

ANION SENSING: RECEPTOR CHARACTERIZATION AND THE
DEVELOPMENT OF A QUICK-SCREEN SENSING ASSAY

by

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A THESIS

Presented to the Department of Chemistry
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

June 2017

An Abstract of the Thesis of

Annie K. Gilbert for the degree of Bachelor of Science
in the Department of Chemistry to be taken June 2017

Title: Anion Sensing: Receptor Characterization and the Development of a Quick-Screen Sensing Assay

Approved: _____

Dr. Darren W. Johnson

Anion sensing is an integral field of research in order to regulate and detect high concentrations of anions that are harmful to the environment. A dominant field of anion sensing research is through the development of supramolecular receptors. Typically, a single receptor is designed to exhibit selectivity, affinity, and a response towards one particular anion. In an attempt to find an all-encompassing receptor, the Darren W. Johnson and Michael M. Haley collaborative lab has developed a wide library of receptors based on an arylolethynyl bisurea scaffold. This research looks to provide a better understanding of the binding interactions and preferences of this scaffold, characterize and screen previously synthesized receptors, and work towards developing a sensing assay utilizing the library of receptor molecules of the Johnson/Haley Lab.

Acknowledgements

This project was supported by the University of Oregon Presidential Undergraduate Research Scholars program (PURS), NIH grant R01-GMO87398, and NSF-INFEWS grant CHE-1607214.

I would like to thank Professors Darren W. Johnson and Michael M. Haley for giving me the opportunity to work on various projects in their lab and guiding me through this research process. Additionally, the work I've done in this lab has been relentlessly supported by Lisa Eytel. A deep debt of gratitude is owed for introducing me to research, teaching me valuable skills in the laboratory, and including me in her research projects.

Furthermore, I would like to thank Professor Geraldine L. Richmond, Karl Reasoner, Bri Gordon, and Andrew Carpenter for their roles in PURS. Many thanks to Professor Barbara Mossberg and Miriam Jordan for their guidance with the Robert D. Clark Honors College thesis process. Lastly, my friends and family have done an excellent job at preserving my sanity through a very trying final year of university. None of this would have been possible without any of their support.

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Background

Anions are small negatively charged atoms or molecules that have critical roles in our everyday lives. For example, nitrate (NO_3^-), a key component of fertilizer, is an essential nutrient for crops.¹ Other anions like chloride (Cl^-) and phosphate (PO_4^{3-}) are key to the regulation of cells and metabolic processes.² Chloride is the most prevalent anion in the body and crucial for the regulation of cell volume, stabilization of the membrane potential, and various other biological processes.³

While nitrate is crucial for crops, excess nitrate can cause algal blooms from water runoff to nearby bodies of water^{1,1}. These algal blooms lead to low oxygen environments that kill off animals and plants.¹ This is a common occurrence in large scale agriculture because of farmers overfertilizing their crops. In an attempt to avoid these detrimental environmental impacts, SupraSensor², a spin-off company from a University of Oregon anion sensing research lab, develops sensing devices for farmers to measure nitrate concentrations in their soil. Knowing the concentration of nitrate not only prevents algal blooms, but also helps farmers save money through a better understanding of when to fertilize.

In addition to the regulation of fertilizing, anions have been implemented in the diagnosis of diseases like cystic fibrosis and have aided in the discovery of the history of water on Mars.^{4,5} Anion sensing is a significant field of research due to the abundance of anions in nature that present several opportunities to discover and problem solve through their detection.

¹ Underlined terms defined in glossary.

² SupraSensor was recently purchased by the Climate Corporation, a subsidiary of Monsanto Company.

Supramolecular Receptors

The science behind the nitrate sensing devices started in a collaborative lab between Professors Michael M. Haley and Darren W. Johnson. Their lab designs and analyzes supramolecular receptors.

Supramolecular chemistry is the study of molecules that interact or bind other molecular units without forming strong, irreversible bonds. A good analogy for a supramolecular receptor is a baseball mitt and a baseball. When the baseball is caught in the mitt, the mitt changes color or glows (fluoresces). This optical response allows for recognition of binding occurring at the molecular level between the receptor and anion. The color change or fluorescence response determines the presence of an anion, and the intensity of the optical response can also be used to detect the concentration of the anionic “guest” molecule.

Supramolecular receptors (also known as host molecules) interact with guest molecules (i.e. an anion) using reversible binding interactions, such as hydrogen bonding, weak- σ interactions, and ion- π interactions.⁶ The Johnson and Haley lab has developed a library of receptors utilizing an arylethynyl bisurea scaffold (Figure 1). This scaffold breaks down into three main components: aryl units, ethynyl linkers, and urea anchors. A rigid alkyne linkage between two arene rings and urea hydrogen bond donors create a binding cavity for anionic guests.⁷ Conjugative communication between aryl units induces a fluorescent response upon anion binding, making it an effective receptor model for anion detection.⁸

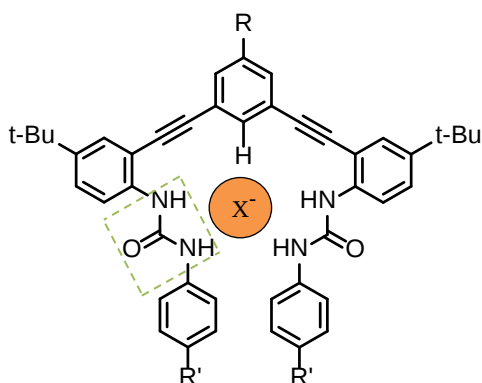


Figure 1: Arylethynyl bisurea scaffold.

Carbons are omitted from the structure for clarity. A carbon atom is represented by each junction. “R” and “R’” groups stand for any atom or functional group. A urea hydrogen donor is outlined in green. The orange circle labeled “X⁻” is used to denote an anion.

Lock-and-Key Model

The ideal receptor is one that exhibits selectivity, affinity, and a detectable response towards a specific anion of interest. This single receptor approach is referred to as the lock-and-key model where a single receptor is modeled to fit with a certain anion better than the rest.⁹ To find this “perfect” receptor, various electron-withdrawing and electron-donating groups have been substituted in the R and R’ positions of the arylethynyl bisurea scaffold (Figure 1) to alter the acidity of the hydrogen bond donors.⁷

Obtaining the perfect combination of affinity, selectivity, and response necessary in the lock-and-key model is difficult to accomplish in a small molecule receptor, let alone discover any unique anion recognition. In the process of searching for an all-encompassing receptor, a large library of arylethynyl bisurea receptors has been developed by the Johnson/Haley Lab. This wide selection of receptors provides a solid foundation for developing a quick-screen sensing assay.

Sensing Assay Model

The sensing assay model utilizes a composite response like taste receptors.¹⁰ Rather than a single receptor, a selection of receptors with varying responses is used to detect a specific anion. Data from all receptors is collected and processed through pattern recognition algorithms, such as principle component analysis (PCA). This allows for detection of unknowns and the presence of various anions within a single screening. In addition, an assay also provides the opportunity for quick determination of any unexpected or interesting responses to any certain anion from a group of unscreened receptors.

Materials and Methods

This research breaks down into three sections: 1) attempt to better understand the binding preferences of the arylethynyl urea scaffold, 2) characterization of previously synthesized receptors, and 3) work towards developing a sensing assay utilizing the library of receptor molecules from the Johnson/Haley Lab. This process involves a variety of analytical techniques and various receptor molecules that will be described in the following sections.

Materials

In order to better understand the binding preferences of arylethynyl urea scaffolds, two arylethynyl monourea scaffolds were synthesized (Figure 2).¹¹ Unlike the two urea “arms” in the traditional arylethynyl bisurea scaffold, the single “armed” receptors provide more rotational freedom for the core arene to position into the preferred binding state.¹¹ Potential binding states include anion- π , aryl CH hydrogen bond, or weak- σ interactions.

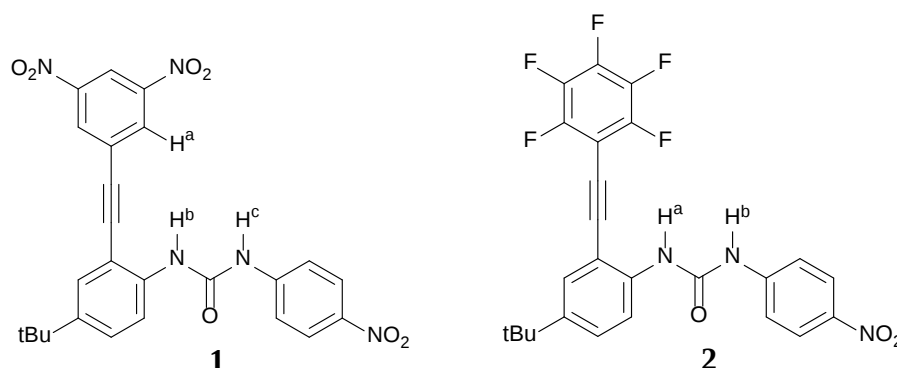


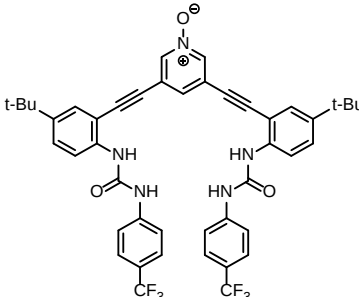
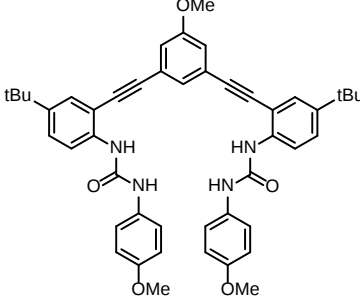
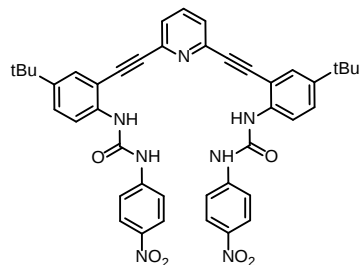
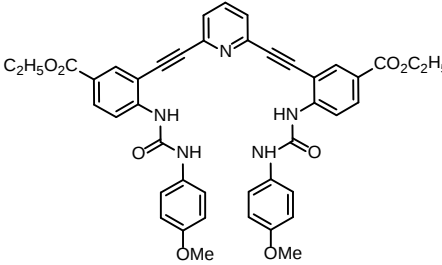
Figure 2: Dinitro and pentafluoro monopodal receptors.

Two monopodal arylethynyl monourea scaffolds, including the dinitrobenzene scaffold (1) and the pentafluorobenzene scaffold (2).

Based off previous research, the dinitro receptor (**1**) was hypothesized to interact with anionic guests via an anion- π or weak- σ binding motif, as of the steric interactions from the nitro groups were predicted to prevent aryl CH hydrogen bonding.¹² The pentafluoro receptor (**2**) was hypothesized to interact via an anion- π binding motif due to the presence of an extremely electron deficient arene.¹⁶

In addition to the monopodal receptors, a large library of receptors of the Johnson/Haley lab has yet to be screened for various oxoanions. This research looks to characterize arylethynyl bisurea receptors with phenyl, pyridine, and inverted pyridine cores (Table 2). The library mainly focuses on tert-butyl groups on the side arene position because of synthetic accessibility and ease of spectroscopic elucidation. The central and pendant arene groups are commonly modified with electron donating groups and electron withdrawing groups including methyl ethers (OMe) and electron withdrawing groups like nitro (NO₂) and trifluoromethyl (CF₃) groups.

