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# Role of CH/π interactions in substrate binding by *Escherichia coli* β-galactosidase

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**Abstract**—Interactions between carbohydrates and aromatic amino-acid residues are often observed in structures of carbohydrate–protein complexes. They are characterized by an orientation of the pyranose or furanose ring parallel with the aromatic ring of amino-acid residues. An important role in the formation of these complexes is supposed to be played by CH/π interactions. This paper presents an ab initio quantum chemistry study of CH/π interactions between β-galactosidase from *E. coli* and its substrates and products. The energy stabilizing the interaction between Trp999 residue and substrate bound in the shallow binding mode was calculated at the MP2/6-31+G(d) level as 5.2 kcal mol$^{-1}$ for the glucose moiety of allolactose, 2.4 kcal mol$^{-1}$ for the galactose moiety of allolactose and 5.0 kcal mol$^{-1}$ for the glucose moiety of lactose. The energy stabilizing the interaction between Trp568 residue and galactose in the deep binding mode was calculated as 2.7 kcal mol$^{-1}$. Interaction energies at the HF/6-31+G(d) and B3LYP/6-31+G(d) levels were small or repulsive; therefore, highly correlated ab initio methods were necessary to study these interactions. These unexpectedly strong interactions give a rationale for allolactose formation and illustrate the role of the Trp999 residue. In addition, this illustrates the importance of CH/π interactions for the function of carbohydrate-binding proteins and carbohydrate-processing enzymes.


*Keywords:* CH/π interactions; β-Galactosidase; Ab initio; Carbohydrate; Molecular recognition

## 1. Introduction

CH/π interactions have been studied by means of supramolecular and theoretical chemistry for many years.[1–3,†] These interactions are sometimes referred to as improper blue-shifting H-bonds (together with C–H···O and C–H···halogen interaction) because they reduce the X–H bond length and increase its vibrational sequence, contrary to usual X–H…Y H-bonds. CH/π interactions for a number of model systems, such as T-shaped benzene dimer or benzene–methane complex, were studied at various levels of theoretical chemistry including the second-order Møller–Plesset perturbation theory (MP2),[4–7] coupled cluster (CC)[5–7] and configuration interaction (CI).[8] These interactions were also studied by combined database mining and quantum chemical study.[9] The stabilizing energy of minimized methane–benzene dimer calculated at the CCSD(T) (single, double and perturbative triple excitation coupled cluster) level was 1.42 kcal mol$^{-1}$ (as an estimated basis set limit).[7] Energy decomposition showed that electrostatic contribution is significantly reduced when compared to the usual H-bonds.[7] The system of benzene dimer was also studied by statistical thermodynamic methods.[10]

CH/π interactions play an important role in intramolecular interactions in proteins[11] and in protein–ligand interactions.[12–14] A large number of carbohydrate-binding proteins and carbohydrate-processing enzymes contain one or more aromatic amino-acid residues in their binding sites. Aromatic rings of these residues are

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† See also http://www.tim.hi-ho.ne.jp/dioniso

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usually oriented parallel to the carbohydrate rings, and axial C–H groups of carbohydrates point onto an aromatic ring. The role of CH/π interactions in substrate binding by human lysozyme and wheat-germ agglutinin was studied by site-directed mutagenesis and chemical modification.[12] As far as we know, ab initio methods have not been applied in studies of CH/π interactions in carbohydrate–protein complexes.

β-Galactosidase (β-D-galactoside galactohydrolase, EC: 3.2.1.23) from *E. coli* catalyzes hydrolysis of lactose as well as of a wide range of synthetic substrates. It is also capable of synthesizing allolactose by isomerization of lactose and other oligosaccharides and glycosides by transfer of galactose from a substrate to other saccharides or aglycones. The crystal structure of this enzyme is known for a number of complexes with substrates, products, transition state analogues and trapped reaction intermediates.[15] There are two tryptophane residues involved in interaction with ligands in two different binding modes. The initial substrate binding is characterized by interaction with Trp999 in the shallow mode. Such an interaction is found in complexes with a noncovalent inhibitor (PDB:1JYX) and in structures of substrates bound to inactive E537Q mutant.[15] A substrate then tends to move deeper into the binding site where the catalysis is supposed to take place. This deep binding mode is characterized by interaction of the galactose moiety with Trp568 residue. This interaction is found in published crystal structures of complexes of β-galactosidase with galactose (PDB:1JZ7), transition state analogue (PDB:1JZ6) and in a number of trapped reaction intermediates.[15]

