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Efficient and Accurate Theoretical Methods To Investigate Anion-π Interactions in Protein Model Structures

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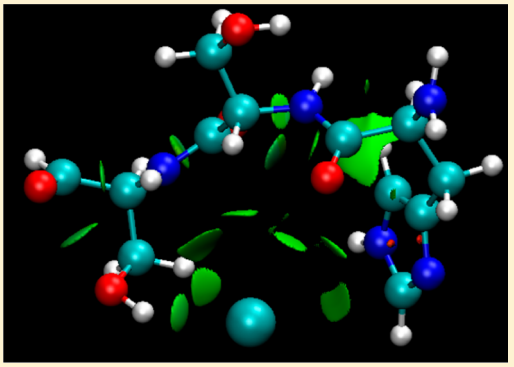
ABSTRACT: Anion-π interactions are attractive interactions between electron-poor aromatic rings and electron-rich negative ions or groups. Predicted by theoretical studies in the late 1990s, the first experimental evidence of anion-π interactions was provided by two independent studies in 2004. Since then, the role of these interactions in chemical and in biochemical systems has been investigated. In this work we report benchmark interaction energies, estimated from the extrapolated MP2 basis set limit with CCSD(T) correction, for several model complexes. These are then used to assess faster, more approximate methods including MP2 and its local approximation, along with various forms of Density Functional Theory (DFT). The most promising of these are then used to describe the geometries and interaction energies of a series of complexes between electron-poor substituted benzene rings and anions as well as examples of such interactions in models of proteins.



INTRODUCTION

In 2004 two experimental studies provided the first evidence of an attractive interaction between anions and electron-poor aromatic rings. Such an interaction is now defined as anion-π interaction.[1,2] Several years before these experiments, theoretical studies[3] had predicted the interaction between anions, or electron-rich groups, and aromatic rings such as hexafluorobenzene or triazine. Since then, anion-π interactions are widely accepted by the community, and new studies are aimed at understanding the role of this novel interaction in supramolecular chemistry,[4] in anion (Cl^-) channels,[5,6] in proteins, and DNA/RNA.[7]

More than ten years ago, the first theoretical investigation,[3] based on Hartree−Fock (HF), Møller−Plesset (MP), and Density Functional theory (DFT) calculations, predicted that hexafluorobenzene can weakly bind electron-rich molecular groups such as F−H, H−Li, N═CH, and C═NH, interaction energies being typically smaller than 4 kcal/mol. Inspired by this work, in 2002 three computational studies predicted that anions may also bind electron-poor aromatic rings.[8−10] Bartberger and co-workers,[9] for instance, studied complexes of 1,3,5-triazine rings with fluoride, chloride, and azide anions using MP2. The computed interaction energies, inclusive of zero-point vibration energies (ZPE) and basis set superposition error (BSSE) corrections, range between −4.8 and −48.8 kcal/mol. Alkorta et al.[8] explored the structural and electronic properties of several perfluoro-aromatic model compounds with anions, including fluoride, chloride, bromide, and cyanide. The geometries of the complexes were fully optimized using HF, B3LYP, and MP2. Interaction energies and anion-ring distances range between −5.0 and −19.0 kcal/mol and between 2.5 and 3.3 Å, respectively

Since these early studies, it has been proposed that the source of these interactions is mainly electrostatics, as the quadrupole moment of an electron-deficient aromatic rings such as hexafluorobenzene is positive.[11] When the quadrupole moment is close to zero or even negative, a weak interaction may still occur, such as in bromide-1,4,5,8,9,12-hexaazatriphenylene rings.[11] In these cases, dispersion plays an important role. A recent paper has confirmed that "standard" DFT underestimates interaction energies in complexes where dispersion becomes important.[12] Notably, it was also shown that Truhlar's MPWB1K functional[13] performs well in such cases. Recently, Houk and co-workers proposed an alternative explanation for the stabilization in anion-π interactions. Based on extensive high-level calculations, they suggest that in substituted benzene the stabilization is mainly due to interactions of the anion with the local dipoles induced by substituents.[14]

While widely studied in supramolecular assemblies, the search for anion-π interactions in biological macromolecules has just begun: in 2011, three different studies indicated that such interactions may be of importance in protein structures. A systematic search through the Protein Data Bank (PDB) showed for the first time that anion-π close contacts exist in experimental protein structures between the standard aromatic residues (Trp, Phe, Tyr, His) and anions, such as chloride and

