Spectroscopic Studies of Halogen Bonding in Model Systems

From one end of the electromagnetic spectrum to the other

by

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B.Sc., California Institute of Technology, 2011

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Abstract

At its simplest, chemical bonding involves a combination of two dominant contributions: direct electrostatics (ionic) and electron sharing (covalent). The relative importance of these contributors has been the subject of significant study in primary (intramolecular) chemical interactions. For example, the relevance and importance of covalent contributions has been a primary focus of transition metal chemistry for decades. For weaker secondary chemical interactions such as hydrogen bonding (HB) and halogen bonding (XB), the prevailing view in the literature is that electrostatic interactions are so dominant that covalent contributions are negligible. A notable exception is that of so-called symmetric hydrogen bonds, which exhibit large covalent contributions.

With X-ray Absorption Spectroscopy (XAS), we have provided the first direct experimental evidence of covalency in XB. From such studies, we observe that XB exhibit a significantly higher degree of covalency compared with HB counterparts of similar bond strength. Notably, the degree of covalency in certain XB is equivalent to that observed in transition metal halides. Our studies provide information of the electronic changes that occur in both the charge donor and charge acceptor in model systems, affording us a unique experimental view of these weak interactions. We also demonstrate the importance of covalent contributions in XB by showing the effect of covalency in the electron transfer properties in XB-modified dye sensitised solar cells. These results lead us to conclude that XB should more generally be classified as coordinate bonds (and thus identified using an arrow) to distinguish them from significantly less covalent HBs and other weak interactions.
Lay Summary

Understanding weak transient attractions between two different chemicals can play a major role in explaining the behaviours of these chemicals. In this thesis, the weak chemical interaction known as a halogen bond has been studied in depth to resolve an argument within the scientific literature about the nature of this interaction. It has been shown that this bond can play a key role in some systems like solar cells, which use chemical dyes to generate electricity, while in other environments, specifically experiments designed to model Alzheimers Disease, other weak chemical interactions are more important. Redefining the nature of this interaction has helped enable us to explain its importance in these environments.
Preface

Chapter 2

The work in section 2.2 is based on a publication in the Journal of the American Chemical Society: Sean W. Robinson, Chantal L. Mustoe, Nicholas G. White, Asha Brown, Amber L. Thompson, Pierre Kennepohl, and Paul D. Beer. Evidence for halogen bond covalency in acyclic and interlocked halogen-bonding receptor anion recognition. 137(1):499-507, 2015. The published manuscript was written in collaboration with S. Robinson, P. Kennepohl and P. D. Beer. The complexes analysed in sections 2.2 and 2.4 were prepared by Paul Beer’s lab, University of Oxford. The Beer lab verified the structure of these complexes by NMR (not included in this thesis) and measured the binding energies. The XAS data collection and analysis for these complexes was done by the author. The data in section 2.3 was previously published in Faraday Discussions: Chantal L. Mustoe, Mathusan Gunabalasingam, Darren Yu, Brian O. Patrick, and Pierre Kennepohl. Probing covalency in halogen bonds through donor K-edge X-ray absorption spectroscopy: polyhalides as coordination complexes. 203:79-91, 2017. The published manuscript was written in collaboration with P. Kennepohl. The samples analysed in section 2.3 were prepared by undergraduate mentee, D. Yu. The XAS data collection and analysis for these complexes were done by the author. The work in section 2.4 is further analysis of the complexes in section 2.2. The XAS data collection and analysis was done by the author. This work has not been published previously.

Chapter 3

Chapter 4

The sample preparation, data collection, data analysis, and python script writing for Chapter 4 was done by the author. This work has not been published previously. Jake Lever helped visualise the output from the author’s NMR analysis scripts for figure 4.19. While none of her data is presented in this thesis, undergraduate mentee Melanie Backer should also be acknowledged for her many attempts to determine why the amyloid beta peptide initially failed to aggregate.
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<tr>
<td>#—X</td>
<td>see figure 2.2</td>
</tr>
<tr>
<td>←</td>
<td>charge transfer through either electron transitions or covalency</td>
</tr>
<tr>
<td>Aβ</td>
<td>amyloid beta peptide</td>
</tr>
<tr>
<td>bpy</td>
<td>bipyridine</td>
</tr>
<tr>
<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>DSSC</td>
<td>Dye Sensitised Solar Cell(s)</td>
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<tr>
<td>Dye-X</td>
<td>see figure 4.1</td>
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<tr>
<td>EOB</td>
<td>Eosin B</td>
</tr>
<tr>
<td>EOY</td>
<td>Eosin Y</td>
</tr>
<tr>
<td>EPR</td>
<td>Electron Paramagnetic Resonance</td>
</tr>
<tr>
<td>ERB</td>
<td>Erythrosine B</td>
</tr>
<tr>
<td>FLN</td>
<td>Fluorescein</td>
</tr>
<tr>
<td>HB</td>
<td>hydrogen bond(s)(ing)</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<td>NOESY</td>
<td>Nuclear Overhauser Effect Spectroscopy</td>
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<td>PHB</td>
<td>Phloxine B</td>
</tr>
<tr>
<td>ROB</td>
<td>Rose Bengal</td>
</tr>
<tr>
<td>SSE</td>
<td>Sum of Squares Error</td>
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<tr>
<td>SSRL</td>
<td>Stanford Synchrotron Radiation Lightsource</td>
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<tr>
<td>TDDFT</td>
<td>Time Dependent Density Functional Theory</td>
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<td>TFE</td>
<td>TetraFluoroEthanol</td>
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<td>TOCSY</td>
<td>TOTal Correlation Spectroscopy</td>
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<td>UV-Vis</td>
<td>Ultra Violet - Visible</td>
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<tr>
<td>X</td>
<td>halogen</td>
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<tr>
<td>XAS</td>
<td>X-ray Absorption Spectroscopy</td>
</tr>
<tr>
<td>XB</td>
<td>halogen bond(s)(ing)</td>
</tr>
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<td>Z</td>
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Firstly, I would like to thank my PhD supervisors, Pierre Kennepohl and Suzana Straus for allowing me to be a part of their research groups. I am very grateful to both of them for their flexibility, support, and encouragement. I would like to thank Suzana for giving me the opportunity to delve into the details of a field which I’ve always loved, Nuclear Magnetic Resonance (NMR), and I would like to thank Pierre to opening my eyes to the fascinating power of X-ray Absorption Spectroscopy (XAS).

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I am very lucky to have worked with beamline scientists Matthew Lat-
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Finally, I would like to thank my family and my partner Jake for their endless support.
Dedication

This thesis is dedicated in equal parts to my dad, my mum, my sister, and my partner, Jake:

- To my dad for teaching me that forces cannot be seen, and inspiring me with “discrete elephants”, engineering demos, and coffee-shop calculus.

- To my mum for inspiring me with her love of numbers, puzzles, and poetry.

- To my sister, Elisha, for inspiring me with her humour, love, and creativity.

- And to Jake for the laughs, the love, and sharing and encouraging my passion for science.
Chapter 1

Introduction

Many different types of chemical bonds exist from the very strong to the very weak, and from exclusively electrostatic to predominantly covalent. While many factors can affect the properties of a chemical bond including effective nuclear charge, competing interactions, and orbital sizes, there are only two phenomena which cause the distance between two atoms to be less than the sum of their van der Waals radii: electrostatics and orbital overlap. Another school of thought combines these two effects when understanding bonding properties. This competing theory represents atoms as point charges to explain electron distribution within a bond, thus avoiding the concept of electron ownership and covalency [1, 2]. While this simplification is attractive, it fails to explain electron transition allowedness and probabilities. Therefore, this thesis will focus on discussing chemical bond properties using both electrostatics and covalency.

While both electrostatics and covalency can influence chemical bonds, some bonds are exclusively electrostatic. A chemical bond which results only from electrostatic attractions involves atoms whose electronic structures are independent from one another. Some examples of these include ionic bonds and van der Waals attractions, notably the strongest and weakest chemical bonds known. Bonds in which the electronic structures of the atoms are interdependent have some degree of covalency (also referred to as electron sharing, charge donation and orbital overlap). Unlike exclusively electrostatic bonds, bonds which exhibit at least partial covalency have bonding and antibonding orbitals with wavefunctions that include contributions from both bonding atoms (equation 1.1).

\[ \Psi = \alpha |\psi_A\rangle - \sqrt{1 - \alpha^2} |\psi_B\rangle \]  

(1.1)

Where:

- \( \Psi \) is the wavefunction for the bonding orbital
- \( \psi_A \) and \( \psi_B \) are the wavefunctions for the orbitals involved in bonding
- \( \alpha \) is the degree of charge donation from atom A to atom B
Examples of these bonds include traditional covalent bonds and coordinate bonds [3-5]. As defined by the International Union of Pure and Applied Chemistry (IUPAC), a coordinate or dative bond is a bond involving two atoms, one atom which donates an electron pair to the bond and another atom which acts as an acceptor. In other words, unlike a covalent bond which preferentially results in homolytic cleavage of the electron pair upon bond dissolution, breaking a coordinate bond will result in heterolytic cleavage of the electron pair [6].

Figure 1.1: Bond strength variability for common primary and secondary chemical bonds [7]. Reprinted with permission from author.

Figure 1.1 includes both primary bonds and secondary bonds, i.e. bonds which are often referred to as intermolecular forces. This thesis uses the term ‘bond’ to apply to any force holding to atoms together to emphasise that these interactions exist on a continuum. In bonds, electrostatics and covalency determine the bond strength and the role of a bond in a system. The dependency of bond strength on electrostatics as well as covalency means that while bond strength varies drastically for different types of bonds (figure 1.1), it is not directly dependent on the relative distribution of the atoms’
1.1 Halogen Bonding

electrons. For example, covalent bonds have the highest degree of covalency followed by coordinate bonds and finally ionic bonds, a trend which is not mirrored in bond strength.

This difference in covalency causes the two strongest types of bonds, ionic and covalent, to exhibit very different chemical properties in polar solvents. The exclusively electrostatic ionic bonds are easily dissolved in a polar solvent by competing electrostatic interactions. On the other hand, covalent interactions cannot be broken by using the competing electrostatic interactions arising from dissolving the compound in a solvent. Here, although overall bond strength of a covalent bond is similar and often weaker than an ionic bond, the overlap of electron wavefunctions in the covalent bond ensures that this bond remains intact while an ionic bond is often broken.

Although secondary bonds are considerably weaker than primary bonds (figure 1.1), these bonds can also play an important role in the behaviour of a system. While figure 1.1 illustrates the most well known types of secondary chemical bonds, there are many other types of weak chemical bonds. Other secondary bonds not represented in figure 1.1 include tetrel, pnictogen, chalcogen, and halogen bonds which involve elements in groups 14-17 on the periodic table, respectively. These four types of bonds are generally similar to hydrogen bonds in that they involve covalently-bonded electron-deficient atoms (specifically a covalently-bonded tetral, pnictogen, chalcogen or halogen atom) interacting with an electron-rich species [8]. The involvement of a tetral, pnictogen, chalcogen or halogen atom in a covalent bond results in an area of electron depletion opposite the bond (this area is referred to as a sigma hole and will be discussed further in the next section). These interactions form when an electron-rich species interacts with the sigma hole [8]. Like hydrogen bonds, these interactions are commonly referred to as non-covalent interactions despite an ongoing debate over the relative contributions of electrostatics and covalency[1, 9–12].

This thesis will focus on identifying and characterising halogen bonds in different systems, specifically in terms of electrostatics and covalency. The importance of these bonds in each system will also be discussed.

1.1 Halogen Bonding

Halogen bonds (XB) are interactions between an electron donor and a covalently-bonded halogen acting as an electron acceptor [13, 14]. The applications of XB include but are not limited to ion recognition [15–17], crystal engineering [18, 20], catalysis [21, 22], and molecular self-assembly [23].
In understanding the nature of a bond, it is useful to compare to other like interactions. Theoretically, the most straightforward way to compare two bonds would be to determine the importance of electrostatic and covalent contributions in each bond. However, as different contributions to bond strength are not experimental observables, it is very difficult to determine the relative importance of electrostatics and covalency in bond strength. In fact, even computationally, we cannot separate electrostatic and covalent contributions as different computational models yield different results. Two experimental observables that are useful in comparing bonds are (1) percent covalency in terms of maximum possible orbital overlap and (2) overall bond energy or bond strength. These properties can be used to determine the degree of importance of electrostatics and covalency in bonding properties by comparing bonds which have similar values for (1) but different values for (2) or vice versa.

While there are many different types of bonds with which XB can be compared, a comparison of XB with HB and coordinate bonds will prove most useful in this thesis. The comparison with the HB is interesting not only because this is a common comparison in the literature [16, 23–26], but also because these two bonds have analogous structures and are comparable in strength [27].

To the author's knowledge, the comparison between XB and coordinate bonds is not present in the literature outside of the work presented in this thesis. This comparison is nonetheless useful because while these two bonds are not comparable in strength, they exhibit similar degrees of charge donation (as will be shown in chapters 2 and 3).

While tetrel, pnictogen, and chalcogen bonds are also structurally similar to XB, this comparison is not as illuminating simply due to the fact that, especially in comparison to HB and coordinate bonds, the properties of these bonds are not as well understood. Indeed, most research on these bonds is crystallographic or computational [12, 28]. Other more delocalised secondary bonds such as dipole-dipole and van der Waals interactions are structurally dissimilar to XB and a comparison with these bonds is less informative as a result.

Two conventions regarding the depiction and discussion of XB in the literature are worth discussing. First, both XB and HB are often discussed in terms of a bond donor, referring to the covalently-bonded halogen or hydrogen respectively, and a bond acceptor, the electron-rich species involved in the bond. As this thesis is mainly concerned with the distribution of electrons throughout these bonds, the terms donor and acceptor will be used to refer to the electron donor and electron acceptor (halogen or hydrogen...
1.1. Halogen Bonding

**Hydrogen Bond**

![Diagram of a Hydrogen Bond](image)

R = N, O, F  \[ D = N, O, S, Cl, Br, I, etc. \]

**Halogen Bond**

![Diagram of a Halogen Bond](image)

R = C, N, halogen, etc.  \[ D = N, O, S, Se, Cl, Br, I, Cl^-, Br^-, I^- \]

X = Cl, Br, I

Figure 1.2: Schematic of a HB and a XB. D has been used to represent the electron donor in each bond. As this thesis is concerned with the distribution of electrons within these bonds, the terms electron donor and electron acceptor will be used rather than bond donor and bond acceptor, as is more common in the literature.

As mentioned previously, XB are analogous to hydrogen bonds (HB), in that they are also weak interactions involving an electron-rich and an electron poor species (figure 1.2). However, a notable difference between XB and HB is the range of possible bond angles. Unlike HB which can have highly variable bond angles [29], XB usually exhibit bond angles of 170 – 180° [13, 14]. This discrepancy in geometries can be explained by
1.1. Halogen Bonding

the presence of a region of electron deficiency present in covalently-bonded halogens referred to as the σ-hole [13, 14] (figure 1.3). Unlike the positive region on the H in CH$_3$Br (seen in figure 1.3 blue) which is surrounded by an area of slowly decreasing positive charge, the σ-hole on the covalently-bonded halogen is surrounded by a donut shaped ring of negative charge (red). While the surrounding donut of negative charge is expected as halogens are more electronegative than carbon, the σ-hole can also be explained as it reflects the location of both the filled σ$_{\text{C-Br}}$ orbital (electron density between C and Br) and the unoccupied σ$_{\text{C-Br}}^*$ orbital. The location of this electron deficient σ-hole surrounded by an area of negative corresponds with a preferred bond angle of 180°.

Figure 1.3: Electrostatic potential map of bromomethane showing an area of relative positive charge on the covalently-bonded bromine known as the σ-hole. Electrostatic potentials were generated with Gaussian04 using the B3LYP functional and Basic(3-21G) basis set and then visualised with WebMO[30]. Two different views included for clarity. Note: while bright blue and bright red do represent -0.035V and 0.040V, respectively, the change in electrostatic potential depicted by change in hue as depicted by the scale is approximate for all electrostatic potential maps visualised with WebMO [30] in this thesis.

Due to the size and polarisability of the different halogens, the size of σ-hole of a covalently-bonded halogen increases with the atomic radius (figure 1.4). As covalently-bonded fluorine lacks a discernable σ-hole, it is rarely able to act as the electron acceptor in an XB. On the other hand, covalently-bonded iodine with the largest and most positive σ-hole is capable of forming the strongest XB. [14].

The above explanation of XB focuses on the electrostatic nature of the XB, and until recently such interactions were generally considered to be
1.1. Halogen Bonding

Figure 1.4: Electrostatic potential maps of halomethanes showing the increase in size of the $\sigma$-hole as the atomic radius, and thus polarisability, of the halogen increases. Images generated with WebMO\cite{30} molecular orbital calculations of fluoro-, chloro-, bromo- and iodomethane.

predominantly electrostatic \cite{13, 20, 25}. Indeed, the current widespread view in the literature is that the bonds are an electrostatic interaction in which an electron-rich species is attracted to the positively charged $\sigma$-hole formed in a covalently-bonded halogen \cite{13, 14, 31}. However, a competing and less widespread view is that these bonds are partially covalent due to an overlap between the $\sigma^*$ orbital of the covalently-bonded halogen and an orbital of the same symmetry on the electron donor. In figure 1.5b, the electron donor is a halide and thus the $\sigma^*$ orbital is overlapping with the X^- LUMO $p_z$ orbital. This partially covalent model of the XB could further explain the discrepancy between the geometries of HB and XB as the stability gained by orbital overlap between $\sigma^*_R-X$ and $p_z$ (for example) orbitals is lost as the bond angle decreases. It is important to note that this explanation assumes that XB are more covalent in nature than HB; this assumption will be explored further in chapter 2. Indeed, this thesis aims to address the validity of using the partially covalent model to describe XB.

Despite the appearance of XB in the literature several decades ago, the ability to identify and characterise these bonds is mostly, though not entirely, limited to X-ray crystallography \cite{12, 32}. X-ray crystallography can be used to identify when the distance between a halogen and its neighbour is less than the sum of the atomic radii, and if this is true for two or more atoms near a particular halogen, the halogen is involved in one or more XB. The use of NMR to indirectly detect XB has also been investigated \cite{32}. This thesis aims to establish a new method to detect XB in non-crystalline systems,
1.2. X-ray Absorption Spectroscopy

X-ray Absorption Spectroscopy (XAS) is an element-specific technique which enables us to probe the nature of low-lying unoccupied (or partially occupied) orbitals using high energy X-ray radiation from a synchrotron to excite core electrons. In XAS, specific wavelengths of X-ray radiation produced by the acceleration of electrons are selected using a double-crystal monochromator. The resulting beam of X-ray radiation passes through the sample to (an) ion chamber detector(s). The relative angles of the two crystals in the monochromator can be changed in order to scan over a range of energies. The use of the synchrotron as a lightsource yields a high flux of photons at a wide range of energies. For example, XAS experiments in this thesis were conducted at various energies ranges within 2 keV to 15 keV.

This ability to select energies of high energy photons enables the excitation of core electrons. XAS experiments are conducted at the ionisation
1.2. X-ray Absorption Spectroscopy

energy of a particular core electron of a particular element. For example, to probe the transitions available to a Br$_{1s}$ electron, the researcher starts the experiment approximately 100 eV below the ionisation energy of Br$_{1s}$ electron and slowly increases the energy of the incident photon beam until at least 100 eV above the ionisation energy. The detection of one of four of the following (1) transmission of incident beam, (2) total electron yield from sample, (3) partial X-ray fluorescence yield, or (4) total X-ray fluorescence yield results in a spectrum similar to that seen in figure 1.7. In an XAS spectrum, a sudden increase in signal intensity reflects the ionisation of electrons from an orbital of interest (figure 1.6). The inflection point of this feature is referred to as the edge (figure 1.7).

It is important to note that in XAS peak intensity refers to the area under the peak rather than the height of the peak by convention. This terminology is used as peak area rather than peak height is directly proportional to the probability of the associated transition. When peak width and shape remain constant, peak height is also proportional to associated transitions but this is not the case in XAS. In XAS, peak shapes can be Gaussian distributions (arises from experimental noise), Lawrencian distributions (arises from Heisenberg’s uncertainty principle) or Voight functions, a combination of the two. As peak shape changes based on the different contributions of experimental noise, line-broadening from Heisenberg’s uncertainty principle, and physical phenomena contributing to the peak (photon/electron emission and/or scattering), it is convention in XAS to use peak intensity to refer to the ‘area under the peak’ rather than the ‘height of the peak’.
1.2. X-ray Absorption Spectroscopy

Figure 1.6: Mass extinction coefficients for Si, Cl, and Fe. The almost stepwise increase in intensities correspond to the ionisation of the following electrons: Si\textsubscript{2s} at 150 eV, Si\textsubscript{1s} at 1839 eV, Cl\textsubscript{2s} at 270 eV, Cl\textsubscript{1s} at 2822 eV, Fe\textsubscript{3s} at 91 eV, Fe\textsubscript{2s} at 845 eV and Fe\textsubscript{3s} at 7122 eV. Figure reprinted with permission from the director of the Centre for X-ray Optics [33].
1.2. X-ray Absorption Spectroscopy

Figure 1.7: Br K-edge XAS spectrum of NaCl. Edge, pre-edge region and Br\textsubscript{5p}$\rightarrow$1s peak have been labelled for clarity. Data was collected at beamline 7-3 of the Stanford Synchrotron Radiation Lightsource (SSRL).