The role of the Trp999 residue was studied by site-directed mutagenesis, and it was shown to be crucial for binding of substrate in the shallow mode and for formation of allolactose (β-D-galactopyranosyl-(1→6)-β-D-glucopyranose)—a natural inductor of *Lac* operon. Mutation of this residue leads to decrease of affinity of the enzyme towards its inhibitors and to decrease its activity towards its substrates.[14]

Our homology-based model of β-galactosidase from Antarctic bacteria *Arthrobacter* sp. C2-2[16] showed that Trp999 residue of *E. coli* β-galactosidase is replaced by cysteine (Cys999) in the enzyme from *Arthrobacter* sp. C2-2. Because this exchange could be responsible for the difference in kinetic parameters of these enzymes (different temperature profile of activity, altered substrate specificity, etc.), we decided to focus on this site and on the role of CH/π interactions.

## 2. Results

### 2.1. Structure of complexes

In this section, we describe the geometry of selected complexes involved in this study and results of their geometry optimization by ab initio energy minimization. Shallow and deep binding modes of β-galactosidase from *E. coli* as defined by Juers et al.[15] are illustrated in Figure 1. The initial structures of individual complexes studied by ab initio methods were taken from published crystal structures[15] as described in the methodology section and are illustrated in Figure 2. Ab initio energy minimization was carried out on complexes B–D and F. In the shallow binding mode, both pyranose rings of allolactose are oriented parallel to the indole ring of tryptophane (Fig. 2A). Allolactose was divided into both monosaccharide moieties to make ab initio calculations straightforward (Fig. 2B and C). The glucopyranose moiety interacts with the Trp999 residue mainly via one of the hydrogen atoms at C-6 (Fig. 2B). This C–H bond points towards the benzene ring of tryptophane. The distance between the centre of the benzene ring and the point of intersection of the plane of the benzene ring and the axis of the C–H bond is 0.67 Å for the initial complex and 0.52 Å for the minimized complex. The distance between the centre of the aromatic ring and C-6 of glucopyranose was 3.91 Å for the initial complex and 4.21 Å for the minimized complex. The hydrogen at C-4 of glucose interacts with the tryptophane pyrrole ring. Other hydrogen atoms of the glucose moiety can also be involved in interactions with the Trp999 residue. The root-mean-square deviation between experimental and minimized complex B was 0.45 Å for all atoms and 0.29 Å for nonhydrogen atoms.

The galactose moiety of allolactose as well as of lactose interacts with Trp999 residue via the hydrogen

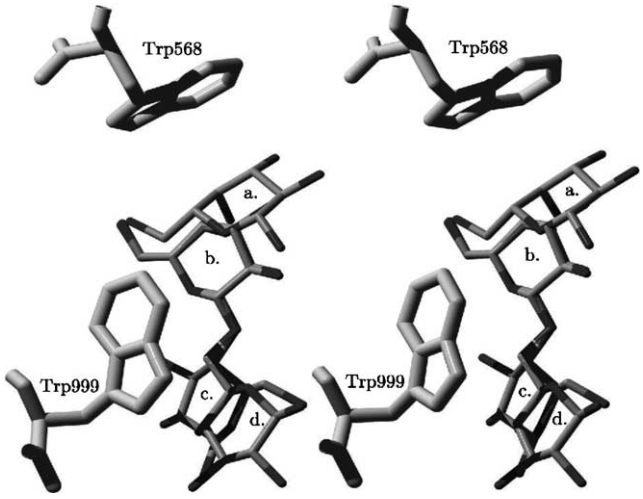


**Figure 1.** Stereoview of different binding modes (deep and shallow) of β-galactosidase from *E. coli* as found in crystal structures.[15] Trp568 Residue interacts with galactose bound in the deep binding mode (a). Trp999 Residue interacts with lactose and allolactose bound in the deep binding mode. Position of galactose moiety (b) in the shallow binding mode is the same for lactose and allolactose, while position of the glucose moiety of lactose (c) and allolactose (d) are different.

