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HIGHLIGHT

Recent advances in anion–π interactions†

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Over the past 10 years, anion–π interaction has been recognized as an important weak force that may occur between anionic systems and electron-deficient aromatics. Lately, this supramolecular contact has experienced a rapidly growing interest, as reflected by numerous recent literature reports. The present paper highlights the tremendous progress achieved in the field by emphasizing three important studies involving anion–π interactions published in 2010. In addition, a pioneering search of the Protein Data Bank (PDB) reveals short anion–π contacts in some protein structures.

1. Introduction

Supramolecular chemistry focuses on self-assembled systems whose spatial organization involves weak and reversible noncovalent interactions.[1–3] These intermolecular non-covalent bonding contacts include hydrogen bonds,[4,5] π–π stacking,[6,7] CH–π[8,9] and cation–π[10,11] interactions, with energies ranging from 2 to 120 kJ mol^{-1} (Fig. 1).

During the past decade, a new potential supramolecular bond involving aromatic moieties, namely the anion–π interaction (and more generally the lone pair–π interaction), has been revealed by theoretical investigations[12–16] and has been observed in single-crystal X-ray structures.[17–21] The bond energies for such anion–π supramolecular pairs are in the range 20–70 kJ mol^{-1},[15,22,23] and therefore are close to those characterizing cation–π interactions (Fig. 1).

Taking into account all these pioneering studies, several thorough searches on anion–π interactions at the Cambridge Structural Database (CSD) have been carried out.[24–30] These CSD examinations have clearly shown that a number of anion–π close contacts can be observed in solid-state structures. For instance, two illustrative examples have been selected that contain a 1,3,5-triazine ring and a fluorinated phenyl ring, respectively. These two electron-deficient rings have been comprehensively explored, theoretically.[15,16,31]

The triazine-based case is represented by the salt 1-fluoro-2,4,6-trimethoxy-1,3,5-triazinium hexafluoroantimonate (CSD refcode MACHAA).[32] Views of its

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† Electronic supplementary information (ESI) available: Tables S1 and S2 illustrating two examples of compounds exhibiting anion–π interactions found in the CSD; Figures S1–S4 showing anion–π contacts found in the PDB. See DOI: 10.1039/c0ce00819b

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Dr Arturo Robertazzi currently works as a postdoctoral researcher in Prof. Knapp's group at the Free University of Berlin. He completed his PhD degree at Cardiff University (UK) under the supervision of Dr J. Platts in 2006. There, he employed quantum chemical calculations to study the interactions of transition metals with DNA bases. In recent years, he applied several computational methods, from docking to molecular dynamics, to investigate systems of biological relevance, such as heme proteins, nitrogen-fixation biocatalysts and DNA cleavers.

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Florian Krull is a PhD candidate supervised by Professor Knapp at the Institute of Chemistry and Biochemistry of the Freie Universität Berlin. He was born 1980 in Lüneburg, Germany, and holds a diploma degree obtained in 2006 at the Centre for Bioinformatics Hamburg. Currently he is working on scoring functions applied to protein–protein docking predictions.

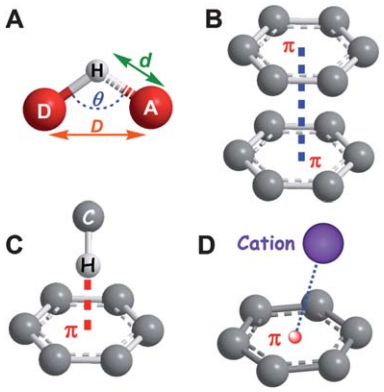

Fig. 1 (A) Hydrogen-bond (energy: 12–120 kJ mol^{-1}),[5] (B) π–π (energy: 2–10 kJ mol^{-1}),[7] (C) CH–π (energy: 6–13 kJ mol^{-1})[8] and (D) cation–π (energy: 5–80 kJ mol^{-1})[11] interactions.

crystal structure are depicted in Fig. 2. The hexafluoroantimonate anion strongly interacts with the triazine through three fluoride atoms (F2, F4 and F5; Fig. 2A and Table S1†). The F···$C_{triazine}$ separation distances vary from 2.831(5) to 2.886(5) Å (Table S1†), which are well below the sum of the van der Waals (vdW) radii of the F and C atoms, which is 3.17 Å.[24] The top view of this supramolecular pair clearly illustrates the almost perfect position of the SbF_6^- ion over the π-acidic ring (Fig. 2B).