The use of the term edge feature can be somewhat ambiguous as it can refer to either the sudden increase in signal intensity or to the first peak at or after the energy of ionisation. For clarity, in this thesis the term edge will refer to the sudden increase in signal intensity, and the term edge peak will refer to the peak immediately following this rise. For the spectrum in figure 1.7, the energy of the edge is 13474 eV, and the edge peak predominantly corresponds to the peak identified as the Br\textsubscript{5p}$\rightarrow$1s transition. All peaks to the left of the edge peak in an XAS spectrum are referred to as pre-edge features or pre-edge peaks. Other physical phenomena can also contribute to the edge feature including other high energy transitions, here Br\textsubscript{6p}$\rightarrow$1s for example, and scattering. Thus, this feature can be very broad (approximately 10 eV) and is not used to quantify the probability of associated transitions.

In XAS, the nomenclature K-edge, L-edge, and M-edge are used to refer to the excitation of electrons from the n = 1, n = 2 and n = 3 shells, respectively. For edges with more than one possible angular momentum, a numerical subscript is used to clarify the resulting excited state. Table 1.1 includes all edges discussed in this thesis.

The two main criteria which dictate the intensity of features correspond-
1.2. X-ray Absorption Spectroscopy

<table>
<thead>
<tr>
<th>Edge</th>
<th>Orbital</th>
<th>Resulting Excited State</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-edge</td>
<td>1s</td>
<td>( ^2S_{1/2} )</td>
</tr>
<tr>
<td>L(_1)-edge</td>
<td>2s</td>
<td>( ^2S_{1/2} )</td>
</tr>
<tr>
<td>L(_2)-edge</td>
<td>2p</td>
<td>( ^2P_{1/2} )</td>
</tr>
<tr>
<td>L(_3)-edge</td>
<td>2p</td>
<td>( ^2P_{3/2} )</td>
</tr>
</tbody>
</table>

Table 1.1: Orbitals from which an electron is excited and resulting excited states for relevant XAS edges. Atomic state configuration given as \( ^{2S+1}L_J \) where \( S \) denotes spin, \( L \) denotes angular momentum and \( J = L – S, L – S + 1, ... L + S \)

The first criteria tells us that the acceptor orbital must be at least partially located on the same atom as the initial orbital. The second criteria arises from the fact that the change in the angular momentum of the electron must equal that of the incident photon (\( \Delta l = \pm 1 \) where \( l \) is the quantum number for orbital angular momentum). For example, the intensity of an electron transition from an s orbital to an acceptor orbital will directly correlate to the \( \%p \) character of the acceptor orbital.

One of the strengths of XAS is that due to the large separation of the edges of core electrons in different elements and different edges within the same element, XAS is an element-specific technique. For the spectrum in figure 1.7, all signal intensity is a direct result of excitation of only Br 1s electrons. The next closest ionisation edges are the Se K-edge (12658 eV) and the Kr K-edge (14326 eV). Thus, any contamination in the sample would be clearly visible as a second edge jump >700 eV before/after the Br K-edge edge jump. The element specificity of XAS enables experiments which probe the properties of a single element within a sample, albeit the signal is an average of all atoms of that particular element within a sample. This particular strength is what has enabled the use of XAS to probe XB in this thesis.

Although XAS has not been used to probe the nature of XB prior to the work done in this thesis, XAS is a well established method to probe the covalency of ligand-metal donor-acceptor interactions [4]. The first use of XAS to probe the covalency of donor-acceptor systems was Solomon and co-workers’ use of Cl K-edge XAS to evaluate the degree of delocalization in chloro-metal bonds [3, 4, 34]. In such cases, a pre-edge feature is observed in the spectrum that formally corresponds to excitation of a Cl 1s electron.
1.2. X-ray Absorption Spectroscopy

Figure 1.8: (Left) Cl K-edge XAS of CuCl$_4^{2-}$ and ZnCl$_4^{2-}$ complexes. (Right) A molecular orbital diagram depicts the transition corresponding to the pre-edge feature observed in the XAS spectrum of the CuCl$_4^{2-}$ complex. Figure adapted with permission from [3] © 2000 American Chemical Society.

There are various other experimental methods which can be used to evaluate covalency of coordinate bonds including analysis of the g values and coupling in electron paramagnetic resonance spectroscopy (EPR) and ligand to metal charge transfer transitions in UV-Vis spectroscopy, and XAS [3, 35]. Each of these techniques has associated limitations. EPR can only be used for a sample with at least 1 unpaired electron. In UV-Vis, the existence of ligand and metal character in the orbital of excitation requires that the probability of the transition depends on both the ligand and metal character.

Solomon and co-workers also pioneered the use of XAS to quantify the degree of covalency in coordinate bonds. Evaluation of coordinate bond covalency in XAS is possible as the intensity of the pre-edge feature in the ligand K-edge XAS is directly proportional to the amount of np ligand
1.2. X-ray Absorption Spectroscopy

Figure 1.9: (Left) Cl K-edge XAS pre-edge features of chlorometal bonds in different [MCl$_4$]$^{n-}$ complexes (Right) Calculated Cl$_{3p}$ character for one M–Cl coordinate bond for each complex. Figure adapted with permission from [3] © 2000 American Chemical Society.

character in the acceptor orbital where np denotes the frontier p orbital ligand. We can show this relationship using the following equations.

The intensity of the pre-edge feature corresponding to the $\Psi^* \leftarrow \text{Cl}_{1s}$ transition is defined as follows:

$$I_{\Psi^* \leftarrow \text{Cl}_{1s}} = k |\langle \Psi^* | \vec{r} | \text{Cl}_{1s} \rangle|^2$$  \hspace{1cm} (1.2)

where $\Psi^*$ is the antibonding orbital of a metal-coordinate bond, I is the intensity of the peak corresponding to this transition, k is a constant, and $\vec{r}$ is the electric dipole operator.

We can also rewrite equation 1.1 for the wavefunction of metal-chloride coordinate antibonding orbital:

$$\Psi^* = \sqrt{1 - \alpha^2} |\text{M}_{\text{nd}}\rangle - \alpha |\text{Cl}_{3p}\rangle$$ \hspace{1cm} (1.3)

where nd are the valence d orbitals on the metal (M).

As $\Psi^* \leftarrow \text{Cl}_{1s}$ transition only occurs due to spatial overlap between the Cl$_{3p}$ and Cl$_{1s}$ orbitals, we can rewrite the intensity of this transition as follows:

$$I_{\Psi^* \leftarrow \text{Cl}_{1s}} = \alpha^2 k |\langle \text{Cl}_{3p} | \vec{r} | \text{Cl}_{1s} \rangle|^2 = \alpha^2 (I_{\text{Cl}_{3p} \leftarrow \text{Cl}_{1s}})$$  \hspace{1cm} (1.4)

We can see in equation 1.4 that the intensity of the pre-edge feature is directly proportionate to the amount of charge transfer ($\alpha^2$) from the Cl$_{3p}$
orbital, as the donor in this example, to the electron acceptor (in this case a metal d orbital). Solomon and coworkers took advantage of the fact that the $\text{ICl}_3p \leftarrow \text{Cl}_1s$ term is not affected by different electron acceptors. Thus, the ratio of metal-chloride charge transfer to the intensity of the XAS pre-edge feature corresponding to the $\Psi^* \leftarrow \text{Cl}_1s$ transition will be the same for all metal-chloride bonds, and indeed for any bond in which a chloride acts as electron donor.

Providing a relevant calibration standard is available, comparing the intensity of the pre-edge feature of interest to the pre-edge feature in the calibration standard is a convenient method of obtaining a quantitative measure of the degree of ligand and metal character in the acceptor orbital [3–5]. Using EPR, XAS and density functional theory (DFT) calculations, Solomon and coworkers established calibration standards for evaluating covalency in metal-chloride and metal-sulfide bonds (figure 1.9 for chlorometal bond covalencies). As the intensity of the pre-edge feature is directly proportional to the percent $\text{Cl}_3p$ in the acceptor orbital of the $\text{MCl}_2^-$ complex, these complexes can be used as calibration standards for systems in which a comparison to EPR is not possible. Specifically, with its 1 electron hole and well-defined pre-edge feature (no overlap with edge), $\text{CuCl}_2^-$ will be used as a reference for this thesis.

1.3 Thesis Aims

XAS will be used to probe for XB in three different applications in this thesis: ion trapping complexes, dye-sensitized solar cells (DSSC) and Alzheimer-associated amyloid $\beta$ peptide aggregation (chapters 2, 3, and 4 respectively). Despite the dissimilitude between these three systems, they are all united by the importance of secondary chemical bonds. Both halogen bonds and hydrogen bonds can be used to bind ions in ion recognition complexes [27]. DSSCs require an interaction between the light sensitive dyes and a redox active electrolyte species to regenerate the excited dyes [36]. The amyloid $\beta$ peptide also aggregates via hydrogen bond formation, amongst other interactions, and this aggregation can be modulated by halogenated fluorescein derivatives [37]. XAS will be used to elucidate the role, if any, of XB in these 3 different systems. Other model systems with less apparent applications will also be employed to characterise these bonds.

The aim of thesis is three fold:
1.3. Thesis Aims

1. To establish the use of XAS to probe the properties of halogen bonds in different systems: In chapter 2, XAS will be employed to study XB in systems through XAS of both the XB electron donor and the XB electron acceptor. To the author’s knowledge, XAS has not been previously used to study XB. The benefits and drawbacks of both approaches will be discussed. Some of this work has been previously published by the author [27].

2. To provide evidence that halogen bonds are partially covalent in nature using XAS: In chapter 2, an XAS method analogous to that used to probe coordinate bond covalency will be used to both provide evidence for and directly quantify the degree of charge transfer in XB. This work has been previously published by the author [27, 38].

3. To probe systems in which halogen bonds may play an important role: In chapters 3 and 4, the methodology established in chapter 2 will be applied to systems in which XB have not previously been identified. It is known that halogens play a key role in both DSSC [39] and in the modulation of amyloid β peptide aggregation by halogenated fluorescein derivatives [37]. In DSSC, it has been shown that both the presence and identity of halogen substituents can affect DSSC efficiency [39]. The halogenation of fluorescein derivatives has been shown to modulate amyloid β aggregation [37]. The work done in chapters 3 and 4 of this thesis will establish whether XB play a role in the importance of halogen substituents in these two systems. Some of this work has been previously published by the author [36].
1.3. Thesis Aims

When looking at chemical bonds,

*We care if they’re short or they’re long.*

*Bond strength is key*

*In this inquiry*

*But so are the shared electrons.*
Chapter 2

Evidence of Covalency in Halogen Bonds

As presented in chapter 1, the nature of the XB is debated within the literature with the purely electrostatic representation being the predominantly accepted theory. In the current chapter, direct evidence will show that XB in model compounds synthesised by the Beer group at Oxford have partial covalency (figure 2.2). This is the first direct evidence of XB covalency in the literature.

XAS will be used to assess the degree of covalency of both XB and HB in these systems. To establish the nature of the interactions between the halide (Cl\(^{-}\) or Br\(^{-}\)) donor and the electron acceptor, we have employed Cl and Br K-edge XAS. As mentioned previously, Solomon and co-workers established Cl K-edge XAS as a technique for probing the covalency of metal-chloride coordinate bonds via the Cl\(^{-}\) ligands \([3, 4]\). However, using XAS to probe the covalency of bonds between two non-metals has not been done previously. This method will be used to assess the covalency of both XB and HB present in Beer and co-workers’ model systems, thus enabling a comparison of the properties of these two weak interactions. As XB do not necessarily have halide electron donors, I L\(_{1}\) and L\(_{3}\)-edge XAS will also be used to probe the XB via the XB electron acceptor. If successful, this method could be generalisable to all XB.

2.1 Calibration: XAS of Halide Salts

XAS requires the use of calibration standards for calibration of the energy of electron excitation. As XAS is an element-specific technique, it is usually necessary to calibrate using a standard containing the same element. A generally accepted calibration standard for metal XAS is the metal itself in

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*Section 2.2 is based on the author’s contributions in previously published work \([27]\). The data in section 2.3 was also previously published by the author \([38]\). Unless noted, all work was done by the author. See Preface for more details.*
2.1. Calibration: XAS of Halide Salts

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_0$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>2825.75</td>
</tr>
<tr>
<td>NaCl</td>
<td>2825.95</td>
</tr>
<tr>
<td>KCl</td>
<td>2825.35</td>
</tr>
<tr>
<td>RbCl</td>
<td>2825.15</td>
</tr>
<tr>
<td>CsCl</td>
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<tr>
<td>CaCl$_2$</td>
<td>2825.65</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>2825.25</td>
</tr>
<tr>
<td>NBu$_4$CuCl$_4$</td>
<td>2824.70</td>
</tr>
</tbody>
</table>

Table 2.1: Cl K-edge XAS $E_0$ values for Chloride Salts

elemental form. As this is not possible for XAS at halide edges, different halide salts have been used for calibration.

Although CuCl$_2^-$ salts are the generally used for Cl K-edge XAS due to their distinct pre-edge feature at 2820.2 eV (figure 2.1(b)) [3, 4], other Cl$^-$ containing compounds can be used. A variety of Cl$^-$ salts have been analysed and referenced to CuCl$_2^-$. The first inflection point in the XAS spectrum has been reported for all salts analysed (table 2.1). As both KCl and NaCl were used as calibration standards in this thesis, these experiments were needed to ensure calibration consistency. Furthermore, as it is reasonable to use any of these salts as calibration standards, publishing a consolidated table of these values was deemed useful for future researchers.

As expected, XAS of halide salts shows that there is no pre-edge feature in Cl K-edge XAS of Cl$^-$ (figures 2.1(a) and 2.1(b)). The only salt which exhibits a pre-edge feature is tetrabutyl copper chloride. In CuCl$_4^-$, chloride is involved in a partially covalent coordinate bond with copper. The covalency of this bond results in the new allowed transition ($\sigma^*_{\text{Cu} \leftarrow \text{Cl}} \leftarrow \text{Cl}_{1s}$), and this transition corresponds to the pre-edge feature visible at 2820.2 eV.

XAS experiments were also performed at the Br K-edge and the I $L_3$-edge. Similar studies for different iodide and bromide salts were not done due to time constraints at the synchrotron. KBr was used as a standard for Br K-edge XAS (figure 1.7) and KI was used a calibration standard for I $L_3$-edge. The energy values used to calibrate the spectra of these compounds are the ionisation energies of the Br$_{1s}$ ($E_0 = 13474$ eV) and I$_{2p}$ ($E_0 = 4557$ eV) electrons as reported in the X-ray Data Booklet [33]. The true ionisation energies for the reference compounds KBr and KI are unlikely to be exactly 13474.0 eV and 4557.0 eV as ionisation energies for two electrons in the same element at the same energy level in different compounds are
2.2 Using XAS to probe for XB: XAS of a halide electron donor

Figure 2.1: Cl K-edge XAS data for a variety of chloride salts including (a) the alkali metals and (b) alkaline earth metals and tetrabutyl copper chloride.

not the same (table 2.1). However, as all spectra are calibrated using the same method (unless otherwise noted), any energy discrepancy between the ionisation energies reported in the X-ray Data Booklet for the Br$_{1s}$ and I$_{2p}$ electrons and the true ionisation energies for KBr and KI will not affect conclusions drawn from comparing multiple XAS spectra collected at the same edge.

2.2 Using XAS to probe for XB: XAS of a halide electron donor

The systems described in this chapter (figure 2.2) were synthesised by the Beer Group at Oxford to investigate the use of XB and HB to trap halides. The Beer group synthesised these model compounds to demonstrate the applications of XB in controlling the specificity of halide trapping systems. Despite the similarity of the strengths of XB and HB [24], the limited examples of using these bonds to coordinate anions in competing solvents show different behaviour between the halogen and HB systems [15, 26, 40]. These model systems are designed to compare the anion selectivity of these weak
2.2. Using XAS to probe for XB: XAS of a halide electron donor

interactions. Specifically, these systems allow for three different comparisons: (1) 1 XB (compound denoted as 1-X in figure 2.2) vs. 2 XB (3-X) (2) 2 HB (2-X) vs. 2 XB (3-X) and (3) 2 XB and 2 HB in direct competition (4-X) vs. 2 XB and 2 HB in isolation (2-X and 3-X, respectively).

Iodine was chosen as the electron acceptor in the XB because, as the largest and most polarisable of the halogens, iodine substituents are more electron deficient than Br or Cl substituents in analogous systems. Thus iodine is capable of forming the strongest XB. Beer and coworkers analysed the anion coordinating properties of these systems for Cl\(^-\), Br\(^-\), I\(^-\), C\(_2\)H\(_3\)O\(_2\), H\(_2\)PO\(_4\), NO\(_3\), and SO\(_4^2\). However, only the systems using Cl\(^-\) and Br\(^-\) were analysed with XAS as XAS of elements with Z < 17 is particularly difficult to analyse via XAS due to atmospheric signal attenuation and self absorption [41]. Furthermore, as XAS is an element-specific technique, the XAS spectrum results from signal from all atoms of a particular element. This means that the use of I\(^-\) as both the electron donor and the electron acceptor in the halogen would result in a spectrum with competing information from both the electron donor and electron acceptor of the XB. The use of different elements for the electron donor and electron acceptor reduces the complexity of each spectrum and allows for the separate analysis of the electron donor and acceptor.

Prior to the XAS studies, Beer and co-workers characterised these systems by NMR spectroscopy and X-ray crystallography [27]. NMR was used for product confirmation and to determine binding constants of halides to the different systems. X-ray crystallography confirmed both the structure of the systems and the presence of strong XB as the bond distances between halogen substituents and free halides were shown to be 84-86% of the sum of the van der Waals radii of the atoms of interest. My contribution focuses on the use of Cl K-edge and Br K-edge XAS to investigate the nature of these bonds.

2.2.1 Qualitative XAS Analysis

If XB were purely electrostatic interactions, one would expect a halide ion (Cl\(^-\) or Br\(^-\)) to exhibit K-edge XAS spectra with no pre-edge features. Electric-dipole allowed Cl\(_{3p}\) ↔ Cl\(_{1s}\) (or Br\(_{4p}\) ↔ Br\(_{1s}\)) transitions would not be observed since these valence p-states are filled (ns\(^2\)np\(^6\)). Covalent delocalization of the filled Cl\(_{3p}\) orbital with an empty acceptor orbital (e.g. via HB or XB) results in the possibility of a new allowed transition corresponding to charge transfer from the chloride to its bonding partner (\(\sigma_{RX} \leftrightarrow Cl^* \leftrightarrow Cl_{1s}\)).
2.2. Using XAS to probe for XB: XAS of a halide electron donor

Figure 2.2: Chemical structures of the halide trapping molecules in which XB covalency was identified by XAS (figures 2.3(a) and 2.3(b)). The compounds analysed in these studies contained either Cl or Br as X as indicated by the naming scheme (e.g. 1-Cl vs. 1-Br). It should be noted 3-X is the HB analogue of 2-X. The compounds have been drawn in colours that correspond to the XAS data presented throughout the rest of the chapter.

Given that the intensity (i.e. area under the peak, section 1.2) of such transitions is directly proportional to the amount of Cl$_{3p}$ in the final state’s wave function, $\sigma_{RX \leftrightarrow Cl^*}$, the intensity of any observable pre-edge feature provides us a direct measure of the charge transfer in a XB.

For the complexes containing XB, 1-Cl, 2-Cl and 4-Cl, the near-edge regions of the XAS spectra exhibit an intense pre-edge feature that is not present in ionic chloride salts (figure 2.3(a)). The presence of this intense feature can only result from charge transfer between the chloride donor and
2.2. Using XAS to probe for XB: XAS of a halide electron donor

its partners \[3\, 4\]. These results clearly demonstrate charge transfer from the donor via a degree of covalency in the XB.

For comparison, data was also collected on the HB analogue of 2-Cl (3-Cl). The more intense pre-edge feature of 3-Cl shows that, with a chloride donor, XB interactions are significantly more covalent in character than comparable HB interactions. The comparison of these two bonds is relevant as Beer and coworkers showed that the analogous XB and HB of complexes 2-Cl and 3-Cl are similar in bond strength despite their different covalencies (see \[2.2\]). These findings are consistent with previous reports that charge transfer is an important factor in XB bonds \[31\].

The presence of a low-energy shoulder is also evident in the Br K-edge XAS data, indicating a pre-edge feature similar to that observed in the Cl K-edge data. The pre-edge shoulder also appears larger for the X-bonded systems than that of the H-bonded system (3-Br).