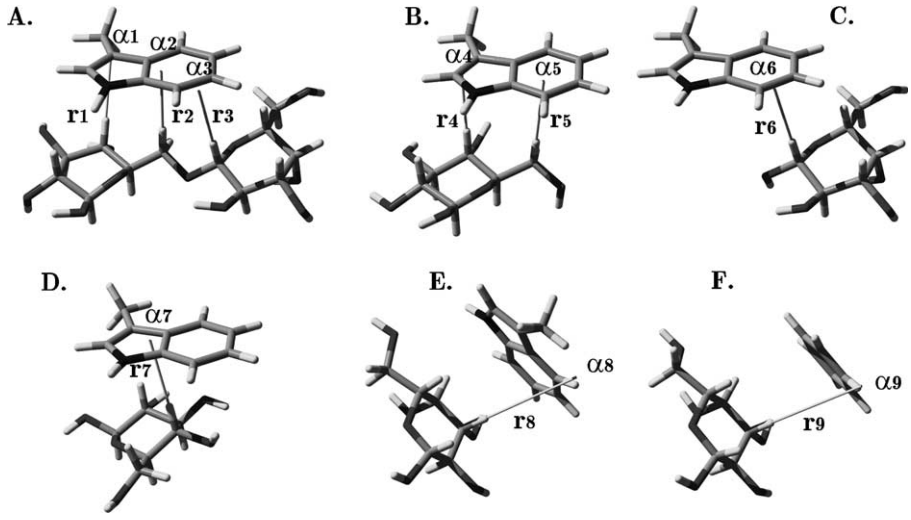


**Figure 2.** Structures of complexes representing CH/π interaction of β-galactosidase from *E. coli* with carbohydrates: interaction of Trp999 with allolactose (A) and with glucose (B) and galactose (C) moieties of allolactose bound in the shallow mode; interaction of Trp999 with the glucose moiety of lactose (D) and interaction of Trp568 with galactose in the deep mode (E, F). Conformations of complexes A and E were taken directly from experimental structures.[15] Complexes B–D and F are shown in a conformation after ab initio minimization. Axes of key C–H bonds are drawn as sticks. Parameters $r_1$–$r_9$ are distances between the hydrogen of the donor C–H bond and the plane of an aromatic ring measured in the direction of the C–H bond. Parameters $\alpha_1$–$\alpha_9$ are angles between the donor C–H bond and the plane of an aromatic ring. Values of $r_1$–$r_9$ and $\alpha_1$–$\alpha_9$ are listed in Table 1.

atom bound at C-1 (Fig. 2C). This C–H bond points to one of the C–C bonds on the benzene ring of tryptophane. The distance between C-1 of galactose and the closest carbon atom of Trp999 (CH2) was 3.74 Å for the initial and 3.78 Å for minimized conformation of the complex. The effect of ab initio energy minimization on the extended conformation was small. Root-mean-square deviations between experimental and minimized complexes were 0.12 Å for all and 0.09 Å for nonhydrogen atoms. The position of the galactose moiety is entirely the same in the complex with allolactose and lactose (root-mean-square deviation between galactose moiety in allolactose and lactose is 0.11 Å). Therefore, we can apply the results of the galactose moiety of allolactose to the galactose moiety of lactose.

The glucose moiety of lactose (Fig. 2D) is rotated with respect to the glucose moiety of allolactose. The position of atoms O-4–C-4–C-5 of lactose corresponds with the position of O-6–C-6–C-5 of allolactose. The C–H bond at C-4 is mainly involved in interaction with Trp999. This bond points onto the centre of the indole system. The distance between C-4 and the point of intersection an axis of its C-4–H bond and the plane of the indole system was 3.79 Å for the initial and 4.04 Å for the minimized complex. Root-mean-square deviations between experimental and minimized complex D was 0.29 and 0.22 Å for all and nonhydrogen atoms, respectively.

In the deep binding mode, galactose interacts mainly via the hydrogen of carbon C-3. Orientation of the carbohydrate ring and the benzene ring of tryptophane is not exactly parallel. The angle between the axis of the benzene ring and the C-3–H bond is relatively high (49° for the initial and 41° for the minimized complex). The distance between the hydrogen atom bound at carbon C-3 and the centre of the benzene ring of Trp568 was 2.82 Å for the initial and 3.42 Å for the minimized complexes. Root-mean-square deviations between experimental and minimized complexes were 0.44 and 0.37 Å for all and nonhydrogen atoms, respectively. Geometrical parameters of the complexes studied are summarized in Figure 2 and in Table 1. Parameters $r_1$–$r_9$ are distances between the hydrogen of the donor C–H bond and the plane of the aromatic ring measured in the direction of the C–H bond. Parameters $\alpha_1$–$\alpha_9$ are angles between the donor C–H bond and the plane of the aromatic ring.