The anion–π association involving an electron-poor perfluoro arene is nicely exemplified by the solid-state structure of the salt bis(pentafluorophenyl)bromonium

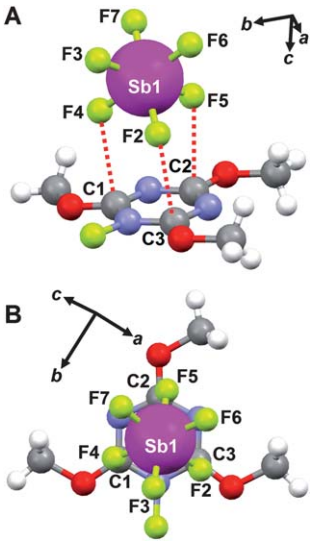

Fig. 2 (A) Side view and (B) top view of the molecular structure of 1-fluoro-2,4,6-trimethoxy-1,3,5-triazinium hexafluoroantimonate (CSD refcode MACHAA).[32] The red dotted lines symbolize the close F···C contacts; C1···F4 = 2.858(6) Å, C2···F5 = 2.886(5) Å, and C3···F2 = 2.831(5) Å.

hexafluoroarsenate (CSD refcode HOHKAQ).[33] Views of its molecular structure are depicted in Fig. 3. The hexafluoroarsenate anion is in close contact with two pentafluoroaryl rings (atoms F6, F8, F6d and F8d; Fig. 3A and Table S2†). This π–anion–π supramolecule is characterized by short F···C_{C6F5} distances ranging from 2.906(9) to 3.167(9) Å (Table S2†).[24] The

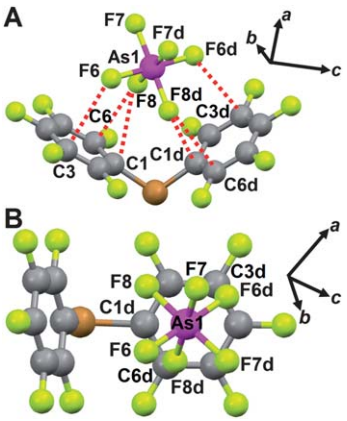

Fig. 3 (A) Side view and (B) top view of bis(pentafluorophenyl)bromonium hexafluoroarsenate structure (CSD refcode HOHKAQ).[33] The red dotted lines symbolize the close F···C contacts; C1···F8: 3.035(8) Å, C3···F6: 3.167(9) Å, C6···F8: 2.906(9) Å. The symmetry operation between the two aromatic rings is given as $d = -1/2 + x, 3/2 - y, 7/4 - z$.

AsF_6^- ion is embraced by the aromatic moieties (see Fig. 3 and Table S2†).

Most anion–π close contacts found in the CSD have not been described as such by the authors in the corresponding publications. Actually, the interest of the scientific community in this supramolecular interaction has increased rapidly after the first two explicit crystallographic reports on this non-covalent contact, in 2004.[34,35] Since then, numerous crystallographic observations of anion–π

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Patrick Gamez

Patrick Gamez received his DPhil at the University of Lyon and was awarded the French Chemical Society Prize for his thesis work. After a Marie Curie postdoctoral stay at the MPIfürKohlenforschung and at the University of Strasbourg, he spent 10 years at Leiden University. Since 2010, he is ICREA research professor in bioinorganic chemistry at the Universitat de Barcelona. His current research interests are coordination and supramolecular chemistry applied to biomimetic oxidation catalysis, the design of multidentate ligands for crystal engineering and the preparation of magnetic switchable materials (spin-crossover). He is involved in the preparation of MOFs for applications in catalysis. As a pioneer in the field, he is actively investigating the supramolecular anion–π interaction. He is the (co-)author of over 177 publications.

interactions (obtained serendipitously) have been described in the literature.[12] In recent years, the anion–π interaction is increasingly regarded as a potential non-covalent interaction for the design of anion receptors.[36]

In the present highlight paper, the future prospects of anion–π interactions for potential applications in anion recognition are examined through the discussion of representative examples reported during the year 2010. In addition, an analysis of the Protein Data Bank (PDB) has been carried out, which shows that such interactions may occur as well in biological macromolecular structures.

2. Anion–π interactions at work

Anions are omnipresent in living cells where they play important roles in biological processes.[37–39] The significance of anionic species in biochemical systems therefore has triggered the design of artificial anion-binding hosts, for instance to treat diseases such as channelopathies (chloride transporters).[40]

Recently, Matile and co-workers have reported artificial systems to transport anions across lipid bilayer membranes.[41,42] These "anion-slides" are based on anion–π interactions between the ion and π-acidic, forming rod-shaped Oligomeric NaphthaleneDiImides (O-NDIs). Thus, the linkage of naphthalenediimide units through tetramethylbenzyl moieties produces an unbendable scaffold with a string of electron-deficient binding sites for anions to move cooperatively across a lipid bilayer (Fig. 4).[41] The involvement of anion–π interactions in these synthetic channels could not be experimentally proven. Actually, the participation of the amide functions or potentially charged peptide chains (R groups in Fig. 4) in the binding of anions could not be excluded.[41]

Lately, Matile and co-workers have undertaken important investigations aimed at trying to observe anion–π interactions at work.[43] For this purpose,