<table>
<thead>
<tr>
<th>Compound</th>
<th># of XB</th>
<th># of HB</th>
<th>$K_a$ (m$^{-1}$)</th>
<th>$\Delta G^0$ of Binding (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl</td>
<td>2</td>
<td>0</td>
<td>387 ± 20</td>
<td>-15 ± 3</td>
</tr>
<tr>
<td>3-Cl</td>
<td>0</td>
<td>2</td>
<td>206 ± 11</td>
<td>-13 ± 2</td>
</tr>
<tr>
<td>2-Br</td>
<td>2</td>
<td>0</td>
<td>238 ± 12</td>
<td>-14 ± 2</td>
</tr>
<tr>
<td>3-Br</td>
<td>0</td>
<td>2</td>
<td>106 ± 3</td>
<td>-11.6 ± 0.6</td>
</tr>
</tbody>
</table>

Table 2.2: Association constants ($K_a$) of 2-Cl, 3-Cl, 2-Br, and 3-Br as measured by Beer and coworkers by WinEQNMR \[42\]. The solvents used were CD$_3$Cl and d$_6$DMSO for 2-X and 3-X, respectively. Binding energies were calculated from association constants measured by Beer and coworkers. Binding energy uncertainties ($\varepsilon\Delta G$) were calculated according to the rules of error propagation (note: although uncertainties would normally be denoted by $\sigma$, $\varepsilon$ is used to avoid confusion with $\sigma$ orbitals) \[43\].
2.2. Using XAS to probe for XB: XAS of a halide electron donor

Figure 2.3: (a) Cl K-edge and (b) Br K-edge XAS data for Cl\textsuperscript{−} and Br\textsuperscript{−} trapping molecules, respectively. In plot (a), the overlay of the four spectra makes the pre-edge feature of 4-Cl\textsuperscript{−} difficult to see so the peak shoulder corresponding to the $\sigma_{XB}^{*} \leftarrow \text{Cl}_{1s}$ is indicated by an arrow for clarity.
2.2. Using XAS to probe for XB: XAS of a halide electron donor

2.2.2 Quantitative XAS Analysis

SixPack [44] and BlueprintXAS [45, 46] were used in order to obtain values for the pre-edge feature intensities. SixPack is used to calibrate and average spectra [44]. Spectra with high levels of noise and abnormalities due to faulty channels in the 30-channel germanium detector were removed from the dataset. NaCl ($E_0 = 2820.2$ eV) and KBr ($E_0 = 13474$ eV) spectra were acquired at the same time and used as calibration standards for Cl K-edge and Br K-edge spectra, respectively. BlueprintXAS version 2.7 was used for background subtraction and normalisation. Although SixPack is standard software used to workup XAS data, BlueprintXAS is not as frequently used and does not have an associated standard protocol. Thus the methodology used for BlueprintXAS analysis is described below.

To minimise the user bias introduced during data work-up, BlueprintXAS fits the spline, peaks and background concurrently (figures 2.4 and 2.5) [45, 46]. While the parameters for each variable are user defined (see below), the fits were run in AUTO mode as in this mode, a Monte Carlo methodology is used to choose the starting point of each fit to further remove user bias. Each fit contained the following components: “piecewise spline + edge”; “peak” for the edge peak; “normalised peak” for the pre-edge peak. These components can be visualised in figures 2.4 and 2.5. In these figures, data has yet to be normalised. Additionally, the “piecewise spline + edge” component has been split into the “edge peak” and “background + spline” to aid visualisation. The final values for each parameter are given in appendices (appendix A).

Background subtraction and normalisation are standard methods used in XAS data to allow for comparison of spectra [44]. Background subtraction involves fitting linear functions to the data before and after the edge jump. A sum of these linear functions is considered the “background” and is subtracted from the data. The spectra in figures 2.4 and 2.5 have already been background subtracted to aid visualisation. Data is considered normalised when the height difference between the background before and after the edge jump equals 1. In figure 2.4, the edge jump is the height difference between the data below 2820 eV in energy and above 2830 eV (energies are approximate). In the Br K-edge spectra (figure 2.5), the edge jump is the height difference between the data below 13465 eV in energy and above 13510 eV in energy. As the height of the edge jump will depend on the intensity of the incident beam and number of atoms of the element of interest in the sample, normalisation allows for the comparison of the spectral features of different samples.
2.2. Using XAS to probe for XB: XAS of a halide electron donor

Figure 2.4: The average of the calculated fits for: (a) 1-Cl, (b) 2-Cl, (c) 3-Cl and (d) 4-Cl after fits had been filtered. The ‘acquired data’ plotted is neither normalised nor background subtracted as this is done post-fitting by subtracting the ‘background + spline’ from the ‘acquired data’ and dividing the result by the intensity of the edge jump. Consequently, the intensity of the edge jump for ‘acquired data’ is proportional to the number of atoms excited and is thus different for each dataset. Finally, the energy of the edge jump for these fits does not correspond to the energy of the edge jump in figure 2.3(a) as the calibration of the chloride datasets was done after the fitting.
2.2. Using XAS to probe for XB: XAS of a halide electron donor

Figure 2.5: The average of the calculated fits for: (a) 1-Br, (b) 2-Br (c) 3-Br and (d) 4-Br after fits had been filtered. 50 additional fits were run for the bromide data sets due to a larger number of failed fits (i.e. fit reached maximum number of iterations without determining a valid solution). The ‘acquired data’ plotted is neither normalised nor background subtracted as this is done post-fitting by subtracting the ‘background + spline’ from the ‘acquired data’ and dividing the result by the intensity of the edge jump. Consequently, the intensity of the edge jump for ‘acquired data’ is proportional to the number of atoms excited and is thus different for each dataset.
2.2. Using XAS to probe for XB: XAS of a halide electron donor

Fits were filtered, i.e., removed from final set if (a) any error occurred during fit calculation, (b) the fit significantly deviated from spectra, i.e., sum of square errors (SSE) was too large, and/or (c) if fit is a significant outlier for peak height, peak energy or E0. The “time average” option, a feature of BlueprintXAS (used for all fits except 1-Cl and 3-Cl) calculates the average time taken to calculate each fit and filters any fits above this value. This setting was used to remove failed fits as the fits with the longest calculation times usually corresponded with the fits that failed. Failed fits for 1-Cl and 3-Cl were removed from the dataset manually (table 2.3).

<table>
<thead>
<tr>
<th>Dataset</th>
<th># Fits Run</th>
<th># Fits Remaining after Filtering</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Cl</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>2-Cl</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>3-Cl</td>
<td>100</td>
<td>46</td>
</tr>
<tr>
<td>4-Cl</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td>1-Br</td>
<td>150</td>
<td>104</td>
</tr>
<tr>
<td>2-Br</td>
<td>150</td>
<td>38</td>
</tr>
<tr>
<td>3-Br</td>
<td>150</td>
<td>70</td>
</tr>
<tr>
<td>4-Br</td>
<td>150</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 2.3: Filtering Fits: A total of a 100 and 150 fits were run for the chloride and bromide datasets, respectively. Due to the large number of failed fits for 2-Br and 4-Br, the total number of fits run was increased to obtained on the order of 50 fits after filtering (recommended by the BlueprintXAS’s developer). To ensure consistency of workup for all Br K-edge data, the total number of fits run for all Br compounds was increased. Although fits were calculated for the Br spectra, the resulting fits were not considered viable as explained in the main text. In fact, the significant number of failed fits was the first indication of issues with these fits.

As the purpose of fitting the data is to quantify the pre-edge feature intensity for each spectrum, the validity of the fits was assessed by looking at the coefficient of variation for the pre-edge intensity. As the ratio of the standard deviation to the mean, the coefficient of variation is a measurement of the precision. The coefficient of variation is lowest for the datasets for 1-Cl, 3-Cl, 4-Cl, and 4-Br (table 2.4), indicating a larger degree of confidence in the calculated pre-edge intensity values for these fits.

The fitted pre-edge intensities of 3-Cl and 3-Br exhibit the two highest coefficients of variation and thus require further inspection into the validity of these fits. A qualitative inspection of the pre-edge feature for 3-Cl reveals
2.2. Using XAS to probe for XB: XAS of a halide electron donor

<table>
<thead>
<tr>
<th>Dataset</th>
<th>( \mu_{I_1} )</th>
<th>( \sigma_{I_1} )</th>
<th>CV (_{I_1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Cl</td>
<td>0.90</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>2-Cl</td>
<td>1.59</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>3-Cl</td>
<td>0.13</td>
<td>0.03</td>
<td>0.23</td>
</tr>
<tr>
<td>4-Cl</td>
<td>1.00</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>1-Br</td>
<td>0.15</td>
<td>0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>2-Br</td>
<td>0.62</td>
<td>0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>3-Br</td>
<td>0.66</td>
<td>0.23</td>
<td>0.35</td>
</tr>
<tr>
<td>4-Br</td>
<td>0.19</td>
<td>0.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 2.4: The average \( (\mu) \), standard deviation \( (\sigma) \), and the coefficient of variation \( (CV = \sigma/\mu) \) are reported for the pre-edge feature intensity. Units are in normalised intensity for all values except the coefficient of variation which is unitless.

that it is significantly smaller than the pre-edge features 1-Cl and 2-Cl which corresponds to the trend observed in the fitted values. Moreover, the separation of the pre-edge and edge peaks is larger than all other spectra except the peak separation in 1-Cl and 2-Cl, which are comparable. Thus, the high coefficient of variation for 3-Cl is likely due to the fact that as this pre-edge feature has the smallest intensity of pre-edge features in all 8 datasets, it will be most affected by experimental noise.

The high coefficient of variation is significantly more concerning as a qualitative inspection of the XAS spectrum of 3-Br reveals that, due to the large overlap between the pre-edge and edge peaks, there are very few data points which define the shape of the pre-edge feature. This means that a small change in shape of the edge feature could result in large changes in the calculated intensity of the unresolved pre-edge intensity feature. Similar degrees of large overlap and lack of peak resolution are a cause for concern in all Br fits. Indeed, 1-Br and 2-Br had the next highest coefficients of variation.

Due to a combination of the large degree of peak overlap, lack of peak resolution, higher coefficient of variations and large number of failed fits for 1-Br, 2-Br, 3-Br, these fits were deemed inconclusive. While the fit for 4-Br had a low coefficient of variation, indicative of a viable fit, the values obtained for this fit are not useful as there is no data to compare it to. Consequently, a quantitative comparison of the pre-edge intensities for the four bromide data sets was not possible. This result is not unexpected as lifetime broadening at the higher energy Br K-edge, as well as smaller
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energy separation between features, leads to poorer resolution of the pre-edge features of interest. However, it is interesting to note that if the pre-edge intensity value for 3-Br is discarded (the fitted pre-edge intensity value with the highest coefficient of variation), we observe a similar trend in pre-edge intensities for both the Cl and Br compounds (2-X >> 4-X > 1-X, i.e. % X electron donation is largest when the halide is involved 2 XB, followed by 2 XB in competition with 2HB, followed by 1 XB, as is expected - this trend is discussed in more detail later).

The chloride fits were deemed sufficiently successful to continue with further quantitative analysis. Having obtained intensity values for the pre-edge features from the fits, the degree of covalency in the XB bond can be quantified, and the degree of charge transfer in different systems can be compared. This direct comparison of charge transfer in XB and HB in analogous systems is unprecedented in the literature. Percent charge transfer of a metal-chloride coordination bond was determined by Solomon and co-workers [3] and a similar method is employed for this dataset. As discussed in Chapter 1, equations [1.2][1.4], the ratio of the charge-transfer of bond in which a chloride acts as as the electron donor metal-chloride bond to the normalised intensity of pre-edge feature in Cl K-edge XAS corresponding to the D ← Cl⁻ transition. To calculate the percent covalency ($\alpha^2$) for our systems, we will use the ratio calculated by Solomon and coworkers with CuCl$_4^{2-}$ [3].

$$\frac{\alpha^2_{\text{CuCl}_4^{2-}}}{I_{\text{Cu}←\text{Cl}^-}} = 12.5 = \frac{\alpha^2_{\text{RI}←\text{Cl}^-}}{I_{\text{RI}←\text{Cl}^-}}$$  \hspace{1cm} (2.1)

where $\alpha^2$ is the charge transfer (percent covalency) and $I$ is the intensity of the Cl K-edge XAS pre-edge feature. The data presented in this thesis is the first calculation of bond covalency in non-metal systems involving halide salts.

When quantifying the charge transfer of each interaction, the total number of XB and HB interactions in each complex must be considered. In 1-Cl, where only a single XB interaction is possible, we find that the degree of donation is consistent with 6% charge transfer to the iodinated triazole acceptor. In complex 2-Cl where two XB are present, the total charge donation from the halide ion almost doubles to 11%, which implies that the two XB in the complex are mostly independent and additive. By contrast, replacement of the iodine acceptors for protons in the (3-Cl) leads to an almost complete loss of intensity in the Cl K-edge XAS pre-edge feature, reflecting very poor charge donation through HB in this system. Data for the catenane 4-Cl, where both HB and XB are present, indicate that charge
2.2. Using XAS to probe for XB: XAS of a halide electron donor

donation from the chloride anion decreases substantially (as compared to 2-Cl). This presumably reflects weakened XB due to competition with amide H-bond interactions.

The degree of charge transfer observed for these systems by XAS is also well supported and substantiated by TDDFT simulations for the same systems. Moreover, the TDDFT simulations indicate that the degree of covalency is essentially the same for both Cl⁻ and Br⁻ donors [27].

<table>
<thead>
<tr>
<th>Compound (XAS data)</th>
<th>Interactions</th>
<th>Pre-Edge Energy (eV)</th>
<th>Normalised Pre-Edge Intensity</th>
<th>% Cl₃p charge donation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Cl</td>
<td>XB 1</td>
<td>2816.9 ± 0.1</td>
<td>0.90 ± 0.05</td>
<td>6.4 ± 2.1</td>
</tr>
<tr>
<td>2-Cl</td>
<td>HB 2</td>
<td>2817.3 ± 0.1</td>
<td>1.59 ± 0.04</td>
<td>11.3 ± 2.0</td>
</tr>
<tr>
<td>3-Cl</td>
<td></td>
<td>2816.2 ± 0.2</td>
<td>0.13 ± 0.03</td>
<td>0.9 ± 1.9</td>
</tr>
<tr>
<td>4-Cl</td>
<td></td>
<td>2818.4 ± 0.2</td>
<td>1.00 ± 0.06</td>
<td>7.1 ± 2.3</td>
</tr>
</tbody>
</table>

Table 2.5: Total Cl₃p charge donation as calculated by Blueprint-XAS Analysis. Calculated errors are obtained from statistical distribution of fit results for >50 fits for each data set. *Total donation is calculated in comparison with Cl K-edge XAS data on a CuCl₂⁻ reference; errors include an estimate of the error in charge transfer of this reference compound.

Notably, the magnitude of charge donation/covalent character in the XB in 1-Cl, 2-Cl, and 4-Cl is similar to that which is commonly observed in transition metal complexes where covalent contributions are considered to be of great importance in defining chemical properties. For example, in simple divalent metal chlorides ([MCl₂⁻], where M = Cu, Ni, Co, Fe), bond covalencies have been determined to range from 6% to 9% [3]. To put these values into perspective, it is important to note that in a fully symmetric covalent bond, the charge donation would be an equal 50% from each atom. Thus both coordinate bonds and XB can be described as having up to one fifth of the maximum possible covalency.

To emphasise the comparable degree of covalency between XB and co-ordinate bonds, we propose that XB be depicted with an arrow reflecting the direction of charge transfer rather than a dotted line. Although XB are usually represented by a dotted line, the choice of the arrow is based on an improved understanding of the electronics of these bonds. The use of arrows to depict XB is used in this thesis from this point forwards.
To test this new methodology of using XAS to probe XB, Br K-edge XAS experiments were conducted on model systems involving KBr and I\textsubscript{2}. The purpose of these experiments is to determine when XB interactions are at a maximum for the electron donor (in this case Br\textsuperscript{−}). To do this the ratio of KBr:I\textsubscript{2} was increased in each successive experiment until the pre-edge feature corresponding to the $\sigma_{XB}^* \leftarrow Br_{1s}$ no longer increased.

There are two possible phenomena which explain the lack of increase in pre-edge feature intensity from the KBr:I\textsubscript{2} 1:1.3 spectrum to the KBr:I\textsubscript{2} 1:3 spectrum. This data could indicate that for these systems, each Br\textsuperscript{−} can be involved in XB with a maximum of 1.3 I\textsubscript{2} molecules on average. In other words, when the Br\textsuperscript{−} is involved in two XB with two different I\textsubscript{2} molecules,
2.4 Using XAS to probe for XB: XAS of the XB electron acceptor

Each XB exhibits less charge transfer than a Br\(^-\) involved in a single XB with an I\(_2\). Alternatively, the Br\(^-\) could be involved in more than two significantly weaker XB. Either way, we know that the charge transfer from Br\(^-\) to I\(_2\) is maximised at a ratio of approximately KBr:I\(_2\) 1:1.3. This experiment both provides a satisfying proof of concept for our methodology, and it illustrates that this methodology can provide stoichiometry information about XB too.

We can also see that the XB between Br\(^-\) and I\(_2\) exhibits more covalency and a greater orbital overlap between the Br\(_{4p}\) and the \(\sigma^*_{I-I}\) than the XB involving Br\(^-\) in the previous sections. This can be seen in the larger pre-edge feature intensity.

An interesting though more difficult experiment would be to run a similar experiment at an iodine edge, slowly increasing the amount of KBr until no more changes are observed in the pre-edge feature of the I L\(_3\)-edge data. The feasibility of studying XB using XAS of the XB electron acceptor will be explored in the remaining sections of this chapter.

2.4 Using XAS to probe for XB: XAS of the XB electron acceptor

In section 2.2, XAS was established as a method to probe the degree of covalency of an XB, specifically for XB involving a Cl\(^-\) or Br\(^-\) as the electron donor. While the purpose of these XAS experiments was to probe the electronic properties of XB, XAS can also be able to probe for the presence XB in any systems involving a halide electron donor (this will be explored in chapters 3 and 4).

The ability to use XAS in addition to X-ray crystallography to probe for XB allows us to study a wide range of systems for which obtaining single crystals would be difficult if not impossible. However, the main drawback of this XAS diagnostic approach is that it can only be used to study XB which have a halide ion acting as the XB electron donor. In XB involving other electron donors such as covalently-bound oxygen, nitrogen and sulphur, XAS of the electron donor is not possible. This is because XAS of low-Z elements (Z<17, i.e. elements with atomic number less than 17) is much more difficult due to atmospheric signal attenuation and self absorption [41].

In this section, we explore the use of XAS to probe XB via the covalently-bound halogen that acts as the XB electron acceptor. If successful, this technique could be generalisable to all XB systems as, by definition, all XB involve a covalently-bound halogen.

To explore this approach, the halide trapping complexes discussed in
section 2.2 (1-X, 2-X, and 4-X) are analysed by XAS. As all compounds involve an iodine acting as the XB electron acceptor only XAS experiments at I edges were necessary. Both I L$_3$-edges and I L$_1$-edges were studied (excitation of the I$_{2s}$ and I$_{2p}$ electrons, respectively), but only the I L$_3$-edges are reported here. The non-linear nature of the post-edge region of our I L$_1$-edge data rendered normalisation of the spectra difficult and unambiguous intercomparison of the spectra impossible. Consequently, all I L$_1$-edge data was deemed inconclusive.

XAS data of the electron acceptor for 3-X is not included in this section. As 3-X involves HB rather than XB, it is not possible to conduct XAS experiments on the H electron acceptor.

I L$_3$-edge XAS experiments for the KBr:I$_2$ systems discussed in the previous section is also not reported. This is due to the fact that experiments showed that I L$_3$-edge XAS data for I$_2$ is significantly affected by slight changes in sample preparation method (i.e. more/less grinding with a mortar and pestle). This is most likely due to the many different possible polyiodides which can form [47]. The changes resulting from a slight change in sample prep resulted in far more significant changes in the edge region than would likely arise from XB.

However, XAS data of the synthetic precursors of 1-X, 2-X, and 4-X, (1-PF$_6$, 2-PF$_6$, and 4-PF$_6$, respectively) in which PF$_6$ acts as the counterion is included in this section. These systems do have a covalently-bound iodine but do not involve XB and therefore allow us to compare the XAS of iodine substituents in analogous compounds in the presence and absence of XB. The lack of XB in these compounds can be assumed as the steric bulk of PF$_6$ suggests that this counterion is unable to fit into the relatively small binding pockets of the various complexes. All compounds were synthesised by the Beer group [27].

To facilitate data interpretation: all XAS data of 1-X (1 XB present) and 1-PF$_6$ is blue, all XAS data of 2-X (2 XB present) and 2-PF$_6$ is green, and all XAS data of 4-X (2 XB and 2 HB present) and 1-PF$_6$ is red. This colour scheme is the same as figure 2.3 which shows the Br K-edge and Cl K-edge XAS data for these same systems.