Table 1: Receptor library¹³

3		4	
5		6	

7		8	
9		10	
11		12	
13			

Methods

A typical characterization for a synthesized molecule includes structural elucidation through nuclear magnetic resonance (NMR), X-ray crystallography, mass spectrometry, amongst various other techniques. Supramolecular anion receptor characterization also includes determining the association constant (K_a) and host-to-

guest stoichiometry. An association constant describes the affinity of a receptor towards a selected anion, so the larger the association constant, the more likely the receptor and anions are to be together in solution. The host-guest stoichiometry describes the ratio of receptor to anion in a binding event. The following sections describe the methods in determining these values.

*Titration*s

Association constants can be experimentally determined via titrations. This involves the gradual addition of guest (anion) to a host solution at constant concentration while monitoring some physical property of the system.¹⁴ This research utilizes specific chemical resonance through ¹H NMR and absorption band through ultraviolet visible spectroscopy (UV-Vis). As additional equivalents of anion are introduced to the host solution, the spectra of the receptor changes due to the interactions taking place in the binding cavity.

¹H NMR titrations are commonly employed by the Johnson/Haley lab because urea proton resonance peaks noticeably shift due to the hydrogen bond interaction with the introduced anion (Figure 3). With UV-Vis titrations, the absorption band or wavelength increases or decreases in absorbance from these same weak interactions in the binding cavity. The changes in the resonance of the proton peaks or the absorption band are tracked and used to determine the strength of the interactions by calculating the K_a values with non-linear regression methods.

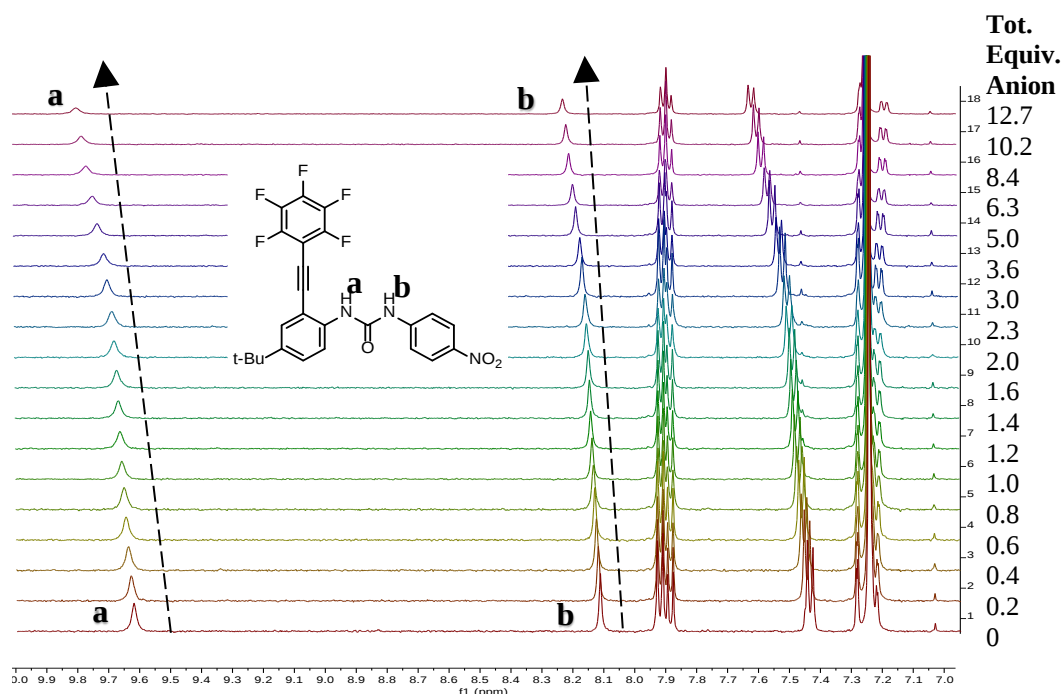


Figure 3: Stacked ^1H NMR titration of **2** with iodide.

From bottom to top the spectra represents a sample that contains only receptor (0 equivalents of anion) with gradually increasing equivalents of anion (equivalents displayed on the right). The urea proton peaks **a** and **b** gradually shift downfield (to the left) as more anion is added to the initial receptor solution, indicating the nearby presence of a more electron-rich species.

Job's Plot Analysis

In a binding event between a receptor and anion, the receptor may bind the anion in conjunction with additional receptors or multiple anions. This ratio of host-to-guest is analyzed for the two monopodal receptors through Job's plot analysis. This involves the variation of the mole fraction of one species while keeping the total molar concentration of both species constant.¹⁵ In this research, the mole fraction of host (receptor) is varied as the total concentration of host and guest (anion) is held constant.

As the mole fraction of host is varied, some physical property, P , is measured.¹⁵ This research utilizes ^1H NMR proton resonance shifts of the host molecule. This data is plotted against the mole fraction of host. A local maximum at a 0.5 mole fraction indicates a one-to-one host-to-guest binding, while local maxima at 0.33 and 0.66 indicate one-to-two and two-to-one host-to-guest binding stoichiometry, respectively (Figure 4).

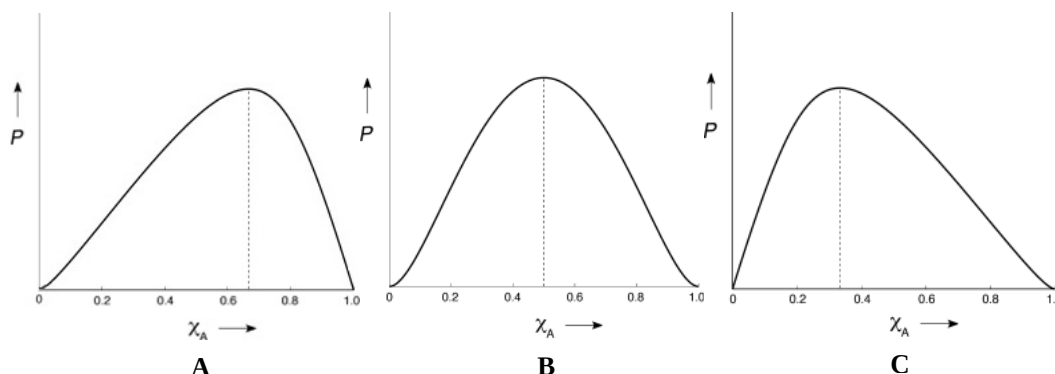


Figure 4: Job's plot analysis binding stoichiometry examples.

P represents some physical property and X_A is equal to the mole fraction of A. Graphs representing two-to-one binding stoichiometry (A), one-to-one binding stoichiometry (B), one-to-two binding stoichiometry (C).¹⁵

Fluorescence Characterization

Another method for examining the responses of a receptor with different anions involves utilizing fluorescence spectroscopy. This process first requires the determination of the λ_{max} (maximum wavelength in the absorption spectrum) for the receptor which can be found through UV-Vis spectroscopy. This λ_{max} value is used as the excitation wavelength for obtaining the emission spectrum of the receptor. Since λ_{max} is the energy level at which the maximum amount of light will be absorbed by the receptor, exciting at this wavelength will thus provide a maximum amount of emission.

Qualitative fluorescence screening of host-guest interactions involves an excess of anion, introduced as a tetrabutylammonium (TBA) salt, added to receptor solutions at a set concentration. For qualitative means, the fluorescence spectrum of each anion with the receptor is measured using the same excitation wavelength as with the blank receptor.

This process can also be done for several receptors at once using a plate reader. In this research, eleven receptors were screened with a selection of oxoanions and halides in chloroform (Table 1). Solutions for each receptor were prepared with approximately the same concentration (0.033 mM). In addition, six solutions of anions (tetrabutylammonium salts of each anion) were prepared with approximately the same concentration (0.0012 mM). Target anions included dihydrogenphosphate (H_2PO_4^-), nitrate (NO_3^-), perchlorate (ClO_4^-), bisulfate (HSO_4^-), bromide (Br^-) and iodide (I^-).

Receptor solutions were pipetted into columns of a 96-well microplate and the anion solutions were pipetted into the rows at 2 equivalents of anion per receptor (Table 2). For later processing of the data, one column was devoted to blank anion solutions and one row to blank receptors. A single well was used as a blank for solvent.

Table 2: 96-well plate set-up.

		Receptor												
		1	2	3	4	5	6	7	8	9	10	11	Blank	
Anions	Blank													Blank Solvent
	ClO_4^-													Blank Anions
	NO_3^-													
	H_2PO_4^-													
	Br^-													
	I^-													
	HSO_4^-													
	Empty													

Experimental Results and Discussion

Monopodal Receptors¹¹

This project was done in collaboration with Lisa M. Eytel (University of Oregon chemistry graduate student) and is published in *Chemistry: A European Journal*.¹¹ In this research, we determined association constants for receptors **1** and **2** with Cl⁻, Br⁻, and I⁻ using ¹H NMR titrations (Table 2). Values were calculated using non-linear regression, non-cooperative fitting models in MatLab. This was accomplished through simultaneously fitting both urea protons in **2** and both urea protons and the aryl CH proton in **1** (Figure 5).

Table 2: Anion association constants (K_a) for receptors **1** and **2**.

Host	Cl ⁻ (M ⁻¹)		Br ⁻ (M ⁻¹)		I ⁻ (M ⁻¹)	
	K_{a1}	K_{a2}	K_{a1}	K_{a2}	K_{a1}	K_{a2}
1	300	740	320	1040	360	6570
2	118000	10200	930	2500	130	750

Values are an average of three ¹H NMR titrations with $\pm 15\%$ error. Anions from tetrabutylammonium salts in 10% *d*₆-DMSO/CDCl₃.

The titrations were initially fit to a 1-to-1 host-to-guest model, however, the residual errors were significant. Subsequently the ratio of host to guest was analyzed through Job's plot analysis to investigate this source of error. The local maxima shifted towards a mole fraction value of 0.66 for both receptors, suggesting a 2-to-1 host-guest model is more appropriate for these scaffolds (Figure 6). As expected, the titration data fit to a 2-to-1 host-guest model with minimal residual error for both receptors.

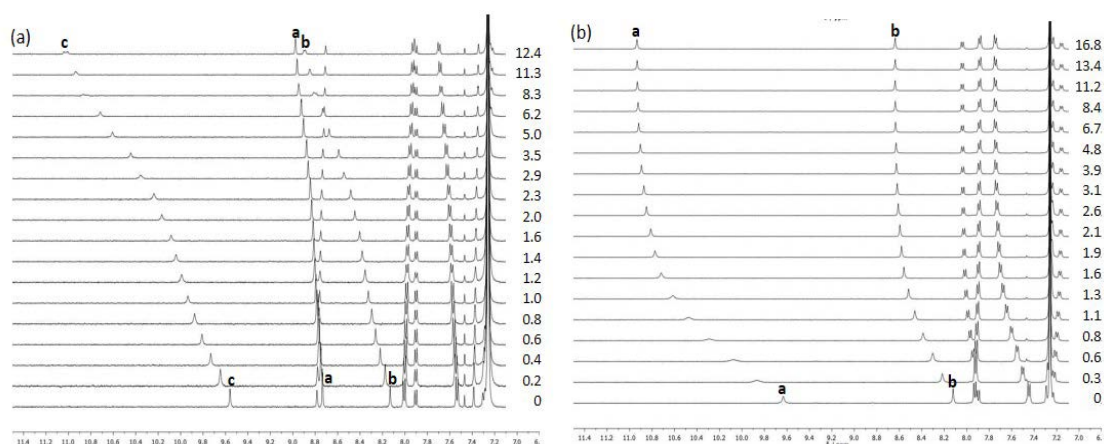


Figure 5: ^1H NMR titrations of **1** and **2**.