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phosphate.[15] Hinde and co-workers also performed a PDB search focusing on interactions between Phe and negatively charged residues such as Asp and Glu. While edgewise interactions (in which the angle between the anion group and the plane of the ring ranges between 0 and 40 deg) were found to be very common and significantly attractive (estimated energies range between −8 and −2 kcal/mol), anion-π interactions were found less frequently and with energies close to zero, e.g., weakly attractive or slightly repulsive.[16] Also, by a systematic search of protein structures followed by ab initio calculations, Deyà and co-workers showed that anion-π interactions are likely to occur in flavin-dependent enzymes.[17]

In this work, several theoretical methods were tested in order to establish an efficient and accurate procedure to be applied to a systematic theoretical characterization of the anion-π close contacts of the protein structures previously found in the PDB search.[15] In order to do so, the ability of DFT, MP2, LMP2, and CCSD(T) to describe the geometries and interaction energies between a series of electron-poor substituted benzene rings and anions, such as F^-, Cl^-, and Br^-, was analyzed. Notably, it was found that LMP2 is an excellent method to reproduce benchmark interaction energies, as are two DFT methods. These methods were then applied to four model systems extracted from real protein structures containing a possible anion-π interaction between the aromatic residues Phe, Trp, Tyr, and His and a Cl^- ion. Results obtained in this work confirm the elusive character of the anion-π interactions. Decomposing the protein model complexes into their components, aromatic ring, protein group, and anion suggests a destabilization effect due to the aromatic ring. However, electron density analysis indicates that anion-π interactions may be possible, albeit weak, in proteins. Finally, this work provides accurate and efficient theoretical tools to describe these interactions and represents the first step toward a systematic theoretical investigation of the anion-π interactions in systems of biological relevance.

METHODS

Ab initio calculations were performed by using Molpro[18] and employed augmented correlation consistent aug-cc-*pVn*Z basis sets.[19−21] All MP2 calculations employed the density fitting (DF) approximation for calculation of both underlying HF and correlation energy[22,23] with the corresponding fitting basis sets for HF and MP2. CBS(T) data were obtained from extrapolation of DF-MP2 energies with aug-cc-pVTZ and aug-cc-p-AVQZ by using the formula of Helgaker et al.[24] The MP2 basis set limit was then corrected for the known shortcomings of MP2 by applying a ΔCCSD(T) correction, that is, the difference in correlation energy between MP2 and CCSD(T), obtained with aug-cc-pVDZ, to give an estimate of the CCSD(T) interaction at the basis set limit, denoted CBS(T). All such data were corrected for Basis Set Superposition Error (BSSE) by using the counterpoise method.[25]

DFT calculations, both single point and optimizations, and MP2 optimizations were performed with the G09 package.[26] B3LYP,[27] PBE0,[28] TPSSh,[29] w-B97X-D,[30] B97-D,[31] BHandH (as implemented in the G09 package),[32] M05-2X,[33] M06-2X,[34] DFT-D3,[35] and MPWB1K[13] were employed in combination with the 6-31++G(d,p) basis set. BHandH has been shown[36] to reproduce geometries and energies of complexes governed by dispersion forces. This DFT functional was also employed in previous investigation to study anion-π interactions in supramolecular assemblies.[4,37] MPWB1K was designed to describe systems dominated by dispersion and has previously been shown to work well for anion-π interactions.[12] M05-2X and M06-2X are meta-GGA functionals whose excellent performance for a wide variety of noncovalent interactions has been demonstrated by many studies.[38−40] DFT interaction energies were corrected from BSSE using counterpoise technique. We note that, as found in previous studies,[8] not all the complexes are global minimum of the potential energy surface.

We have also carried out local MP2 (LMP2) calculations, exploiting the short-range nature of electron correlation.[41] After localization of the canonical HF orbitals, only excitations to virtual orbitals that are spatially close are considered. Coupled with DF methods, this approach can lead to significant savings in computational resources,and can approach linear scaling with system size in favorable cases.[22,23] This DF-LMP2 method, coupled with aug-cc-pVTZ, has been successfully applied to various intermolecular interactions[42] and also effectively eliminates BSSE and the need for counterpoise correction. The localized orbitals required for DF-LMP2 were generated through the Pipek-Mezey method,[43] and the orbital domain selection followed the procedure of Boughton and Pulay[44] with merging of rotationally invariant π-domains where appropriate. All domains were calculated with a large intermolecular separation and then fixed for calculations on the interacting system. The use of localized orbitals also allows decomposition of total correlation energies into intra- and intermolecular contributions and from interactions between specific orbitals on separate molecules, as set out by Schütz.[45]