Probing XB through the XB electron acceptor instead of a Cl$^-$ or Br$^-$ acting as a XB electron donor is significantly more challenging. In the XAS spectrum of a halide involved in an XB, we observe a new peak corresponding to the $\sigma^*_{XB} \leftarrow \text{Cl}^-$ or $\sigma^*_{XB} \leftarrow \text{Cl}^-$ to indicate XB formation. In the XAS spectrum of a covalently-bound halogen, there are no new transitions allowed upon XB formation. Therefore, we expect changes due to XB in the XAS spectrum of the electron acceptor to manifest as changes in peak intensity
2.4. Using XAS to probe for XB: XAS of the XB electron acceptor

and shifts in peak energy. The electron transition which is expected to be most affected in the systems studied is the $\sigma_{C-I}^{\star} \leftarrow I_{2p}$ (figure 2.7).

Figure 2.7: The molecular orbital diagram of a covalently-bound iodine involved in an XB. The transitions of interest arising from an I L$_{3}$-edge XAS experiment for the complex in the absence and presence of XB with Cl$^{-}$ are shown in purple and green, respectively.

Upon partially covalent XB formation the antibonding orbital of the C-I bond ($\sigma_{C-I}^{\star}$) overlaps with the Cl$_{3p}$ (in the case of Cl$^{-}$ acting as the XB electron donor) to form two new orbitals: $\sigma_{XB}$ and $\sigma_{XB}^{\star}$ (figure 2.7). The electron transition which is lowest in energy is therefore the $\sigma_{C-I}^{\star} \leftarrow I_{2p}$ in the absence of XB and $\sigma_{XB}^{\star} \leftarrow I_{2p}$ in the presence of XB. The energy of the $\sigma_{XB}^{\star} \leftarrow I_{2p}$ is expected to be higher than $\sigma_{C-I}^{\star} \leftarrow I_{2p}$, due to the stabilisation of the $\sigma_{XB}$ orbital, and the probability of the $\sigma_{XB}^{\star} \leftarrow I_{2p}$ would expected to be lower than $\sigma_{C-I}^{\star} \leftarrow I_{2p}$ due to the decreased iodine character in the $\sigma_{XB}^{\star}$ orbital. This will be discussed further in sections 2.4.1 and 2.4.2.

2.4.1 I L$_{3}$-edge XAS: Comparing different systems with the same counterion

Figure 2.8 plots the I L$_{3}$ edge spectra for the 9 compounds of interest. These 9 spectra are grouped in order to visualise the changes between the
2.4. Using XAS to probe for XB: XAS of the XB electron acceptor

spectra of three different iodine XB electron acceptor compounds when these compounds are involved in XB with the same halide.

An inspection of the XAS spectra suggests that the feature at approximately 4560 eV corresponds to the $\sigma^*_{C-I} \leftarrow I_{2p}$ or $\sigma^*_{XB} \leftarrow I_{2p}$ transitions ($\sigma^*_{C-I} \leftarrow I_{2p}$ for systems with no XB and $\sigma^*_{XB} \leftarrow I_{2p}$ for systems with XB). The relatively small size of the pre-edge feature when compared with the edge is not unexpected as the relative electronegativities of iodine and carbon suggests that $\sigma^*_{C-I}$ orbital will be localised on the C atom. Furthermore, this transition is only dipole allowed due to the presence of I$_{5s}$ character in the $\sigma^*_{C-I}$ orbital.

Although all of these systems involve iodine substituents, the direct comparison of systems which such widely different scaffolding may seem ambitious. However, as the pre-edge and edge regions of XAS are affected predominantly by the electronics of the atom studied and its immediate bonding partners, a comparison between these systems which all contain an iodine bound to an $sp^2$ hybridised carbon is reasonable, particularly if the identity of the halogen bond electron acceptor remains the same. Indeed, the pre-edge and edge features are nearly identical for the three different systems (1-PF$_6$, 2-PF$_6$, and 4-PF$_6$) (figure 2.8(c)). However, when the iodine substituents of these three systems are involved in different degrees of XB (figures 2.8(a) and 2.8(b)), the pre-edge features change. Specifically, the intensity of the pre-edge feature is arguably largest for the 4-X compounds, very closely followed by the 2-X compounds with the pre-edge features of the 1-X compounds having the smallest intensity. The energy of the pre-edge features of the 1-X compounds are also noticeably higher (1-2 eV) than the energies of the corresponding features in the 2-X and 4-X spectra.

To understand these changes, it is useful to think about the degree of covalency for each system, and thus we return to the results from section 2.2. There are three ways to count the number of XB in each system: the number of XB in which Cl$^-$ participates, the number of XB in which each iodine substituent participates and the total number of XB in each system (table 2.6).

<table>
<thead>
<tr>
<th></th>
<th>1-X</th>
<th>2-X</th>
<th>3-X</th>
<th>4-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bonds</td>
<td>1 XB</td>
<td>2 XB</td>
<td>2HB</td>
<td>2 XB &amp; 2 HB</td>
</tr>
<tr>
<td>Bonds per I</td>
<td>1 XB</td>
<td>1 XB</td>
<td>–</td>
<td>1 XB</td>
</tr>
<tr>
<td>Bonds per X</td>
<td>1 XB</td>
<td>2 XB</td>
<td>2HB</td>
<td>2 XB &amp; 2 HB</td>
</tr>
</tbody>
</table>

Table 2.6: The number of intermolecular bonds per system/per iodine substituent or per chloride ion for 1-X, 2-X, 3-X, and 4-X.
2.4. Using XAS to probe for XB: XAS of the XB electron acceptor

If we take the total %Cl\textsubscript{3p} contribution in each $\sigma^*_\text{XB}$ (table 2.5) and divide by the number of XB in each system in which Cl$^-$ participates (table 2.6), we calculate the degree of %Cl\textsubscript{3p} contribution per XB as follows: 6.4% for 1-Cl, 5.7% for 2-Cl, and 3.6% for 4-Cl. These values tell us that XB in 1-Cl are the most covalent while the XB in the 4-Cl are least covalent. As a single Cl$^-$ ion serves as the sole electron donor for each system, the competing bonds in 2-Cl and 4-Cl decreases the %Cl\textsubscript{3p} contribution per XB.

<table>
<thead>
<tr>
<th></th>
<th>1-Cl</th>
<th>1-Br</th>
<th>1-PF\textsubscript{6}</th>
<th>2-Cl</th>
<th>2-Br</th>
<th>2-PF\textsubscript{6}</th>
<th>4-Cl</th>
<th>4-Br</th>
<th>4-PF\textsubscript{6}</th>
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<tbody>
<tr>
<td>Energy</td>
<td>4560.1 eV</td>
<td>4560.1 eV</td>
<td>4559.4 eV</td>
<td>4559.5 eV</td>
<td>4559.7 eV</td>
<td>4559.5 eV</td>
<td>4559.5 eV</td>
<td>4559.3 eV</td>
<td>4559.2 eV</td>
</tr>
</tbody>
</table>

Table 2.7: The first maximum of the first derivative of the I L\textsubscript{3}-edge XAS for all compounds in figures 2.8 and 2.9. These values can be used to approximate the location of the centre of the pre-edge feature for each spectrum.

With this information in mind, we return to understanding XB from the perspective of the iodine electron acceptor. As the XB in these systems form via the overlap of the Cl\textsubscript{3p} and $\sigma^*_{\text{C-I}}$ orbitals, we expect the trend in % contribution of the $\sigma^*_{\text{C-I}}$ to the $\sigma^*_\text{XB}$ to be inverse of %Cl\textsubscript{3p} contribution. Thus, 4-Cl is expected to have the highest % contribution of the $\sigma^*_{\text{C-I}}$, followed by 2-Cl, and then 1-Cl. As the probability of the transition $\sigma^*_\text{XB} \leftarrow$ I\textsubscript{2p} transition is directly proportional to the amount of iodine character of the $\sigma^*_\text{XB}$ orbital (which is determined by the % contribution of the $\sigma^*_{\text{C-I}}$), we expect the trend in probability of the $\sigma^*_\text{XB} \leftarrow$ I\textsubscript{2p} transition to be as follows: 4-Cl $>$ 2-Cl $>$ 1-Cl. Finally as the intensity of the feature corresponding to this transition is proportional to the probability, we expect the same trend in the intensities of the pre-edge features in the XAS spectra. Indeed, a visual approximation of the intensities of pre-edge features for figure 2.8(a) involving the Cl$^-$ donor and figure 2.8(b) involving the Br$^-$ donor seems to agree with this expected trend. Note: while the pre-edge feature of 2-Cl appears to be to the left of 1-Cl, the actual location of the pre-edge feature as determined by where the second derivative is zero is approximately the same (table 2.7).

Furthermore, a similar logic can explain the observed shift in the pre-
2.4. Using XAS to probe for XB: XAS of the XB electron acceptor

edge features for both figure 2.8(a) and figure 2.8(b). As orbital overlap improves in the XB, i.e. higher %Cl\textsubscript{3p} contribution in the $\sigma^*_{\text{XB}}$ and higher % contribution of the $\sigma^*_C$ to the $\sigma_{\text{XB}}$, the energy of the $\sigma^*_C$ is expected to increase as the energy of the $\sigma_{\text{XB}}$ decreases. Thus as % contribution of the $\sigma^*_C$ to the $\sigma^*_{\text{XB}}$ (seen as the decrease in intensity of the $\sigma^*_C \leftarrow I_{2p}$ feature), the energy of this feature is expected to increase (shift right). Again this approximately corresponds with what we observe for both the XAS data in figure 2.8(a) involving the Cl\textsuperscript{−} donor and figure 2.8(b) involving the Br\textsuperscript{−} donor.

In conclusion, I L\textsubscript{3}-edge XAS of the XB electron acceptor can be used to observe changes in XB bond covalency, i.e. the overlap between donor and acceptor orbital, for as overlap increases, the pre-edge feature corresponding to the $\sigma^*_{\text{XB}} \leftarrow I_{2p}$ will decrease in intensity and increase in energy as predicted by the molecular orbital diagram.
2.4. Using XAS to probe for XB: XAS of the XB electron acceptor

Figure 2.8: I L₃-edge XAS: Comparing different systems with the same counterion as follows (a) Cl⁻ (b)Br⁻ (c)PF₆⁻
2.4. Using XAS to probe for XB: XAS of the XB electron acceptor

2.4.2 I L\textsubscript{3}-edge XAS: Comparing analogous systems with different counterions

In this section, we will compare the same 9 compounds as the previous section. Figure 2.9 includes the same 9 XAS spectra as figure 2.8. However, these 9 spectra are grouped differently in order to visualise the changes between the spectra of each iodine XB electron acceptor compounds as the counterion is changed.

In comparing these spectra, we observe similar trends to those discussed in section 2.4.1. The systems with PF\textsubscript{6} counterions exhibit the highest pre-edge feature intensity indicative of the least (or in this case absence of) XB. The difference in intensities of the pre-edge features for the Br\textsuperscript{-} and Cl\textsuperscript{-} analogues is difficult to analyse qualitatively as all features are very small. However, we can say that for at least 1-X, the Br\textsuperscript{-} and Cl\textsuperscript{-} analogues appear to exhibit nearly identical pre-edge feature intensities. Interestingly, this agrees with the TDDFT calculations reported for these compounds [27], as the calculations suggest that a similar degree of XB covalency is expected for the Cl\textsuperscript{-} and Br\textsuperscript{-} analogues for all systems.

Finally, there is a noticeable trend in the intensity of the edge peak for all three acceptor systems (specifically edge peak intensity is largest for the PF\textsubscript{6} analogues > Br\textsuperscript{-} > Cl\textsuperscript{-}). This change is worth mentioning as it is consistent for the 3 different series (1-X, 2-X, 4-X). Due to their proximity in energy, both I\textsubscript{6s} ← I\textsubscript{2p} and I\textsubscript{5d} ← I\textsubscript{2p} transitions could contribute to the intensity of this peak. Thus, the decrease in intensity of this feature suggests the involvement of either or both of these orbitals in XB as orbital overlap with an electron donor would result in a decrease in % iodine character, and thus a decrease in the probability of these transitions and decrease in intensity of the associated feature in the XAS spectrum. It also suggests that the involvement of these orbitals would be most significant for the Cl\textsuperscript{-} systems. As other physical phenomena can contribute to the intensity of the edge feature including scattering off nearby atoms, this is merely one of many possible explanations.
Figure 2.9: I L3-edge XAS: Comparing analogous systems with different counterions (a) 1-Cl, 1-Br, and 1PF6; (b) 2-Cl, 2-Br, and 2PF6; (c) 4-Cl, 4-Br, and 4PF6
2.5 Conclusions

Beer and coworkers demonstrated the importance of XB in halide recognition systems as $2-X$ display a marked enhancement in anion recognition over $3-X$, their HB analogues. Cl and Br K-edge XAS revealed the presence of intense pre-edge features characteristic of charge transfer between the halide donor and the XB acceptor. Quantitative fitting of the pre-edge features in the Cl K-edge XAS data provided a direct measure of the degree of covalency in the XB interaction, which is comparable to that observed in coordinate bonds transition metal complexes. Furthermore, XAS data showed that perpendicular XB interactions in the Cl$^-$ donor systems are independent and additive, but that the degree of XB covalency can be mitigated through the presence of HB donors. I L$_3$-edge XAS studies of the iodine substituents showed that as XB covalency increases, the peak corresponding to the $\sigma_{XB}^+ \rightarrow I_{2p}$ transition decreases in intensity and increases in energy, as predicted by molecular orbital theory.

Most importantly, these results offer (1) the first experimental evidence of covalency in XB, revealing that although XB are comparable to HB in terms of bond energy, they are comparable to coordinate bonds in terms of covalency and (2) a methodology to study XB in a wide variety of systems without the requirement for single crystals.

2.6 Methods

2.6.1 XAS Sample Preparation

Samples were received from Beer and coworkers at Oxford and used as received. For Cl K-edge and Br K-edge XAS analysis, samples were mounted as a finely ground powder diluted (1:1) with boron nitride (BN) dusted on sulfur-free Kapton tape across the window of an Al plate. The resultant homogeneous, finely dispersed powders were pressed into a 0.5 mm thick aluminium spacer, sealed on both sides with Kapton tape.

2.6.2 XAS Data Collection

Br K-edge XAS data was collected at SSRL on beamline 7-3 under ring conditions 80-100 mA at 3.0 GeV. This beamline has a 20-pole, 2 T wigglers, 0.8 mrad beam, and a Si (220) double crystal monochromator that was detuned by 50% intensity to attain harmonic rejection. The incident X-ray intensity (I0), sample absorption (I1), and Br reference absorption (I2)
were measured as transmittance using argon-filled ionization chambers. Six to eight sweeps were taken for each sample, and all data were measured at $19 \pm 7$ K within an Oxford Instruments CF1208 continuous-flow liquid helium cryostat. KBr calibration scans were performed concurrent with each sample.

Cl K-edge and I L$_{3}$-edge XAS data was collected at beamline 4-3 SSRL using a modified low Z setup allowing for low temperature data acquisition under ring conditions of 3 GeV and 60-100 mA. The setup is a 54-pole wiggler beamline operating in high field (10 kG) mode with a Ni coated harmonic rejection mirror and a fully tuned Si (111) double crystal monochromator. Signal was detected with a N$_{2}$ fluorescence (Lytle) detector at ambient temperature and pressure (1 atm, 298 K). Calibration scans for the Cl K-edge data were performed before and after every data set to ensure stable monochromator readings.

The I L$_{3}$-edge XAS data in section 2.4 was not calibrated due to failed calibration scans. However, this does not pose a problem for the intercomparison of this data as the overall stability of the beam during the beamtime in question was very good, i.e. the calibration energy did not drift during the Cl K-edge experiments performed on the same beamline before and after the I L$_{3}$-edge experiments. Thus, while the overall energies of the I L$_{3}$-edge XAS data is only approximate, this data can still be accurately compared to other data collected during the same run. As it is standard to only compare XAS data collected at the same beamline during the same beamtime, the overall stability of the beam means the inability to calibrate each experiment individually has no effect on conclusions drawn from comparing this data.
2.6. Methods

*XB nature is often debated,*

*But all of us can now be sated,*

*For halogen bonds,*

*Have shared electrons*

*As XAS data has stated.*
Chapter 3

Using XAS to Probe XB in Dye-Sensitised Solar Cells

In this chapter, the role of XB in dye-sensitised solar cells (DSSC) is explored. The DSSC studied involve a photosensitive dye with halogen substituents (Dye-X series) (figure 3.1). As we have showed in chapter 2 that XAS can be used to characterise XB, particularly XB involving halide electron donors, XAS will be used to probe for the existence of XB in DSSC between the dyes and halide electrolytes. Previous work provided computational and indirect experimental evidence that XB may play an important role in these systems [39, 48]. The work for this chapter was a collaborative effort and completed as follows: XAS data was collected by the author and Fraser Parlane. XAS data analysis was done by the author. Photosensitive dyes were synthesised by Cameron Kellett. DSSC were built by Fraser Parlane.

3.1 Dye-Sensitised Solar Cells - Background

In avoiding the use of silicon and instead using dyes to enhance the photosensitivity of the semiconductor, DSSC are emerging as a cheaper alternative to silicon based solar cells and a more efficient alternative to other thin film solar cells [49]. These systems involve photosensitive dye molecules which are adsorbed onto a titanium dioxide (TiO$_2$) layer (figure 3.1). Excitation of electrons in the dyes creates a driving force for electron transfer from the dye to the TiO$_2$ layer, and from there to the cathode via connected transparent conducting oxide surfaces. Regeneration of the oxidised dye occurs via a redox process involving an electrolyte [49]. One method of improving the efficiency of DSSC is to improve the dye regeneration rate.

*$^*$The work in sections 3.1 and 3.2 is based on the author’s contributions to previously published work [36]. The breakdown of work for these sections is described in the text. Section 3.3 has not been published previously and comprises of additional analysis of the dyes in section 3.2. All data collection and analysis for this section was done by the author. See Preface for more details.
Berlinguette and coworkers have explored the role of halogenated dyes with an I$_3$/I$^-$ electrolyte in DSSC. These dyes have been designed so that there is a physical separation between the HOMO and LUMO of the dye. This physical separation allows for better charge separation, thus minimising charge recombination upon excitation. The LUMO is located on the section of the dye closest to the surface while the HOMO is located farther from the surface. Thus electron excitation moves the electron physically closer to the titanium dioxide surface and away from the electrolyte.

Previous work from Berlinguette and coworkers noted that the regeneration rate of the dye changed as the identity of the halogen substituent on the dye changed. Specifically, the dye regeneration rate increased as the polarisability of the halogen increased (from F to Cl to Br to I) (figure 3.2, orange line). Differences in the thermodynamic properties of these dyes was unable to explain this trend. Indeed, when the electrolyte was changed from I$^-$/I$_3$ to a cobalt complex (figure 3.2, blue line), the increase in regeneration rate tracked with $\Delta G_{\text{rxn}}$ instead [36, 39, 48].

As all other structural and electronic components of the synthesised dyes were the same, the presence of XB between dye and electrolyte was proposed to explain the difference in the regeneration rates of the Dye-X series [39]. As XB strength between the electrolyte and the halogenated dye series is expected to increase as the size and polarity of the halogen substituent increases, XB between dye and electrolyte could explain the dependency of
3.2. XAS of DSSC

To probe for the existence of XB between dye and electrolyte, we set out to use XAS as a tool to directly measure the electronic coupling (or orbital overlap) between the dye and an electrolyte species. Although the DSSC involve suspected XB formation between an I$^-$ and the halogen substituents of the dye, experiments in this chapter focus on XB formation between Cl$^-$ and the halogen substituents of the dye. Cl$^-$ is an effective surrogate for the regeneration rate on halogen substituent size, i.e. as the size of the σ-hole on the halogen substituent increases (figure 3.3), the strength of the XB interaction increases which results in faster regeneration rates. This rationale was supported with DFT calculations showing that the interatomic distances between the I$^-$ electrolyte and Dye-X were smaller than the sum of the van der Waals radii for Dye-Br and Dye-I, an indication of XB. Furthermore, DFT calculations predicted that oxidation of the Dye-X series increases the charge donation of the I$^-$ to the σ$^*$ of the Dye-X, another indication of XB.

3.2 XAS of DSSC

Figure 3.2: Regeneration rate constants ($k_{\text{reg}}$) for the reaction of Dye-X$^+$ on TiO$_2$ substrates with 0.5-10mM of I$^-$ (orange) or 10-150mM of [Co(bpy)$_3$]$^{2+}$ (blue). Figure used with coauthors’ permissions (©Creative Commons) [36, 39, 48].
3.2. XAS of DSSC

Figure 3.3: Increasing $\sigma$-hole on Dye-X series. DFT models of the singly-oxidised dyes, Dye-X$^+$ (where X is F, Cl, Br, and I), reveal an increasingly electropositive $\sigma$-hole on the terminus of the halogen substituents as the size of the halogen increases. The electrostatic potential is plotted over a sphere corresponding to the van der Waals radius of the respective halogen substituent. Figure used with coauthors’ permissions (©Creative Commons) [36].