(a) ^1H NMR titration of **1** with TBA·Cl (three proton peaks monitored). (b) ^1H NMR titration of **2** with TBA·Br (two proton peaks monitored). Labels refer to protons depicted in Figure 2.¹¹

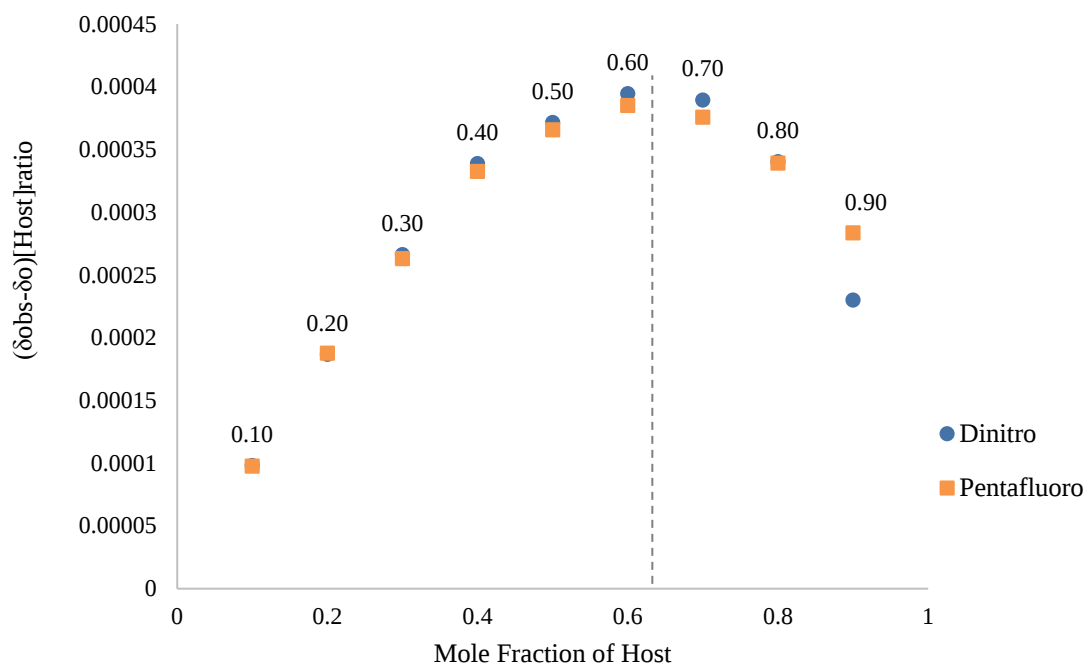


Figure 6: Job's plot of receptors **1** and **2** with bromide.

Local maxima shifted towards a mole fraction of 0.66 supports a 2-to-1 host-guest binding conformation.

In addition, the 2-to-1 host-guest model was confirmed in the solid-state through X-ray crystallography. An X-ray crystal structure of **1** with Br⁻ shows two receptors binding the Br⁻ anion with six weak hydrogen bond contacts in total between the two receptors (Figure 7). Appropriate crystals of **2** have yet to be isolated for X-ray crystallography, however, a 2-to-1 binding is supported by the Job's plot analysis, K_a values obtained through ¹H NMR titrations, and similar possible binding interactions as seen in **1**.

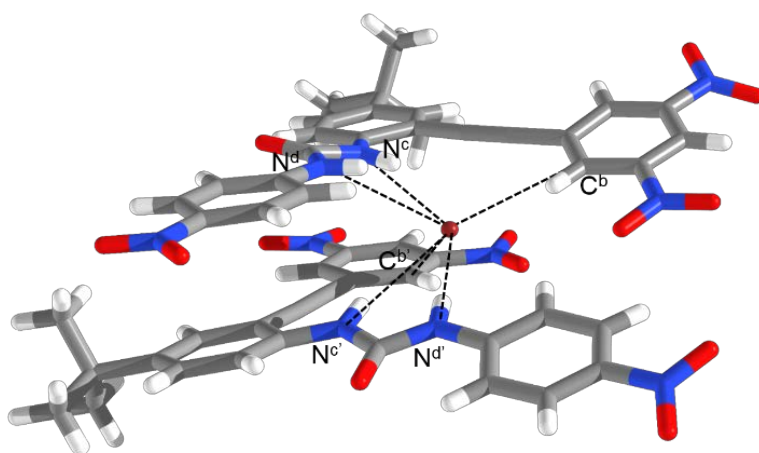
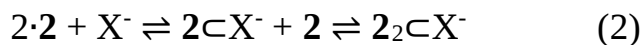
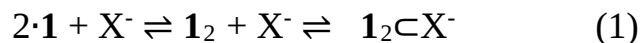


Figure 7: X-ray crystal structure of **1** with bromide.

Two equivalents of **1** encompass a single Br⁻ anion. Hydrogen bond interactions are shown as dotted lines.

Due to the differences across K_a values, the formation of this 2-to-1 host-guest complex is concluded to have two different mechanisms for receptors **1** and **2**. The K_a values of **1** are similar in magnitude across the three different anions, while the K_a values for **2** vary drastically across anions. The K_a for **1** is also on the same magnitude of the dimerization constant found in the absence of anion. These factors suggest the K_a for **1** is independent of the anion present and two host molecules dimerize before

the anion binds (equation 1). On the other hand, due to the anion dependent nature of K_{a1} for **2**, it was concluded that receptor **2** binds the anion first and then an additional receptor binds the one-to-one host-guest complex (equation 2).



The combination of X-ray diffraction crystallography and proton shifts of an aryl CH proton suggest aryl CH-anion hydrogen bonding is the preferred binding interaction of **1**. The binding motif of **2** is more difficult to assign, however. A color change is not seen upon anion binding, suggesting a weak- σ interaction is not occurring. The lack of aryl CH hydrogen bond donors present in the core phenyl eliminates the possibility of aryl CH hydrogen bonding. These factors, alongside literature precedence for the interactions of electron deficient arenes, suggest an anion- π binding motif.¹⁶

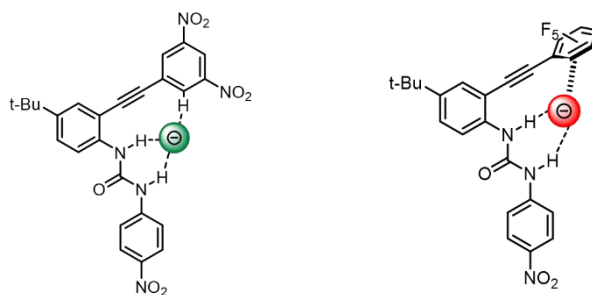


Figure 8: Illustrated simplistic binding motifs of **1** and **2**.

Dinitro receptor (**1**) with aryl CH-anion hydrogen bond motif (left) and the pentafluoro receptor (**2**) with an anion- π binding motif (right). These figures are simplified to show the binding interactions taking place in the binding cavity and do not accurately depict the 2-to-1 binding taking place.

The evidence towards a 2-to-1 host-guest complex formation suggests the necessity of two urea arms for a strong arylethynyl urea-based anion receptor. The aryl

CH hydrogen bonding interaction in **1** proved to be a dominating binding interaction regardless of steric effects with the nitro groups. In addition, the different binding motifs of the monopodal receptors appear to influence the mechanism in which the 2-to-1 host-guest complex forms.

Receptor Characterization

Subsequent to the published monopodal receptor work, turn-on fluorescence of the inverted pyridine (N-confused) N-oxide receptor (**3**) was discovered in the process of analyzing receptors for a sensing assay. The receptor is turn-on fluorescent for Cl^- , ClO_4^- , Br^- , and H_2PO_4^- in chloroform (CHCl_3) (Figure 9).

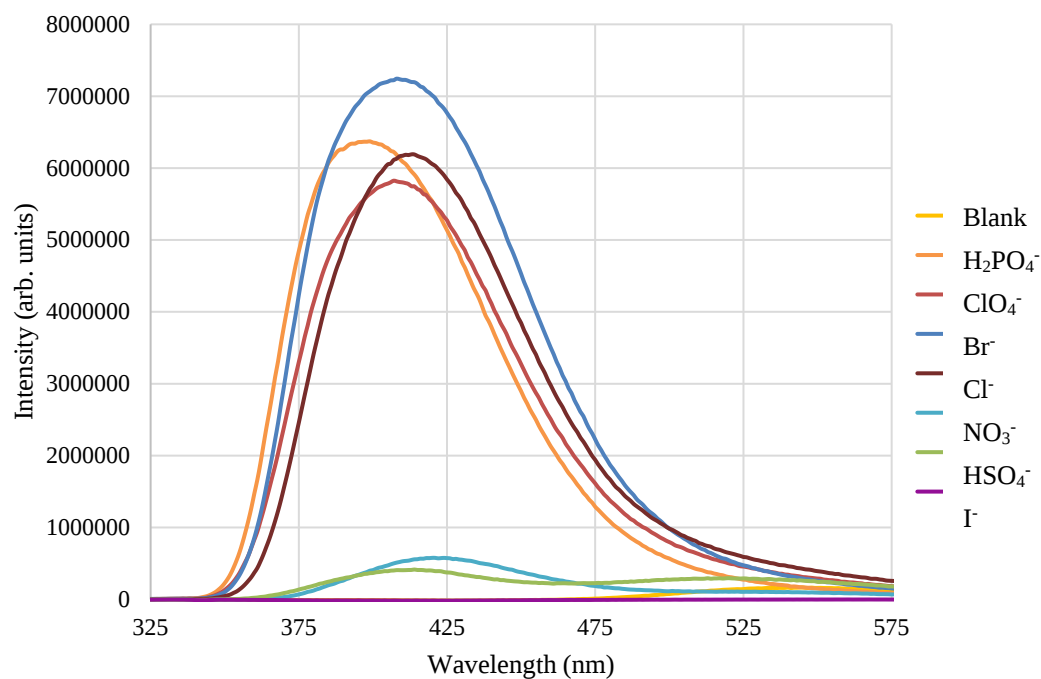


Figure 9: Fluorescence emission spectrum of **1**.

Excitation at 310 nm. Increase in intensity from blank receptor (along baseline in yellow) to receptor with Cl^- , ClO_4^- , Br^- , and H_2PO_4^- shows turn-on fluorescent properties of **3**.

The determination of the association constants for the halide anions (Cl^- , Br^- , and I^-) had been previously attempted by ^1H NMR titration. These attempts were

unsuccessful with significant residual error in fitting the data. Thus, the association constant for the anions eliciting this turn-on fluorescence response (Cl^- , Br^- , ClO_4^- , and H_2PO_4^-) were attempted to be determined through UV-Vis titrations. An association constant for ClO_4^- of 680 M^{-1} ($\pm 17\%$ error) was successfully determined in triplicate using absorbance peaks at 310 and 364 nm.

