REVIEWS

Halogen bonding (X-bonding): A biological perspective

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Abstract: The concept of the halogen bond (or X-bond) has become recognized as contributing significantly to the specificity in recognition of a large class of halogenated compounds. The interaction is most easily understood as primarily an electrostatically driven molecular interaction, where an electropositive crown, or σ-hole, serves as a Lewis acid to attract a variety of electron-rich Lewis bases, in analogous fashion to a classic hydrogen bonding (H-bond) interaction. We present here a broad overview of X-bonds from the perspective of a biologist who may not be familiar with this recently rediscovered class of interactions and, consequently, may be interested in how they can be applied as a highly directional and specific component of the molecular toolbox. This overview includes a discussion for where X-bonds are found in biomolecular structures, and how their structure–energy relationships are studied experimentally and modeled computationally. In total, our understanding of these basic concepts will allow X-bonds to be incorporated into strategies for the rational design of new halogenated inhibitors against biomolecular targets or toward molecular engineering of new biological-based materials.

Keywords: halogen bond; molecular recognition; protein–inhibitor complexes; drug design

Introduction

Nature is highly adept at taking advantage of the laws of chemistry and physics to evolve highly complex biological systems. For the most part, however, terrestrial biology makes rather restrictive use of the elements from the periodic table, being based primarily on six elements (C, H, O, P, N, and S), along with a smattering of Group I and II and transition metals. Halogens (the Group VII elements) are not widely discussed in biology, except in terms of the effects of their anionic (fluoride, chloride, bromide, and iodide) forms on properties such as the osmolarity and ionic strengths of solutions. In chemistry, however, molecular halogens are important for their high reactivity; consequently, halogenation is seen as an important step in synthetic organic chemistry. The prevalence of halogenated compounds has made them widely used as inhibitors against biomedically important targets, and with the halogens often providing several orders of magnitude in specificity for these targets. For most of the history of biochemistry and medicinal chemistry, however, halogens have been treated primarily as electron-rich, lipophilic atoms that do not, in themselves, participate in specific molecular interactions that contribute to the recognition of ligands by proteins.

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The recent "rediscovery" of halogen bonds (or X-bonds, Fig. 1) as highly directional, short-range electrostatic interactions with electron-rich atoms (oxygens, nitrogens, and sulfurs) provides us with a renewed appreciation for the role that this class of elements plays in recognition and, potentially, as a new tool for biomolecular design and engineering. In this review, we will discuss the physicochemical basis for X-bonds and how their energies are estimated from both theoretical and experimental bases. We will then explore the types of X-bonds seen in biomolecular complexes and how they are being applied in the design of new inhibitors and to control molecular structures. We will start, however, with a brief history of this unique interaction and why it has become interesting from a biological perspective.

The first reports of halogens potentially serving as Lewis acids came in the mid to late 1800s with the description of complexes formed between molecular halogens (I_2, Br_2, and Cl_2) and ammonia and methylamines.[1] The detailed physical descriptions of such interactions came from the studies of Odd Hassel[2] in the mid-20th century on the crystal structures of molecular halogens in complex with organic Lewis bases, where it was observed that the interatomic distance from, for example, the bromine of Br_2 to the oxygen of dioxane could be as short as 2.7 Å, or >20% shorter than the sum of their respective van der Waals radii ($\sum r_{vdW}$). At that time, the interactions were called "charge transfer bonds," referring to a bonding model in which the charge from the lone pairs of an electron-rich atom, such as an oxygen or nitrogen, is transferred to a Lewis acid, in this case the halogen, in a manner similar to what is commonly observed with transition metal complexes. From that point on, however, the field appeared to be relatively quiet until about 1990, when a new term (halogen bond) started to appear in the chemical literature.[3–5] These short-range interactions were being used to control the assembly of organic molecules in crystals and in solution, among other things. This new name now reflects the more electrostatic character of the interaction, similar to classic hydrogen bonding, rather than their charge-transfer nature.

The re-emergence of X-bonds in chemistry has, until recently, been largely invisible to the biological community. Indeed, when short-range interactions between halogens and Lewis bases were first noted in complexes of proteins and nucleic acids,[6,7] they were unexplainable from a simplistic understanding of periodic chemistry. A survey of the Protein Data Bank (PDB[8]) by Auffinger *et al.*[9] in 2004, however, showed that such interactions were common in the crystal structures of biomolecular systems, particularly in complexes of halogenated ligands with their protein targets, but had remained ignored as significant contributors to specificity in molecular recognition. There was, at the time, at least one group trying to apply the concept of X-bonds for the rational design of new inhibitors against Factor Xa to serve as anticoagulants—unfortunately, this work had not been published.[10] Since then, the literature to characterize biological X-bonds, particularly as potential tools for rational drug design and molecular engineering has grown exponentially. Still, the contribution of X-bonds to any particular biomolecular structure is typically realized only in hindsight, and the biological studies to engineer X-bonds for such applications have lagged significantly behind the chemical and material sciences fields. This can be attributed to, until recently, a dearth of accurate and accessible methods to model the interaction in macromolecular systems.

For this review, we will focus on recent advances in the study of X-bonds from a perspective that helps to inform the biological community of their relevance and potential in conferring specificity. We will not attempt to summarize the advances made in the areas of the chemical, theoretical, and material sciences—there are already a number of recent reviews that focus on these particular areas of study.[5,11–13] We will, instead, discuss the current understanding of X-bonding, where the interactions are found in biology, their structure–energy relationships, how these relationships are modeled, and how this is now starting to come together in a manner that allows X-bonds to potentially become a powerful tool for molecular design.

Fundamentals of Halogen Bonding

The basic principles that underlie the X-bonding concept come from quantum mechanical (QM) analyses of complexes of halogenated organic molecules with various types of Lewis bases. The first question that comes to mind when we try to describe the concept of X-bonding is: How can halogens, considered to be electron rich in themselves, form short-range, stabilizing interactions with electron-rich Lewis bases? Fluorine, for example, is often used as a substitute for hydrogen-bond acceptors in carbohydrate chemistry,[14] which would appear to contradict the ability of halogens to serve as Lewis acids. To address this problem, we need to consider a more detailed description of the shape and charge distributions of halogens that are covalently bonded to other atoms (typically carbon in a C—X bond, where X is a polarizable halogen), as predicted by current QM models.

