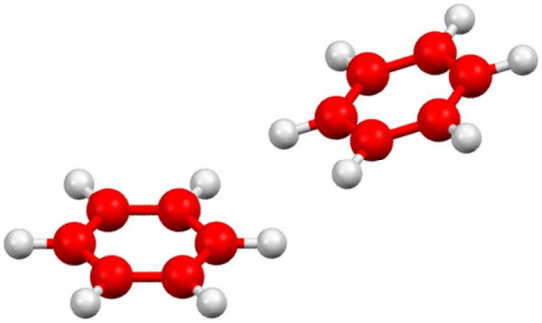

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