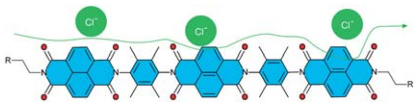

Fig. 4 Anion–π slide for chloride transmembrane transport.[41]

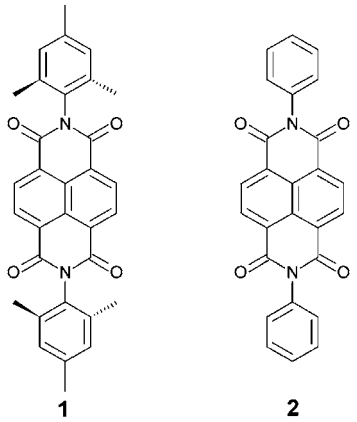

Fig. 5 Examples of NDI monomers designed by Matile and co-workers to study anion–π interactions at work.[43]

a series of simple monomeric naphthalenediimides (such as NDI **1** and NDI **2** in Fig. 5) have been prepared.

Advanced laser-induced ESI-MS-MS (electrospray ionization tandem mass spectrometry) measurements provided direct evidence for anion binding by these π-acidic compounds.[43] An equimolar mixture of NDI monomers **1** and **2** (Fig. 5) was used for competition experiments with Cl^-. Thus, a solution of the NDIs and one equivalent of NEt_4Cl was electrosprayed, and the corresponding heterodimer **1** + **2** + Cl^- could be detected (Fig. 6A).

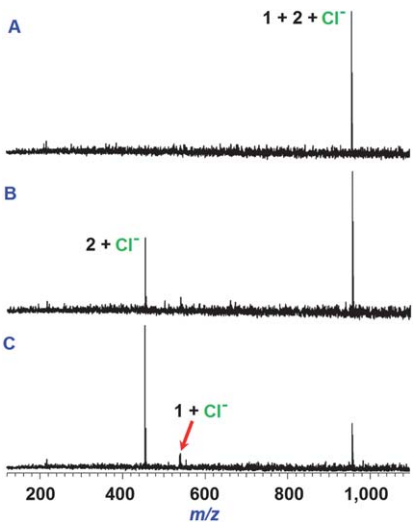

Fig. 6 Laser-induced ESI-MS-MS experiments:[43] Spectra of heterodimer complexes of **1** and **2** with Cl^- anions, (A) before fragmentation and after fragmentation induced by a (B) 100 ms and a (C) 200 ms laser pulse.

Next, fragmentation of the heterodimer **1** + **2** + Cl^- was induced by irradiation with a 25 W infrared laser. After 100 ms irradiation, the complex **2** + Cl^- was observed (Fig. 6B). After 200 ms irradiation, a new peak corresponding to **1** + Cl^- was noticed, while the peak for the heterodimer **1** + **2** + Cl^- significantly decreased (Fig. 6C). These mass spectrometry data clearly indicate that NDI **2** has a higher affinity for Cl^- than NDI **1**. In addition to these experiments in the gas phase, computational studies showed that increasing the π-acidic character of the naphthyl group of the NDI monomers, along with relieving the steric hindrance near the assumed anion binding site (namely the electron-poor surface), increased the magnitude of the interaction between the anion and the π-acidic ligand.[43]

Next, the anion transport activity of each monomeric NDI of the series examined was determined in phospholipid liposomes using various fluorescence techniques. The anion transport results were in good agreement with the anion-binding trends observed by mass spectrometry and theoretical investigations. Hence, the compounds showing higher anion-binding affinities exhibited the best anion-transport activities. This relationship therefore supports the hypothesis that anion–π interactions are indeed involved in the anion-transport properties displayed by previous systems reported by Matile and co-workers,[41,42] and by the monomeric NDIs described in the latest paper.[43]

The most efficient transporter in the group, a NDI bearing two electron-withdrawing cyano substituents at the naphthyl core, showed remarkable anion-transport activity for chloride, even at nanomolar concentrations. Interestingly, some of the π-acidic NDIs exhibited a notable selectivity for the nitrate anion. This uncommon nitrate selectivity was ascribed to the ability of the electron-rich π-orbitals of the nitrate anion to form specific π–π stacking interactions with the electron-poor π-surface of the aromatic part of the artificial transporter.