The original concept that charge transfer is the primary physicochemical basis for X-bonding has largely been replaced by electrostatic models based on the polarization of halogens that participate in covalent bonds. QM calculations applied at various levels, from Hartree–Fock method, to density functional theory (DFT), to Møller–Plesset perturbation

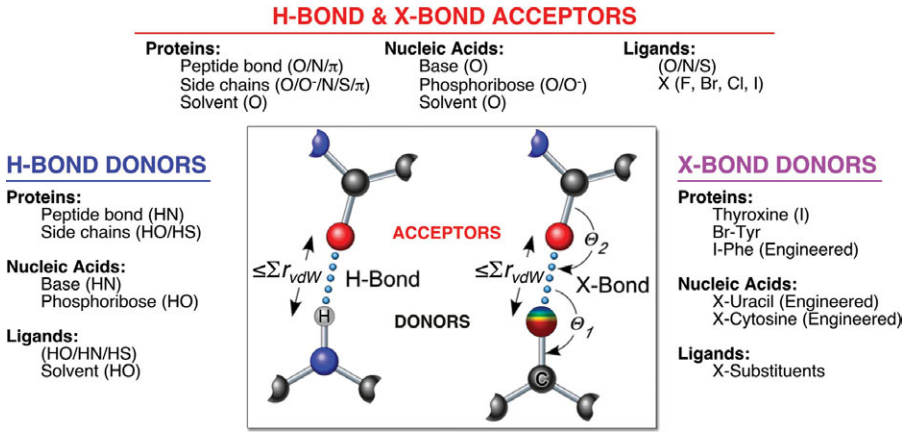

Figure 1. Hydrogen and halogen bonds. The geometries and types of donor and acceptor atoms are compared for classic hydrogen bonds (H-bonds) and halogen bonds (X-bonds) seen in biomolecular systems. Each interaction is characterized as being shorter than the sum of the van der Waals radii ($\sum r_{vdW}$) of the respective atoms. The halogen of the X-bond is shown with the electropotential polarized from positive (blue) to neutral (green) to negative (red). The approach of the acceptor to the halogen and halogen to the acceptor are labeled as Θ_1 and Θ_2, respectively. Acceptors that include the delocalized electrons of the amide peptide bond or the ring of an aromatic amino acid are listed as π.

(MP2) theory on simple halogenated organic compounds indicate that the distribution of electrostatic potential across the surface of the halogen is nonuniform. This anisotropic distribution of charge results in a crown of positive electrostatic potential directly opposite a covalent C—X bond, whereas the expected electronegative potential is manifested as a ring that encircles the halogen's girth perpendicular to the covalent bond.[15–19] Accompanying this anisotropic charge distribution is distortion to the halogen's shape, referred to as "polar flattening," where the effective radius of the halogen is shorter in the direction of the covalent bond[20,21] by as much as 16% relative to the standard r_{vdW}.[22] Together, the effects of flattening and charge depletion opposite the C—X bond provides a rationale for how halogens, such as bromine and iodine, can attract electron-rich oxygens and nitrogens to form "bonds" that are similar to classic H-bonds.

The σ-hole model

This leads to the next question, which is why are halogens polarized to such an extent that they form X-bonds? Perhaps the most accessible description, because of its simplicity, for how polarization affects the charge and shape properties of halogens is the σ-hole model (Fig. 2) as formulated by Politzer, Murray, and Clark.[17,18] In this model, the apparently unique nature of halogens has its roots in the fundamental properties of the covalent σ-bond between atoms. To understand this model, recall that Group VII atoms have five electrons residing in the *p*-atomic orbitals of the valence shell and that, according to molecular orbital theory, it is the single valence electron of the p_z orbital that participates in forming a covalent σ-bond to a carbon atom. Consequently, the depopulation of this orbital opposite the C—X σ-bond leaves a hole that partially exposes the positive nuclear charge. This σ-hole accounts for the electropositive crown and polar flattening associated with the polarization effects predicted from the QM calculations, whereas the four electrons remaining in the p_x, p_y orbitals account for the electronegative ring lying perpendicular to the σ-bond. In analogy to H-bonds, we can consider the σ-hole to be the donor to the electron-rich Lewis base acceptor in an X-bond.

One would expect that σ-bond formation to any atom would have this same type of polarization effect and, indeed, this has been predicted for other

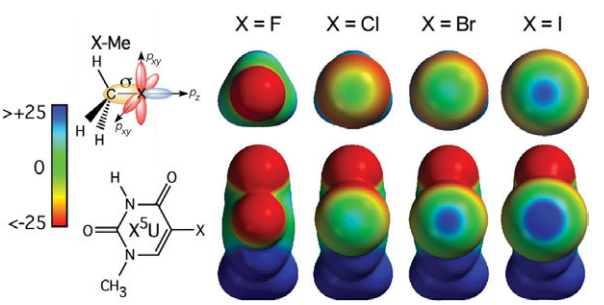

Figure 2. The σ-hole model and polarization of the electrostatic surface potential. The σ-hole resulting from redistribution of the valence electron in the p_z-atomic orbital (blue) to form the covalent C-X σ-bond (yellow) of a halomethane (X-Me) molecule results in depopulation of the p_z orbital, but maintaining the electrons of the p_x and p_y orbitals (red). The resulting polarization of the electrostatic potential of the halogen surfaces increases as the size of the halogen increases, from F to Cl to Br to I (viewed down the X-C bond). The halogen attached to a more electronegative molecule (e.g., a uracil base, X^5U) exaggerates the polarization effects. Electrostatic potential surfaces were calculated by DFT calculations at the 3-21G* level.[9]

atoms, including those in the Group VI atoms of the periodic table. Thus, the X-bond is simply one example of a larger class of interactions that are now referred to as σ-hole bonding.[23] Halogens, however, are unique in that the electropositive crown is not masked by other covalently bonded groups or by lone pair electrons in nonbonding orbitals that extend in approximately the same orientation as the σ-hole. We should note, however, that this relatively straightforward electrostatic explanation of X-bonding may not tell the entire story, as there remains debate concerning the relative contributions of dispersion and even the original charge transfer concept to the interaction. Attempts have been made to deconvolute the various components of the interaction using symmetry-adapted perturbation theory and natural bond orbital (NBO) analyses;[24–27] however, the conclusions from such studies are highly dependent on the model system the method of analysis.[24,28]

Geometry of X-bonds

The basic concept of the σ-hole makes the X-bond a highly directional interaction, as reflected in the angle of approach of the X-bond acceptor to the halogen relative to the direction of the σ-bond (Θ_1, Fig. 1). Surveys of Θ_1 angles for small molecule structures in the Cambridge Database[29] as well as biomolecular structures in the PDB[9] indicate a strong preference for a near linear approach of the acceptor toward the electropositive crown of the σ-hole, with a significant drop-off as the acceptor approaches the crossing point between the positive and negative electrostatic potentials ($\Theta_1 \approx 140^\circ$). The balance between the maximum positive electrostatic potential at $\Theta_1 = 180^\circ$ with the increase in available surface area of the halogen atom as Θ_1 approaches 90° accounts for the preference for $\Theta_1 \approx 160^\circ$–$165^\circ$.