Atoms in Molecules[46] (AIM) analysis on BHandH calculated electron densities was carried out using the AIM2000 package,[47] allowing visual identification of all bond critical points (bcp's) involved in intermolecular interactions and properties at these bcp's, most notably the electron density, were calculated. However, it is not always clear from AIM data whether an individual interaction acts to stabilize or destabilize. We have also used the reduced density gradient s(r) and related properties, as defined in Johnson et al's program NCIPLOT.[48] It has been shown that s(r) identifies noncovalent interactions and that the sign(s) of the curvature of the density at relevant points can distinguish strongly stabilizing (e.g., hydrogen bonds), weakly stabilizing (e.g., van der Waals), or destabilizing (e.g., steric clash) contacts.

RESULTS AND DISCUSSION

Tables 1a and 1b report benchmark CBS(T) interaction energies for nine complexes, whose geometry was taken from ref 12. The former reports *ab initio* data, while the latter gives values from DFT. To the best of our knowledge, these are the first reported benchmark data for such complexes that includes both the effects of electron correlation at the CCSD(T) level and of extrapolation to the basis set limit, allowing us not only to assess the strength of the interactions present but also to examine the performance of more approximate methods. For a given aromatic, interaction with fluoride is significantly stronger than with chloride or bromide, an effect that is greater with trifluorotriazine (TFZ) than with hexafluorobenzene (HFB) or trifluorobenzene (TFB). Chloride's interaction with either aromatic is only slightly stronger (1 to 2 kcal/mol) than that of bromide. For a given anion, interaction with TFZ is stronger than with HFB, which in turn is more strongly bound than TFB.

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Table 1a. *Ab Initio* Interaction Energies of Model Complexes at Literature Geometry (kcal/mol)

	CBS(T)	MP2/CBS	LMP2	RI-MP2[a]
HFB···F$^-$[b]	−20.1	−19.4	−18.9	−18.8
HFB···Cl$^-$	−15.4	−16.0	−15.3	−12.9
HFB···Br$^-$	−14.1	−15.1	−14.2	−12.6
TFZ··· F$^-$[c]	−27.8	−26.9	−26.2	−24.3
TFZ···Cl$^-$	−17.7	−18.3	−17.7	−15.1
TFZ···Br$^-$	−15.7	−16.5	−15.8	−14.2
TFB··· F$^-$[d]	−9.0	−8.5	−8.4	−7.7
TFB···Cl$^-$	−7.0	−7.4	−6.6	−4.8
TFB···Br$^-$	−6.4	−7.1	−6.6	−5.0
MUE[e]		0.69	0.48	1.98

[a]Taken from ref 12. [b]HFB = hexafluorobenzene. [c]TFZ = trifluorotriazine. [d]TFB = trifluorobenzene. [e]Mean unsigned error, relative to CBS(T) data.

Table 1a also reports interaction energies at the same geometry using several faster but more approximate approaches and the overall mean unsigned error (MUE) resulting from each. The difference between extrapolated MP2 basis set limit (MP2/CBS) and benchmark data is the CCSD(T)/aug-cc-pVDZ correction applied, and in general this is rather small. However, the sign of this correction is not uniform: MP2/CBS underestimates the strength of fluoride complexes but overestimates those of all other anions. This appears to be a function of the relative importance of electrostatic and dispersion forces, since MP2 is known to overestimate binding of dispersion-bound complexes (discussed in more detail below). Local MP2 with aug-cc-pVTZ (without counterpoise correction) performs slightly better than MP2/CBS for significantly reduced computational cost. As with conventional MP2, this method underestimates the binding of the fluoride complexes, but errors for all other complexes are less than 1 kcal/mol leading to MUE = 0.5 kcal/mol. The importance of the basis set in MP2 calculations is apparent on comparison of literature RI-MP2 data, obtained with the 6-31++G** basis set, with complete basis set limit values. The relatively small basis set used in ref 12 leads to significant errors, in most cases 1.3 to 3.5 kcal/mol.