3. π-Accepting arene as halide receptor

Anion binding and sensing is a topical field of contemporary supramolecular

chemistry with potential applications in pollutant sequestration and biomedical and environmental monitoring.[44]

In highly electron-deficient aromatic molecules, like tetracyanopyrazine[45] or tetracyanobenzene,[46] the corresponding π-acceptor/anion interactions involve charge transfer,[47] which is often associated with the appearance of highly coloured compounds in solid state or solution.[48] Consequently, such CT complexes may be used to design and prepare anion-sensing receptors.[44]

Very recently, Dunbar and co-workers have investigated the anion-binding properties of electron-deficient 1,4,5,8,9,12-hexaazatriphenylenehexacarbonitrile (complex **3** in Fig. 7).[49] The potential interactions between **3** and the halide salts [nBu$_4$N][X] (X=Cl, Br, I) have been studied both in solution and solid state. The formation of the CT complexes **3** + X (X=Cl, Br, I) in solution (THF or nitromethane) has been undoubtedly evidenced by UV/vis, ^{13}C and halogen NMR, and ES-MS experiments. All these characterization techniques support the spontaneous generation of highly stable $\{[\mathbf{3}]_2[X]_3\}^{3-}$ CT species (the stability constant values, $K_{CT,X}$, range from 20 to 71 M^{-1}).[49]

The CT complexes **3** + Br$^-$ and **3** + I$^-$ could be isolated as single crystals from THF solutions of [nBu$_4$N][X] and **3** treated with benzene;[50] therefore, their solid-state structures could be determined by X-ray diffraction studies.

The structures of **3** + Br$^-$ and **3** + I$^-$ involve four layers **ABCD** that assemble along the crystallographic c axis with units of **3** alternately interspersed with anions (three or one per layer; red and orange balls in Fig. 8, respectively). The propagation of the linear chains $\{[\mathbf{3}]_2[\mathbf{X}]_3\}_3^-\cdots[\mathbf{X}]^-\cdots\{[\mathbf{3}]_2[\mathbf{X}]_3\}_3^-\cdots[\mathbf{X}]^-$ is governed by supramolecular contacts between the anions and electron-deficient units of **3**, with a **3**-to-**X**$^-$ ratio of 2 : 3.

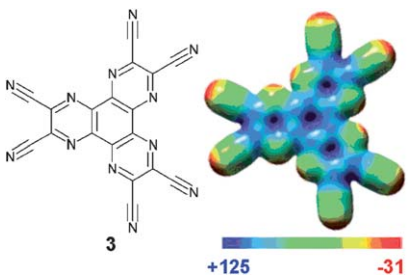

Fig. 7 Structure of 1,4,5,8,9,12-hexaazatriphenylenehexacarbonitrile (**3**) and its electron spin polarization (ESP) map (in kcal mol^{-1}).[49]

The single anion in layer **A** establishes two anion–π contacts with the central ring of two units of **3**, in layers **B** and **D** (Fig. 8). The anion-to-centroid distances, dX$_{int-centroid}$ (Table 1), are both shorter than the corresponding sums of the vdW radii (*i.e.* 3.55 Å for Br$^-$ and 3.68 Å for I$^-$).[24] The X$_{int}\cdots$C$_{int}$ distances are in the range of the sum of the vdW radii (Table 1), hence indicating the occurrence of anion–π interactions in both CT complexes.

Besides, in **3** + Br$^-$ and **3** + I$^-$, three crystallographically equivalent **X**$^-$ ions form layer **C**. Each anion of this layer is located over the periphery of entities of **3** in layers **B** and **D**, and is equidistant from the pyrazine external carbon atoms C$_{ext}$ of **3**, in an η^2,η^2-fashion (Fig. 8). The X$_{ext}\cdots$C$_{ext}$ separation distances are significantly shorter than the corresponding sums of the vdW radii (Table 1). The observed off-center geometries of the anions **X**$^-$, their close contacts to C$_{ext}$, together with the formation of highly colored compounds, suggest that CT interactions are dominant in **3** + 3Br$^-$ and **3** + 3I$^-$ of layer **C**. The shorter X$_{ext}\cdots$C$_{ext}$ distances, as compared to the X$_{int}\cdots$C$_{int}$ distances of Br$^-$ and I$^-$ in layer **A** clearly indicate that the anion–π interactions are weaker for the latter anions.

The high stability of these anion–π complexes along with the distinct anion-specific colours are highly desirable features for the design and preparation of anion-sensing receptors.

4. Anion–π contacts in supramolecular assemblies

Hydrogen-bonds,[5,51] π–π stacking,[52,53] cation–π[10,11] and CH–π interactions[8,54] are common noncovalent contacts in supramolecular chemistry and crystal engineering. Anion–π[55] interactions constitute a new species of supramolecular bonds.[12,17,56–58]

During the past five years, we have been involved in investigations aimed at systematically studying this type of noncovalent bonding interactions observed in new crystal structures, to gain knowledge in this topical field, both theoretically and experimentally.[59–63]

Thus, a few months ago, we have obtained a supramolecular assembly including anion–π and lone pair–π interactions.[64] The reaction of magnesium(II) perchlorate with malonic acid and 2-aminopyridine in water produced the compound $(C_5H_7N_2)_4[Mg(C_3H_2O_4)_2(H_2O)_2](ClO_4)_2$ (**4**). The single-crystal