The geometry of the X-bond in terms of the angle of approach of the halogen toward the acceptor atom (Θ_2, Fig. 1) shows that, for the most part, the σ-hole is attracted to the nonbonding electrons of the acceptor,[9] with $\Theta_2 \approx 120^\circ$ and consistent with the geometries seen in small molecule structures.[29] The exceptions for biological X-bonds are when delocalized π-electrons are available, for example, from the side chain of an aromatic amino acid or the peptide bond of a protein backbone. Once again, such π-X-bonds were first identified from surveys of small molecule crystal structures in the Cambridge Database,[30] showing the halogen to be directed perpendicular to the aromatic ring. Examples of π-X-bonds in biomolecules were first observed to the aromatic ring of Phe residues in the complexes of inhibitors to the protein kinases CDK2 and CK2.[31] This was further extended to a more comprehensive survey demonstrating the broad range of aromatic π-X-bonds in both proteins and small molecules structures, which, when coupled with MP2 calculations, could be attributed to upward of 2.5 kcal/mol toward the energy of interaction.[32] Once again, we see an obvious analogy between X- and H-bonds, in this case the π-X-bonds to π-H-bonds[33] to aromatic side chains of proteins.

The peptide bonds of most amino acids participate in H-bonds that help to define the secondary and tertiary structures of a folded protein. In these cases, the nonbonding orbitals of the carbonyl oxygen of the peptide bonds are occupied by H-bonds; thus, the only electronegative potential available for X-bonding comes from the π orbitals.[34] This type of interaction is the predominant type of X-bond seen in the crystal structures of protein–ligand complexes, and was shown to be oriented perpendicular to and energetically independent of the accompanying H-bond.[34] These observations lead to the hypothesis that biological X-bonds are orthogonal interactions to H-bonds when both share the carbonyl oxygen of a peptide bond as the common acceptor, suggesting that an X-bond can be introduced as an interaction for recognition without disrupting the H-bond stabilized structure of the protein target.

Tunability of X-bonds

The energy associated with any particular X-bond is dependent on several intrinsic properties of the interaction, including the polarizability of the donor halogen, the electron-withdrawing ability of the molecule that the halogen is covalently bound to, and the basicity of the acceptor atom. The most direct effect on not only the energy, but also the directionality of X-bonds is the polarizability of the halogen, which follows the series F <Cl< Br < I, for common halogens. The electrons of fluorine are not so easily redirected to the σ-bond;[17,28] there is not a significant depopulation of the p_z-atomic orbital opposite the σ-bond and, consequently, F shows only a minimum σ-hole.[17,28] A study using NBO analysis on the molecule CF_3X (where X = F, Cl, Br, I) found that 71.4% of the σ-bond electron density was shifted toward the fluorine, whereas Cl, Br, and I tended to split the electrons equally with the carbon.[17] Thus, fluorine is generally considered to be a poor X-bond donor, except in cases where there is a very strong electron-withdrawing ability of the molecule that it is bonded to.[35]

For Cl, Br, and I, the electron-withdrawing ability of the molecule they are bound to helps tune the size of the σ-hole, which then affects the interaction energy [Fig. 3(A,B)] as well as the size of the electropositive crown. A series of QM studies on various fluorinated benzene in complex with acetone demonstrated the degree of "tunability" of X-bonds.[36] We have repeated this type of calculation here for a

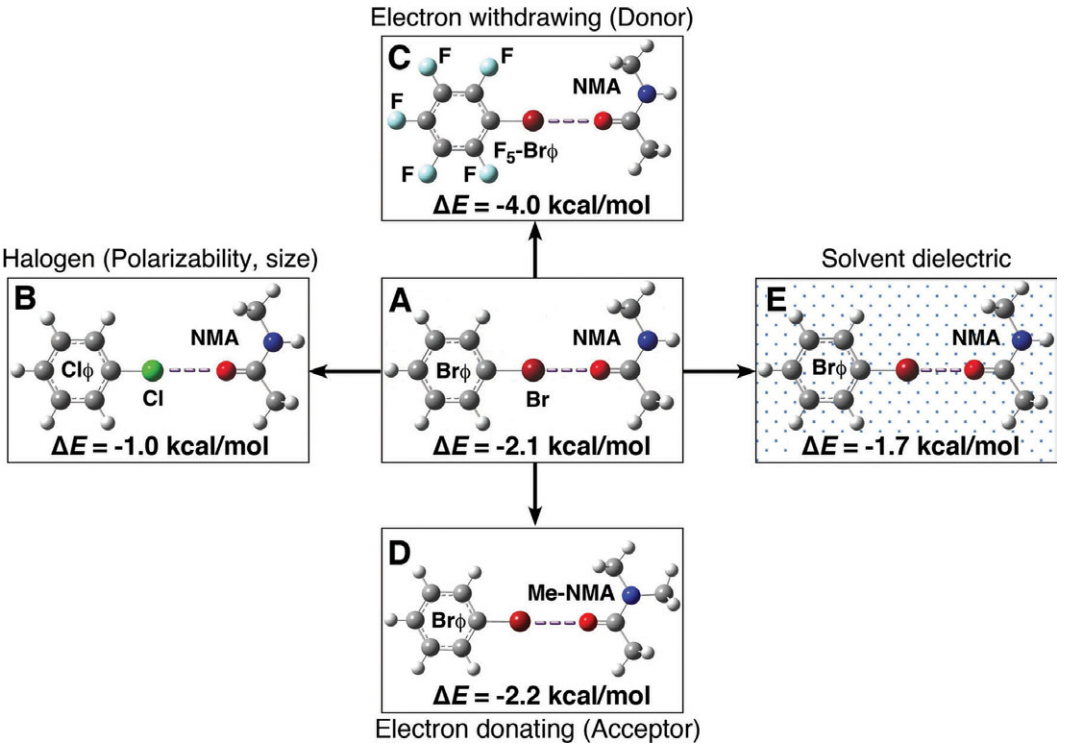

Figure 3. Tunability of halogen bonding energies. MP2 calculations at the 6-31G(d) level compares the effects on the energies ($\triangle E$) for bromobenzene (Brϕ) interacting with the carbonyl oxygen of *N*-methyl acetamide (NMA) in the gas phase (A) as the bromine is replaced by a less polarizable chlorine (B), with electron-withdrawing fluorine substituents added to the Brϕ donor (C), an electron donating methyl added to the NMA acceptor (D), or as it is transferred to solvent (cyclohexane, with a dielectric constant = 2.023, panel E).