Table 1b reports analogous data from numerous DFT methods; the improper description of dispersion in conventional DFT should lead to poor description of weakly bound complexes. Our data show that this is indeed true for the popular B3LYP method that actually gives worse results than HF (data not shown), while other hybrid methods TPSSh and PBE0 also perform poorly. MPWB1K was recommended by Garau et al.[12] on the basis of comparison with RI-MP2 data, which as noted above is not particularly close to our benchmark data. When tested against CBS(T), this method gives a reasonable description of most complexes, with errors in the range of 1 to 3 kcal/mol. M05-2X and especially M06-2X perform rather better than this, resulting in overall errors of close to 1 kcal/mol. Both methods overestimate binding of the strongest complexes but perform very well for weaker ones. Three other DFT methods perform slightly better than MPWB1K, namely BHandH, DFT-D3, and ωB97X-D. BHandH performs reasonably well for chloride and bromide complexes but significantly overestimates the binding of fluoride ions. DFT-D3 systematically underestimates the binding (average error equal to 1.3 kcal/mol), while ωB97X-D gives a more balanced description of all complexes with errors of 1 to 2 kcal/mol throughout.

The data in Tables 1a and 1b were obtained at single geometries taken from the literature, but the shape of the potential energy surface (PES) around minima is as important as the interaction energy. We have therefore carried out PES scans of selected complexes to evaluate the performance of different methods for this aspect of anion-π interactions, in which the anion was constrained to lie on the line perpendicular to the aromatic ring, directly above its centroid. The large computational requirements of the CBS(T) approach meant that we cannot use this as a benchmark for the entire PES. Instead we employed LMP2 as a reliable and efficient *ab initio* method, against which we compared four DFT methods.

Figure 1 shows two typical scans, namely for fluoride and chloride with TFB; scans for other anions and aromatics (not shown) display similar behavior. In both cases, the inability of B3LYP to give reasonable interaction energies or geometries is immediately apparent. For the fluoride complex that is expected to be more stabilized by electrostatic forces, ωB97X-D and especially MPWB1K closely follow the LMP2 curve across all separations, whereas M06-2X and BHandH substantially overestimate stabilization throughout. For the chloride complex, however, the excellent performance of M06-2X and BHandH relative to LMP2 is striking, the curves lying within 0.5 kcal/mol across the range considered and both reaching a minimum at 3.3 Å, whereas ωB97X-D and MPWB1K lie well above the LMP2 curve and reach minima at slightly longer separations.

While one-dimensional PES data are useful for symmetrical model complexes, such an approach cannot be generally applied to nonsymmetrical cases. In Table 2 we therefore

Table 1b. DFT Interaction Energies of Model Complexes at Literature Geometry (kcal/mol)

	MPWB1K[a]	B3LYP[a]	BHandH	PBE0	TPSSh	ωB97X-D	B97-D	M05-2X	M06-2X	DFT-D3
HFB···F$^-$	−19.7	−17.5	−23.4	−17.6	−16.6	−19.0	−17.2	−20.9	−21.7	−19.7
HFB···Cl$^-$	−13.0	−11.0	−15.4	−11.6	−10.8	−13.2	−12.2	−13.9	−14.7	−14.2
HFB···Br$^-$	−11.9	−9.4	−14.3	−10.4	−9.7	−12.4	−12.0	−13.0	−13.8	−13.4
TFZ··· F$^-$	−26.3	−23.0	−33.4	−25.5	−24.3	−26.1	−22.9	−29.6	−30.2	−25.1
TFZ···Cl$^-$	−15.6	−13.0	−19.4	−14.6	−13.7	−15.8	−14.8	−17.3	−18.3	−16.0
TFZ···Br$^-$	−13.7	−10.9	−17.4	−12.5	−11.6	−13.9	−13.4	−15.5	−16.5	−14.4
TFB··· F$^-$	−7.7	−6.6	−9.8	−6.8	−6.3	−8.0	−7.2	−8.4	−9.2	−8.0
TFB···Cl$^-$	−4.6	−3.3	−5.9	−3.9	−3.5	−5.3	−4.6	−5.2	−5.9	−5.1
TFB···Br$^-$	−3.7	−2.1	−6.4	−3.2	−2.6	−5.1	−4.8	−5.2	−6.4	−5.5
MUE	1.89	4.04	1.61	3.01	3.78	1.61	2.67	1.05	0.86	1.30

[a]Taken from ref 12.