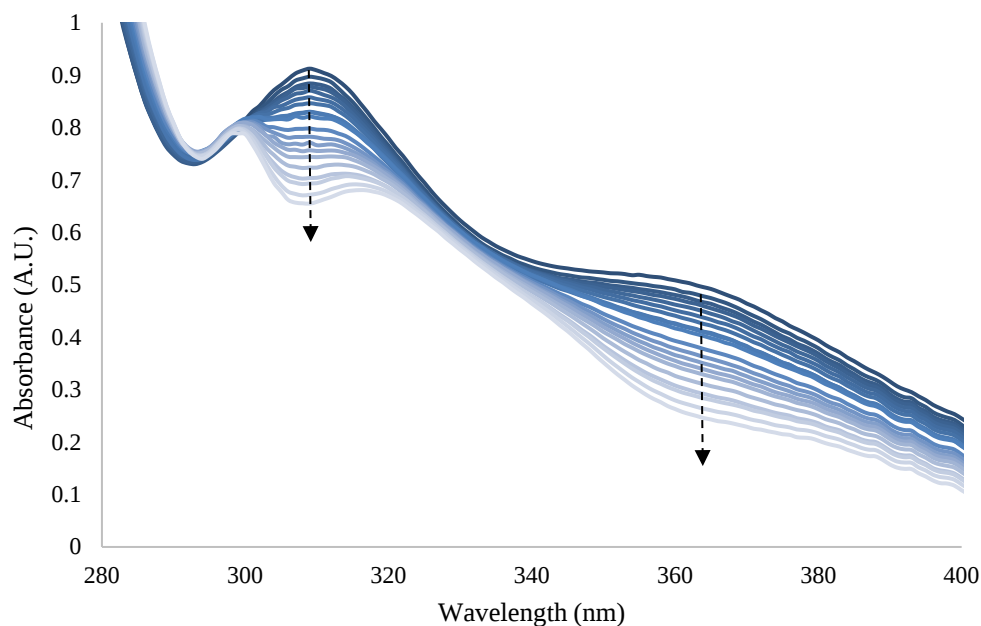


Figure 10: UV-Vis titration of **3** with perchlorate.

From top to bottom the spectra represents a sample that contains only receptor (0 equivalents of anion) with gradually increasing equivalents of anion. The change in absorbance intensity indicates the binding of an anion. Top spectrum has 0 equivalents of ClO_4^- and bottom has 76 equivalence.

A unique splitting pattern of the receptor peak at 310 nm occurred upon the gradual addition of anion to the receptor solution (Figure 10). This splitting also occurred in Br^- , but started at a much lower anion equivalence (0.48 compared to 12.41 for ClO_4^-) which interfered with the fit of the data. These splitting patterns were

hypothesized to be from the receptor being in an initial dimer state and breaking apart upon addition of anion.

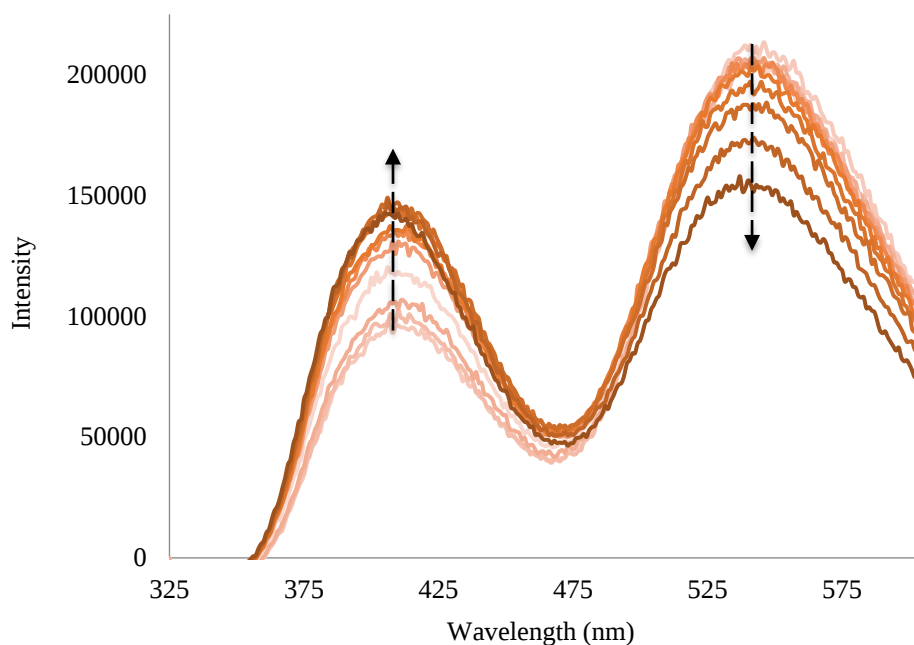


Figure 11: Fluorescence emission spectra of the dilution of **3** in chloroform.

Excitation at 310nm. Two separate peaks appear in the fluorescence emission spectra can be attributed to the monomer (left, $\lambda=405$) and the dimer (right, $\lambda=545$) of **3**. As solution of **3** diluted, the monomer peak increases in intensity as the dimer peak decreases in intensity.

A fluorescence dilution experiment was performed to test this hypothesis. A solution of **3** was prepared to mimic the same concentration used in the titration experiments (0.033 mM) and chloroform solvent was gradually added to the receptor solution to dilute the concentration over time. As more solvent was added, the left (red-shifted) peak increased in intensity as the right (blue-shifted) peak decreased in intensity (Figure 11). The two peaks are hypothesized to be from the monomer (left) and dimer (right). As this concentration is decreased, the dimer gradually breaks apart, decreasing

its intensity, and the monomer is formed, thus increasing its intensity. This would suggest that the dimer is quenching the fluorescence in chloroform at the concentration at which titrations are performed.

Sensing Assay Preliminary Results

The plate reader screening gave data output for fluorescence and absorbance intensities. Only one scan was completed due to the time constraint of this project. These results and analysis are therefore preliminary but provide an interesting insight for discovering any unique responses from a series of receptors with a wide array of anions. This section focuses on the qualitative analysis of the fluorescence data obtained. The UV-Vis data is currently being analyzed and processed by another lab member.

The large data set was first analyzed as a heat map to look at which receptors provided the most intense and noticeable response (Figure 12). The heat map is composed of several thin lines, each representing the relative intensity of one spectrum of an individual receptor paired with a certain anion. An accurate depiction of a single spectrum is labeled in Figure 12 (b). Receptors **3** and **12** had the highest intensity responses from the screening of the eleven receptors of Table 2 (regions **a** and **b** in Figure 12). A side view of this heat map is shown in Figure 13 that demonstrates the magnitude of these intensity differences with the other receptors, as well as what the color differences represent.

This heat map was then split into six plots, separating each emission spectrum by anion tested (Figures 14-19). This gives a clearer visual of the response patterns produced by the array of receptors towards each anion.

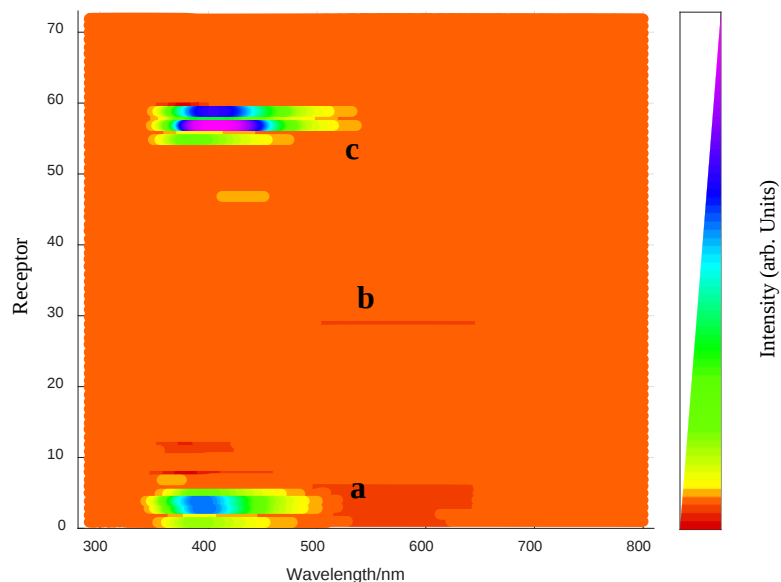


Figure 12: Heat map of fluorescence emission intensities of every receptor + anion combinations.

Fluorescence emission intensities of receptor with each anion. Each line represents a unique spectrum of a certain receptor with an anion (a single line represented at **b**). Greatest intensities come from receptors **3** and **12** (**a** and **c** respectively).

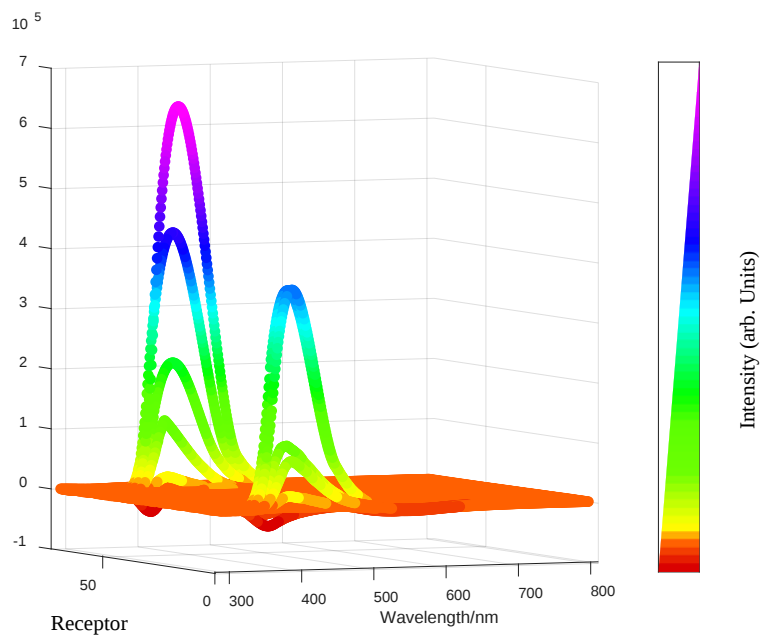


Figure 13: Side-view of fluorescence emission spectra.