### 2.2. Calculation of interaction energies

Interaction energies were calculated at three different levels of theory for conformations found in the crystal structure (referred to as experimental complexes) and for conformation after the ab initio energy minimization (referred to as minimized complexes). At the HF and B3LYP levels, the interaction of Trp999 with allolactose bound in the shallow binding mode (Fig. 2A) as well as with both of its carbohydrate moieties (Fig. 2B and C) was calculated as repulsive for all experimental complexes. Similar results were obtained for experimental complexes of the glucose moiety of lactose bound in the shallow binding mode (Fig. 2D). Energy of interaction of galactose in the deep mode and Trp568 (Fig. 2E) calculated at both HF and B3LYP levels were slightly attractive (0.2 and 0.6 kcal mol$^{-1}$ for HF and B3LYP

**Table 1.** Geometrical parameters of complexes A–F. Symbols $r_1$–$r_9$ and $\alpha_1$–$\alpha_9$ correspond with distances and angles shown in Figure 2

| A (experimental) | B (minimized) | C (minimized) | D (minimized) | E (experimental) | F (minimized) |
|---|---|---|---|---|---|
| $r_1 = 2.99$ Å; $\alpha_1 = 72°$<br>$r_2 = 2.75$ Å; $\alpha_2 = 84°$<br>$r_3 = 2.81$ Å; $\alpha_3 = 75°$ | $r_4 = 2.90$ Å; $\alpha_4 = 85°$<br>$r_5 = 3.11$ Å; $\alpha_5 = 79°$ | $r_6 = 2.79$ Å; $\alpha_6 = 75°$ | $r_7 = 2.89$ Å; $\alpha_7 = 80°$ | $r_8 = 4.33$ Å; $\alpha_8 = 41°$ | $r_9 = 4.43$ Å; $\alpha_9 = 49°$ |

energies, respectively, for experimental structures). We proposed that the N–H group of the indole ring can form a proper H-bond with one of the hydroxyl groups of galactose; therefore, we decided to represent the Trp568 residue by a benzene ring (Fig. 2F). The geometry of such a proper H-bond is unfavourable, and its effect on total interaction energy is small, but this was the easiest way to focus only on CH/π interactions in this complex. The energies for experimental structure of the complex where Trp568 residue was represented by benzene were calculated as repulsive at the HF and B3LYP levels. For all experimental structures, energies calculated at the B3LYP level were shifted towards attractive interactions, when compared with HF energies.

Energies calculated by the method of second-order Møller–Plesset perturbation theory were computed for complexes B,C,D and F. All interactions were calculated as attractive for all tested experimental complexes. The highest value (4.5 kcal mol$^{-1}$) was obtained for the glucose moiety of allolactose bound in the shallow mode. The value for the glucose moiety of lactose was also high (3.2 kcal mol$^{-1}$). Interaction energies for the galactose moiety in the shallow binding mode and the galactose moiety in the deep binding site were smaller (2.2 and 2.6 kcal mol$^{-1}$, respectively). Interaction energies for minimized complexes at MP2 levels were even higher than for experimental complexes. We obtained values of 5.2, 2.4, 5.0 and 2.7 for complexes B,C,D and F, respectively. Results are summarized in Table 2.

Since geometries of C–H/π complexes can be disturbed by fluctuations and additional interactions, we decided to explore the profile of interaction energy as a function of intermolecular distance. For the most favourable complex (Trp999—glucose moiety of allolactose, complex B), the indole ring of tryptophane was shifted in the direction of the donor C–H bond (C-6 of glucose) and interaction energies were calculated at HF/6-31+G(d) and MP2/6-31+G(d) levels. The results (Fig. 3) show that optimal distance is approximately 2.5–3 Å even though calculation of the accurate value of optimal distance would require more interaction energies to be computed. It is also clear that the Hartree–Fock method overestimates optimal intramolecular distance when compared with second-order Møller–Plesset perturbation theory.