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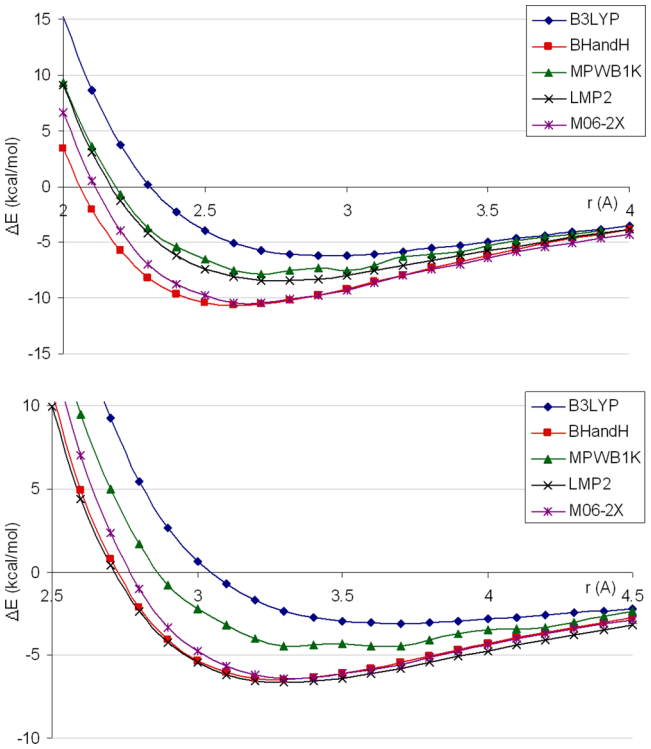

Figure 1. PES scans of TFB···F (top) and TFB-Cl (bottom). The distance of the anion from the centroid of the arene (Å) is on the *x*-axis and interaction energy (kcal/mol) on the *y*-axis.

Table 2. Details of MP2 Optimizations (Å and deg)

arene	anion	r	$r_{X\text{-}C}$ [a]	α [b]
C_6F_6	F	2.50	2.90	86.5
	Cl	3.16	3.45	90.0
	Br	3.12	3.47	84.7
C_6F_5	F	2.55	2.79	84.0
	Cl	3.15	3.40	88.4
	Br	3.17	3.43	88.6
C_6F_4	F	2.64	2.98	89.9
	Cl	3.22	3.51	85.7
	Br	3.24	3.52	89.6
C_6F_3	F	2.72	3.06	90.0
	Cl	3.31	3.60	90.0
	Br	3.46	3.62	89.0
C_6F_2	F	2.82	3.12	86.4
	Cl	3.40	3.67	86.5
	Br	3.41	3.58	79.5

[a]The distance between the anion and the closest atom of the ring. [b]The angle between the mean plane of the ring and the centroid···anion vector.

report the results of MP2/6-31++G(d,p) geometry optimization, performed without symmetry constraint. As expected, decreasing fluorination of the aromatic ring typically leads to longer centroid···anion distances (r), although there is little change between C_6F_6 and C_6HF_5. There is little difference in distances to chloride and bromide, despite the significantly greater van der Waals radius of the latter (1.850 vs 1.725 Å),[49] and in some cases bromide is actually closer to the ring centroid than is chloride. Not all complexes optimize to an exactly perpendicular orientation, so Table 2 also reports the closest anion···C distance ($r_{X\text{-}C}$) and the angle between the mean plane of the ring and the centroid···anion vector, denoted α. Distances follow the same trend noted above, but angles vary between exactly 90° to less than 80° in the case of bromide with $C_6H_4F_2$. This orientation is shown in Figure 2, which demonstrates that the bromide is disposed toward the fluorines in a symmetrical fashion.

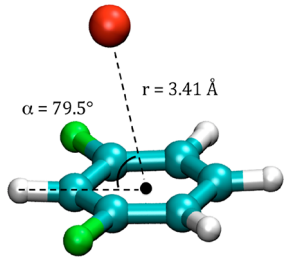

Figure 2. MP2 optimized geometry of bromide···1,3-difluorobenzene.

The difference in behavior for fluoride complexes compared to chloride and bromide suggests that complexes of these anions may be stabilized by different effects. To probe this in more detail, we have used the local correlation approach to examine the importance of dispersion and ionic terms and the underlying Hartree−Fock energies to represent electrostatic effects. Table 3 contains contributions to the overall LMP2

Table 3. Contributions to LMP2 Binding Energy (kcal/mol)

arene	anion	HF	dispersion	ionic
C_6F_6	F	−18.7	−3.5	−2.8
	Cl	−10.5	−4.3	−3.0
	Br	−7.7	−4.8	−4.3
C_6F_5	F	−14.7	−3.4	−2.7
	Cl	−7.8	−4.1	−2.8
	Br	−5.3	−4.8	−3.6
C_6F_4	F	−11.0	−3.0	−2.3
	Cl	−5.3	−3.9	−2.4
	Br	−3.1	−4.5	−3.3
C_6F_3	F	−7.4	−2.8	−1.9
	Cl	−2.8	−3.6	−2.1
	Br	−1.2	−4.2	−2.8
C_6F_2	F	−3.5	−2.5	−1.5
	Cl	−0.3	−3.2	−1.8
	Br	1.4	−4.0	−2.4