$I^-$ species used in the DSSC because the nucleophilicity of Cl$^-$ is comparable with I$^-$ [50]. Also, as the reduction of the photosensitive dyes by Cl$^-$ is less favourable than by I$^-$ (equations [3.1]–[3.3] [50, 51], longer timescales are available for measuring XB interactions between the oxidised dyes and Cl$^-$ than the analogous experiment with I$^-$.  

\[
\begin{align*}
2\text{Cl}^- & \rightarrow \text{Cl}_2 + 2e^-  & E^o = -1.36 \text{ V} \\
2\text{I}^- & \rightarrow \text{I}_2(s) + 2e^-  & E^o = -0.54 \text{ V} \\
3\text{I}^- & \rightarrow \text{I}_3^- + 2e^-  & E^o = -0.53 \text{ V}
\end{align*}
\]

Equations [3.1]–[3.3]. Oxidation potentials of Cl$^-$ and I$^-$. The lower $E^o$ value for the Cl$^-$ oxidation reaction illustrates that this oxidation is less favourable than the oxidation of I$^-$. [52]

As shown in chapter 2, Cl K-edge XAS can be used to quantify the degree of covalency in XB donor-acceptor systems, specifically for XB involving a
3.2. XAS of DSSC

Cl\(^-\) electron donor. Charge transfer from the Cl\(^-\) to a XB electron acceptor is detected by a lower energy, pre-edge feature in the Cl K-edge XAS spectrum that arises from charge depletion of the filled Cl\(_{3p}\) orbital (chapter 2.2). The integrated intensity of this pre-edge feature is directly proportional to the degree of mixing between the Cl\(_{3p}\) orbital and the electron-accepting orbital.

Cl K-edge XAS is used to probe for the presence of XB in these systems using the method established in chapter 2. Specifically, a pre-edge feature with a distinctively lower energy than the Cl\(_{4p}\) ← Cl\(_{1s}\) transition most likely results from an electron transition from the Cl\(_{1s}\) orbital to the antibonding orbital of a partially covalent XB (σ\(_{\text{RX}}^*\) ← Cl\(_{1s}\)), as seen in figure 3.4. Thus, if present, a pre-edge feature in a Cl K-edge spectrum of Cl\(^-\) with the dyes likely indicates the presence of XB.

The XAS spectra recorded for Dye-I on TiO\(_2\) does not show a pre-edge feature (figure 3.5, black line) due to either poor orbital overlap or the absence of XB. Both possibilities lead to the same interpretation that minimal XB occurs between Dye-I and the Cl\(^-\). A poor interaction between Cl\(_{3p}\) and the σ\(_{\text{RX}}^*\) may be due to the σ\(_{\text{RX}}^*\) being significantly higher in energy than the Cl\(_{3p}\) electron donor orbital, or the σ\(_{\text{RX}}^*\) being localised more towards the

Figure 3.4: Relevant electron transitions induced by Cl K-edge XAS if XB present between Dye-X and Cl\(^-\). Figure used with coauthors’ permissions ([Creative Commons]) [36].
3.2. XAS of DSSC

carbon rather than the halogen substituent. Previous XB experiments have shown the importance of placing strong electron-withdrawing substituents around the XB donor to realize the σ-hole, particularly when in solution [53]. It is therefore not surprising that Dye-I would not participate in a XB significantly enough to result in a detectable XAS signal due to their lack of electron-withdrawing units in proximity to the halogen.

To determine if XB formation occurs with the photoexcited dyes, the XAS experiment was repeated using dyes which had been oxidised using NOBF$_4$ to obtain a radical species (Dye-\(X^+\)) that mimic the effects of photoexcitation. This system is worth investigation as the increased electron deficiency in the photoexcited/oxidised dyes could facilitate XB with the electrolyte. Unlike the Dye-I + Cl$^-$ system, The Dye-$X^+$ + Cl$^-$ series does exhibit this feature at approximately 2824 eV (figure 3.5, red line). Upon oxidation of the dye, the $\sigma^*_{RX}$ is lowered in energy and the increase in polarization of the carbon-halogen bond causes the $\sigma^*_{RX}$ to be more localized towards the halogen. This scenario is more compatible with XB formation, resulting in a new dipole-allowed $\sigma^*_{RX\leftarrow Cl} \leftarrow Cl_{1s}$ transition. Note that Cl K-edge XAS data is not presented for Dye-Cl as this data would yield a combination of features from both the electrolyte and the dye that renders analysis more difficult.
3.2. XAS of DSSC

Figure 3.5: Cl K-edge XAS data of Dye-I in the ground (Dye-I) and oxidised (Dye-I\textsuperscript{+}) states in the presence of Cl\textsuperscript{−}. The presence of the pre-edge feature at 2825 eV in the Dye-I\textsuperscript{+} system is indicative of XB formation. Likewise, the absence of a pre-edge feature in the Dye-I system is indicative of no XB formation.
Figure 3.6: The average calculated fits for the Cl K-edge XAS data of (a) NOBF$_4$ + Cl$^-$, (b) Dye-F$^+$ ← Cl$^-$, (c) Dye-Br$^+$ ← Cl$^-$ and (d) Dye-I$^+$ ← Cl$^-$. 
3.2. XAS of DSSC

The pre-edge features in the XAS spectra of the oxidised dyes were quantified using the same method described in chapter 2 (figure 3.6 for fitting functions and table 3.2 for values). Two distinct mechanisms are responsible for the pre-edge features that arise upon oxidation of the surface-adsorbed dyes. Firstly, Cl\(^{-}\) may form XB with the oxidised dye (Dye-X\(^{+}\) ← Cl\(^{-}\)). Secondly, the oxidation of the TiO\(_2\) surface (with NOBF\(_4\)) generates defect sites that result in some covalent binding of halide ions to the surface as observed in the control experiment. Notably, there also exists a pre-edge feature in the XAS spectrum of NOBF\(_4\) + Cl\(^{-}\). We also observe two pre-edge features in the Cl K-edge spectrum for (Dye-Br\(^{+}\) ← Cl\(^{-}\)), a smaller feature at higher energy most probably corresponding to Cl\(^{-}\) interactions with TiO\(_2\) defect sites and NO\(^{+}\), and a larger feature at lower energy that only occurs in the presence of the oxidised dye.

We can thus estimate the minimum pre-edge intensity arising from XB interactions, and thus minimum \%Cl\(_{3p}\) contribution in the XB, by subtracting contributions from Cl\(^{-}\) interactions not involving the dyes from the intensity of the observed pre-edge features (table 3.2). The pre-edge intensity observed in the absence of dyes (NO\(^{+}\) and TiO\(_2\) with Cl\(^{-}\)) allows us to estimate charge transfer arising from Cl\(^{-}\) interactions not directly involving dyes. The use of this spectrum as a baseline is justified by the fact that, upon addition of dyes to the system, interactions of Cl\(^{-}\) with either the defect sites or NOBF\(_4\) will likely decrease due to the competing XB interactions. Using this method of estimation, we note that the pre-edge intensity from XB interactions is essentially zero in Dye-F\(^{+}\), and quite significant in both Dye-Br\(^{+}\) and Dye-I\(^{+}\). This experimental trend matches the behaviour observed in our computational modelling.

The XAS data presented in this study is evidence of an interfacial XB interaction between Cl\(^{-}\) and Dye-Br\(^{+}\) and Dye-I\(^{+}\). At first glance, these results seem to contradict the trend observed in figure 3.2 as the XAS data indicates higher charge transfer between Cl\(^{-}\) and Dye-Br\(^{+}\) compared with that between Cl\(^{-}\) and Dye-I\(^{+}\) (table 3.2). As Cl\(^{-}\) is significantly smaller than I\(^{-}\), it is reasonable to expect greater orbital overlap between the bromine substituents on Dye-Br and Cl\(^{-}\) than Dye-I and Cl\(^{-}\). In the DSSC where I\(^{-}\) is used, greater I\(^{-}\) orbital overlap should exist with Dye-I\(^{+}\) rather than with Dye-Br\(^{+}\) resulting in the observed trend in \(k_{\text{reg}}\) values (figure 3.2).

In conclusion, XAS was used to directly confirm an interfacial XB interaction between a soluble halide species and immobilized dyes bearing different halogen substituents. This work confirms that these intermolecular interactions only exist when the dyes bear polarizable halogen substituents like bromine and iodine, as opposed to non-polarizable substituents like fluo-
### 3.2. XAS of DSSC

<table>
<thead>
<tr>
<th>XAS Experiment (compound)</th>
<th>Normalised XAS pre-edge feature</th>
<th>XAS pre-edge feature energy (eV)</th>
<th>Percent contribution of Cl$_3p$ in $\sigma^*_XB$</th>
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</thead>
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<tr>
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<td>No contribution</td>
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<td>1.0-3.5%</td>
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<tr>
<td>Dye-Br$^{+}+$+Cl</td>
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<td>2818.3</td>
<td>5.2-7.7%</td>
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<tr>
<td>Dye-I$^{+}+$+Cl$^-$</td>
<td>0.43 ± 0.02%</td>
<td>2818.5</td>
<td>3.2-5.6%</td>
</tr>
</tbody>
</table>

Table 3.1: Experimental contribution of Cl$_3p$ in $\sigma^*_XB$ and relevant information as determined by analysis of pre-edge feature in XAS data for the Dye-X series. Ranges for percent contribution of the Cl$_3p$ orbital to the $\sigma^*_XB$ was determined as follows: the maximum value was calculated assuming 100% of the pre-edge feature intensity arose from the XB interactions and the minimum value was calculated assuming the same contribution from background interactions as observed for NOBF$_4$ + Cl$^-$ sample. For example: minimum %Cl$_3p$ contribution for Dye-F$^{+}+$+Cl$^-$ is equal to pre-edge feature intensity (0.27) - NOBF$_4$ pre-edge feature intensity (0.19) multiplied by %Cl$_3p$ contribution in CuCl$_2$$^{2-}$ (9.8) then divided by pre-edge feature intensity for CuCl$_2$$^{2-}$ (0.75) [3, 4].

...as expected from XB theory [23, 54]. This work also demonstrates that these XB interactions occur after electron injection into the TiO$_2$, and thus can be an important factor only during the short lifetime of the oxidised dye. Furthermore, computational methods show that these XB interactions are not significantly affected by the identity of the nucleophilic halide species [36], and therefore we believe that the results presented here with Cl$^-$ can be extended to the dye-iodide interactions that occur within DSSCs. As such, the previously observed increases in dye regeneration rate by I$^-$ [55] can be attributed to the increased XB donating ability of Dye-Br$^{+}+$ and Dye-I$^{+}+$. It is particularly striking that such a weak intermolecular interaction involving a distinctively minority species can have such a huge effect on the measured photovoltage, and it suggests that encouraging other intermolecular interactions between oxidised dye and electrolyte species may have similar beneficial effects on dye performance.
3.3 Solution XAS of Dye-Br

Dye-Br in solution with and without Cl\(^-\) was further studied by XAS to determine if the presence of XB corresponded with changes in the XAS spectrum of the XB electron acceptor (Br K-edge XAS). These XAS experiments are analogous to the I L\(_3\)-edge experiments in section 2.4. Similar to the I L\(_3\)-edge experiments, the changes observed in this XAS data are expected to be small as this data reflects changes in an electron transition (figure 3.7) rather than the appearance of a new allowed transition (as is the case for XAS analysis of XB through the electron donor). As can be seen in the molecular orbital diagram, the \(\sigma^*_{XB} \leftarrow Br_{1s}\) is expected to be higher in energy with less Br character. This change corresponds to a decrease in pre-edge peak intensity and a shift to higher energy for this feature.

Figure 3.7: The molecular orbital diagram of a covalently-bound bromine in Dye-Br\(^+\) involved in an XB with Cl\(^-\). The transitions of interest in Br K-edge XAS are shown in orange and green for ground state Dye-Br (absence of XB) and oxidised DyeBr (presence of XB), respectively.

The combination of Dye-Br and Cl\(^-\) was chosen for these experiments as this pairing exhibited the highest degree of overlap as shown by the sample yielding the most intense pre-edge feature (figure 3.6(c)). Furthermore, the different XAS instrumentation used for each experiment yields data with less
3.3. **Solution XAS of Dye-Br**

noise for the Br K-edge. This is due to a larger number of detector channels (30) available for Br K-edge experiments. By comparison, the instrumentation used for the I L3-edge only has 1-4 detector channels (1 channel for the Lytle fluorescence yield detector and 4 channels for the Vortex fluorescence yield detector).

As the aim of these experiments is to elucidate the effects of XB present in solution, the adsorbance of the dye to TiO$_2$ was deemed unnecessary. Thus, due to instrument constraints for the Br K-edge XAS, the samples were not prepared on a mesoporous TiO$_2$ film deposited in glass (as in section 3.2). Instead, all samples analysed are acetonitrile solutions. As the sample prep was changed from the experiments in section 3.2, XAS data was also collected for the XB electron donor (Cl K-edge XAS) to confirm that XB are present in these systems.

The effects on XB formation from chemical oxidation of Dye-Br by NOBF$_4$ is also explored. This experiment is challenging as the oxidised dye is only briefly present in solution (the dark green colour change observed upon addition of saturated NOBF$_4$ in acetonitrile lasted for approximately 2 seconds before the solution returned to the initial orange colour). The solutions were flash frozen immediately following the addition of NOBF$_4$. However, green and orange areas were both visible in the frozen solutions indicating that both ground state and oxidised were present. Therefore, for all solution XAS data reported in this section for oxidised Dye-Br (Dye-Br$^+$), it is important to note that Dye-Br is also present in these samples in unknown ratios.

### 3.3.1 Solution XAS of Dye-Br: Br K-edge

Br K-edge XAS data of Dye-Br with and without Cl$^-$ shows no change for the ground state dye (figure 3.8(a)). This is expected as the results discussed earlier suggested that there was no XB formation between Cl$^-$ and the ground state dyes. Dye-Br and the oxidised dye, Dye-Br$^+$, show little difference indicating no significant charge transfer between the oxidised dye and any unused oxidant NOBF$_4$ or side products. As there is little difference between the edge energies for Dye-Br and Dye-Br$^+$, it can also be assumed that there is not significant charge delocalisation onto the Br substituent as to change the oxidation state of the Br.

By contrast, the addition of NBu$_4$Cl to the oxidised dye results in a decrease in intensity of the peak at approximately 13475 eV and the appearance of a small shoulder peak at approximately 13460 eV (figure 3.8(b)). The peak at approximately 13475 eV corresponds to the $\sigma^*_{C-Br}$ of the Dye-Br
3.3. Solution XAS of Dye-Br

Figure 3.8: Br K-edge XAS data of (a) Dye-Br with and without NBu₄Cl, and oxidised Dye-Br (Dye-Br⁺) and oxidised Dye-Br (Dye-Br⁺) with and without (x2 repeats) NBu₄Cl. The data for oxidised Dye-Br (Dye-Br⁺ - blue line) is included in both graphs for the sake of comparison.

(for ground state dyes). A decrease in the intensity of this peak is expected upon XB formation as orbital overlap between the covalently-bound Br in Dye-Br and the Cl⁻ would result in a decrease of Br character in the resulting orbital and thus a decrease in the probability of the transition. This orbital overlap should also result in a change in the energy of the transition (a peak shift). A slight peak shift of +0.2 eV upon addition of NBu₄Cl is observed but as this shift in energy is close to the resolution of this technique, it is not conclusive.

The appearance of the small shoulder feature at approximately 13460 eV is also noteworthy. Our preferred explanation for this feature is the presence of π-type character in XB. Upon one electron oxidation of Dye-Br, a lower energy transition (πₓB ← Br₁s) is now possible (figure 3.9). The existence of XB π-type character is further supported by data in section 3.3.2 which suggests that the Cl₁s electron has an additional allowed transition. It should be noted that this small shoulder feature could also result from Dye-Br acting as both a donor and acceptor in an XB or cation-π interactions between the NBu₄⁺ and the oxidised Dye-Br. Both of these interactions may result in a new low energy feature for the Br K-edge XAS, but neither
3.3. Solution XAS of Dye-Br

account for the changes observed in the Cl K-edge XAS in the following section.

Figure 3.9: The molecular orbital diagram of a covalently-bound bromine in Dye-Br$^+$ involved in an XB exhibiting $\pi$-type character with Cl$^-$. The transitions of interest in Br K-edge XAS are shown in orange and green for ground state Dye-Br (absence of XB) and oxidised DyeBr (presence of XB), respectively.

Figure 3.8(b) shows two different repeats of the oxidation of Dye−Br$^+$ in the presence of NBu$_4$Cl. Although the difference between the two spectra is initially concerning, it can be explained by the limitations of the experiment. As mentioned earlier, upon oxidation at room temperature in the presence of trace amounts of water, the dark green Dye-Br$^+$ returns to the ground state (orange Dye-Br) in less than two seconds. Although the samples analysed were flash frozen in liquid nitrogen less than one second after oxidation, an appearance of both green and orange indicate the presence of both the oxidised and ground state dyes. Thus, the different intensities observed between the two repeats of the same sample can be explained by the different ratio of ground state and excited state dyes present in solution.
3.3. Solution XAS of Dye-Br: Cl K-edge

To confirm the presence of XB in acetonitrile solutions of Dye-Br$^{+}$ and Cl$^-$, XAS data was also collected for the XB electron donor (Cl K-edge XAS).

As the instrument set up (liquid helium flow onto the sample into a plastic bag under vacuum) was insufficient to keep the samples frozen for the duration of the data acquisition (3-10 min/scan for these experiments), the data acquired gives insight into changes in the system as the oxidised dye returned to the ground state. While samples were not visible during analysis, no green solution (oxidised dye) was visible when the sample was removed from the sample chamber.

In figure 3.10, the first XAS data collection scan shows the diagnostic pre-edge feature (approximately 2821 eV) indicative of partially covalent XB. By this point in the second scan (10 minutes later), this feature has disappeared suggesting that there is no longer significant XB formation. Interestingly, there is a second less intense pre-edge feature at a lower energy (approximately 2818 eV) than the first pre-edge feature. This feature can be explained by several phenomena. Similar to the pre-edge feature observed in the Cl K-edge spectrum of NOBF$_4$ with Cl$^-$ in the previous section, this pre-edge feature could merely be a result of interactions between the Cl$^-$ and other non-dye species. However, the pre-edge feature in figures 3.10 and 3.11 is at 2818 eV while the 'background' feature corresponding to interactions between NOBF$_4$ and Cl$^-$ in figure 3.6 is at 2824 eV.

The second possible explanation for the feature at 2818 eV is the presence of $\pi$ character in the XB. Any $\pi$-type interactions contributing the XB would be expected to be lower in energy and less intense than the pre-edge feature corresponding to the $\sigma$ character of the XB.

The experiment shown in figure 3.10 was repeated with shorter scans, approximately 3 min/scan (figure 3.11). The first four scans of this experiment also show a pre-edge feature at approximately 2821 eV indicative of partially covalent XB. Additionally, the first two scans also show a feature at approximately 2818 eV. However, in both of these scans, this feature is defined by a single data point and cannot be regarded as conclusive.

To verify if these two pre-edge features are indicative of $\pi$-type interactions in halogen bonding, there are two follow up experiments: (1) repeat experiments (figures 3.10 and 3.11) in a cryostat to prevent the sample melting and (2) perform Cl K-edge XAS of NOBF$_4$ and Cl$^-$ in acetonitrile to determine if interactions between NOBF$_4$ and Cl$^-$ pre-edge give rise to one of the pre-edge features present in figures 3.10 and 3.11. These experiments have not yet been conducted due to the standard time constraints associated
3.3. Solution XAS of Dye-Br

Figure 3.10: Cl K-edge XAS data of oxidised Dye-Br (Dye-Br\(^{+}\)) returning to the ground state dye in the presence of NBu\(_4\)Cl as the sample melts. As the sample is only visible before the first scan and after the final scan, it is not possible to determine when the sample was completely melted. However, as there is little change after the second scan, it is reasonable to assume that the sample was fully melted by the end of the first scan (10 minutes). Each scan lasts approximately 10 minutes (283 data points/scan).
3.3. Solution XAS of Dye-Br

Figure 3.11: Cl K-edge XAS data of oxidised Dye-Br (Dye-Br$^{+}$) returning to the ground state dye in the presence of NBu$_4$Cl as the sample melts. Similar to the data collected in figure 3.10, the sample is only visible before the first scan and after the final scan. Thus, it is not possible to determine when the sample was completely melted. However, as there is little change after the fourth scan, it is reasonable to assume that the sample was fully melted by the end of the third scan (9 minutes). Each scan lasts approximately 3 minutes (81 data points/scan).
3.4 Conclusions

with allocated beamtime at synchrotrons. Although this is only preliminary data, this Cl K-edge XAS data could be the first direct experimental evidence that an XB can have \( \sigma \) and \( \pi \)-type contributions.

3.4 Conclusions

In this chapter, XAS is used to show that only the oxidised dyes are able to form partially covalent coordinate bonds with the Cl\textsuperscript{–} electrolyte. Our data suggests that increasing the covalency of these interfacial XB is directly responsible for the increased dye regeneration rate as the radius of the dye’s halogen substituents increase. These results are particularly suggestive due to the short lifetime of the oxidised dye. The strength of using XAS to probe XB is particularly evident in these systems as the XB occur at a solid-liquid interface which would be difficult to characterise by other spectroscopic techniques.