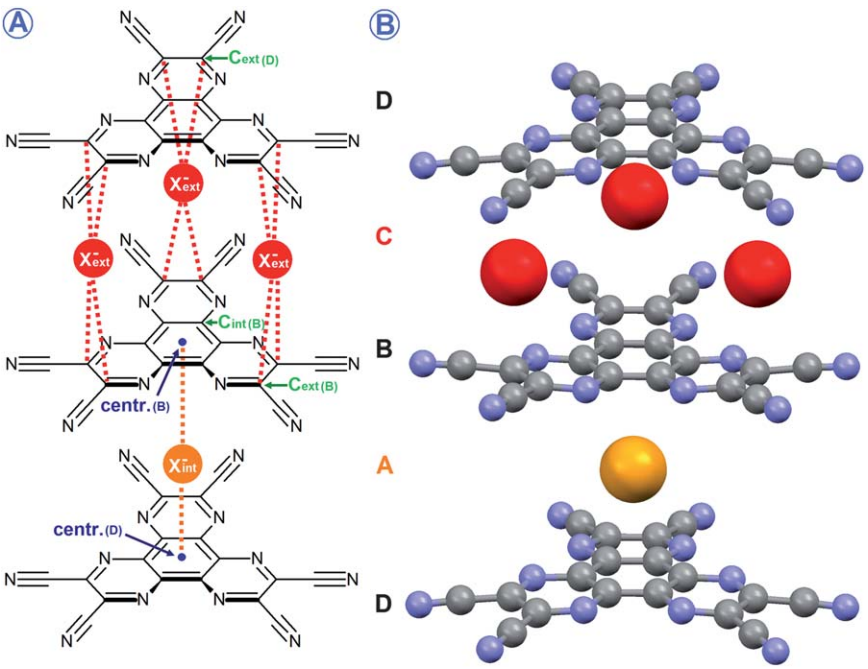

Fig. 8 (A) Multi-site anion contacts in the CT complexes **3** + X$^-$; each halide ion is in contact with two units of **3**. (B) **ABCD** layers along the crystallographic c axis forming 1D vertical stacks.[49]

Table 1 Intermolecular close contacts (Å) for **3** + Br^- and **3** + I^- (see Fig. 8)

X^-	$dX_{int-centroid}$	$X_{int}\cdots C_{int}$	$X_{ext}\cdots C_{ext}$
3 + Br^-	3.282[a]	3.579[a]	3.354[a]
	3.245[b]	3.542[b]	3.239[b]
3 + I^-	3.419[a]	3.666[a]	3.506[a]
	3.337[b]	3.635[b]	3.334[b]

[a] Distance to layer **B**. [b] Distance to layer **D**.

X-ray structure of **4** exhibits monomeric anionic units $[Mg(C_3H_2O_4)_2(H_2O)_2]^{2-}$ that are interlinked to each other *via* strong self-complementary O_{water}–H$\cdots$ $O_{C=O}$ hydrogen bonds, giving rise to an $R_2^2(12)$ motif (Fig. 9).

This assembly generates an infinite 1D chain along the crystallographic *a* axis. Furthermore, in complex **4**, each $[Mg(C_3H_2O_4)_2(H_2O)_2]^{2-}$ unit interacts with four aminopyridinium cations ($C_5H_7N_2^+$; apyr) through N_{apyr}–H$\cdots O_{mal}$ hydrogen bonds (mal = malonate), leading to $R_2^2(8)$ motifs (Fig. 10).

The lattice perchlorate anions are implicated in the creation of 2D sheets *via* perchlorate$\cdots$perchlorate interactions ($O_{perchlorate}\cdots O_{perchlorate}$ = 2.803(3) Å) and hydrogen bonding with the coordinated water molecules of $[Mg(C_3H_2O_4)_2(H_2O)_2]^{2-}$ moieties (O_{water}–H$\cdots O_{perchlorate}$: 2.813(2) Å, O_{water}–H–$O_{perchlorate}$: 173(3)°). Moreover, two of the perchlorate oxygen atoms are involved in anion–π contacts with two different neighbouring aminopyridinium cation (shortest $O_{perchlorate}\cdots$ring distances of 3.085(3) and 3.150(3) Å).

One of the noncoordinating oxygen atoms of the malonate ligand is orientated toward the π-face of a 2-aminopyridine moiety (Fig. 11).[64] The distance between this O atom and the centroid of the aminopyridine ring is 3.2104(18) Å. This 2-aminopyridine ring is further π-stacked over a second aminopyridine molecule in a head-to-tail fashion, with the amino nitrogen atoms lying only 3.46 and 3.29 Å above the ring centroids. Finally, the aminopyridine ring which is in anion$\cdots$π contact with one of the perchlorate oxygen atoms is further interacting with a noncoordinated malonate oxygen atom, generating an additional lone pair$\cdots$π association.