binding energy from Hartree−Fock (including electrostatic terms) as well as dispersion and ionic intermolecular correlation effects. These data show that, for a given arene, fluoride has much stronger electrostatic but rather weaker dispersion and ionic interactions than do chloride or bromide. The changes in correlation energy contributions are rather small when compared to the electrostatic ones, dispersion energies changing by 1 to 1.5 kcal/mol between anions for a given arene, and by even less for a given anion interacting with different arenes. Thus, it seems clear that the trends in overall binding energy are driven largely by electrostatic effects, with small but important modulation by electron correlation. Within the intermolecular correlation energy, ionic terms (in which one electron is excited from an occupied orbital one monomer into a virtual orbital on the other monomer) are almost as large as dispersion ones (in which excitations are within each monomer). Most discussion of the importance of electron

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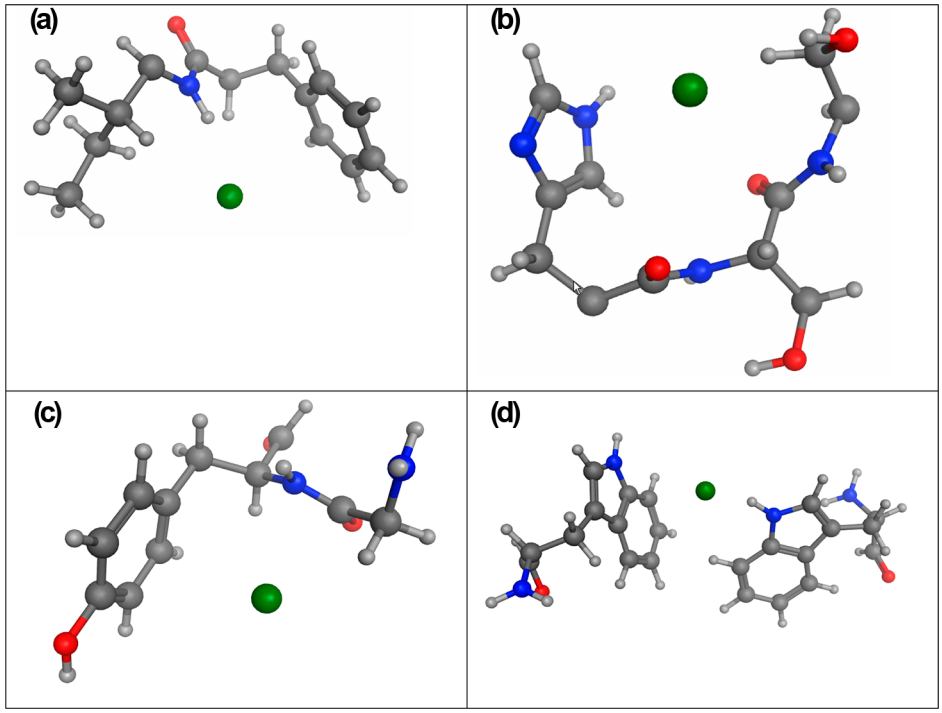

Figure 3. Anion-π interactions in protein structures: a) 1GKZ, b) 2GFG, c) 3FP4, and d) 2VQU.

correlation in noncovalent interactions tends to focus on the latter, but this analysis makes it clear that both terms require consideration for accurate description of anion-π interactions.

Having established some guidelines for successful theoretical description of anion-π interactions, we now apply some of those methods to some examples of these interactions found in proteins. In our previous PDB search,[15] we found more than 200 protein structures showing a Cl^- in close contact to an aromatic residue. Four protein structures 2GFG (contact with His), 1GKZ (contact with Phe), 2VQU (contact with Trp), and 3FP4 (contact with Tyr) were selected that contained a Cl^- ion above an aromatic ring with geometries close to the "ideal" models such as those of Alkorta and co-workers.[3] In our protein structures the anion distance from the ring centroid ranges between 3.3 and 4.5 Å, the angle between 70 and 86°. Hydrogens were added to X-ray structures using the "Protonate 3D" command in MOE; for three structures this is unambiguous, but for 2GFG different protonation states of histidine were examined, indicating that the lowest energy form has Nδ deprotonated and Nε protonated.

Coordinates of chloride plus relevant residues were extracted, as shown in Figure 3, and binding energy calculated using DFT and LMP2, with results summarized in Table 4, indicating substantial stabilization in both cases and from all methods considered. Agreement between methods is generally good, indicating stabilization over 10 kcal/mol in all cases, and rather more than this in two examples. As found for the model systems considered above, M06-2X performs very well when compared to LMP2. BHandH is in slightly better accord with LMP2 data than ωB97X-D: the former tends to overestimate stabilization, while the latter underestimates it.