XAS of the XB acceptor was also conducted on the oxidised Dye-Br in solution. As in section 2.4, a decrease in the pre-edge feature of the these XAS spectra corresponded to the presence of XB. The limitations of the technique prevent us from determining if this decrease in intensity is also accompanied by the expected increase in the energy of the peak (as predicted by molecular orbital theory). An additional feature in the Br K-edge spectra is potentially indicative of \( \pi \)-type character in these XB. This observation is further supported by preliminary Cl K-edge XAS of the XB donor. While these experiments should be repeated for validation, this data could be the first direct experimental evidence of \( \pi \)-type character in XB.

3.5 Methods

Sample Preparation  Sample analyses on beamline 14-3 were prepared on a sensitized, mesoporous TiO\textsubscript{2} film deposited on glass. Each film was stained with Dye-F, Dye-Br, or Dye-I, then sealed with X-ray-transparent polypropylene before being filled with an acetonitrile solution of 100mM NBu\textsubscript{4}Cl. The oxidised forms of the dyes on TiO\textsubscript{2} were obtained via chemical oxidation by washing the films with a saturated solution of NOBF\textsubscript{4} in acetonitrile prior to filling with electrolyte. To account for possible chloride-nitrosonium interactions, an undyed film was also washed with NOBF\textsubscript{4} before filling with electrolyte to serve as a control.

Samples analyses on beamlines 4-3 and 7-3 were prepared as acetonitrile solutions with 1mM dye and 2mM NBu\textsubscript{4}Cl. For samples involving the ox-
3.5. Methods

idised dye (Dye-Br$^+$), a saturated NOBF$_4$ acetonitrile solution was added to a solution of 2mM NBu$_4$Cl and 1mM of the Dye-Br using a syringe. Oxidation of the relevant dye was instantaneous (dark green colour) and to prevent the dye from returning to its ground state (orange colour), solutions were frozen in liquid nitrogen less than 1 second after was NOBF$_4$ solution added.

**XAS data collection**  Cl K-edge XAS data was collected at beamlines 14-3 (TiO$_2$ films), 7-3 (acetonitrile solutions) and 4-3 (acetonitrile solutions) at the SSRL. For beamline 14-3, the samples were analyzed at ambient temperatures and pressures in a helium environment. The beam is unfocused over a size of 1 mm x 6 mm with an energy resolution of approximately $1 \times 10^4 \Delta E E^{-1}$ with ring conditions of 3 GeV and 500 mA to allow for high energy resolution measurements on homogeneous samples. Data points were taken at several points across the surface of the cell to ensure homogeneity. Cl K-edge data collection at beamline 4-3 used a modified low Z setup allowing for low temperature data acquisition under ring conditions of 3 GeV and 500 mA. The setup is a 54-pole wiggler beamline operating in high field (10 kG) mode with a Ni coated harmonic rejection mirror and a fully tuned Si (111) double crystal monochromator. Calibration scans were performed before and after every data set to ensure stable monochromator readings. Signal was detected with a N$_2$ fluorescence (Lytle) detector at ambient temperature and pressure (1 atm, 298 K). Br K-edge XAS data were collected at SSRL on beamline 7-3 under ring conditions 500 mA at 3.0 GeV. This beamline has a 20-pole, 2 T wigglers, 0.8 mrad beam, and a Si (220) double crystal monochromator that was detuned by 50% intensity to attain harmonic rejection. The incident X-ray intensity ($I_0$), sample absorption ($I_1$), and Br reference absorption ($I_2$) were measured as transmittance using argon-filled ionization chambers. Signal was detected with a 30-channel Ge detector. All data was measured at 20 $\pm$ 15 K within an Oxford Instruments CF1208 continuous-flow liquid helium cryostat.

**XAS data analysis**  SixPack was used to calibrate and average XAS spectra [44]. NaCl ($E_0 = 2825.95$ eV) and KBr ($E_0 = 13474$) spectra acquired at the same time as sample data were used as a calibrant for Cl K-edge and Br K-edge spectra, respectively. For data collected on the beamline 14-3 (TiO$_2$ films), BlueprintXAS version 2.7 was then used for background subtraction and normalization. To minimize the user bias introduced during data work-up, BlueprintXAS fits the spline, peaks, and background con-
3.5. Methods

currently [45, 56], while the parameters for each variable are user defined (tables A.5-A.8 for final values for each parameter) The fits were run in AUTO mode as in this mode, a Monte Carlo methodology is used to choose the starting point of each fit to further remove user bias. Each fit contained the following components: edge peak fit; spline + edge peak fit; pre-edge peak fit.
3.5. Methods

Again we probe for XB,

Twixt solid and liquid they be,

And now we can prove,

That they help to move

Charge through the DSSC.
Chapter 4

Using XAS, NMR and CD to Probe Amyloid β Aggregation

The importance of XB is increasing across many fields including biochemistry. While halogenated molecules are less commonly associated with biological systems, halogen substituents are surprisingly common in both biological molecules and drug candidates. There are over 3500 metabolites containing halogens, and both nucleic acids and proteins can undergo halogenation in response to oxidative stress [57–63]. It is likely that some of these systems involve XB. Indeed, upon discovering that XB were responsible for an unexpected structure of brominated DNA, Auffinger and collaborators embarked upon an analysis of Protein Data Bank structures which identified XB in 72 additional structures: 66 protein and 6 nucleic acid systems where intermolecular halogen-oxygen bond distances less than that of the sum of the Van der Waals radii [57]. Other researchers have demonstrated the importance of XB in biological systems by replacing hydrogens with halogen substituents to improve drug binding specificity [64–66] and using XB to stabilize cancer-associated p53 mutants [67].

In this chapter, we will probe one biological system which may benefit from the presence of XB: the inhibition of amyloid β peptide aggregation by fluorescein derivatives. The aggregation of various forms of this peptide into β-sheet oligomers and fibrils is associated with the onset and diagnosis of Alzheimer’s Disease [68, 69]. The two most common forms of the aggregated peptide are Aβ40 and Aβ42, respectively the first 40 or 42 amino acid residues in the Amyloid Precursor Protein. In the search for molecules which prevent Aβ40 aggregation, recent work reveals that small halogenated dyes can modulate Aβ40 aggregation [37]. Furthermore, these studies show that an analogous molecule with two nitro electron withdrawing groups in place of the two halogen substituents is unable to modulate Aβ40 aggregation (discussed further in section 4.1). This result suggests that the electron
4.1 Background: Aβ40 aggregation modulation by fluorescein derivatives

Withdrawing nature of the halogen substituents by itself is insufficient to modulate Aβ aggregation. To investigate whether the importance of halogen substituents is a result of XB in the systems, we will use the XAS methodology discussed in Chapters 2 and 3.

Using XAS to probe for XB in peptide aggregation is particularly advantageous as it removes the need for single crystals, XAS sample requirements being either a solid powder or frozen solution containing the element of interest. Aβ40’s secondary structure is very sensitive to different sample preparation; indeed Aβ40 can interconvert between random coil, α-helix, or β-sheet secondary structures with small changes in pH, temperature, and solvent [70]. This complex behaviour of Aβ40 makes probing for XB in this system an ideal experiment for study by XAS.

In this section, XAS will be coupled with 2D Nuclear Magnetic Resonance (NMR) spectroscopy. As probing Aβ40 aggregation with XAS only provides information about the electronic environment of the halogen substituents, NMR will be used to investigate the binding site of these dyes.

4.1 Background: Aβ40 aggregation modulation by fluorescein derivatives

As mentioned previously, the importance of halogen substituents in the modulation of Aβ40 aggregation was initially identified by Wong and collaborators [37]. This study shows that addition of halogen substituents to fluorescein derivatives (figure 4.1) changes their effectiveness in modulating Aβ40 aggregation when incubated with the peptide. Circular dichroism (CD) experiments reveal that after incubation with fluorescein (FLN) and eosin b (EOB), the secondary structure of Aβ40 is predominantly β-sheet, indicative of aggregation. By comparison, after incubation with eosin y (EOY), phloxine B (PHB), erythrosine B (ERB), and rose bengal (ROB), the secondary structure of the peptide is predominantly random coil, indicative of free monomer (figure 4.2).

This CD data is further supported by transmission electron microscopy and cytotoxicity studies [37]. Transmission electron microscopy images show that same dyes which prevent β-sheet formation in the CD spectra also reduce Aβ fibril formation [37]. The dyes’ reduction of peptide β-sheet formation also correlates with a reduction in the cytotoxicity of the peptide [37].
4.1. Background: \(\alpha_\beta\)40 aggregation modulation by fluorescein derivatives

Figure 4.1: Chemical structures of the six dyes used in \(\alpha_\beta\)40 aggregation modulation studies [37]. Chemdraw structures were produced by the author.
Figure 4.2: CD spectra of Aβ monomer and Aβ aggregates modulated by fluorescein derivatives. (a) CD spectra of Aβ40 monomer and Aβ40 incubated with and without EOB and PHB for 5 days at 37°C. Aβ40 is shown to aggregate in the presence of EOB, while PHB partially prevents aggregation. (b) CD spectra of Aβ40 incubated with and without EOY, ROB and ERB for 5 days at 37°C. Incubation with EOY, ROB and ERB is shown to prevent aggregation. (c) CD spectra of Aβ40 incubated with and without FLN 5 days at 37°C. FLN does not prevent aggregation (© Creative Commons) [37].
4.2 XAS Studies of Aβ40 Aggregation

A follow-up study from the same group investigated the binding site of these dyes via fluorescence quenching and competitive sequence-specific antibody binding studies \[71\]. These studies suggest that the dyes interact with the first 16-22 residues of the Aβ40 peptide. Fluorescence quenching resulting from dye-peptide binding is observed for ERB, EOY, PHB and ROB in the presence of the Aβ11-22 fragment. Furthermore, ERB, EOY, PHB and ROB were shown to inhibit antibody-Aβ40 binding for the first 16 residues of Aβ40 only.

The aim of this chapter is to probe the nature of these dye-peptide interactions by probing for the presence of XB by XAS and examining the location of the binding site with Nuclear Magnetic Resonance (NMR) spectroscopy.

4.2 XAS Studies of Aβ40 Aggregation

Although chlorine, bromine and iodine substituents are all present in the dyes, only Br K-edge XAS is used to probe for XB interactions between the dye and the peptide. To mimic biological environments, the Aβ40 aggregation is studied in a salt buffer solution which includes NaCl. The presence of Cl\(^-\) in the samples at 20 times the concentration of the dyes precludes the use of Cl K-edge XAS. The resulting Cl K-edge XAS spectrum would be difficult to interpret as it would be an average of all chlorine atoms present, not just the chlorine substituents on the dye. Using Br K-edge XAS to probe for XB in these systems is also preferred to I L\(_3\)-edge XAS as the higher energy Br K-edge is associated with less self-absorption and reduced instrument noise given the available beamlines: 30-channel detector for Br K-edge beamline compared with the 1-4 channel detector used at I L\(_3\)-edge beamline. Br K-edge XAS also allows us to probe one of the most interesting outcomes of Wong and collaborators: the fact that EOY can inhibit Aβ40 β-sheet formation while EOB, with two less bromine substituents, cannot.

To investigate these systems, previously published procedures were followed \[72\] with the additional step of agitation at 200 rpm during incubation as Aβ40 is found to only aggregate with the addition of this condition (figure 4.3). As found by Wong and collaborators, β-sheet formation is observed after 5-day incubation at 37\(^\circ\)C for both the peptide alone in the buffer solution and when incubated with EOB. No β-sheet formation is observed when Aβ40 is incubated with EOY and PHB, and the CD data for both is indicative of random coil structure. Interestingly, this data differs slightly from Wong and collaborators’ CD
4.2. XAS Studies of Aβ40 Aggregation

data indicates PHB only partially prevents aggregation (figure 4.2) while the PHB CD spectrum collected by the author is more similar to that of EOY, indicating that PHB is just as effective as EOY at preventing aggregation (figure 4.3). This similarity between the two different dye systems is repeatable (figure 4.4).

With the repeatability of the CD experiments confirmed, Br K-edge XAS analysis of Aβ40 incubated with EOB, EOY, and PHB were then conducted (figures 4.5, 4.6, and 4.7), with XAS of the dyes alone in buffer solution serving as the negative control and the EOB system, in which β-sheet formation is not prevented, serving as the positive control. Samples were incubated for 5 days with daily aliquots removed and flash-frozen for XAS analysis. A lack of significant changes in the pre-edge features of all XAS spectra indicates that no XB formation is observed in these systems. CD spectra are presented for these samples to illustrate that using a different incubator, daily aliquoting, and flash-freezing do not affect peptide aggregation (figure 4.10). As no change was observed in the XAS data over the course of 5 days, and the time-dependent secondary structure of Aβ42 in the presence of these dyes has been extensively studied [72], the time-dependent secondary structure of Aβ40 in the presence of EOB, EOY, and PHB was not investigated.

The procedure outlined by Wong and collaborators uses 10 equivalents of the dyes to prevent β-sheet formation in Aβ40, but any unbound dye will make changes in the XAS spectra smaller and more difficult to observe. Thus, Br K-edge spectra of Aβ40 in the presence of 0.86 equivalents of EOB and EOY were acquired to minimise spectral effects of unbound dyes present in the samples. Small decreases in pre-edge feature intensity is observed for both Aβ40 co-incubation with EOY (figure 4.8) and for the positive control, Aβ40 co-incubation with EOB (figure 4.9). This small decrease observed may be insignificant due to the limitations of the experiments. However, if the decrease in pre-edge feature intensity is attributable to XB formation, it is observed for both systems, and thus XB alone cannot be responsible for EOY’s prevention of Aβ40 β-sheet formation.

As we have shown that Cl− can act as a XB electron donor, XAS data for EOY and PHB in the presence of NBu4Cl was also acquired. These studies investigate whether XB formation with Cl− may prevent XB formation between the dyes and Aβ40. While small changes in the edge and post-edge regions are present in the spectra (figure 4.11), there is no change in the pre-edge feature. The small changes in the post-edge region suggests that the solvation environment of the bromine substituents is changing as the signal in the edge-region arises from scattering. The lack of change in the pre-edge features indicates no change in the electronics of the bromine.
substituents. The lack of change in the electronic environment suggests that there is no XB between solvated Cl\textsuperscript{−} and the dyes.
4.2. XAS Studies of $A\beta_{40}$ Aggregation

Figure 4.3: CD spectra for 30$\mu$M $A\beta_{40}$ incubated at 37°C, 200 rpm, for 5 days with and without 300$\mu$M of EOY, EOB, PHB. The CD spectra of $A\beta_{40}$ in the absence of the dyes and in the presence of EOB is indicative of $\beta$-sheet formation suggesting that aggregation has occurred. The CD spectra of $A\beta_{40}$ in the presence of EOY and PHB is indicative of random coil suggesting that aggregation has been prevented. These experiments were performed to confirm Wong and collaborators findings [37]. Due to high HT (high tension) voltage values (>500V), data below 205 nm was not included. The spectra in this figure are noisier than the rest of the CD data in this thesis due to the age of the light source in the CD instrument. The light source was replaced before the collection of the other spectra.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.4: CD spectra for 30μM Aβ40 incubated at 37°C, 200 rpm, for 5 days with and without 300μM of FLN, EOY, PHB. The CD spectra of Aβ40 in the absence of the dyes and in the presence of FLN is indicative of β-sheet formation suggesting that aggregation has occurred. The CD spectra of Aβ40 in the presence of EOY and PHB is indicative of random coil suggesting that aggregation has been prevented. Due to high HT (high tension) voltage values (>500V), data below 205 nm was not included.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.5: Br K-edge XAS spectra of Aβ40 incubated at 37°C, 200 rpm, with 10x EOY over the course of 5 days. As XAS samples are not recoverable, each spectrum represents a new aliquot from a stock sample. Each aliquot was flash frozen in liquid nitrogen after being removed from the bulk solution. The calibration of these spectra was particularly difficult due to the beamline instability during these samples. These difficulties are evident in the approximately 0.3eV shifts between the spectra in this figure and to a lesser extent in figures 4.6 and 4.7. Despite this, it is evident that there is no significant change in the intensity of the pre-edge feature (13473 eV) indicating no XB formation.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.6: Br K-edge XAS spectra of Aβ40 incubated at 37°C, 200 rpm, with 10x EOB over the course of 5 days. There is no significant change in the intensity of the pre-edge feature (13473 eV) indicating no XB formation. As XAS samples are not recoverable, each spectrum represents a new aliquot from a stock sample. Each aliquot was flash frozen in liquid nitrogen after being removed from the bulk solution. The day 5 sample was lost during this process.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.7: Br K-edge XAS spectra of Aβ40 incubated at 37°C, 200 rpm, with 10x PHB over the course of 5 days. There is no significant change in the intensity of the pre-edge feature (13473 eV) indicating no XB formation. As XAS samples are not recoverable, each spectrum represents a new aliquot from a stock sample. Each aliquot was flash frozen in liquid nitrogen after being removed from the bulk solution. The day 4 sample was lost during this process.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.8: Br K-edge XAS spectra of 1.2 equivalents of Aβ40 incubated with EOY at 37°C, 200 rpm, for 2 days. The length of the beamtime at SSRL prevented a longer incubation time. A small decrease in peak intensity is observed. This is also observed in the EOB positive control (figure 4.9), and could be attributed to noise for both experiments. Thus, if this small decrease is attributable to XB formation, it is not responsible for EOY’s prevention of Aβ40 β-sheet formation.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.9: Br K-edge XAS spectra of 1.2 equivalents of Aβ40 incubated at 37°C, 200 rpm, with EOB for 2 days. The length of the beamtime at SSRL prevented a longer incubation time. A small decrease in peak intensity is observed. This is also observed in the Aβ40 sample incubated with EOY (figure 4.8), and could be attributed to noise for both experiments.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.10: CD spectra for 30 μM Aβ40 incubated at 37°C, 200 rpm, for 5 days with and without 300 μM of EOY, EOB, PHB. These experiments were conducted to probe whether the changes required for the experiments conducted at SSRL affected aggregation. Changes include: incubation at SSRL and daily removal from incubation/agitation to flash freeze aliquots for XAS analysis. Samples were flash frozen and transported back to UBC for CD analysis. As expected, the CD data indicates that Aβ40 incubated with EOB has β-sheet structure while Aβ40 incubated with EOY and PHB has random coil structure. The lack of β-sheet formation in the Aβ40 sample is concerning but as this sample was not analysed by XAS, this is tentatively attributed to the fact that while the Aβ40 sample was removed from the incubator daily, it was not perturbed by aliquoting by syringe. As β-sheet formation was observed in the EOB positive control, the lack of aggregation in the Aβ40 sample was not investigated further due to the resource-intensive nature of repeating this experiment at SSRL. Due to high HT (high tension) voltage values (>500V), data below 205 nm was not included in this thesis.
4.2. XAS Studies of Aβ40 Aggregation

Figure 4.11: Br K-edge XAS spectra of EOY and PHB in presence and absence of Cl⁻.
4.3 Nuclear Magnetic Resonance Spectroscopic Studies of Aβ Aggregation

As XAS reveals that the interaction between EOY and Aβ40 is unlikely due to the presence of XB, the dye-peptide interactions are investigated by 2D proton Nuclear Magnetic Resonance (\(^1\)H-NMR) Spectroscopy. Both Total Correlation Spectroscopy (\(^1\)H-TOCSY) and Nuclear Overhauser Effect Spectroscopy (\(^1\)H-NOESY) data was collected. These two experiments are complementary as TOCSY NMR reveals which protons are in the same amino acid, and NOESY NMR reveals which amino acids are neighbours. In correlation NMR spectroscopy, the transfer of magnetisation, or coupling, between nuclei occurs if the two nuclei are connected through bonds. Total correlation NMR spectroscopy (TOCSY) enables the identification of spin systems, groups of these coupled nuclei where the nuclei in the group are coupled to at least one other nucleus within the group and no nuclei outside of the group. In TOCSY NMR of peptides, these spin systems correlate with amino acids. By contrast, in \(^1\)H-NOESY NMR, magnetisation is transferred through space rather than through bonds. Thus, crosspeaks arise in a \(^1\)H-NOESY spectrum if two protons are within approximately 5 Å.