This intricate network of supramolecular bonds generates a 3D structure that is assembled through a combination of hydrogen-bonds, lone pair$\cdots$π, π$\cdots$π, and anion$\cdots$π interactions (Fig. 11). A thorough AIM analysis of this supramolecular architecture has been subsequently carried out.[64] The computational results obtained corroborate the supramolecular interactions initially proposed while describing the solid-state structure of **4**.[64]

5. Anion–π interactions in proteins

In order to assess whether anion–π interactions play a role in proteins, a thorough search of the Protein Database (PDB, www.pdb.org[65] has been carried out, following a procedure previously proposed.[24]

A program specifically written for this study has been used to look for contacts occurring in proteins between an anion (Cl^-, Br^-, F^-, NO_3^-, ClO_4^- and PO_4^{n-})[66] and any of the aromatic residues tryptophan (Trp), phenylalanine (Phe), tyrosine (Tyr) and histidine (His). A contact between an anion and an aromatic ring is considered as a potential anion–π interaction when the two following geometrical criteria are fulfilled: (i) the distance ($D_{A-\pi}$) between the centroid[67] of the aromatic ring and the anion[68] is smaller than 5 Å; (ii) the angle ($\alpha_{A-\pi}$) formed by the vector connecting the ring centroid with the anion and the plane of the ring ranges between 60 and 90°. These criteria are slightly looser than those applied in previous studies on small molecules found in the CSD.[24] One reason is that structural variations are generally larger in crystal structures of proteins than of small molecules. Thus, even structures featuring longer distances $D_{A-\pi}$ may be relevant for the purpose of this study. Only crystal structures with a resolution below 2.5 Å were included in the analysis. For the most relevant cases (those with higher statistical occurrences), further analyses were performed in line with previous studies.[24] In particular, the shortest distance ($d_{A-\pi}$) between the anion (or the closest negatively charged atom of the anionic group) and any atom of the aromatic ring was monitored. If $d_{A-\pi}$ is in the range of the sum of vdW radii of the ring atom (typically N or C) and the corresponding anionic atom, the contact between the two groups is defined as an "anion–π interaction".[24] For the relevant atom pairs, the sums of vdW radii are r_{N-Cl} = 3.30 Å, r_{C-Cl} = 3.45 Å, r_{N-O} = 3.07 Å, r_{C-O} = 3.22 Å.[24] The results for all anions are collected in Table 2.

It may not be surprising[12–16,24] that the number of anion–π contacts fulfilling the search criteria is small. In particular, the total number of chloride ions found in the PDB is 9824. Of these, 244 chlorides were found in close contact with an aromatic ring, corresponding to an occurrence of 2.5%. In addition, out of a total of 18 635 phosphate anions found in proteins, 80 of these were close to an aromatic ring, the occurrence being thus equal to 0.4%. All other anions represent less than 20 anion–π contacts. Hence, only the structures containing a chloride or a phosphate anion close to an aromatic ring were further analyzed.

For the structures containing chloride or phosphate anions, histidines are the aromatic residues which are the most represented (Table 2). Notably, His is aromatic at all pH values, *i.e.*, it is aromatic even when protonated. In proteins, histidines are usually uncharged. However, those in close contact with an anion are likely to be protonated, and are therefore positively charged. This is an important fact since protonated histidines are electron-poor aromatic rings, such as the electron-deficient arenes investigated theoretically,[12–16] or the positively charged rings found in supramolecular assemblies for which strong evidences of anion–π interactions have been provided.[18]

The other aromatic residues (Phe, Trp, Tyr) generally remain neutral. The question is: can these charge-neutral aromatic residues really bind an anion? Simple models based on quantum-mechanical calculations *in vacuo* have shown that electron-withdrawing substituents are necessary to invoke a significant attraction between an aromatic ring and an anion.[12–16] None of the aromatic residues found in proteins exhibit

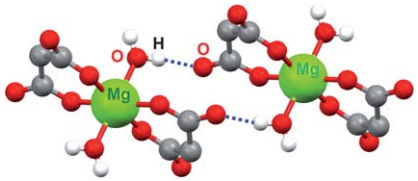

Fig. 9 Association of $[Mg(C_3H_2O_4)_2(H_2O)_2]^{2-}$ units through O_{water}–H···$O_{C=O}$ hydrogen bonds (O_{water}···$O_{C=O}$ = 2.677(2) Å, O_{water}–H – $O_{C=O}$ = 169(3)°.[64]

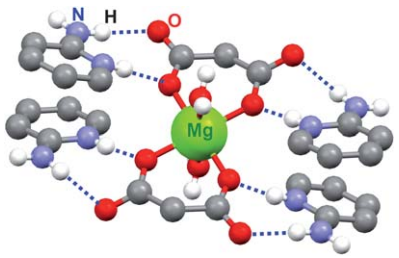

Fig. 10 $[Mg(C_3H_2O_4)_2(H_2O)_2]^{2-}$ unit connected to four 2-aminopyridinium cations by means of N_{apyr}–H···O_{mal} hydrogen bonds (N_{apyr}···O_{mal}: 2.773(2)–2.893(2) Å, N_{apyr}–H–O_{mal}: 169(3)–175(2)°.[64]