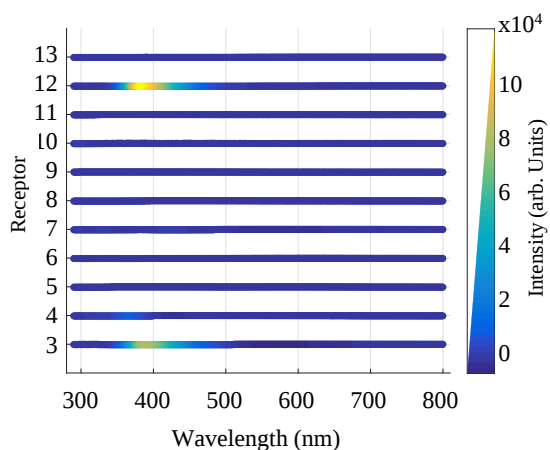


Figure 14: Fluorescence of receptors with ClO_4^-

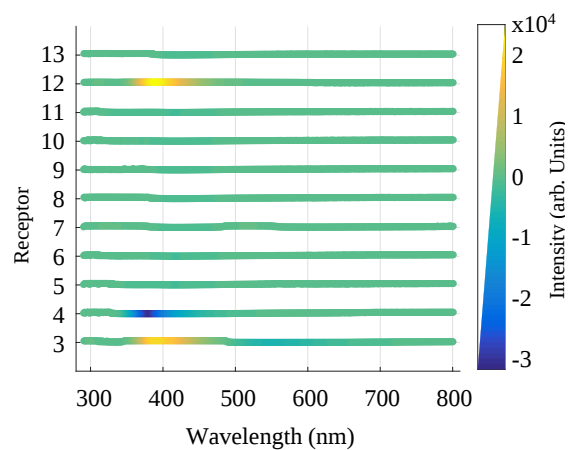


Figure 15: Fluorescence of receptors with NO_3^-

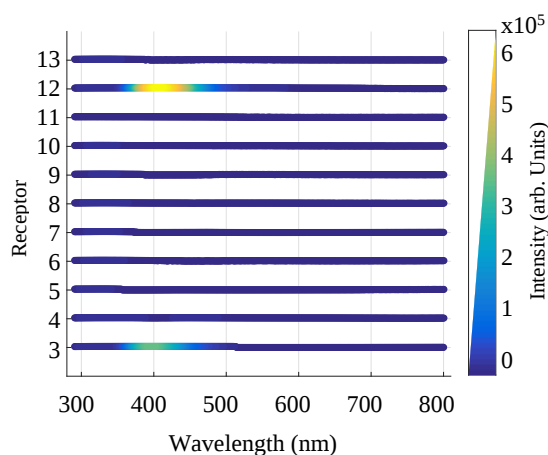


Figure 16: Fluorescence of receptors with H_2PO_4^-

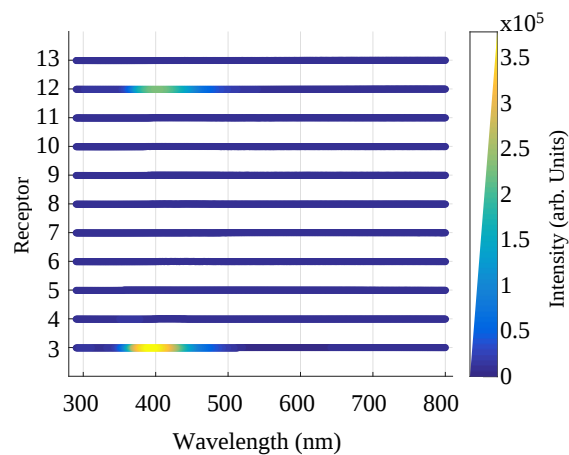


Figure 17: Fluorescence of receptors with Br^-

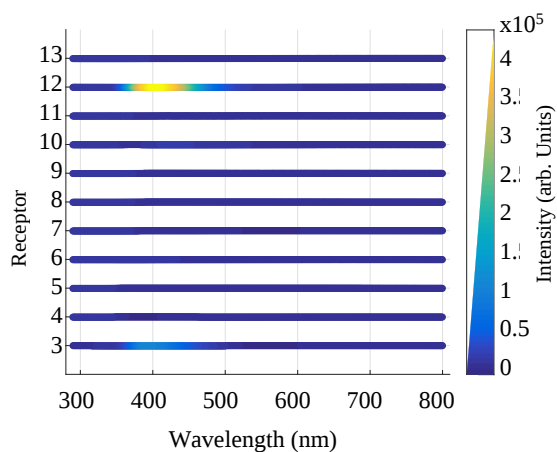


Figure 18: Fluorescence of receptors with I^-

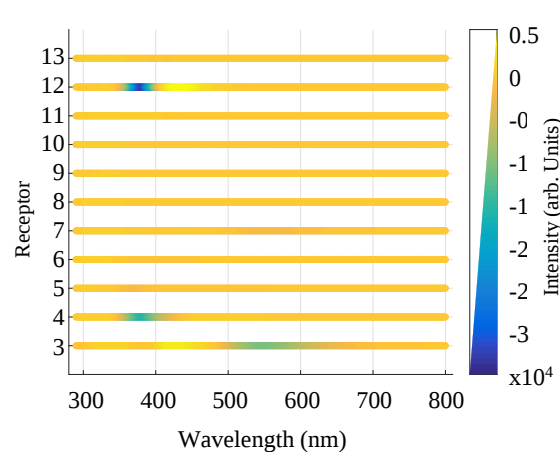


Figure 19: Fluorescence of receptors with HSO_4^-

Very similar response patterns are seen for all anions except HSO_4^- and NO_3^- .

These composite spectra show negative (turn-off) fluorescence responses towards these

particular anions (Figure 20 and Figure 21). The unique patterns produced by receptors **3**, **5**, and **12** suggest further investigation of these receptors with HSO_4^- and NO_3^- and possible incorporation of these receptors in a sensing assay.

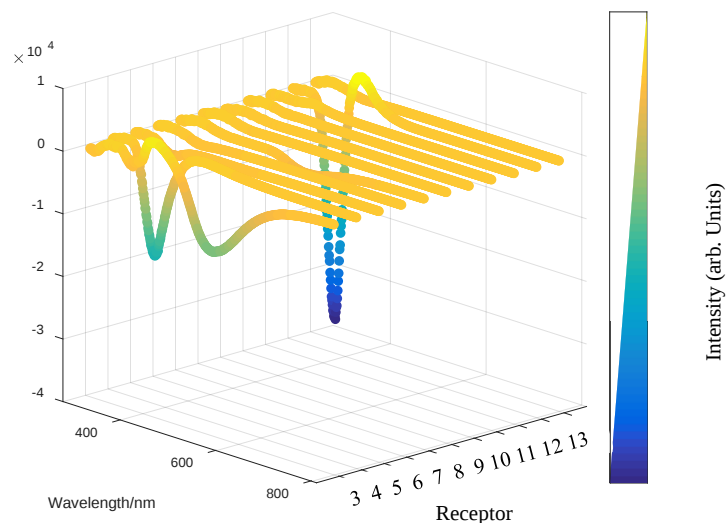


Figure 20: Side-view of fluorescence emission spectra with bisulfate.

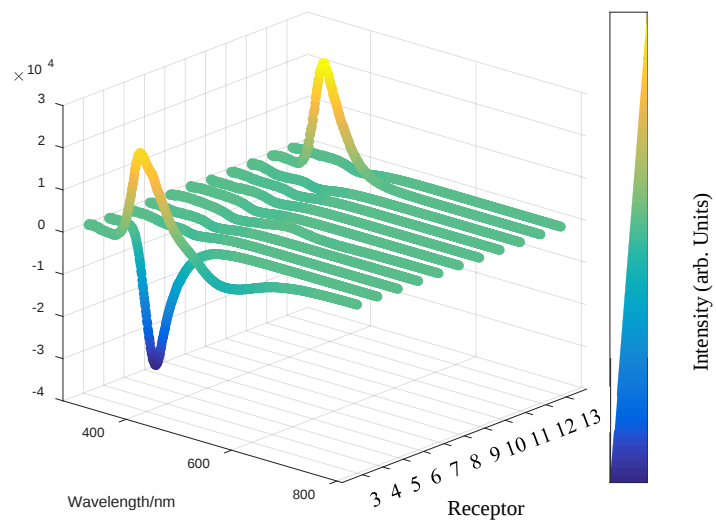


Figure 21: Side-view of fluorescence emission spectra with nitrate.

Future Directions

This project provided insight toward the preferred binding mode and limitations of the main receptor platform for the Johnson/Haley lab (arylethynyl urea scaffolds). The 2-to-1 host-guest binding stoichiometry of **1** and **2** suggests the necessity of having two “arms” in a strong arylethynyl urea receptor.

Future directions for the monopodal project include further study of the cooperativity demonstrated in these single arm receptors when forming the 2-to-1 host-guest complex. This research could involve the study of different single armed receptors exhibiting aryl CH hydrogen bond interactions and anion- π interactions to compare to the previous receptor binding mechanisms. The design and analysis of single arm receptors exhibiting weak- σ interactions or halogen binding would also provide further insight into the relationship between other binding interactions and the assembly mechanism.

Receptor **3** shows promising turn-on fluorescent properties in chloroform that could be employed in a sensing assay. Binding constants for other oxoanions and halides need to be determined. Fluorescence titrations should be performed for solutions in chloroform since the UV-Vis titrations and ^1H NMR titrations have been unsuccessful for anions other than ClO_4^- in this solvent. Titrations and fluorescence characterization of **3** also need to be performed in various solvents to better understand the effects of solvent on the binding properties of this receptor. It is hypothesized that in chloroform the receptor is dimerizing, quenching its fluorescence response, and upon anion binding, the dimer breaks apart to provide a drastically different fluorescent

response. Furthermore, determining the dimerization constant for **3** will provide an insight into the binding behavior of this receptor.

Research developing a sensing assay is in progress. In the initial screening of potential receptors, the excitation wavelength for all receptors were normalized to complete a quick screen. The intensities of other receptors could be increased by using exact values of λ_{max} . This can be done using the UV-Vis data of the blank receptors and developing a program for the plate reader to excite each column at the λ_{max} for the corresponding receptor.

The initial screening revealed unique response patterns for receptors **3** and **12** with HSO_4^- and NO_3^- , indicating these receptors may be ideal to include in a sensing assay. This screening process should be continued to find other receptors with unique response patterns in order to accurately detect other anions. Once this library of receptors is constructed, receptor response data can be processed using pattern recognition algorithms that allow for the detection of unknowns and the presence of various anions within a single screening.

In addition, this project has contributed an efficient way to identify unique binding responses of unscreened receptors that could be applicable to the lock-and-key model. This scanning technique could be employed with the future characterization of other receptors to focus on receptors that have promising responses with a particular anion.

Supporting Information

Monopodal receptor supporting information from published paper.¹¹

X-Ray Crystallography

General details. Diffraction intensities were collected at 173 K on a Bruker Apex2 CCD diffractometer using CuK α radiation, $\lambda = 1.54178$ Å. Space group was determined based on systematic absences. Absorption correction was applied by SADABS.¹⁶ Structure was solved by direct methods and Fourier techniques and refined on F^2 using full matrix least-squares procedures. All non-H atoms were refined with anisotropic thermal parameters. All H atoms were refined in calculated positions in a rigid group model. There are two symmetrically independent molecules in the crystal structure. One of the terminal –Me groups in the $[N(n\text{-Bu})_4]^+$ cation is disordered over two positions in the ratio 0.69/0.31. The Br $^-$ anion forms H-bonds to the two main molecules. Crystals of the investigated compound were very small needles and even using a strong *Incoatec I μ S Cu* source the diffraction data were collected only up to $2\theta_{\text{max}} = 100^\circ$. The reflections at high angles were very weak and as a result reflection statistics at high angles is poor and value of R_{int} is high. While the found X-ray structure is not precise, it provides clear chemical information about the formed complex. All calculations were performed by the Bruker SHELXL-2013 package.¹⁸

Crystal structure $1_2 \cdot (n\text{-Bu})_4\text{NBr}$. $\text{C}_{68}\text{H}_{84}\text{BrN}_{11}\text{O}_{15}\text{S}$, $M = 1407.43$, $0.23 \times 0.01 \times 0.01$ mm, $T = 173$ K, Monoclinic, space group $P2_1/c$, $a = 20.6647(10)$ Å, $b = 10.3192(5)$ Å, $c = 35.0367(16)$ Å, $\beta = 104.992(3)^\circ$, $V = 7217.0(6)$ Å 3 , $Z = 4$, $D_c = 1.295$ Mg/m 3 , $\mu(\text{Cu}) = 1.630$ mm $^{-1}$, $F(000) = 2960$, $2\theta_{\text{max}} = 100.0^\circ$, 25199 reflections, 7300 independent reflections [$R_{\text{int}} = 0.3631$], $R1 = 0.1166$, $wR2 = 0.2657$ and $\text{GOF} = 0.974$ for 7300 reflections (850 parameters) with $I > 2\sigma(I)$, $R1 = 0.3173$, $wR2 = 0.3633$ and $\text{GOF} = 0.974$ for all reflections, max/min residual electron density $+1.343/-0.516$ eÅ 3 . CCDC 1507418 contains the supplementary crystallographic data for these compounds.

These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Titration

General Titration Procedures. Concentration of receptor was kept constant by preparing a stock solution of the receptor and performing a serial dilution with the receptor stock solution to dissolve the guest. Receptor concentration was maintained constant throughout the titration to avoid concentration effects on the proton chemical shifts. Tetrabutylammonium salts, purchased from TCI America or Sigma Aldrich, were dried by heating to 50 °C *in vacuo* before use. Hamilton gas-tight syringes were used for all titrations. Titrations were performed in triplicate and the reported association constants represent the average fits across all titrations. Representative data are provided for each receptor and halide.