Titration of EOY with Aβ40 results in changes in many NOESY crosspeaks indicative of a binding interaction between EOY and Aβ40. These changes include appearance of peptide-peptide crosspeaks suggesting unstructured regions of the peptide becoming more rigid upon addition of EOY (figure 4.12). The appearance of NOESY crosspeaks between protons with 7.4 to 7.7 ppm with peptide methyl protons (0.65 - 0.8 ppm) are either additional peptide-peptide crosspeaks or dye-peptide crosspeaks (figure 4.14). These crosspeaks may be direct evidence of the residues to which EOY binds. Furthermore, NOESY peptide-peptide crosspeaks appear upon addition of EOY and then disappear as more equivalents of the dye are added (figure 4.13) and peptide-peptide and dye-dye crosspeaks disappear upon addition of EOY (figures 4.13 and 4.15). These changes are indicative of more complex structural changes which require assignments to elucidate.
Figure 4.12: The same region of the NOESY spectra for Aβ40 in the presence of different EOY concentrations (left) and the NOESY spectrum for the apo-peptide (right) is shown. In the right hand-side plot, dark/light blue, dark/light green, dark/light orange and dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40 in the presence of 0.46mM, 0.67mM, 0.89mM and 1.10mM EOY, respectively. In the left hand-side plot, dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40. Peaks which are present in the spectra of dye with peptide but are absent in the apo-peptide spectrum are indicated with arrows. The different colours indicate coupled systems.
4.3 Nuclear Magnetic Resonance Spectroscopic Studies of Aβ Aggregation

Figure 4.13: A comparison of various $^1$H-NOESY spectra illustrating the appearance and then disappearance of crosspeaks upon mixing EOY with Aβ40. The same region of the NOESY spectra for Aβ40 in the presence of different EOY concentrations (right) and the NOESY spectra for the apo-peptide (middle) and the dye (left) is shown. In left hand-side plot, dark/light blue, dark/light green, dark/light orange and dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40 in the presence of 0.46mM, 0.67mM, 0.89mM and 1.10mM EOY, respectively. In middle plot, dark/light blue corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40. In right hand-side plot, dark/light blue corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40. Peaks which are present in the spectra of dye with peptide but are absent in the apo-peptide spectrum are indicated with arrows. Pink arrows indicate peaks which appear upon addition of 1 equivalent of the dye but disappear as more dye is added. Red arrows indicate peptide peaks which disappear as more dye is added.
4.3. Nuclear Magnetic Resonance Spectroscopic Studies of Aβ Aggregation

Figure 4.14: A comparison of various $^1$H-NOESY spectra illustrating the appearance of additional crosspeaks upon mixing EOY with Aβ40. The same region of the NOESY spectrum for the apo-peptide (top) and the NOESY spectra for Aβ40 in the presence of different EOY concentrations (bottom) is shown. In the top plot, dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40. In the bottom plot, dark/light blue, dark/light green, dark/light orange and dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40 in the presence of 0.46mM, 0.67mM, 0.89mM and 1.10mM EOY, respectively. The comparison of these spectra shot that many amide/aromatic proton to methyl proton cross-peaks appear upon addition of the dye.
Figure 4.15: A comparison of various $^1$H-NOESY spectra illustrating the disappearance of apo-dye and apo-peptide crosspeaks upon mixing EOY with Aβ40. The same region of the NOESY spectra for Aβ40 in the presence of different EOY concentrations (right) and the NOESY spectra for the apo-peptide (middle) and the dye (left) is shown. In left hand-side plot, dark/light blue, dark/light green, dark/light orange and dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40 in the presence of 0.46mM, 0.67mM, 0.89mM and 1.10mM EOY, respectively. In middle plot, dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40. In right hand-side plot, dark/light red corresponds to the +/- peak phases of the NMR spectrum of 0.46 mM Aβ40. Arrows indicate peaks that are present in either the apo-dye spectrum or the apo-peptide spectrum but not in the spectra of the mixture. Two arrows of the same colour indicate the above- and below-diagonal crosspeaks for the same two protons.
4.3. Nuclear Magnetic Resonance Spectroscopic Studies of Aβ Aggregation

Overlap in the amide region of the 1D spectrum of the peptide prompted the addition of d$_3$-TFE to the NOESY/TOCSY NMR sample to increase peak separation in amide region as done in the literature [70, 73, 74]. While the separation of amide peaks aids assignment, the broad solvent peaks of d$_3$-TFE and water at 3.75 ppm and 4.75 ppm presumably also hides a significant number of H$_\alpha$ crosspeaks. Consequently, the spin systems identified in the TOCSY spectra are likely incomplete, and each spin systems identified corresponds with a minimum of ten of the forty residues. Thus, assignment by hand was not feasible. In an effort to solve this, python algorithms were written by the author to aid with identifying spin systems in the TOCSY spectrum of the apo-peptide and finding corresponding crosspeaks in the NOESY spectrum (appendices B.2 and B.1). The logic of these programs is depicted in flowcharts (figures 4.16 and 4.17).

Figure 4.16: The algorithm logic for the python program developed for TOCSY analysis. Decisions are in blue.

The data from above and below the diagonal in the TOCSY and NOESY spectra was separated in analysis as this provides an informal validation
An additional assignment tool was developed by the author which can take any NMR spectral data published in the Protein Data Bank and generate a TOCSY spectrum for that peptide or protein (figure 4.18, appendix B.3). The program also overlays the user’s spectrum on top of the simulation of the TOCSY spectrum for the TOCSY data. Despite the similar experimental conditions, there is little overlap between the user’s data and the simulated TOCSY spectrum [75]. However, this tool can be used to generate a few possible assignments including the arginine (R5) residue highlighted in the image.
4.3. Nuclear Magnetic Resonance Spectroscopic Studies of Aβ Aggregation

Figure 4.18: A screenshot of the TOCSY generator program which can take any NMR spectral data published on the Protein Data Bank and generate a TOCSY spectrum for that peptide or protein and overlay the users data (black x’s). The data from the Protein Data Bank is displayed with a rainbow colour scheme to help identify where the residue lies in the amino acid sequence. A mouse-over tool enables the user to identify a peak of interest (here R5 or arginine 5, the 5th residue in Aβ40, is being pointed to by the cursor).
4.3. Nuclear Magnetic Resonance Spectroscopic Studies of A\textsuperscript{\beta} Aggregation

Using the distance restraints from the NOESY data, a network graph of nearby spin systems is generated (figure 4.19). Lowering the constraint from 0.007 ppm to 0.006 ppm for two peaks to be considered as the same proton significantly reduces the number of distance restraints, providing additional validation for the remaining distance constraints. While the assignment of these spin systems is ongoing, the only spin systems present which can correspond to a glycine residue are 15 and 17 due to the presence of either a proton with a chemical shift less than 2 ppm or multiple protons with chemical shifts above 6 ppm in the remaining spin systems. Due to the preponderance of glycine residues in C-terminus half of A\textsuperscript{\beta}40, five glycine residues in total with a maximum of three non-glycine sequential residues in the last 18 residues of the peptide, it is likely that the spin systems in this network graph are in the N-terminus half of the peptide. As these spin systems correspond to the changing NOESY crosspeaks discussed earlier, the current data suggests that the changes observed in the NOESY spectrum result from structural changes in and EOY binding to the first half of the peptide.
4.3. Nuclear Magnetic Resonance Spectroscopic Studies of Aβ Aggregation

Figure 4.19: Network graphs of spin system distance restraints. The constraint for two peaks to be correspond to the same proton is (a) 0.006 ppm and (b) 0.007 ppm. Spin systems were generated with the TOCSY python program (appendix B.1) and the spin system numbers refer to the list of spin systems in the relevant text file (appendix C). Distance restraints were generated using the NOESY python program (appendix B.2). Line thickness correlates to the NOESY crosspeak intensity.
4.4 Conclusion

Previously reported results showing that the aggregation of Aβ40 peptide is modulated by halogenated fluorescein derivatives was verified. Using Br K-edge XAS, we showed that XB interactions are not responsible for the importance of the halogens in these systems. No XB were found in these systems. Although no XB were found in this system, this is nonetheless an illustration that XAS can be used to probe complex biological systems. With the prevalence of halogenated substituents in biological systems, this use of XAS could provide insight into other complex systems.

As XAS suggests that XB are not responsible for EOY’s ability to bind to Aβ40, 1H-TOCSY and 1H-NOESY NMR experiments were used to further probe this interaction. The disappearance of apo-peptide and apo-dye peak in addition to the appearance of peptide-peptide crosspeaks as more equivalents of EOY are added to the Aβ40 sample are a clear indication of a binding interaction between the dye and the peptide. While the Hα region of the spectra was obscured by presence of the TFE solvent peak, headway has been made on the assignment of these spectra using the author’s python programs. While the assignment of these spectra is ongoing, current NMR data supports the literature finding that EOY binds to the first 22 residues of the Aβ peptide.

As the NMR data, supported by the literature, suggests that EOY does bind to Aβ40 while the XAS data suggests there are no XB, the halogen substituents must be responsible for other electrostatic phenomena which enable the dye-peptide interaction. One possible explanation is that the halogen substituents are important in how they change the electronics of fluorescein scaffold to which they are attached, for example in increasing the overall hydrophobicity of the molecule and promoting peptide-dye cation π interactions. However, the lack of binding between EOB which has two non-halogen electron withdrawing substituents and Aβ40 [71] suggests that this isn’t the sole role of the halogens. Instead, the region of negative charge surrounding the positively-charged σ-hole of halogen substituents might interact electrostatically with positively-charged residues on the peptide. Such an interaction in combination with the increased hydrophobicity of the dye could strengthen π-π interactions between the dye and the peptide. We hope to further elucidate the nature of this interaction once the NMR assignments are complete.


4.5 Methods

Preparation of Peptide Thin Films. A\textsubscript{40} was purchased from Anaspec as a lyophilised powder. 1.0 mg of A\textsubscript{40} was dissolved in 1 ml of hexafluoroisopropanol by sonication for 5 minutes. The solution was stored overnight at room temperature and then aliquoted into 12 vials. Aliquots were dried with a gentle stream of compressed air perturbing the surface of the liquid until a thin film was visible. The thin films were left in a vacuum dessicator overnight and then stored at -20\textdegree C until needed for aggregation experiments.

Aggregation of A\textsubscript{40} for all CD and XAS experiments. A\textsubscript{40} preparation was based on published studies [72]. A\textsubscript{40} thin films were reconstituted by sonication for 10 minutes ([A\textsubscript{40}] = 300 \mu M) in the following solution: 300 \mu M CH\textsubscript{3}CN, 300 \mu M Na\textsubscript{2}CO\textsubscript{3}, 250 \mu M NaOH. Buffer solution (8.5 mM NaCl, 14 \mu M Na\textsubscript{2}CO\textsubscript{3}, 0.85mM NaOH, 6.0\% CH\textsubscript{3}CN) and dye stock solution (10 mM dye in buffer solution) were added to the dissolved A\textsubscript{40} to yield a solution with 10 equivalents of dye to peptide (30 \mu M dye and 30 \mu M A\textsubscript{40}). An equivalent volume of buffer solution was used in place of dye stock solution when A\textsubscript{40} was prepared in absence of dyes. Solutions were incubated at 37\textdegree C and agitated at 200 rpm for 5 days. For the experiments in figures 4.8 and 4.9, the initial amount of A\textsubscript{40} dissolved was increased to yield final concentrations of approximately 350 \mu M A\textsubscript{40} and 300 \mu M dye.

CD data collection. Circular Dichroism (CD) data was collected on a JASCO J-815 CD spectrometer with an air cooled xenon arc lamp from 190-250 nm. Due to high HT (high tension) voltage values (>500V), data below 205 nm was not included in this thesis.

XAS data collection and analysis. Br K-edge XAS data was collected at the SSRL beamline 7-3 under ring conditions 500 mA at 3.0 GeV. This beamline has a 20-pole, 2 T wigglers, 0.8 mrad beam, and a Si (220) double crystal monochromator that was detuned by 50\% intensity to attain harmonic rejection. The incident X-ray intensity (I0), sample absorption (I1), and Br reference absorption (I2) were measured as transmittance using argon-filled ionization chambers. Signal was detected with a 30-channel Ge detector. All data was measured at 20 \pm 15 K within an Oxford Instruments CF1208 continuous-flow liquid helium cryostat. SixPack was used to calibrate, average, and normalise XAS spectra [44]. KBr (E\textsubscript{0} = 13474) spectra acquired at the same time as sample data was used as a calibrant for Br K-edge spectra.
**NMR sample preparation.** An Aβ40 thin film was dissolved in dissolving solution by sonication for 10 minutes. Buffer solution, deuterated water (D$_2$O), and deuterated trifluoroethanol (d$_3$-TFE) were added according to table [4.1]

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<td>d$_3$-TFE</td>
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Table 4.1: NMR sample preparation by amount of each component.

**NMR data collection.** $^1$H-TOCSY (TOtal Correlation SpectroscopY) and $^1$H-NOESY (Nuclear Overhauser Effect SpectroscopY) experiments were collected on an Bruker 850MHz instrument. Mixing times were 150 ms and 60 ms for $^1$H-NOESY and $^1$H-TOCSY, respectively. 64 scans ($^1$H-TOCSY) and 128 ($^1$H-NOESY) were collected over a 8150 x 256 (F2 x F1) time domain. A water suppression pulse sequence was used for both experiments.
In bio, it’s found frequently,

That binding is helped by XB,

But for amyloid beta,

The XAS data

Shows there’s no XB to see.
Chapter 5

Conclusions and Future Directions

The strength of this work lies in providing evidence of the partially covalent nature of XB and establishing a technique which is able to probe XB in a wide variety of systems. In XB involving a halide electron donor, a diagnostic pre-edge feature in the XAS spectrum of the XB acceptor is indicative of the XB. This feature requires the presence of XB as it corresponds to an electron transition to the antibonding orbital of the XB. The weakness of this technique is that it requires a halide XB electron donor. Other common XB donors, oxygen, sulphur, and nitrogen, for example, are more difficult to study with XAS due to atmospheric attenuation and self-absorption associated with Z<16 elements.

In an effort to establish a XB characterisation methodology which is universally applicable, XAS of the XB electron acceptor was also investigated. As XB electron acceptors are, by definition, halogens, the problems associated with XAS of lighter elements are avoided. We showed that XAS of XB electron acceptors can also characterise XB. Although the XB-associated changes in the XAS spectra of XB electron acceptors are not as pronounced as the changes in the XAS spectra of XB electron donors, diagnostic trends are still observed. These trends result from electron transitions to the antibonding orbital of the covalently bound halogen changing as orbital overlap with the electron donor changes.

This ability to probe XB with XAS is illustrated by the work in Chapters 2-4 of this thesis. In the halide trapping systems of chapter 2, XAS shows that a XB of comparable strength to a HB exhibits charge transfer more similar to that of a coordinate bond (section 2.2). The charge transfer to each halogen decreases as a single electron donor is involved in more intermolecular interactions (section 2.4). Also in Chapter 2, probing for XB in KBr and I\textsubscript{2} frozen solutions by XAS shows that this technique can explore the stoichiometry of these bonds (section 2.3). Indeed, the Br\textsuperscript{−} charge donation to I\textsubscript{2} reaches a maximum when I\textsubscript{2} is present in 1.3x stoichiometric excess. These results suggest that while each Br\textsuperscript{−} can be involved in multi-
ple XB, the overall charge donation from Br\(^-\) cannot surpass 1.3 times the charge donation of Br\(^-\) involved in 1 XB.

In Chapter 3, the ability of XAS to probe non-crystalline systems enables us to look for XB in DSSC. DSSC are a particularly challenge for many analytical methods as key interactions occur at the solid-liquid interface between the TiO\(_2\)-bound dyes and the electrolyte. We have used XAS to confirm the presence of XB in the DSSC and probe the relationship between XB covalency and regeneration rate. Although it was necessary to use Cl\(^-\) in place of the I\(^-\) electrolyte, our results still suggest that increasing XB covalency increases dye regeneration rate and consequently overall DSSC efficiency.

In Chapter 4, XAS is used to probe another challenging system for XB: the modulation of Alzheimers’-associated A\(_\beta\)40 peptide aggregation by fluorescein-derivatives. This system again poses unique challenges for analysis as the peptide is expected to aggregate. Like the DSSC, XAS allows us to probe this systems despite its complex environment. In this system, the XAS data suggests that partially covalent XB are not present in these systems. Thus, the importance of halogen substituents in preventing aggregation does not arise from halogen bonding. Furthermore, TOCSY and NOESY NMR experiments are used to probe the role of the EOY in inhibiting A\(_\beta\)40 peptide aggregation. This preliminary data supports the current literature findings that these dyes interact with the first half of the A\(_\beta\)40 peptide.

The use of XAS to probe for XB is in its infancy, and there are many interesting systems still to be explored. The author has facilitated two future collaborations to be explored by the Kennepohl group with Dr. Robin Perutz and Dr. Marta Mosquera to use this technique to probe XB in other systems. A recent publication from the Perutz group [76] explores the use of fluorides involved in a metal-fluoride coordinate bond as electron donors in XB. This work investigates trends in XB geometry and strength using X-ray crystallography and \(^{19}\)F solid state NMR. The intended collaboration will explore how charge transfer correlates with bond strength in these systems by looking at the XAS of both the XB electron acceptors and donors.

The collaboration initiated by the author with Dr. Mosquera aims to probe for the presence of XB facilitating backbonding in nearby coordinate bonds. Recent work by Dr. Mosquera probed XB-based networks involving ruthenium complexes in the presence and absence of XB where the halide ligands act as both the electron donor in the XB and in the coordinate bond [77]. In all of the systems studied, with one exception, the ruthenium-halide bond distance was shown to increase in the presence of XB. This
increase in bond length due to the presence of the competing XB interaction. However, an unexpected decrease in the length of the ruthenium-bromide bond length is observed when the bromide ion also acts as an electron donor in a halogen bond with Br₂. We propose this may be due to π backbonding from the Ru to the σ*XB orbital. As the σ*XB orbital is perpendicular to the Ru, it may allow the Br⁻ to act as a π acceptor ligand in the coordinate bond (figure 5.1). We propose to probe the nature of the interaction with Ru and Br K-edge X-ray absorption spectroscopy. If our hypothesis is correct, this will be the first example in the literature of XB facilitating π backbonding in a coordinate bond involving a ligand which is traditionally σ/π donor.

Initial experiments have already been conducted for this project and the project will be continued by the author’s co-worker.

Figure 5.1: (a) XB network involving Br⁻ and Br₂ studied by Mosquera and collaborators. XB networks involving different combinations of X⁻ and X₂ where X = Cl, Br, I were also studied [77]. (b) and (c) Depictions of the σ*XB to illustrate that this orbital is approximately perpendicular to the Ru-Br coordinate bond. This orientation may facilitate an interaction between the unoccupied σ*XB and the occupied Ru dₓz orbital. This interaction may enable the Br⁻ to act as a π acceptor in the coordinate bond, resulting in a decrease in Ru-Br bond length. For clarity, Ru and its orbitals have been depicted in blue and the atoms and orbitals involved in the XB of interest are depicted in green.
In the literature, XB are often described as non-covalent interactions \[13, 25, 67\]. This perceived lack of covalency means that their applications remain structural, i.e. XB in anion recognition \[13, 16, 17, 26, 40\], molecular self assembly \[13, 23\], and facilitating binding in biological systems \[64–67\].

Our work in chapter 3 demonstrating the ability of these bonds to facilitate dye regeneration by electron transfer illustrates that the role XB play in systems is also electronic. It is important to consider the charge transfer in these bonds to fully realise their applications. These bonds may already play important roles in catalysis and electron transfer systems without our realising, and understanding the role of the XB would allow us to further optimise these systems. To change the perception in the literature we propose the use of an arrow to represent XB. An arrow instead of a dotted line emphasises the charge transfer present in XB and could thus facilitate the chemistry community to think of these bonds as weak coordinate bonds which can exhibit \(\sigma/\pi\) donor and acceptor characteristics rather than purely electrostatic interactions facilitating structural changes. Furthermore, XB do in fact align with the IUPAC definition of a dative bond as it is a partially covalent bond involving in two atoms, “one of which serves as a donor and the other as an electron acceptor” and that “minimum-energy rupture... follows a heterolytic bond cleavage” \[6\].
Chapter 5. Conclusions and Future Directions

For two atoms involved in XB,

Electrons are shared partially.

Let’s not think too narrow

And instead use an arrow

To emphasize covalency!
Bibliography


Bibliography


Bibliography


[65] Leo A. Hardegger, Bernd Kuhn, Beat Spinnler, Lilli Ansehn, Robert Ecabert, Martine Stihle, Bernard Gsell, Ralf Thoma, Joachim Diez, Jörg Benz, Jean-Marc Plancher, Guido Hartmann, Yoshiaki Isshiki, Kenji Morikami, Nobuo Shimma, Wolfgang Haap, David W. Banner,


Appendix A

BlueprintXAS Parameters

In this appendix are the final averages of all fitting parameters from Blueprint-XAS for 1-Cl, 2-Cl, 3-Cl and 4-Cl and the Dye-X series. As the fits for 1-Br, 2-Br, 3-Br and 4-Br were deemed inconclusive, the final fitting parameters for these spectra are not included.