Table 2 Anion–π contacts fulfilling the following search criteria. The distance $D_{A-\pi}$ between the anion and the aromatic ring centroid is shorter than 5 Å and the angle $\alpha_{A-\pi}$ formed by the vector connecting the aromatic ring center with the anion and the aromatic ring plane is between 60° and 90°

		Number of contacts[b]			
Anion (X^-)	Relative occurrences[a]	Trp···X^-	Phe···X^-	Tyr···X^-	His···X^-
Cl^-	2.5%	27	49	56	113
PO_4^{n-}	0.4%	17	9	22	29
NO_3^-	0.8%	3	4	7	4
Br^-	0.6%	0	3	1	2
F^-	0%	—	—	—	—
ClO_4^-	0%	—	—	—	—

[a] The occurrence is calculated as the percentage of anions that fulfill the search criteria over the total number of anions found in the PDB. By analyzing the solvent accessible surface area (SASA), the same quantity was calculated only for buried chlorides and phosphates (SASA close to zero). The occurrence slightly increases (data not shown). [b] Absolute number of contacts between the anion (X^-) and the particular aromatic residue.

electron-withdrawing groups. Theoretical studies have also suggested that the anion–π interactions are the result of the interplay between electrostatics and vdW energies.[14] 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. Notably, the most likely amino acids within a distance of 6 Å from the interacting anion (chloride or phosphate) are Cys, Met, Trp, Gln, His, Tyr, Asn, Arg (Fig. S1†). For instance, Arg and Gln can in principle form H-bonds, the aromatic residues, Trp and Hys, may interact through anion–π interactions.

As mentioned above, the shortest distance, $d_{A-\pi}$, between the anion and any of the atoms of the aromatic group was also monitored. If $d_{A-\pi}$ is in the range of the sum of the vdW radii, the contact is defined as an "anion–π interaction".[24] Fig. S2 and S3† illustrate the scatter plots of $d_{A-\pi}$ *versus* $\alpha_{A-\pi}$ for the anion–π contacts that fulfill the search criteria, reported for each aromatic residue. Most points are in the range 3.5–4.5 Å, and about 10% of the hits are below 3.5 Å. These anion–π contacts show a distance which is in the range of the sum of vdW radii of the corresponding atom pairs, suggesting that a "strong interaction" may occur. Fig. S4† shows the number of contacts as a function of this distance. For $d_{A-\pi}$ smaller than 3.5 Å, 22 chloride–π, "strong interactions" were found, 17 involving His, 2 Trp, 2 Phe and 1 Tyr. A similar analysis was performed for the structures containing phosphate anions. In this case, the number of "strong" anion–π interactions ($d_{A-\pi}$ smaller than 3.3 Å) is 6; 1 involves His, 3 Tyr, 2 Phe. If one allows a small tolerance of 1 Å above the sum of vdW radii, the number of interactions increases up to 40% (Fig. S4†).

Among the structures featuring anion–π contacts, two interesting examples are herein briefly discussed (Fig. 12A–D). Fig. 12A displays the six chains of Glutathione-S-Transferase from *Xylella Fastidiosa* (PDB code: 2X64). In each

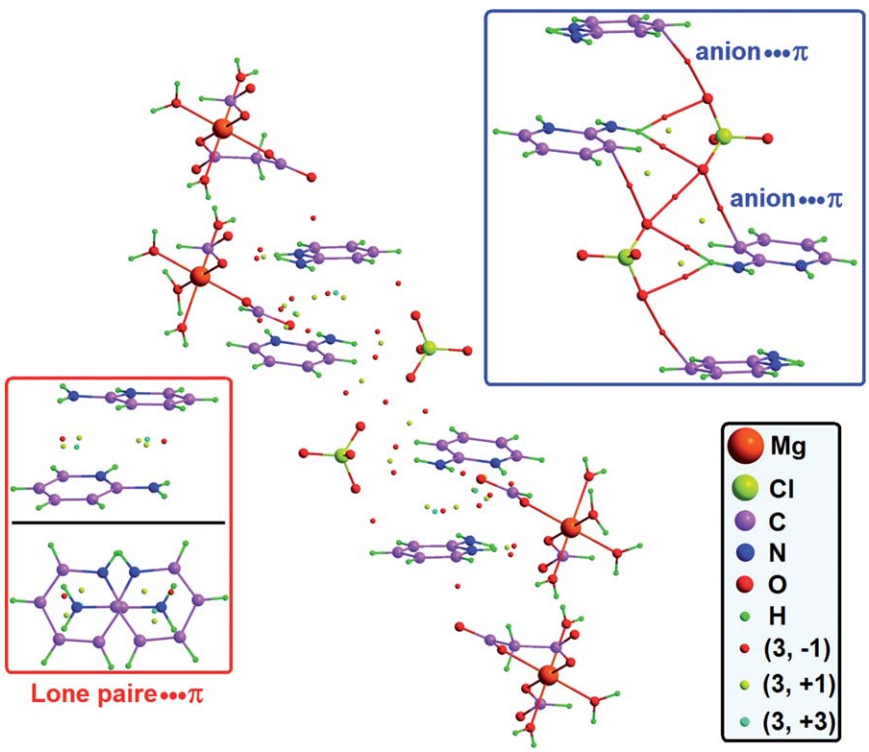

Fig. 11 AIM analysis of a large fragment of **4** showing the occurrence of hydrogen-bonds, lone pair···π, π···π, and anion···π interactions in its crystal packing.[64]