¹H NMR Titrations

General Conditions. ¹H NMR titrations were carried out on an Inova 500 MHz NMR spectrometer (¹H: 500.10 MHz). Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS) using non-deuterated solvent present in the bulk deuterated solvent (CDCl₃, ¹H 7.26 ppm; *d*₆-DMSO: ¹H 2.50 ppm). Mixed solvent systems were referenced to the most abundant solvent. All NMR spectra were processed using MestReNova NMR processing software. Association constants were determined using step-wise non-linear regression fitting in MatLab.¹⁴

Tetrabutylammonium chloride with 1. A concentrated solution of **1** (2.45 mg, [R]=4.87 mM) in 10% *d*₆-DMSO/CDCl₃ (1.00 mL) was prepared. A serial dilution was then performed with 250 μL of 4.87 mM solution of **1** diluted to 3 mL to yield the stock solution of **1** ([R]=0.406 mM). This solution was used in the dilution of TBACl guest

solution (6.53 mg, [G]= 9.98 mM). The remaining stock solution (0.600 mL) was used as the starting volume in the NMR tube.

Table 3: Representative titration data for Cl⁻ with **1**.

	Guest (μL)	[1] (M)	[Cl ⁻] (M)	Equiv.	H ^c δ (ppm)	H ^a δ (ppm)	H ^b δ (ppm)
1	0	4.06E-04	0.00E+00	0.00	9.553	8.725	8.126
2	5	4.06E-04	8.25E-05	0.20	9.634	8.737	8.167
3	10	4.06E-04	1.64E-04	0.40	9.713	8.751	8.208
4	15	4.06E-04	2.43E-04	0.60	9.783	8.759	8.244
5	20	4.06E-04	3.22E-04	0.79	9.844	8.771	8.276
6	25	4.06E-04	3.99E-04	0.98	9.901	8.779	8.304
7	30	4.06E-04	4.75E-04	1.17	9.950	8.785	8.329
8	35	4.06E-04	5.50E-04	1.36	9.998	8.794	8.354
9	40	4.06E-04	6.24E-04	1.54	10.039	8.800	8.375
10	50	4.06E-04	7.67E-04	1.89	10.114	8.813	8.417
11	60	4.06E-04	9.07E-04	2.24	10.186	8.824	8.452
12	80	4.06E-04	1.17E-03	2.89	10.295	8.842	8.508
13	100	4.06E-04	1.43E-03	3.51	10.385	8.857	8.556
14	150	4.06E-04	2.00E-03	4.92	10.550	8.885	8.641
15	200	4.06E-04	2.49E-03	6.15	10.661	8.903	8.709
16	300	4.06E-04	3.33E-03	8.20	10.789	8.929	8.770
17	400	4.06E-04	3.99E-03	9.84	10.873	8.939	8.810
18	600	4.06E-04	4.99E-03	12.30	10.926	8.952	8.840

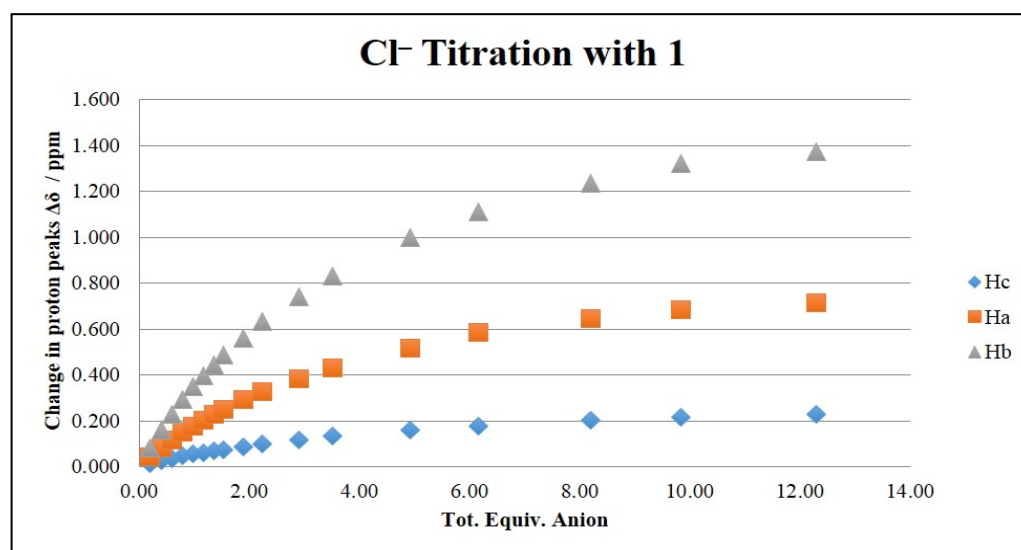


Figure 22: Binding isotherm for Cl⁻ titration with **1**.

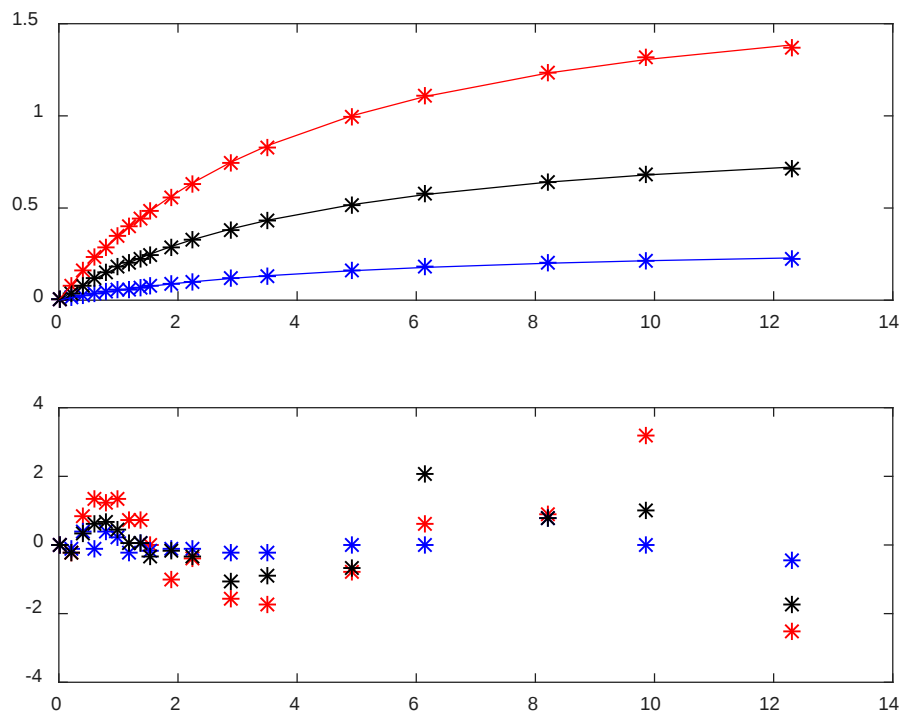


Figure 23: MatLab fit of binding isotherm for Cl^- titration with **1**.

Tetrabutylammonium bromide with 1. A concentrated solution of **1** (5.16 mg, $[\text{R}] = 5.12 \text{ mM}$) in 10% d_6 -DMSO/ CDCl_3 (2.00 mL) was prepared. A serial dilution was then performed with 590 μL of 5.12 mM solution of **1** diluted to 3 mL to yield the stock solution of **1** ($[\text{R}] = 1.01 \text{ mM}$). This solution was used in the dilution of TBABr guest solution (45.03 mg, $[\text{G}] = 6.00 \text{ mM}$). The remaining stock solution (0.600 mL) was used as the starting volume in the NMR tube.

Table 4: Representative titration data for Br⁻ with **1**.

	Guest (μL)	[1] (M)	[Br ⁻] (M)	Equiv.	H ^c δ (ppm)	H ^a δ (ppm)	H ^b δ (ppm)
1	0	1.01E-03	0.00E+00	0.00	9.590	8.730	8.144
2	5	1.01E-03	4.95E-04	0.49	9.743	8.759	8.225
3	10	1.01E-03	9.83E-04	0.98	9.828	8.770	8.270
4	15	1.01E-03	1.46E-03	1.45	9.900	8.784	8.310
5	20	1.01E-03	1.93E-03	1.92	9.943	8.793	8.333
6	25	1.01E-03	2.40E-03	2.38	9.984	8.799	8.356
7	30	1.01E-03	2.85E-03	2.83	10.024	8.805	8.377
8	35	1.01E-03	3.30E-03	3.28	10.058	8.810	8.395
9	40	1.01E-03	3.75E-03	3.72	10.089	8.816	8.412
10	50	1.01E-03	4.61E-03	4.58	10.139	8.825	8.440
11	60	1.01E-03	5.45E-03	5.41	10.194	8.834	8.470
12	80	1.01E-03	7.05E-03	7.00	10.258	8.846	8.506
13	100	1.01E-03	8.56E-03	8.50	10.312	8.855	8.534
14	150	1.01E-03	1.20E-02	11.90	10.415	8.873	8.592
15	200	1.01E-03	1.50E-02	14.87	10.458	8.885	8.616
16	300	1.01E-03	2.00E-02	19.83	10.544	8.896	8.662
17	400	1.01E-03	2.40E-02	23.79	10.581	8.904	8.682
18	600	1.01E-03	3.00E-02	29.74	10.589	8.904	8.693

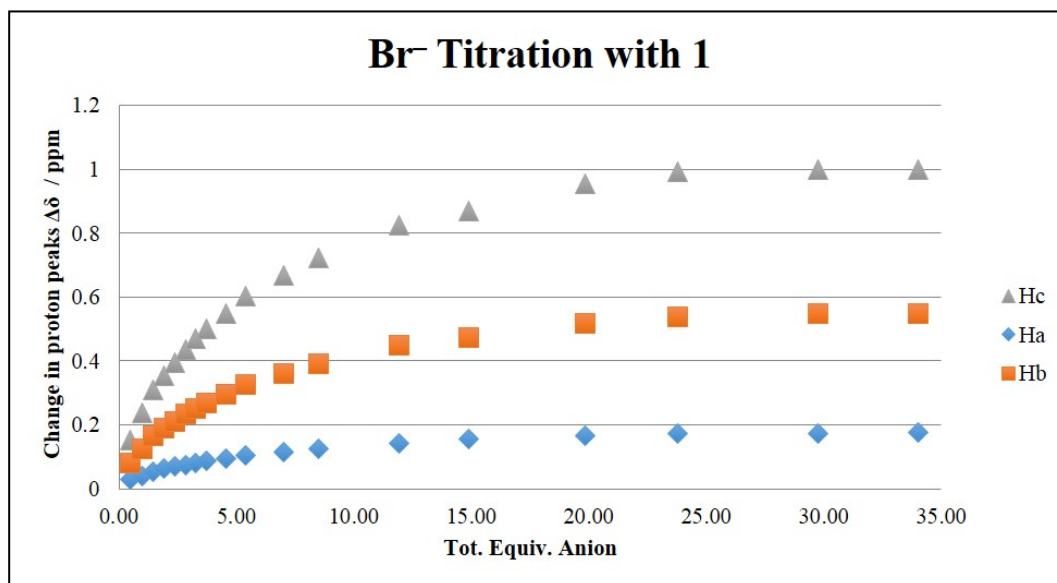


Figure 24: Binding isotherm for Br⁻ titration with **1**.