Inevitably, within a large protein structure numerous interaction modes are possible, and a single binding energy calculation cannot easily isolate which of these are present and their relative importance to overall stabilization. Therefore, calculations were also performed on single components of the

Table 4. Interaction Energies in Proteins (kcal/mol)[a]

protein (residue)	method	ΔE
1GKZ (Phe)	LMP2	−20.2
	BHandH	−21.1
	ωB97X-D	−18.1
	M06-2X	−20.4 (−22.7)
2GFG (His)	LMP2	−12.5
	BHandH	−12.9
	ωB97X-D	−11.2
	M06-2X	−11.9 (−13.8)
2VQU (Trp)	LMP2	−23.8
	BHandH	−23.0
	ωB97X-D	−18.7
	M06-2X	−20.9 (−24.1)
3FP4 (Tyr)	LMP2	−10.0
	BHandH	−12.2
	ωB97X-D	−7.6
	M06-2X	−10.5 (−13.6)

[a]Values in parentheses are for model systems in which aromatics are replaced by hydrogen.

model protein complexes, e.g., the anion and the ring, the anion and a modified amino acid in which the aromatic ring was replaced by a hydrogen atom. As expected, the interaction between the aromatic ring and the anion was found to be slightly repulsive (data not shown), in agreement with previous calculations.[14] When the amino acid was stripped of its aromatic ring and replaced by a hydrogen atom, we observed in all cases a slight increase in stabilization energy, between 1 and 3 kcal/mol (see Table 4). This indicates weak repulsion between the aromatic ring and the anion in fragments extracted from model proteins. However, these calculations neglect any possible cooperative effect between the components of the system.

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In order to further probe that anion-π interactions are present in proteins, the electron density and related properties of the complexes (complete of all components, as displayed in Figure 3) were examined. AIM analysis (Table 5) confirms the existence of bond paths corresponding to anion$\cdots\pi$ interactions in all four structures. However, the electron density at the corresponding bond critical points is low, less than that for N−H$\cdots$Cl, O−H$\cdots$Cl, and even C−H$\cdots$Cl interactions, and similar to those for O$\cdots$Cl and N$\cdots$Cl contacts. In two cases, namely those with the greatest stabilization energy, N−H$\cdots$Cl hydrogen bonds are present and display relatively large values of electron density at the corresponding critical points.

Table 5. Electron Density Analysis of Anion in Protein Structures

protein	type	ρ_c (au)	NCI[a]
1GKZ	Cl$\cdots\pi$	0.003	ws
	N−H$\cdots$Cl	0.020	s
	C−H$\cdots$Cl	0.006	ws
	C−H$\cdots$Cl	0.009	ws
	C═O$\cdots$Cl	0.004	ws
2GFG	Cl$\cdots\pi$	0.001	ws
	O−H$\cdots$Cl	0.009	ws
	C═O$\cdots$Cl	0.003	ws
	N$\cdots$Cl	0.003	ws
2VQU	Cl$\cdots\pi$	0.010	ws
	N−H$\cdots$Cl	0.031	s
3FP4	Cl$\cdots\pi$	0.007	ws
	C−H$\cdots$Cl	0.022	ws
	N$\cdots$Cl	0.013	ws

[a]s = strongly stabilizing, ws = weakly stabilizing.

This analysis is complemented by use of NCIPlot, which indicates that all contacts for which a bond critical point is present act to stabilize the overall complex. Only the N−H$\cdots$Cl hydrogen bonds in 1GKZ and 2VQU meet the criteria for strongly stabilizing interactions; these are colored blue in Figure 4. The remaining contacts exhibit rather low values of λ_H, the eigenvalue of the Hessian matrix that distinguishes strong/weak binding (colored green in Figure 4). Nevertheless, this analysis lends support to the notion of stabilizing anion$\cdots\pi$ interactions in these proteins, placing them on a par with more established interactions such as C−H$\cdots$Cl hydrogen bonds. For example, Figure 4 indicates that the interactions of chloride with side chains of Phe and Ile in 1GKZ are of approximately equal size and stabilizing nature. No evidence for repulsion between chloride and any part of the protein is found in this analysis. Thus our results do not rule out the possibility of anion-π interactions in protein. In fact, they are in line with our previous hypothesis that "polar, charged and H-bond donating residues may interact with an anionic group, reducing the repulsion between the negative charge of the anion and the electron cloud of the aromatic ring, thereby enhancing vdW interactions".[15] Further systematic calculations are needed to explore this important point.