bromobenzene interacting with *N*-methylacetamide (NMA), and their derivatives, to demonstrate the various effects on the energies of X-bonding interactions as we would expect to see them in most biological complexes [Fig. 3(C)]. In this case, pentafluorobromobenzene is seen to have over twice the stabilizing potential (−4 kcal/mol) of the nonfluorinated compound (−2 kcal/mol). A uracil base (Fig. 2) was seen to approximate the electron-withdrawing ability of a tetrafluorobenzene, which would account for the very strong X-bond measured for a bromouracil to a phosphate oxygen acceptor in DNA.[37,38]

As a predominantly electrostatic interaction, we would expect the interaction energy of X-bonds to increase with the basicity of the acceptor group, as reflected in their partial charge. For any given Lewis base, however, substituent effects could greatly affect its basicity [Fig. 3(D)], with electron donating groups predicted to increase the negative potential and withdrawing groups to reduce the potential of the acceptor. As discussed previously, π-electrons can serve as X-bonds acceptors and, although they are not as strong as the nonbonding electrons in terms of their basicity, are seen with aromatic amino acids or with the carbonyl oxygen of amides. Thus, the acidity of the donor and basicity of the acceptor combine to define the overall stabilizing potential of X-bonds. Finally, we would expect the polarizability of the solvent to affect the interaction energy, with an increase in the dielectric constant associated with a less favorable interaction [Fig. 3(E)].

Relationship between hydrogen and halogen bonds

The interplay between X- and H-bonds can be very complicated. In addition to forming the σ-hole, polarization creates a negatively charged annulus perpendicular to the σ-bond. Halogens, therefore, serve not only as X-bond donors in the direction of the σ-hole, but also as acceptors to H- or X-bond donors. A survey of interactions with H-bond donors shows a much broader distribution across the Θ_1 angles than with X-bond acceptors,[39] which might be expected, as donors such as hydroxyl groups can interact in an H-bond or X-bond to the hydrogen, depending on their angle of approach. In addition, it has been shown that the X-bond-donating potential of the σ-hole can be extended to become an H-bond donor through a water bridge.[35] On the acceptor side, we have already seen that a carbonyl oxygen of a peptide bond can form an X-bond that is geometric and thermodynamically orthogonal to a pre-established H-bond. The relationship between X- and H-bonds, therefore, appears to be schizophrenic, being competing, complementary, or orthogonal, depending on the situation. Thus, the nature of

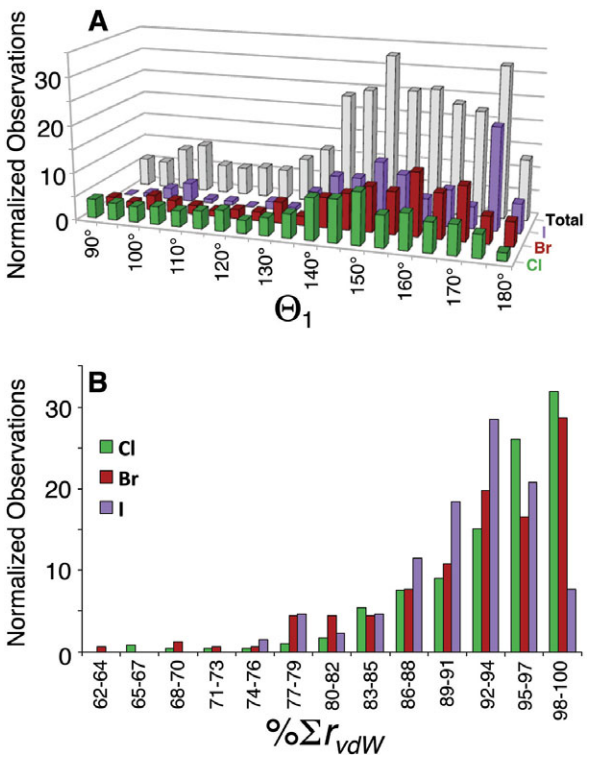

Figure 4. Survey of X-bonds in the PDB. Number of interactions at distances $\leq \Sigma r_{vdW}$ were tabulated for acceptor types that can only form X-bonds, including oxygens, nitrogens, and sulfurs and H-bond donors from $\Theta_1 = 140°–180°$ (up to the neutral point of the electrostatic potential). (A) Number of X-bonds to Cl (477 total), Br (157 total), and I (130 total), normalized for total number of observations for each type of halogen, and the total of these normalized observations. The X-bond distribution is centered at $\Theta_1 \approx 160°$ for all halogen types. (B) Distribution of distances between X-bond donors and acceptors as percentages of the Σr_{vdW} ($\%\Sigma r_{vdW}$).

any particular X-bond is best understood from a detailed analysis for that system.

Where Are Halogen Bonds Seen in Biology?

With the increasing recognition of X-bonding as a significant contributor to specificity, the number of X-bonds observed in a variety of biomolecular systems has increased dramatically. For the most part, X-bonds continue to be recognized in crystal structures only in hindsight; however, there are increasing efforts to design the interaction into complexes to control specificity or the folding in biomolecules.

Survey of biological halogen bonds

The most comprehensive view of the variety of X-bonds seen in biology comes from surveying the PDB. Starting from the very first of such studies, which introduced X-bonds to the biological community,[9] detailed analysis of the crystal structures in the PDB have defined the geometries of and expanded the range of acceptors available for the interaction, and have delineated the complimentary and orthogonal relationships between H- and X-bonds.

The number of biological X-bonds identified in the PDB has increased significantly from 116 in 2004[9] to well over 600 in the current study (Fig. 4), reflecting at some level the growth of structures in the PDB, along with a growing recognition of the interaction. In the current survey, we see acceptor interactions with halogens extending throughout the entire range of Θ_1 from 90° to 180°. As expected, the distributions peak at $\Theta_1 \approx 160°$ for the total of the X-bonds (the three bins from 140° to 150° appear to be unusually high, which we attribute to van der Waals contacts at the point of neutral electrostatic potential as well as contributions from potential H-bonding-type interactions). The X-bonds tend to be ~7% shorter than the $\sum r_{vdW}$ [Fig. 4(B)]. As the types of acceptors become expanded to include all possible H-bond acceptors, including aromatic side chains[31,32] and even anionic halides,[35] the list of biological X-bonds is expected to grow at an even faster rate.