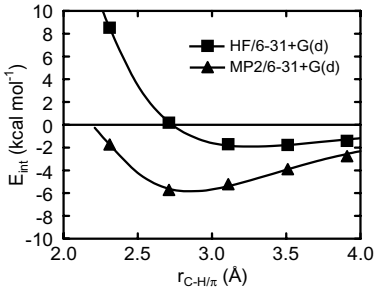


**Figure 3.** Profile of interaction energy as a function intermolecular distance. For complex B, the indole ring of tryptophane was shifted in the direction of donor C–H bond (on carbon C6 of glucose) and interaction energies were calculated at the HF/6-31+G(d) and MP2/6-31+G(d) levels. Points were fitted using the Buckingham potential.

## 3. Discussion

Optimization of geometry of original structures from PDB by ab initio energy minimization slightly increased absolute values of interaction energies, but the gain was not dramatic. In our opinion, this is mainly due to the relatively high accuracy of experimental input structures and the fact that there were no intermolecular overlaps of van der Waals radii. The geometry of our models of CH/π complexes was in good agreement with analogical fragments taken from the Cambridge database[9] and

**Table 2.** Counterpoise-corrected interaction energies (in kcal mol$^{-1}$) for complexes shown in Figure 2

| Complex | Carbohydrate | Amino-acid residue | Experimental | | | Minimized | | |
|---|---|---|---|---|---|---|---|---|
| | | | HF | B3LYP | MP2 | HF | B3LYP | MP2 |
| A | Allolactose | Trp999 | 1.1 | 0.6 | — | — | — | — |
| B | Glucose[a] | Trp999 | 0.1 | 0.0 | −4.5 | −1.7 | −1.5 | −5.2 |
| C | Galactose[a] | Trp999 | 1.2 | 0.6 | −2.2 | 1.0 | 0.4 | −2.4 |
| D | Glucose[b] | Trp999 | 1.9 | 1.5 | −3.2 | −1.7 | −1.2 | −5.0 |
| E | Galactose | Trp568 | −0.2 | −0.6 | — | — | — | — |
| F | Galactose | Trp568[c] | 1.4 | 0.8 | −2.6 | −1.0 | −0.6 | −2.7 |

a—Monosaccharide moiety of allolactose. b—Monosaccharide moiety of lactose. c—Represented as benzene.

with results of ab initio[7,9] studies. Moreover, the profile of interaction energy as a function of intramolecular distance is in agreement with studies in smaller systems.[7,9] This interaction remains attractive over a relatively wide range of distances.

The fact that the Hartree–Fock method is not able to detect CH/π interactions is in agreement with published studies of model systems. Energy profiles for the methane–benzene complex at the Hartree–Fock level were calculated as repulsive with little effect of the size of the basis set.[7] There is no particular experience with application of density functional theory in calculation of interaction energies of CH/π interactions. Despite the rapid development of novel functionals and methodologies, these methods are considered to be unreliable for studying interactions mainly of a dispersion nature.[17] Relatively good results were obtained with Perdew and Wang's exchange and correlation functional (PW91).[18]

Experience with high accuracy calculations in model systems, such as application of coupled cluster method, shows that the second-order Møller–Plesset method tends to slightly overestimate the stabilizing energy.[7] The fact that stabilizing energies of interactions studied in this work were higher than those of the methane–benzene system can be caused by possible involvement of more than one hydrogen atoms in interactions. The other reason can be the presence of an electronegative oxygen bound directly to each carbon atom of the interacting C–H group. Stabilization energy in the chloroform–benzene complex was significantly increased by the effect of electronegative chlorine atoms.[3]

Besides the role of the CH/π interactions, which are of a quantum-chemical nature, there is a possible role of these aromatic residues in interactions of thermodynamical origin. These residues are hydrophobic, but in unliganded enzymes they are highly exposed to solvent. Despite the fact that carbohydrates are hydrophilic, the water interacts mainly with hydroxyl groups in equatorial positions leaving the axial space free for interactions. From the crystal structures of the studied enzyme, it is clear that interaction of the carbohydrate ring with the aromatic amino-acid residue does not affect the interaction between equatorial hydroxyl groups of carbohydrate and other amino-acid residues or water networks. When compared to the normal H-bond, most H-bond donors or acceptors (either of the free ligand or of an unligated protein) are usually solvated and large desolvation penalty is associated with ligand binding. The role of aromatic residues in the formation of the shape of an active site is also important and can cause important thermodynamic consequences. Deciphering the thermodynamic role of these residues in binding the substrate would require application of methods based on molecular mechanics and statistical thermodynamics such as free energy perturbation (FEP) and was not the object of this study.