CONCLUSIONS

Ab initio and DFT studies of prototypical anion-π interactions were performed to probe their strength and optimal geometry as well as testing methods for their suitability in estimating these important properties. Studies at a single geometry allow benchmark data to be calculated, employing extrapolation of MP2 data to the basis set limit, followed by correction with CCSD(T) with a smaller basis set. This allows us to test more approximate methods and shows that CCSD(T) corrections are small such that MP2 and its local variant, LMP2, is suitable for description of these interactions. However, we show that MP2 interaction energies are rather basis set dependent, such that previously reported benchmark data are in significant error.

Relative to MP2, we find that the popular B3LYP DFT method is poor for describing both geometry and interaction energy, as are several other variants of DFT. In contrast, several DFT methods perform well: M05-2X and especially M06-2X perform very well, while MPWB1K, BHandH, and ωB97X-D give reasonable estimates of interaction energy. Potential

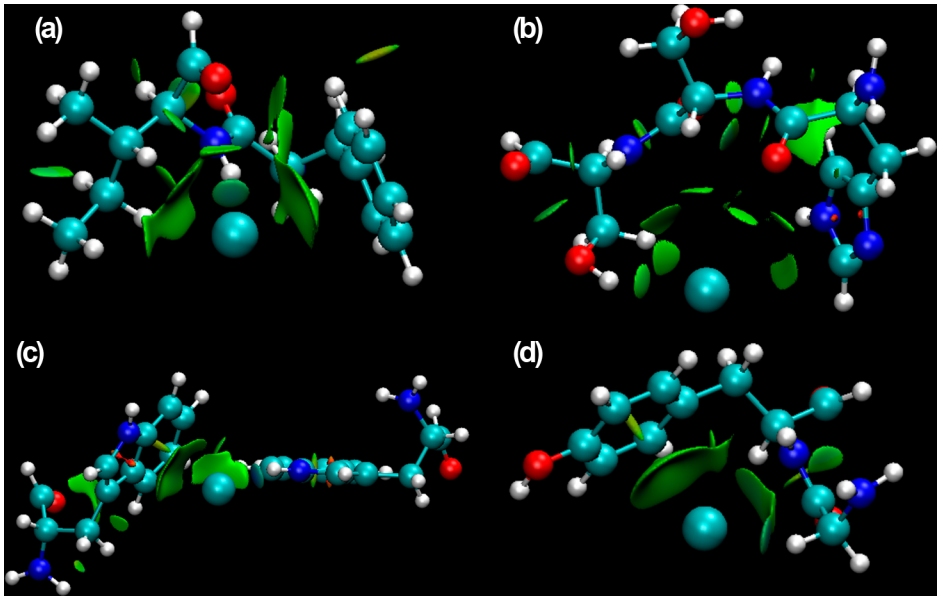

Figure 4. Plot of reduced density gradient s(r) in a) 1GKZ, b) 2GFG, c) 2VQU, and d) 3FP4; plotted at 0.5 au isosurface and colored by magnitude of λ_H. Blue indicates strong stabilization, green weak stabilization, and red repulsion.

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energy scans confirm this picture: B3LYP strongly underestimates energies at all ranges for all complexes considered. For the more strongly bound complexes MPWB1K performs best relative to LMP2, but for more weakly bound complexes this can also underestimate binding. M06-2X and BHandH closely reproduce LMP2 data for a chloride complex, but significantly overestimate binding for a stronger fluoride one, while ωB97X-D gives a well-balanced description of all complexes. Taken together, these studies suggest DFT geometry optimization with MPWB1K, M06-2X, or BHandH, possibly followed by LMP2 single-point binding energy calculation, should give an accurate description of anion-π interactions.

These methods are then applied to four examples of chloride ions found in close proximity to aromatic rings (of Phe, Trp, Tyr, and His) in published protein structures. In all cases, DFT methods indicate substantial stabilization, which, however, very likely originate from H-bonding rather than anion-π interactions. In order to further explore this point, QTAIM analysis was performed. The electron density analysis supports the presence of weak anion···π interactions and reveals no repulsion between the anion and the ring. Finally, this work provides an accurate and efficient methodology, a combination of carefully chosen DFT functionals with accurate energy LMP2, the first step toward a systematic investigation of anion-π interactions in realistic protein models.

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Notes

The authors declare no competing financial interest.

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