Examples of biological halogen bonds

The variety of X-bonds depends on the variety of halogens seen in biology. There are very few examples of naturally halogenated proteins or nucleic acids, except as an oxidative response associated with, for example, asthma.[35] There is, however, an increasing number of halogenated proteins and nucleic acids used to help phase crystallographic data,[40] but these modifications are not entirely benign—it has been shown, for example, that X-bonds can facilitate formation of a number of multi-stranded DNA complexes,[41,42] including the four-stranded Holliday junction.[6]

X-bonds are seen predominantly in protein complexes with halogenated ligands. This is not surprising, given the prevalence of halogenated compounds found as secondary metabolites,[43] including a number that are antibiotics, and incorporated in screens to identify inhibitors against therapeutic targets.[35] At this point, we will consider in some detail the thyroid hormones as examples of naturally halogenated compounds and a set of halogenated inhibitors as anticancer drugs, to demonstrate how X-bonds can be useful in the design of therapeutics as treatments against human disease.

Thyroid hormones

The iodinated thyroid hormones represent a class of naturally occurring ligands where X-bonding plays a role in recognition.[26,44] Thyroxine and thyroid-like hormones are associated with a number of metabolic diseases such as obesity, hypercholesterolemia, diabetes, and amyloidogenesis.[45,46] The role that X-bonds play in the recognition of thyroid hormones

is evident in the short I···O interactions seen in the structure of tetraiodothyroxine with the transthyretin transport protein.[35] A brominated analog of the thyroid-like hormone (2-arylbenzoxazole) was found to bind at a 15-fold higher affinity to transthyretin than its nonhalogenated analog, and to inhibit the formation of transthyretin aggregates, which might lead to a treatment against transthyretin misfolding diseases.[46] In addition, the structures of the thyroid hormones with their receptors are seen to show interactions with geometries that are indicative of X-bonds (Fig. 5), in both the brominated and iodinated forms.[45] Finally, it has been shown that iodination was a requirement in the recognition of thyroxine by RNA aptamers selected to bind to this hormone.[48]

The catabolism of thyroid hormones results in formation of iodotyrosine, which is then processed by iodotyrosine deiodinase to salvage the halogen. The enzyme's specificity for tyrosine analogs follows the order of polarizability of halogens (the series I>Br>Cl>F), suggesting the involvement of X-bonding in the recognition of the substrate. In a more recent study,[49] X-bonds are thought to slightly elongate the cleavable C—I bond, an important step in the enzymatic mechanism.[50] Thus, X-bonds appear to play crucial roles in the biology of thyroid hormones, from its recognition by receptors, to the salvage of iodine during its catabolism and as necessary for subsequent anabolism.

Inhibitors against cancer targets

Halogenated compounds are important inhibitors against proteins, including those that are involved in carcinogenesis. There have been extensive reviews on the role of X-bonds in the recognition of various inhibitors against several classes of protein kinases.[31,51] Two recent examples include a new iodinated inhibitor designed to target the mitogen-activated protein kinase (MEK) and a chlorinated inhibitor to the CDC2-like kinase isoform 1 (CDK1), reinforcing the significance of X-bonds in conferring specificity of inhibitors against protein kinases (Table I).

The structures of halogenated inhibitors in complex with epidermal growth factor receptor and maltripase[54] show that X-bonding can be generalized to other antitumor targets (Table I). Finally, the five-order of magnitude lower K_i of a brominated compared with nonbrominated inhibitor against the tumor suppressor protein aminopeptidases-N (APN) was suggested to be associated with X-bonds rather than general hydrophobic effects,[55] indicating that this concept is becoming invoked even in the absence of specific structural evidence.

Each of the examples discussed so far have implicated X-bonds, again, in hindsight from the structural geometry of interactions or when comparing the efficacies of halogenated to nonhalogenated compounds against protein targets. Two recent studies show that the X-bonding concept can be incorporated at the design stage to increase the affinity of ligands as potential anticancer drugs. Wilcken *et al.*[57] generated a fragment library that was halogen-enriched with the intent of exploiting X-bonding to screen for high-affinity inhibitors against p53. The results of the studies showed that compounds containing iodine had significantly lower affinities compared with similar compounds containing the other halogens. The geometry of the I···O(pCO) interaction (where O(pCO) refers to the carbonyl oxygen of the peptide bond distance of $0.87\sum r_{vdW}$, Θ_1 angle = 172°) was evidence that an X-bond accounts for the halogen selectivity. Finally, Carpenter *et al.*[58] showed that a halogenated benzimidazole carboxamide inhibitor had a 1000-fold higher affinity against integrin $\alpha_4\beta_1$, a newly identified target to fight T- and B-cell lymphomas, than the nonhalogenated analog. We expect that as more is learned about the energy–structure relationship of X-bonds, the rational incorporation of the interaction at the initial stages of inhibitor and drug design will become more commonplace.

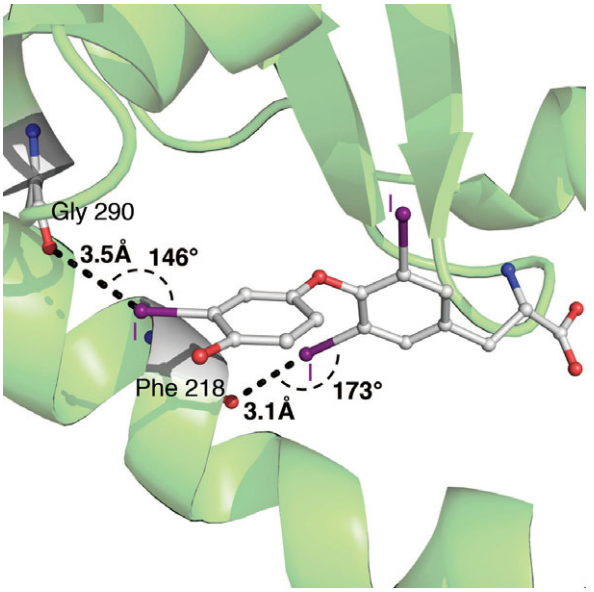

Figure 5. Recognition of 3,5,3′-triiodothyroxine (T3) by human thyroid hormone receptor. X-bonds (dotted lines) are shown from two iodines (purple) of T3 to the carbonyl oxygens (red) of the peptide bonds of the receptor, along with the distances and Θ_1 angles for each interaction (PDB-ID 2H79[47]).