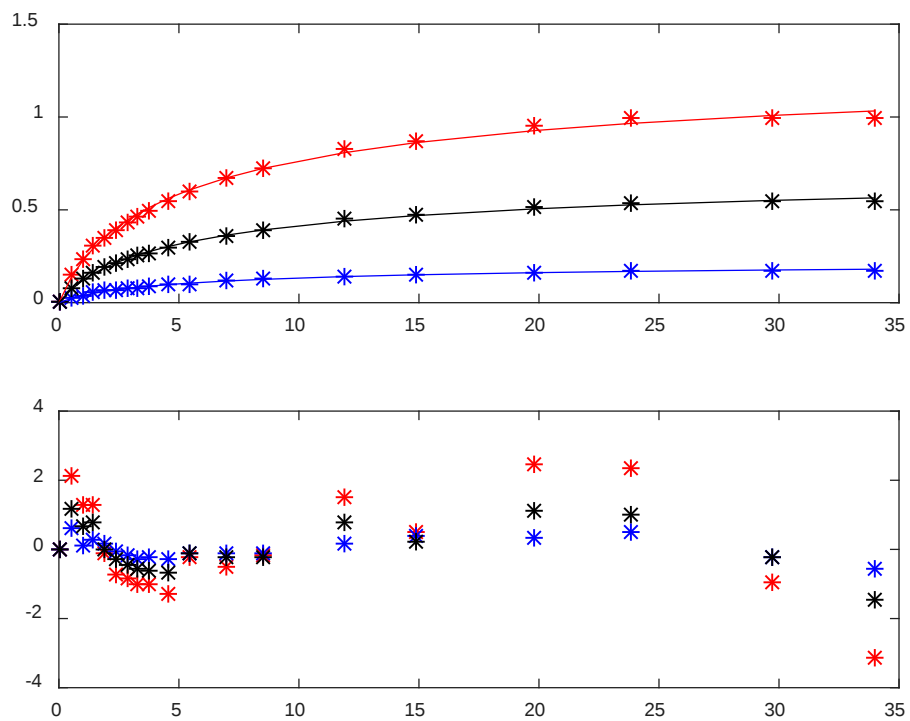


Figure 25. MatLab fit of binding isotherm for Br^- titration with **1**.

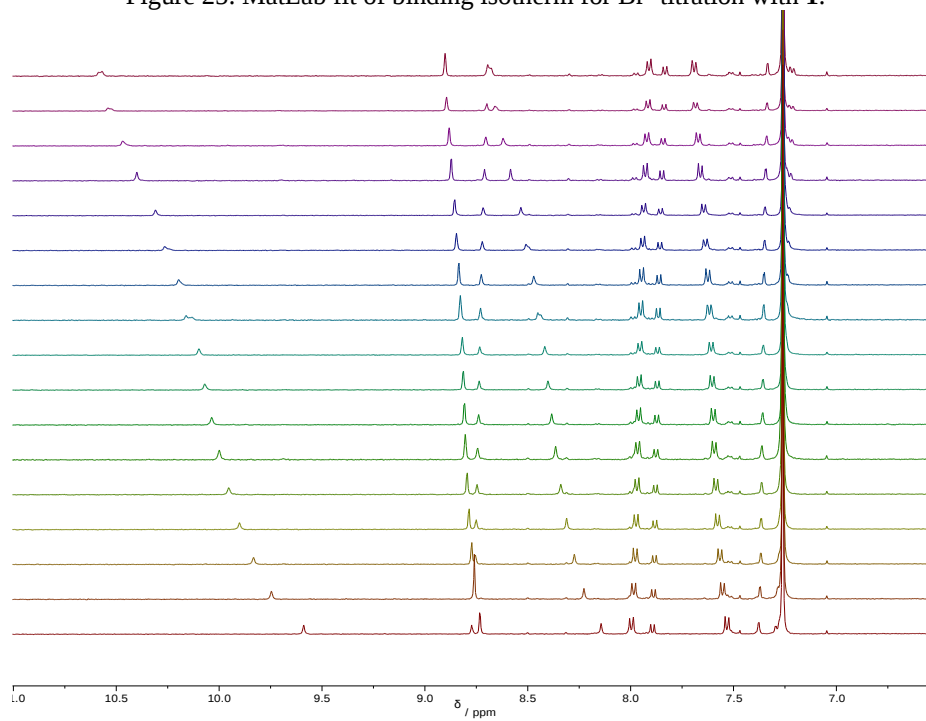


Figure 26: ^1H NMR spectra of Br^- titration with **1**.

Tetrabutylammonium iodide with 1. A concentrated solution of **1** (5.22 mg, [R]=5.18 mM) in 10% d_6 -DMSO/ $CDCl_3$ (2.00 mL) was prepared. A serial dilution was then performed with 590 μ L of 5.18 mM solution of **1** diluted to 3 mL to yield the stock solution of **1** ([R]=1.02 mM). This solution was used in the dilution of TBAI guest solution (51.76 mg, [G]=5.94 mM). The remaining stock solution (0.600 mL) was used as the starting volume in the NMR tube.

Table 5: Representative titration data for I^- with **1**.

	Guest (μ L)	[1] (M)	[I^-] (M)	Equiv.	H^c δ (ppm)	H^a δ (ppm)	H^b δ (ppm)
1	0	1.01E-03	0.00E+00	0.00	9.590	8.730	8.144
2	5	1.01E-03	4.95E-04	0.49	9.743	8.759	8.225
3	10	1.01E-03	9.83E-04	0.98	9.828	8.770	8.270
4	15	1.01E-03	1.46E-03	1.45	9.900	8.784	8.310
5	20	1.01E-03	1.93E-03	1.92	9.943	8.793	8.333
6	25	1.01E-03	2.40E-03	2.38	9.984	8.799	8.356
7	30	1.01E-03	2.85E-03	2.83	10.024	8.805	8.377
8	35	1.01E-03	3.30E-03	3.28	10.058	8.810	8.395
9	40	1.01E-03	3.75E-03	3.72	10.089	8.816	8.412
10	50	1.01E-03	4.61E-03	4.58	10.139	8.825	8.440
11	60	1.01E-03	5.45E-03	5.41	10.194	8.834	8.470
12	80	1.01E-03	7.05E-03	7.00	10.258	8.846	8.506
13	100	1.01E-03	8.56E-03	8.50	10.312	8.855	8.534
14	150	1.01E-03	1.20E-02	11.90	10.415	8.873	8.592
15	200	1.01E-03	1.50E-02	14.87	10.458	8.885	8.616
16	300	1.01E-03	2.00E-02	19.83	10.544	8.896	8.662
17	400	1.01E-03	2.40E-02	23.79	10.581	8.904	8.682
18	600	1.01E-03	3.00E-02	29.74	10.589	8.904	8.693

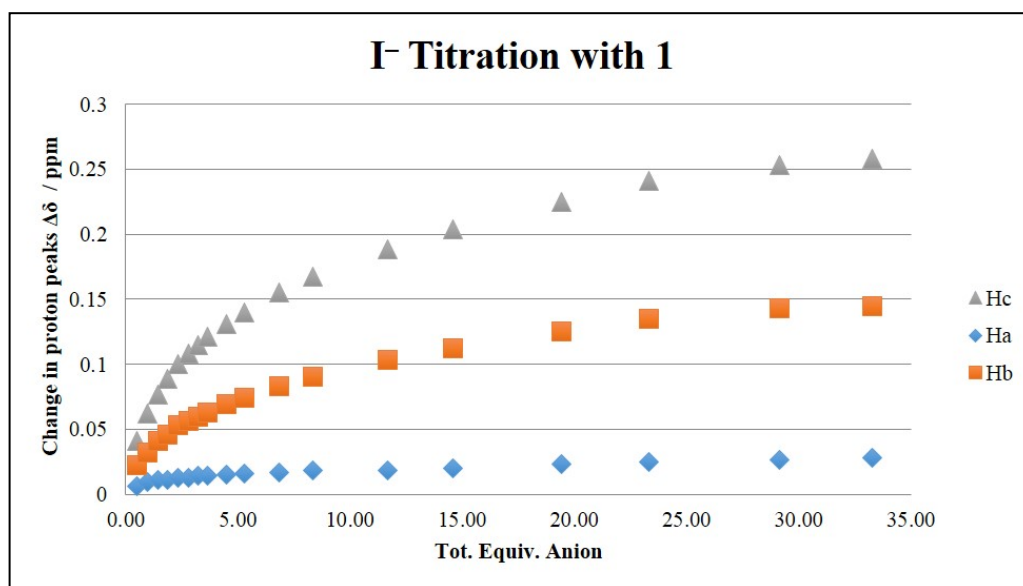


Figure 27: Binding isotherm for I⁻ titration with **1**.

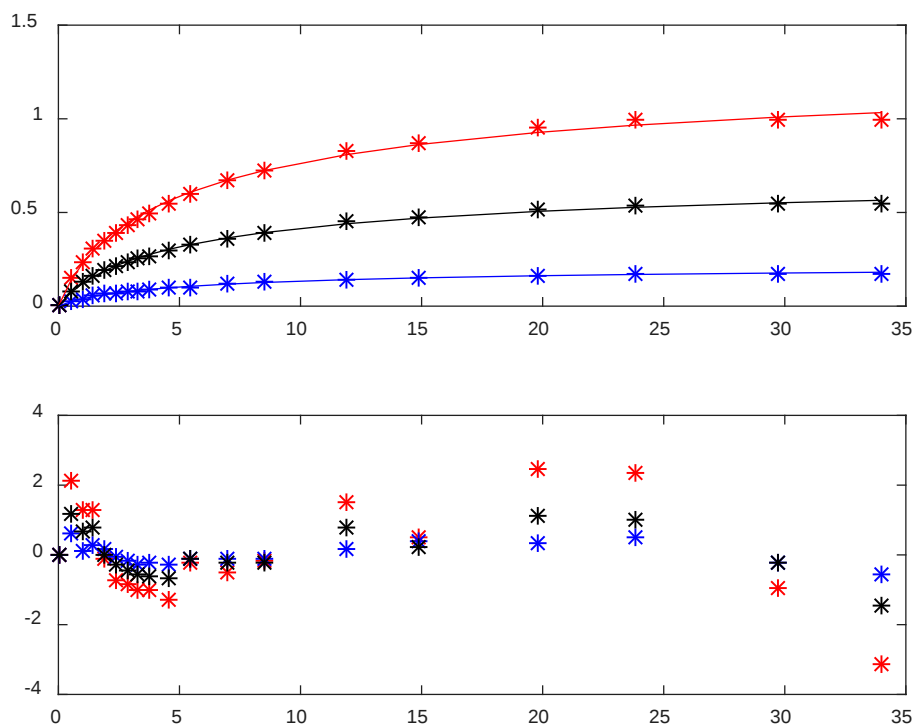


Figure 28: MatLab fit of binding isotherm for I⁻ titration with **1**.