General method for setting parameter restrictions in fits:

- $E_0$ range/spline lower limit: energy of the edge peak +5 eV
- Peak intensity ranges (I1 and I2): limits must be sufficiently large to avoid truncating solution distribution
- Energy of peak: ±1-2 eV from visual peak maximum
- % Gaussian contribution to shape of peak (G#) 40-60%
- Background lower limit: approximately 20 eV range below $E_0$
### Appendix A. BlueprintXAS Parameters

#### Table A.1: XAS fit parameters for $^{13}$Cl

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### Appendix A. BlueprintXAS Parameters

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## Appendix A. BlueprintXAS Parameters

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Table A.3: XAS fit parameters for 3-C1
### Appendix A. BlueprintXAS Parameters

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Table A.4: XAS fit parameters for 4-Cl
### Appendix A. BlueprintXAS Parameters

#### Table A.5: XAS fit parameters for NOBF$_4$ + Cl$^-$

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#### Table A.6: XAS fit parameters for Dye-F$^+$: Cl$^-$

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### Appendix A. BlueprintXAS Parameters

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Table A.7: XAS fit parameters for Dye-Br⁺⁺⁺ → Cl⁻⁻⁻

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Table A.8: XAS fit parameters for Dye-I⁻⁻⁻ → Cl⁻⁻⁻
Appendix B

Python Programs

This appendix consists of a list of the python programs written by me and used to aid in workup of the Aβ peptide NMR data. The code for these programs is reported here with syntax highlighting using the opensource LaTeX package “minted”. A guide for the syntax highlighting is below.

```python
# This is a comment within a program. Comments give the user a
# rough idea of how a program works.
''' 3 apostrophes also denotes a comment '''

"One or two apostrophes denotes a string of text. This is a string"

3 # Numbers and numerical operators are in gray

print "this string"  # Purple denotes Python specific commands

some_variable = 3 # Black denotes user defined
     # variables and their properties

def function: # Light green denotes user defined functions

B.1 TOCSY Program - finding spin systems

The purpose of the TOCSY program is to find all possible amino acid spin systems using a list of the peaks picked by the user in Topspin software. The program uses the peak list file generated by exporting the peak list from Topspin. Changing the numerical values for the _same_pk and _same_sys changes how close in (ppm) two peaks must be to be considered as two peaks which correspond to the same proton (_same_pk) or to be identified as within the same spin system (_same_sys).

#Extracts Spin Systems from TOCSY Peaks
import sys
```
with open("TOCSY_2_2_peak.txt", "r") as file:
    i = 0
    pk_mtrx_0 = list()
    for line in file:
        if i > 7:
            peak = [float(num) for num in line.split()]
            pk_mtrx_0.append(peak)
        i += 1

'''
Change the _same_pk and _same_sys to change
how close in (ppm) two peaks must be to be
considered as the same peak or to be identified as
within the same spin system, respectively.
'''

_same_pk = 0.011
_same_sys_0 = 0.007

file1 = open("generated_spins.txt", "w")

'''
## Use the following 3 lines for debugging ##
for get_pk in pk_mtrx:
    print get_pk[3], get_pk[4]
sys.exit(0)
'''


def get_spin_sys(pk_mtrx, _same_sys):
    spin_sys = list()
    # Get individual peaks from pk_mtrx as list (get_pk)
    for i in range(len(pk_mtrx)):
        get_pk = pk_mtrx[i]
        pk = [get_pk[3], get_pk[4]]
        pk_in_sys = list()
        pk_sys_list = list()
        # Inputs first peak as first spin system
        if i == 0: spin_sys.append(pk)
        # Get individual spin systems from spin_sys as list(get_sys)
        elif i > 0:
new_sys = True
for j in range(len(spin_sys)):
    get_sys = spin_sys[j]
    # Checks if current peak (get_pk) is in
    # current spin system (get_sys)
    if any([abs(get_sys[h] - pk[0]) <= _same_sys
             for h in range(len(get_sys))])
        or any([abs(get_sys[g] - pk[1])
                <= _same_sys for g in range(len(get_sys))]):
        pk_in_sys.append(j)
        pk_sys_list.append(get_sys)
        new_sys = False

# If peak is in 1 or more spin system, this code creates new
# spin system with peak and all spin systems which include
# peak
if new_sys == False:
    get_sys = spin_sys[pk_in_sys[0]]
    for n in range(len(pk)):
        if all([abs(get_sys[p] - pk[n]) >= _same_sys
                 for p in range(len(get_sys))]):
            get_sys.append(pk[n])

    if len(pk_in_sys) >= 2:
        for m in range(1, len(pk_in_sys)):
            check_sys = spin_sys[pk_in_sys[m]]
            for n in range(len(check_sys)):
                if all([abs(get_sys[p] - check_sys[n])
                        >= _same_sys for p in
                        range(len(get_sys))]):
                    get_sys.append(check_sys[n])
                    #spin_sys.remove(spin_sys[pk_in_sys[m]])

for i in range(len(pk_sys_list)):
    if i > 0:
        del_sys = pk_sys_list[i]
        spin_sys.remove(del_sys)
spin_sys[pk_in_sys[0]] = get_sys

# If new peak is not in any spin system,
# create new spin system
if new_sys == True: spin_sys.append(pk)
return spin_sys
# Creates matrix of spin systems which contain # too many values (i.e. biologically impossible)
def too_long(spin_sys):
    long_sys = list()
    for sys in spin_sys_0:
        if len(sys) > 12: long_sys.append(sys)
        # "12" = max # of protons in an AA in Abeta40
    return long_sys

# Identify which peaks belong to unreasonably # large (len>12) spin systems
def extract_pks(pk_mtrx, long_sys, _same_sys):
    new_pk_mtrx = list()
    for sys in long_sys:
        for spin in sys:
            for pk in pk_mtrx:
                if any([abs(spin-pk[x]) <= _same_sys
                         for x in range(3,5))]:
                    new_pk_mtrx.append(pk)
    return new_pk_mtrx

# Prints # of spin system and sorted spin_sys # (from greatest to least), no return value
def print_sys(spin_sys):
    sys_count = 0
    for j in range(len(spin_sys)):
        spin_sys[j].sort(reverse = True)
        if len(spin_sys[j]) > 2: sys_count +=1
    spin_sys.sort()
    for sys in spin_sys:
        print sys
        if len(sys) < 13 and len(sys) > 2:
            for s in sys:
                file1.write(str(s)+"   ")
            file1.write("\n")
        print "number of spin systems which don’t" +
        " correspond to a single peak:" +str(sys_count)

spin_sys_0 = get_spin_sys(pk_mtrx_0,_same_sys_0)
print_sys(spin_sys_0)
B.2. NOESY Program - finding neighbouring spin systems

# Looks for unreasonably large spin systems, # extracts peaks and finds spin systems for # progressively smaller allowed difference between # two peak values
new_spin_sys = list()
check_sys = spin_sys_0
check_val = _same_sys_0
while any(len(sys) > 12 for sys in check_sys) and check_val > 0:
    check_sys = too_long(check_sys)
    redo_pks = extract_pks(pk_mtrx_0, check_sys, check_val)
    check_val -= 0.001
    check_sys = get_spin_sys(redo_pks, check_val)
    print len(check_sys)
for sys in check_sys: sys.sort(reverse = True)
for sys in check_sys:
    if len(sys) <= 12 and any(nsys == sys for nsys
        in new_spin_sys) == False:
        new_spin_sys.append(sys)  # appends sys to new_spin_sys # appends sys to new_spin_sys
        if sys is not in new_spin_sys already
        print_sys(new_spin_sys)

file1.close()

B.2 NOESY Program - finding neighbouring spin systems

The purpose of the NOESY program is to read in a file containing the spin systems identified by the TOCSY program in the previous section, and then determine which of those spin systems must be near enough to generate a peak in the NOESY spectrum. To use the spin systems generated by the previous program, copy/paste the command line output of the TOCSY program into a text file named "generated_spins.txt".

#2D NMR data processing
import matplotlib.pyplot as plt

generate_spins.txt is a text file of the spins system printed
B.2. NOESY Program - finding neighbouring spin systems

by the TOCSY program in the previous program

```
with open("generated_spins.txt", "r") as file0:
    i = 0
    spin_sys = list()
    for line in file0:
        if i > 0:
            shifts = [float(num) for num in line.split()]
            spin_sys.append(shifts)
        i += 1
```

```
## reads in peaklist generated by Topspin for NOESY data
with open("NOESY_1_1.txt", "r") as file:
    i = 0
    pk_mtrx = list()
    for line in file:
        if i > 0:
            peak = [float(num) for num in line.split()]
            pk_mtrx.append(peak)
        i += 1
```

The aim of the following code is to find all peaks in the
NOESY spectrum which belong to a spin system identified
in the TOCSY spectrum by doing the following:

The following code finds F2 values for NOESY peaks
which correspond to a proton in one of the TOCSY spin
systems identified.

The peaks of interest are added to "pks1"
Each entry of "pks1":
[corresponding TOCSY spin system #,
    NOESY F2 value, NOESY F1 value]

The final value for x is the number of peaks
in the NOESY spectrum which correspond
to a TOCSY spin system.
B.2. NOESY Program - finding neighbouring spin systems

```python
x = 0
pks1 = list()
for i in range(len(pk_mtrx)):
    get_pk = pk_mtrx[i]
    for j in range(len(spin_sys)):
        get_sys = spin_sys[j]
        for h in range(len(get_sys)):
            if abs(get_pk[1]-get_sys[h]) <= 0.01:
                print j+2,(get_pk[1],get_pk[2])
                pk = [j+2, get_pk[1],get_pk[2]]
                pks1.append(pk)
                x += 1

print x
```

The aim of the following code is to find neighbouring spin systems by identifying all peaks in the NOESY spectrum with both F2 and F1 values (i.e. chemical shifts) corresponding to spin systems identified in the TOCSY spectrum by doing the following:

The code searches through the peaks in "pks1" to identify which peaks also have an F1 value (chemical shift) corresponding to an identified spin system.

The peaks of interest are added to "pks2"
Each entry of "pks2":
[F2's corresponding TOCSY spin system,
   F1's corresponding TOCSY spin system,
   NOESY F2 value, NOESY F1 value]

The final value of y is the number of peaks in the NOESY spectrum where both F2 and F1 values correspond to a COSY spin system.

```python
neighbours = list()
y = 0
pks2 = list()
```
B.3 Generate TOCSY Spectrum Program

```python
for i in range(len(pks1)):
    get_pk = pks1[i]
    for j in range(len(spin_sys)):
        get_sys = spin_sys[j]
        for h in range(len(get_sys)):
            if abs(get_pk[2]-get_sys[h]) <= 0.005:
                get_pk.insert(0, j+2)
                print(get_pk)
                neighbours.append([get_pk[0],get_pk[1]])
                get_pk = get_pk[1:]
                y += 1

print y
```

The following code graphs the peaks in the NOESY spectrum which give distance restraint information about two of the identified spin systems in the TOCSY data.

```python
AA1,AA2 = zip(*neighbours)
plt.scatter(AA1,AA2, color = "b")
plt.scatter(AA2,AA1, color = "b")
plt.show()
```

B.3 Generate TOCSY Spectrum Program

This program compares user acquired TOCSY NMR data with NMR data published on the Protein Data Bank (PDB). The data from the PDB is reported as chemical shift values for each proton. This program will use these chemical shift values to generate the expected TOCSY spectrum for the published values. This code can be used to compare TOCSY NMR data for any peptide with any available data for the same or a similar peptide published on PDB (provided the proton chemical shifts are reported). The code is currently generates a TOCSY spectrum for any human Aβ40 peptide PDB data, but to use this for another peptide, simply replace the Aβ40 one-letter amino acid sequence with that of the desired peptide. The file names will also need to match the user’s filenames for the PDB chemical shift file and the TOCSY peaklist exported from Topspin.
# Generate TOCSY spectrum program

```python
# Generate TOCSY spectra for Abeta40
# peptide from BRMB 17764 (published on PDB)

import matplotlib.pyplot as plt
import matplotlib.colors as colors
import matplotlib.cm as cmx
from collections import defaultdict

# Input one letter code sequence for peptide of interest here
peptide = "DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV"

jet = cm = plt.get_cmap("jet")
values = range(len(peptide))
cNorm = colors.Normalize(vmin=0, vmax=values[-1])
scalarMap = cmx.ScalarMappable(norm=cNorm, cmap=jet)
print scalarMap.get_clim()

tocsy_pts = defaultdict(list)

# Opens file downloaded from PDB store info in chem_shifts
with open("PDB_shifts.txt", "r") as file0:
  i = 0
  chem_shifts = list()
  for line in file0:
    if i > 62:
      atom_info = [float(value) for value in line.split()]
      chem_shifts.append(atom_info[5:11])
    i += 1

spin_sys = defaultdict(list)
for i in range(len(chem_shifts)):
  atom = chem_shifts[i]
  if len(atom) > 1:
    key = atom[1]+atom[0]
```

Generates dictionary 'spin_sys' with chemical shifts of each AA (key is 3 letter AA code)
B.3. Generate TOCSY Spectrum Program

```python
if "H" in str(atom[3]):
    spin_sys[key].append(float(atom[5]))

'''
Generates dictionary with all F2,F1 points for
TOCSY spectra (key is 3 letter AA code)
'''
for key in spin_sys:
    aa = spin_sys[key]
    f2f1 = list()
    for i in range(len(aa)-1):
        for j in range(len(aa)):
            if j > i: f2f1.append((aa[i],aa[j]))
    tocsy_pts[key].append(f2f1)

def plot_AA(ax1, aa, key, aa_loc):
    key_loc = key + str(aa_loc+1)
    scalarMap = scalarMap.to_rgba(values[aa_loc])
    f2f1 = list()
    f2f1.append(tocsy_pts[key_loc])
    F1 = []
    F2 = []
    for list_j in f2f1:
        for list_k in list_j:
            for point in list_k:
                F2.append(point[0])
                F1.append(point[1])
    ax1.scatter(F2,F1, label = aa + str(i+1),
                color = scalarMap.to_rgba(values[aa_loc+1]),
                s = 10+10/(aa_loc+1))
```

'''Returns data for making invisible mouse over plot'''
label = aa + str(i+1)
xdata_add = F2
xdata_add = xdata_add + F1 #adds points below the diagonal
ydata_add = F1
ydata_add = ydata_add + F2 #adds points below the diagonal
return xdata_add, ydata_add, label

''''Defines mouse over event''''
def hover(event):
    vis = annot.get_visible()
    if event.inaxes == ax1:
        cont, ind = sc.contains(event)
        if cont:
            update_annot(ind)
            annot.set_visible(True)
            fig.canvas.draw_idle()
        else:
            if vis:
                annot.set_visible(False)
                fig.canvas.draw_idle()

def update_annot(ind):
    pos = sc.get_offsets()[ind['ind'][0]]
    annot.xy = pos
    for n in ind['ind']: text = m_label[n]
        #text = "{}, {}".format(" ", join(list(map(str,ind['ind']))),
        " ", join([m_label[n] for n in ind['ind']]))
    annot.set_text(text)
    #annot.get_bbox_patch().set_facecolor
    (cmap(norm(c[ind['ind'][0]])
    #annot.get_bbox_patch().set_alpha(0.4)

fig, ax1 = plt.subplots()

xdata = list()
ydata = list()
m_label = list()

for i in range(len(peptide)):
    letter = peptide[i]
    if letter == "A":
        xtemp, ytemp, ltemp = plot_AA(ax1, "A", "ALA", i)
        for t in xtemp: xdata.append(t)
        for t in ytemp: ydata.append(t)
        for i in range(len(xtemp)): m_label.append(ltemp)
    elif letter == "C":
B.3. Generate TOCSY Spectrum Program

```python
xtemp, ytemp, ltemp = plot_AA(ax1, "C", "CYS", i)
for t in xtemp: xdata.append(t)
for t in ytemp: ydata.append(t)
for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "D":
    xtemp, ytemp, ltemp = plot_AA(ax1, "D", "ASP", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "E":
    xtemp, ytemp, ltemp = plot_AA(ax1, "E", "GLU", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "F":
    xtemp, ytemp, ltemp = plot_AA(ax1, "F", "PHE", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "G":
    xtemp, ytemp, ltemp = plot_AA(ax1, "G", "GLY", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "H":
    xtemp, ytemp, ltemp = plot_AA(ax1, "H", "HIS", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "I":
    xtemp, ytemp, ltemp = plot_AA(ax1, "I", "ILE", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "K":
    xtemp, ytemp, ltemp = plot_AA(ax1, "K", "LYS", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)
elif letter == "L":
```

B.3. Generate TOCSY Spectrum Program

xtemp, ytemp, ltemp = plot_AA(ax1, "L", "LEU", i)
for t in xtemp: xdata.append(t)
for t in ytemp: ydata.append(t)
for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "M":
    xtemp, ytemp, ltemp = plot_AA(ax1, "M", "MET", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "N":
    xtemp, ytemp, ltemp = plot_AA(ax1, "N", "ASN", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "P":
    xtemp, ytemp, ltemp = plot_AA(ax1, "P", "PRO", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "Q":
    xtemp, ytemp, ltemp = plot_AA(ax1, "Q", "GLN", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "R":
    xtemp, ytemp, ltemp = plot_AA(ax1, "R", "ARG", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "S":
    xtemp, ytemp, ltemp = plot_AA(ax1, "S", "SER", i)
    for t in xtemp: xdata.append(t)
    for t in ytemp: ydata.append(t)
    for i in range(len(xtemp)): m_label.append(ltemp)

elif letter == "V":

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B.3. Generate TOCSY Spectrum Program

```python
xtemp, ytemp, ltemp = plot_AA(ax1, "V", "VAL", i)
for t in xtemp: xdata.append(t)
for t in ytemp: ydata.append(t)
for i in range(len(xtemp)): m_label.append(ltemp)
else:
xtemp, ytemp, ltemp = plot_AA(ax1, "W", "TRP", i)
for t in xtemp: xdata.append(t)
for t in ytemp: ydata.append(t)
for i in range(len(xtemp)): m_label.append(ltemp)
else:
xtemp, ytemp, ltemp = plot_AA(ax1, "Y", "TYR", i)
for t in xtemp: xdata.append(t)
for t in ytemp: ydata.append(t)
for i in range(len(xtemp)): m_label.append(ltemp)

# Plots acquired data over simulated data
# User can change file name for data exported from topspin here
with open("Ab40_titration1_2_TOCSY.txt", "r") as file0:
i = 0
peak_list = list()
for line in file0:
    if i > 7:
        peak_info = [float(value) for value in line.split()]
        peak_list.append(peak_info[3:5])
i += 1
F2 = []
F1 = []
F2,F1 = zip(*peak_list)
ax1.scatter(F2, F1, marker = "x", label = "Real",
color = "k", s = 10)
ax1.scatter(F1, F2, marker = "x", color = "k", s = 10)

'''Use this code for mouse over labels for Topspin data.
Comment out if only want AA labels for PDB data'''
simdata_len = len(xdata)
for f in F2: xdata.append(f)
for f in F1: xdata.append(f)
for f in F1: ydata.append(f)
for f in F2: ydata.append(f)
```
B.3. Generate TOCSY Spectrum Program

```python
for i in range(len(F2)): m_label.append("(" + str(F2[i]) + ",
 + str(xdata[simdata_len+i]) + ")")
for i in range(len(F1)): m_label.append("(" + str(F1[i]) + ",
 + str(xdata[simdata_len+i]) + ")")

''''
'''' Creates one invisible plot for all mouseover events ''''
sc = ax1.scatter(xdata, ydata, marker = "o",
                 color = "#999999", s = 10, alpha = 0)

annot = ax1.annotate("", xy=(0,0), xytext=(20,20),
                     textcoords="offset points",
                     bbox=dict(boxstyle="round", fc="w"),
                     arrowprops=dict(arrowstyle="->"))

annot.set_visible(False)

'''' Calls mouseover hover function ''''
fig.canvas.mpl_connect("motion_notify_event", hover)
```
```
Appendix C

Generated Spin Systems

Note: Chemical shifts are given to 4 decimal places as this is the default output from the Topspin software.

Spin System 1: 1.0781 1.0683 0.6726
Spin System 2: 2.143 1.9422
Spin System 3: 2.1918 2.1255 1.9275
Spin System 4: 2.3868 1.8103 1.698 5.1785 1.1011
Spin System 5: 2.7457 1.5174
Spin System 6: 2.7808 1.5076
Spin System 7: 2.7925 1.2879
Spin System 8: 2.8608 1.1853 0.7752
Spin System 9: 2.8705 2.0338 1.9948 1.8348 1.7713 1.3855 1.3709 1.1756 1.117 1.1072
Spin System 10 : 3.2899 0.951
Spin System 11: 3.3387 0.9705
Spin System 12 : 3.4596 3.4498 2.3768
Spin System 13 : 3.4888 1.0096
Spin System 14 : 3.8653 2.9661 1.3514
Spin System 15: 4.0252 1.3221
Spin System 16: 4.0428 1.2146
Spin System 17: 4.3373 2.9041
Spin System 18: 5.1779 1.825
Spin System 19: 6.8905 6.6053
Spin System 20 : 7.3118 7.1616 3.9295 1.9031 1.751 0.7459 0.7312
Spin System 21: 7.3274 7.2156
Spin System 22 : 7.4229 6.615
Spin System 23 : 7.5205 6.8103
Spin System 24 : 7.5751 7.4932 7.1863 3.9832
Spin System 25 : 7.7097 7.2981 1.4246 0.7117
Spin System 26 : 7.7331 4.1053
Spin System 27 : 7.7662 3.91 1.9617 0.785
Spin System 28 : 7.8189 4.3689 2.8943 2.8846
Spin System 29 : 7.8364 7.7994 3.8416
Appendix C. Generated Spin Systems

Spin System 30: 8.0568 4.1248
Spin System 31: 8.3904 7.9632 3.8269
Spin System 32: 8.484 7.6414 3.993