Structure–Energy Relationships

We now have a good understanding for the geometry of X-bonds in various types of protein–ligand interactions, and see some initial successes with intentional design of the interaction into such complexes. To accelerate the incorporation of X-bonds at the initial design stage for engineering new or better inhibitors, however, we need to understand how these

Table I. *Role of X-Bonds in Recognition Specificity of Halogenated Inhibitors Against Anticancer Targets*

Protein target/inhibitor	Geometry		Comments: type of X-bond (PDB-ID), affinity data
	X···O Distance	Θ_1 Angle	
Mitogen-activated protein kinase (MEK)/G-894	$0.94\sum r_{vdW}$	176°	I···O (PDB: 3V04[52])
CDC2-like kinase isoform 1 (CDK1)/KH-CB19	$0.88\sum r_{vdW}$	171°	Cl···O (PDB: 2VAG[53])
Epidermal growth factor receptor (EGFR)/GEFITINB	$1.01\sum r_{vdW}$	163°	Cl···O (PDB: 2ITO[54])
Aminopeptidases N (APN)/1D	NA	NA	$K_i = 60$ p*M* for brominated; 1 μ*M* for nonhalogenated inhibitor[55]
Matriptase/0NW	$0.98\sum r_{vdW}$	158°	Cl···O (PDB: 4E7R[56])

The geometries of interaction of the halogen to the acceptor atom, including the carbonyl oxygen of the peptide backbone (pCO), in terms of the fractional distance relative to the $\sum r_{vdW}$ and Θ_1 angle (if a structure is available).

geometries define the energies of specific X-bonding interactions. The most accurate computational approach to defining the structure–energy of X-bonds is to perform high-level QM calculations on the structures of these complexes; however, these are cumbersome and, in the absence of ultrahigh-resolution crystal structures, are fraught with errors. What we really need is a computational approach that incorporates X-bonding into current molecular mechanics (MM) and their associated docking algorithms, and a set of experimental data to help validate both the QM and MM approaches.

Direct experimental measures of energies of biological X-bonds

There are very few direct ways to measure the energies of X-bonds with specific geometries in biological systems. With small molecules, the interactions between complexes of known geometries can be determined by measuring the melting thermodynamics of the crystalline complex.[59] A similar approach has been applied to directly correlate the energies of X-bonds relative to H-bonds with specific geometries in single crystals of a four-stranded DNA junction.[37,38] In this system, however, the energies were initially estimated through a crystallographic competition assay, in which the stabilizing potential of a bromine X-bond is directly competed against that of an H-bond in 1:1 and 1:2 X- to H-bond ratios (Fig. 6). In this assay, two specific geometries were observed, with a shorter Br···$O^{-1/2}$ interaction being ~5 kcal/mol and the longer interaction being ~2 kcal/mol more stabilizing than the competing H-bond in this system.[37] A study applying differential scanning calorimetry to measure the melting thermodynamics of the interaction in this DNA system showed a similar energy in solution, thereby validating the results from the crystallographic assay.[38]

An X-bond, however, is not always stabilizing. An I···S X-bond to the side chain of a Met designed into a T4-lysozyme/ligand complex was found to have an interaction energy that was not significantly different from what is expected for a simple van der Waals attraction.[60] The contrast to the energies from the DNA studies may be associated with the much weaker basicity of the sulfur in a thiol

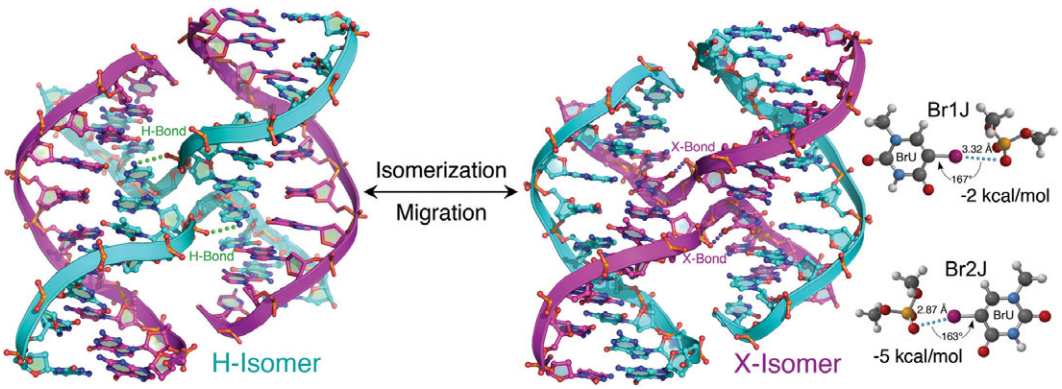

Figure 6. Four-stranded DNA junction as a competitive assay for H-bonding versus X-bonding energies. A four-stranded junction composed of unique DNA strands can isomerize to place either cytosine bases to form stabilizing H-bond (cyan strands) or 5-bromouracil (BrU) bases to form stabilizing X-bonds (magenta strands) to the sharp U-turn of the junction cross-over, adopting the H-isomer and X-isomer forms, respectively. In the X-isomer, the two possible BrU···OPO_3^{-1} interactions have energies that are 2–5 kcal/mol more stabilizing than the competing H-bonds.