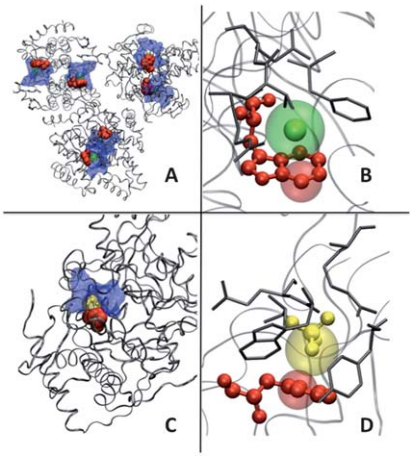

Fig. 12 In (A and B) two views of a Glutathione-S-Transferase, PDB code 2X64: $d_{A-\pi}$ = 3.72 Å, $\alpha_{A-\pi} = 78°$; aromatic ring involved in the interaction: Trp94; closest residues (distance from anion smaller than 6 Å): Thr, Gln, Arg, and Phe. In (C and D), two views of a Glycerol Kinase, PDB code 3H3O: $d_{A-\pi}$ = 2.92 Å, $\alpha_{A-\pi} = 70°$; aromatic ring involved in the interaction: Phe308; closest residues (distance from anion smaller than 6 Å): Trp, Gln, Ser, Arg, Asp, Tyr, and Thr.

chain, an anion–π contact is observed. Interestingly, a chloride is located on top of a Trp residue (Fig. 12B), with $d_{A-\pi}$ = 3.72 Å and $\alpha_{A-\pi} = 78°$. Another aromatic residue (Phe) is found nearby the Cl^- anion, with a distance $D_{A-\pi}$ of 5.66 Å. In addition, Thr, Gln and Arg residues are situated in close proximity to the chloride.

Fig. 12C shows the structure of glycerol kinase (PDB code 3H3O) with a phosphate anion in close contact with one Phe; the separation distance $d_{A-\pi}$ = 2.92 Å is well below the sum of the corresponding vdW radii (3.22 Å), and $\alpha_{A-\pi} = 70°$ (Fig. 12D). The nearest residues are Gln, Ser, Asp, Thr, Arg, Trp and Tyr. Interestingly, the last two amino acids may interact with the phosphate ion through anion–π interactions. Furthermore, Arg can potentially form H-bonds with the anion (the $N_{Arg}\cdots O_{phosphate}$ distance is 2.65 Å).

6. Conclusions

Anion–π interactions are clearly attracting increasing interest among chemists, physicists, theoreticians and material scientists, most likely because anions are ubiquitous in (physico)chemical and biochemical sciences. The three illustrative examples of research investigations involving anion–π contacts published in 2010 definitely demonstrate the importance of the anion–π interaction as a new type of non-covalent bond. Hence, this field of supramolecular chemistry is expected to receive even more attention in the near future.

Regarding the PDB search, the data presented in this study show that anions (especially chloride and phosphate) can be found in close contact with aromatic residues (mostly histidines) in solid-state structures of proteins. The majority of the close contacts, defined by $d_{A-\pi}$ (which is the shortest distance between the anion and any atom of the aromatic ring) are in the range 3.5–4.5 Å. However, only a few cases with $d_{A-\pi} < 3.5$ Å, characterizing a "strong interaction", were found. This search for anion–π contacts in the PDB cannot prove beyond reasonable doubt whether the presumed anion–π interactions found are of significance. Actually, quantum-mechanical calculations are required to quantitatively evaluate the importance of such weak supramolecular interactions in protein structures. Nevertheless, these results show for the first time that anion–π interactions may play also a role in proteins, opening the way to further experimental and theoretical studies, which may shed light on those biological processes in which anions are involved and for which anion–π interactions may be relevant.

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66 The protonation state of phosphate depends on pH. The notation PO_4^{n-} indicates that this group is negatively charged but the exact charge is not in principle known.
67 For Phe, Tyr and His, the centroid is defined as the center of the ring; for Trp the centroid is localized in the center of the C–C bond shared by the two rings contituting the residue.
68 Or, in the case of NO_3^-, ClO_4^- and PO_4^{n-}, the interacting negatively charged atom of the anionic group.