We conclude that CH/π interactions play an important role in the catalytic mechanism of β-galactosidase. These interactions were detected in both the deep and shallow binding modes. The results are in agreement with those of the mutagenesis study on this enzyme.[14] The Trp999 residue acts as a C–H/π acceptor and offers the binding site for the glucose moiety in different orientations including the orientation favourable for formation of allolactose. This interaction between glucose and Trp999 is of quantum-chemical origin rather than being purely hydrophobic. Moreover, the results show the importance of aromatic residues in binding sites of carbohydrate-binding proteins and carbohydrate-processing enzymes. Application of quantum chemical methods together with development of novel empirical potential functions for methods of molecular mechanics that contain terms for such 'nonstandard' interactions seems to be a future trend in structural glycobiology.

## 4. Methods

Structures of complexes were taken from the published crystal structures [15] from Protein Databank: from 1JZ8 for the structure of allolactose in the shallow mode (Fig. 2A–C), from 1JYN for the structure of lactose in the shallow binding mode (Fig. 2D) and 1JZ7 for the complex of Trp568 and galactose in the deep mode (Fig. 2E and F). To apply ab initio quantum chemistry methods it was necessary to reduce the size of the system. Structures of tryptophane residues were represented either as 3-methylindole or benzene. The structure of allolactose was represented either as galactose (Fig. 2B) or glucose (Fig. 2C).

For lactose in the shallow mode, the interaction energy was calculated only for its glucose moiety (Fig. 2D) because the position of galactose moiety is the same as in the complex with allolactose (root-mean-square deviation between galactose moiety in allolactose and lactose is 0.11 Å).

Whereas the enzyme is deposited in PDB files as a tetramer, we used monomer A of each file. Hydrogen atoms were added by Open Babel (http://openbabel.sf.net) with standard distances from heavy atoms. These complexes are denoted as experimental complexes in the text. Conformation of complexes B–D and F was minimized using rational function optimization of GAMESS-US[19] at the HF/MINI level. The minimization was terminated when the largest component of the energy gradient was less than 1/1000 hartree, and the root-mean-square gradient less than 1/3000 Bohr at the same time. The resulting structures are referred to as minimized complexes in the text. The aim of minimization was to optimize the geometry of covalent bonds without disturbing noncovalent interaction; therefore, relatively high convergence criteria were used. We

appreciate that more accurate optimization of the structure would increase absolute values as well as the accuracy of calculated energies, but this would require the minimization of BSSE-corrected (basis set superposition error) energy with inclusion of correlation energy. High number of degrees of freedom together with the absence of useful symmetry makes such an optimization computationally too expensive. It would be also possible to separate both molecules of each complex, minimize them and combine them together, but this procedure could bring other problems and artefacts.

In order to study changes of interaction energy due to geometry fluctuations, we generated four additional conformations of the complex of Trp999 with the glucose moiety of lactose. They were generated from the minimized structure of this complex (Fig. 2B) by shifting of the indole ring of Trp999 in the direction of the donor C–H bond (C-6 of glucose). Distances between the hydrogen of the donor C–H bond and the plane of the ring (measured in the direction of the C–H bond) were 2.31, 2.71, 3.11 (minimized complex B), 3.51 and 3.91 Å.

We calculated interaction energies at three levels of theory: using Hartree–Fock (HF) method, using DFT (density functional theory) approach with B3LYP functional (Becke–Slater-HF exchange with Lee–Yang–Parr correlation functional) and with the second-order Møller–Plesset perturbation theory (MP2). We used split-valence basis with diffusion and polarization function (6-31+G(d)). Calculations were performed with the program Gamess US.[19] The basis set superposition error was corrected by the counterpoise method.[20] B3LYP calculation was carried out in grid-based DFT mode where parameters of a grid can be switched during self-consistent field procedure. Final B3LYP energies were calculated in a finer grid (number of radial grids in the Euler–Maclaurin quadrature set to 96, number of angle theta and angle phi grids in Gauss–Legendre quadrature set to 12 and 24, respectively). Picture representations were created by a Swiss PDB viewer[21] and PoV Ray.[22]

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