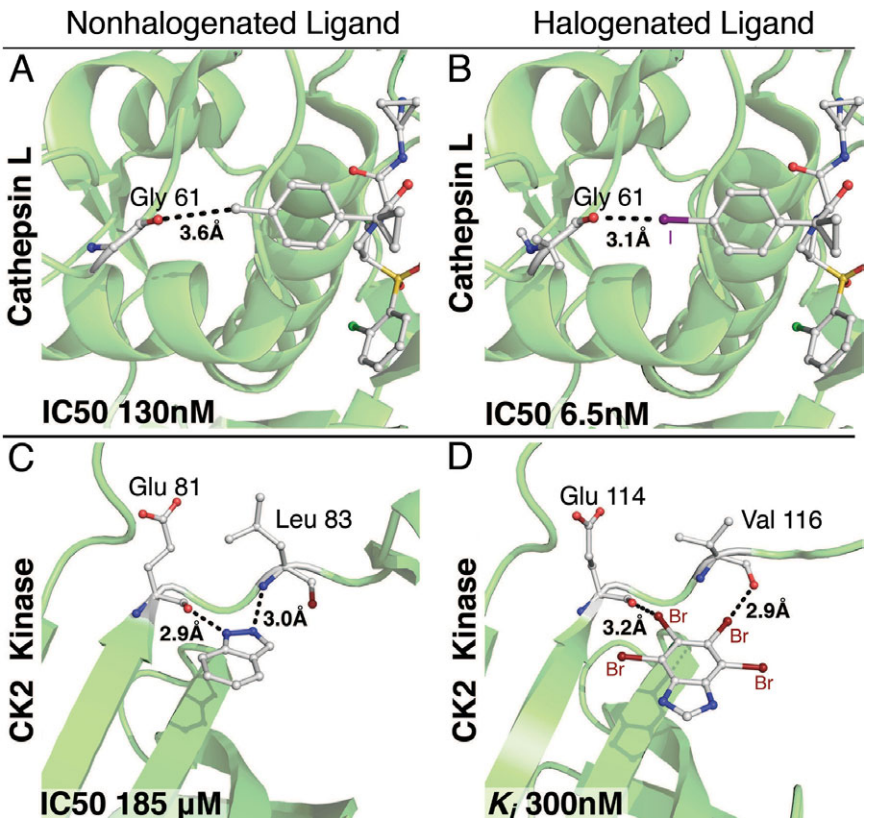

Figure 7. Comparison of halogenated and nonhalogenated inhibitors to protein targets. Cathepsin L in complex with the nonhalogenated ligand (2S,4R)-4-(2-chlorophenyl)sulfonyl-*N*-[1-(iminomethyl)cyclopropyl]-1-[1-(4-methylphenyl)cyclopropyl]carbonyl-pyrrolidine-2-carboxamide (A, PDB-ID 2XU5[61]) and its iodinated analog (B, PDB-ID 2YJ8[62]). Complex of cyclin kinase CK2 with 1H-indazole (C, PDB-ID 2VTA[63]) is compared with casein kinase CK2 bound to tetrabromobenzimidazole (D, 2OXY[64]). The polypeptides backbone are traced as a green ribbon, with amino acids and ligands involved in X-bonding interactions (black dashes) shown as ball stick models with carbons (gray), oxygen (red), nitrogen (blue), iodine (purple), and bromine (brown). The affinities of each ligand are labeled in terms of their IC50 or K_i values.

ether in the protein as opposed to the formally anionic oxygen of the DNA backbone.

Indirect experimental measures of X-bonding energies in protein–ligand complexes

The large number of single crystal structures along with affinity measurements should provide a database of indirect measures of X-bonding energies of ligands in various protein environments. We consider these to be indirect measurements of X-bonding energies, because there is no measure of the energies of the components separately, particularly with the liganded protein in identical conformation as the unliganded form; however, they serve as reasonable estimates.

There are some general trends seen in an analysis of ligand–protein structures and measures of their affinities as reflected in their K_i or IC50 values (Fig. 7), particularly by comparing the affinities of halogenated and the unhalogenated inhibitors toward identical proteins or similar protein domains. The protein cathepsin L is a eukaryotic lysosomal endopeptidase that is associated with antigen processing, tumor invasion and metastasis, bone resorption, and turnover of protein involved in growth regulation. A set of structural studies show that X-bonding was an important contributor to the binding of a series of substituted nitrile inhibitors to a deep pocket in the active site of this enzyme [Fig. 7(A,B)], with a methyl substituent[61] showing a 20-fold increase in the IC50 (equivalent to 1.8 kcal/mol reduction in affinity) relative to the iodinated analog.[62] Similarly, a comparison of casein kinase II (CK2) Ser/Thr kinase structures [Fig. 7(C,D)] shows the imino nitrogens, an indazole inhibitor points toward the loop of the active site.[63] A similar tetrabromobenzimidazole inhibitor is rotated to point its halogens to form two X-bonds to this same loop,[64] which could account for the over 600-fold difference in the IC50 versus K_i for the indazole and tetrabromobenzimidazole, respectively.

Computational approaches to structure–energy relationships of X-bonds

Experimental assays allow us to now develop and validate computational methods at various levels to

Table II. *Experimental and Theoretical X-Bonding Energies of the Br1J and Br2J Conformations (Fig. 6) in the DNA Junction Competitive Assay*

Conformation	$\triangle E_{X\text{-}H(\mathrm{Xtal})}$	$\triangle E_{X\text{-}H(\mathrm{DSC})}$	E_{MP2}	$E_{ff\mathrm{BXB}}$
Br1J	−2.0 ± 0.5	ND	−3.1	−3.2
Br2J	−4.8 ± 0.5	−3.9 ± 1.3	−5.8	−5.5

Experimental X-bond versus H-bond energies as determined crystallographically[37] ($\triangle E_{X\text{-}H(\mathrm{Xtal})}$) or by differential scanning calorimetry[38] ($\triangle E_{X\text{-}H(\mathrm{DSC})}$)are compared with calculated X-bonding energies[22] from quantum mechanical MP2 (E_{MP2}) and by the force field for biological X-bonds ($E_{ff\mathrm{BXB}}$)—all energies are in kcal/mol. The competing H-bond energy is estimated to be ~ -1 kcal/mol.

model specific X-bonding geometries and predict their associated energies. For biologist and medicinal chemists, the goal is to develop algorithms that can be readily applied to the design of new inhibitors against therapeutically important protein targets or new supramolecular complexes from biological systems.

The most accurate means to modeling X-bonds is through QM calculations. For the theoretical chemist, high-level QM calculations have been and are currently used to better understand the foundational principles of X-bonding. The first application of QM showing X-bonding in a biological system (although not recognized as such by the authors) was a DFT calculation[65] based on the 0.66 Å structure of aldose reductase in complex with the brominated IDD594 inhibitor[7]—this ultrahigh-resolution structure provided the accuracy in the atomic coordinates required to minimize errors in the calculation. The crystal structure showed a short 3.0 Å Br···O interaction to the hydroxyl oxygen of the Thr113 side chain, whereas the DFT calculation on the entire complex indicated that this was primarily due to an electrostatic-type interaction. Although the energy of the Br···O interaction was not explicitly estimated in this study, this could account for the 1000-fold specificity of the brominated inhibitor for the aldose reductase over the similar aldehyde reductase.

More direct QM calculations have been performed, however, on models of the Br···$O^{-1/2}$ interaction in the competition assay using DNA junctions.[35] In this case, higher level MP2 calculations were applied to the complex of bromouracil with hypophosphite to model the X-bonding interaction to the phosphate backbone of the DNA.[66] The MP2 calculations yielded energies that accurately mirrored those of the specific geometries seen in the experimental system (Table II), indicating that the QM models were appropriate for this DNA system.

One approach to incorporating the accuracy of QM calculations with MM approaches that are more generally applicable to macromolecules is a hybrid QM/MM method, such as that implemented in the program ONIOM.[67] In this approach, the molecular system is segregated into those parts for which MM calculations can be accurately applied (most of the macromolecule plus the solvent) and those parts which requires a QM calculation (groups immediately around the X-bond donor and acceptor). The application of this QM/MM approach to interactions between halogenated ligands and carbonyl oxygens found in the PDB was able to accurately reproduce the interaction geometry compared with data from crystallographic analysis.[68] The interaction energies, however, were variable depending on the approach implemented for the QM component of the calculation, but they were qualitatively in agreement with general halogen bonding trends.

Perhaps the approach that is most readily accessible to biological and medicinal chemists is the pure MM calculation used to determine both static and dynamic properties of biomolecular systems. There have recently been some significant efforts toward implementing X-bonding into programs such as AMBER[66,69] and OPLS-AA,[70] including the application of a positive extra point (PEP) approach and attempts to derive a set of potential energy force field functions that are specific for X-bonds.

The most straightforward approach to modeling X-bonds in AMBER, which utilizes all of the current functions of the force field, is the PEP. In this method, all of the standard MM parameters are assigned to the center of the halogen being modeled, whereas a pseudoatom having no mass or van der Waals energy, but a defined positive charge, is placed at some distance from the halogen center and diametrically opposed to the σ-bond. The first application by Ibrahim,[71,72] which placed the extra point charge at the halogen surface, showed that the energies calculated by this PEP method correlated well with the affinity of various halogenated benzimidazole inhibitors against CK2 kinase. We note, however, that the distances between the halogen donor and acceptor atoms tend to be on average ∼0.3 Å longer than seen in the crystal structures and the absolute $\triangle G^{\circ}$ for binding are considerably more negative than expected from their dissociation constants.

A recent refinement of the PEP approach from Hobza's group[73] is to place the pseudoatom closer to the halogen center (1.5 Å from the bromine center) and with a compensatory charge of $+0.2e$. This allows a closer approach of the X-bond acceptor to

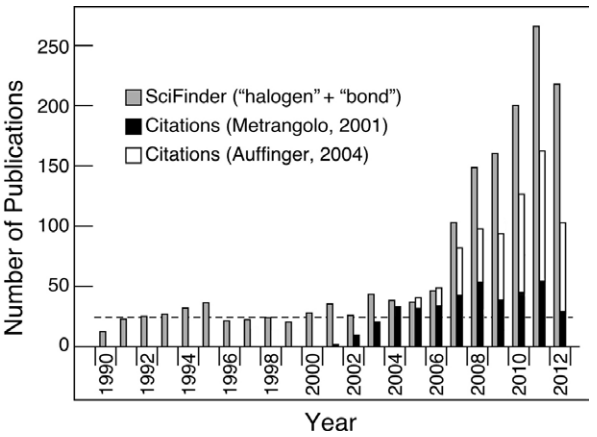

Figure 8. Number of X-bond publications from 1990 to 2012. The number of publications with the words "halogen" and "bond" in the title as found in SciFinder (gray bars) is compared with the number of citations to the publication of Metrangolo *et al.*[11] (as a measure of interest in the material chemistry literature, black bars) and of citations to the publication of Auffinger *et al.*[9] (as a measure of interest in the biological literature, white bars). The horizontal dashed line indicates the average number of publications per year in which "halogen" and "bond" appear in SciFinder, but are not related to X-bonds. The biological literature has accounted for at least half the publications on X-bonds since 2007.

donor, yielding donor–acceptor distances that better mirrored those seen in the crystal structures and energies that are well matched with gas-phase QM calculations.

Our own group took the approach of deriving a halogen-specific set of empirical potential energy functions, based on QM analyses of the DNA junction system, that constitute a force field for biological X-bonds (an *ff*BXB).[22] The *ff*BXB includes a directional model for the aspherical shape of a bromine (consistent with the polar flattening seen in high-resolution crystal structures of complexes of small halogenated compounds) and for the distribution of charges across halogen surface (from positive to negative going from $\Theta_1 = 180°–90°$). This method very accurately reproduces the QM energies for the $BrU\cdots H_2PO_2^-$ model system as well as the experiment energies from the DNA junction competition assay (Table II). When extended to an uncharged oxygen-type acceptor, the *ff*BXB accurately reproduces the QM-calculated energies and geometries for the X-bond interaction between acetone and bromobenzene, and its various fluorinated derivatives. As the *ff*BXB method models the surface potential of the halogen, its energies can be coupled with calculations of the surface area available at each Θ_1 angle to predict a probability for interaction distributed across the angle of approach of the acceptor to the donor halogen relative to the σ-hole. When applied to the acetone–bromobenzene system, these *ff*BXB-calculated probabilities are seen here to predict accurately the distribution of X-bonds to the pCO acceptors found in the PDB. It also shows how H-bonds can interact with the negative annulus that is approximately perpendicular to these X-bonds, thereby, providing a model for the amphimorphic properties of halogens in terms of their electrostatic interactions.

Conclusions and Perspectives

Although short stabilizing interactions involving halogenated complexes have been known for several decades, we have only recently established a good fundamental understanding for why they occur through accurate QM modeling of how electrons distribute between the atomic and molecular orbitals of the covalent bond. What we now call a halogen bond or X-bond has been increasingly recognized as not only being important in hindsight, but also as a potentially powerful tool to engineer specificity in molecular complexes with foresight. Chemists have taken advantage of this concept in designing new halogenated materials with unique properties.[35] Of course, not all halogenated compounds are considered to be beneficial, as evident from the heated debate concerning the potential health risks of polybrominated flame retardants such as decabromodiphenyl ether.[74,75] The perspective for the biological and medicinal chemists, however, is that we can exploit the X-bond to design new or better inhibitors against therapeutic targets, or biologically based materials with definable structural and biochemical properties. The biological applications, however, lag behind those of the small molecule chemist, first, because we were not as quick to recognize the existence of such an interaction and second, because we have not had the computational tools to accurately model the interaction in the large and complex biological molecular systems. However, there has been significant progress in both fronts.

A quick survey of publication and citation databases shows a dramatic rise in the number of halogen bond publications found after 2004 (Fig. 8). This reflects the recognition and acceptance of X-bonds as a distinct molecular interaction that is relevant in biological systems, even if halogens are not commonly found in many naturally occurring proteins or nucleic acids, but are in the ligands that bind to them. The computational tools required to identify and model X-bonds are now being developed and refined. A tool to identify X-bonds in crystal structures of biomolecular systems, based on their geometries, has been incorporated the program HBAT,[76] which will allow structural biologist to better recognize the interaction if it exists.

Both the PEP and *ff*BXB approaches are being incorporated into molecular modeling algorithms to allow us to calculate the energies of the biomolecular

complexes. When fully implemented, these computational tools will allow us to study and better understand the contribution of entropy, both in terms of the conformational entropy within the molecular system and the solvent entropy associated with the hydrophobic effect that together help to define the overall free energy of X-bonds. It is only then that there is the prospect to fully exploit the X-bonding interaction for biomolecular design and engineering using the Group VII atoms.

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