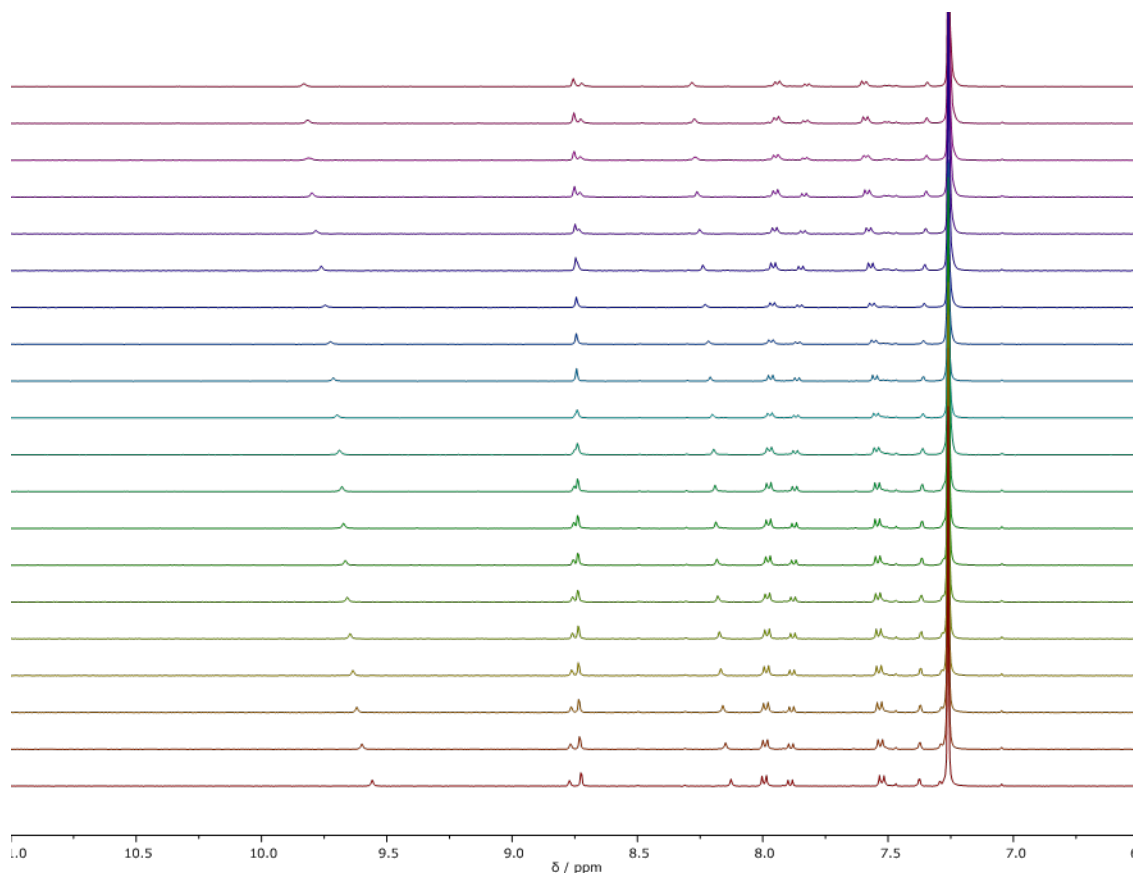


Figure 29: ^1H NMR spectra of I^- titration with **1**.

Tetrabutylammonium chloride with 2. A concentrated solution of **2** (2.96mg, $[\text{R}]=5.88 \text{ mM}$) in 10% d_6 -DMSO/ CDCl_3 (1.00 mL) was prepared. A serial dilution was then performed with 511 μL of 5.88 mM solution of **2** diluted to 3 mL to yield the stock solution of **2** ($[\text{R}]=1.00 \text{ mM}$). This solution was used in the dilution of TBACl guest solution (9.56 mg, $[\text{G}]=14.9 \text{ mM}$). The remaining stock solution (0.600 mL) was used as the starting volume in the NMR tube. The calculated association constants for the titration of TBACl with **2** were at the limits of ^1H NMR titrations, although errors were less than 15% across three titrations. The μM concentrations needed to obtain UV-Vis spectroscopy titration data dilute out the expected 2:1 host-guest model, however, leading to titrations only appropriately fit to a 1:1 host-guest model.

Table 6: Representative titration data for Cl⁻ with 2.

	Guest (μL)	[2] (M)	[Cl ⁻] (M)	Equiv.	H ^a δ (ppm)	H ^b δ (ppm)
1	0	1.00E-03	0.00E+00	0.00	9.617	8.119
2	5	1.00E-03	1.23E-04	0.12	9.888	8.230
3	10	1.00E-03	2.44E-04	0.24	10.145	8.335
4	15	1.00E-03	3.62E-04	0.36	10.379	8.428
5	20	1.00E-03	4.79E-04	0.48	10.562	8.502
6	25	1.00E-03	5.94E-04	0.59	10.695	8.555
7	30	1.00E-03	7.08E-04	0.71	10.775	8.587
8	35	1.00E-03	8.19E-04	0.82	10.819	8.604
9	40	1.00E-03	9.29E-04	0.93	10.847	8.615
10	50	1.00E-03	1.14E-03	1.14	10.877	8.626
11	60	1.00E-03	1.35E-03	1.35	10.892	8.631
12	80	1.00E-03	1.75E-03	1.75	10.910	8.637
13	100	1.00E-03	2.12E-03	2.12	10.919	8.640
14	150	1.00E-03	2.97E-03	2.97	10.929	8.643
15	200	1.00E-03	3.71E-03	3.71	10.933	8.643
16	300	1.00E-03	4.95E-03	4.95	10.939	8.645
17	400	1.00E-03	5.94E-03	5.93	10.941	8.645
18	600	1.00E-03	7.43E-03	7.42	10.944	8.646

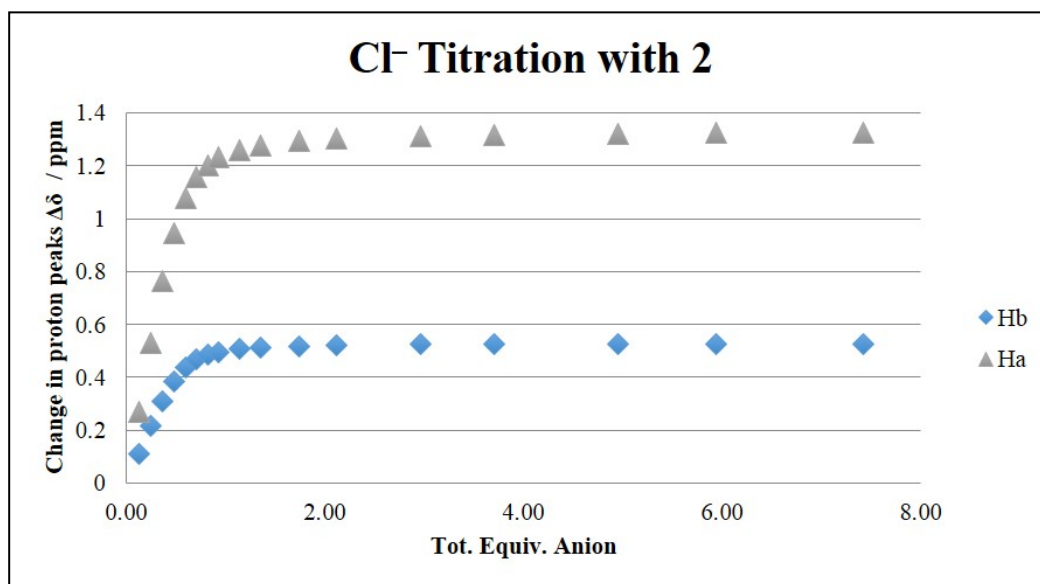


Figure 31: Binding isotherm for Cl⁻ titration with 2.

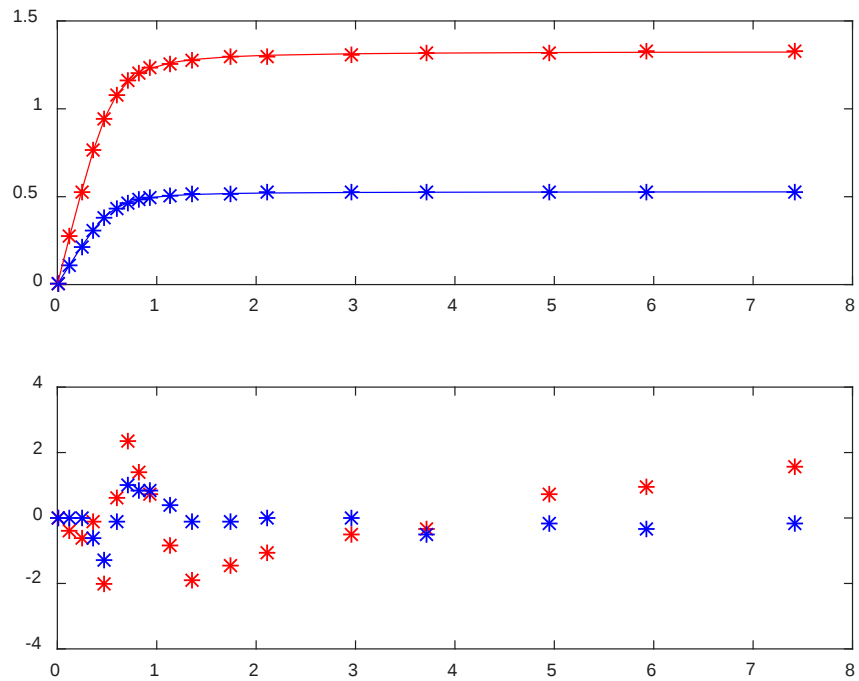


Figure 31: MatLab fit of binding isotherm for Cl^- titration with 2.

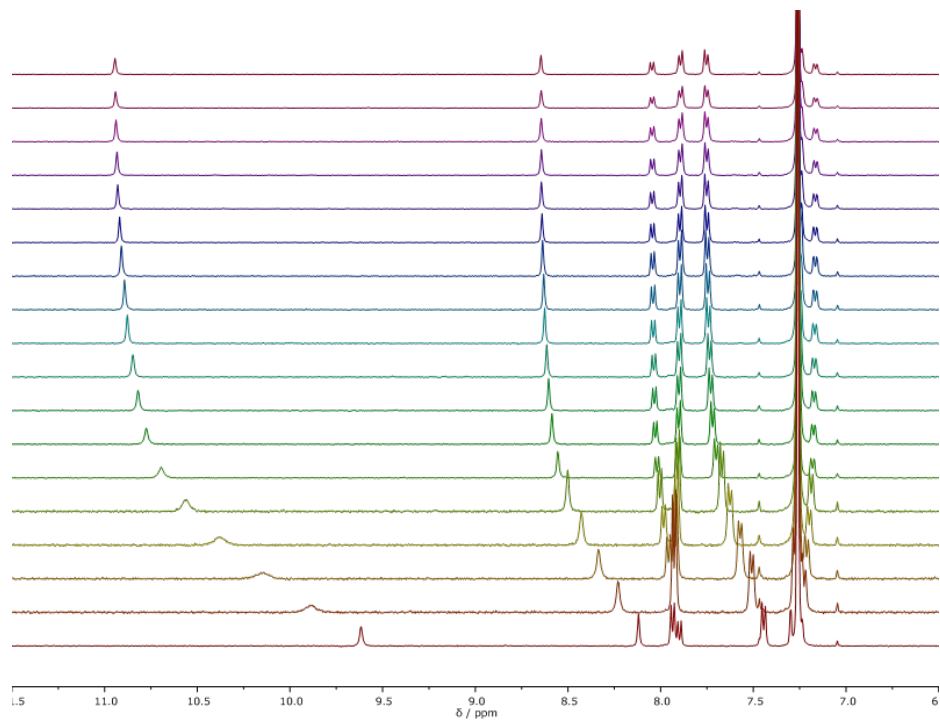


Figure 32: ^1H NMR spectra of Cl^- titration with 2.

Tetrabutylammonium bromide with 2. A concentrated solution of **2** (5.21 mg, [R]=5.17 mM) in 10% d_6 -DMSO/ $CDCl_3$ (2.00 mL) was prepared. A serial dilution was then performed with 590 μ L of 5.17 mM solution of **2** diluted to 3 mL to yield the stock solution of **2** ([R]=1.02 mM). This solution was used in the dilution of TBABr guest solution (23.01 mg, [G]=31.0 mM). The remaining stock solution (0.600 mL) was used as the starting volume in the NMR tube.

Table 7: Representative titration data for Br^- with **2**.

	Guest (μ L)	[2] (M)	[Br^-] (M)	Equiv.	$H^a \delta$ (ppm)	$H^b \delta$ (ppm)
1	0	1.02E-03	0.00E+00	0.25	9.754	8.178
2	5	1.02E-03	2.56E-04	0.50	9.975	8.285
3	10	1.02E-03	5.08E-04	0.74	10.101	8.345
4	15	1.02E-03	7.55E-04	0.98	10.181	8.382
5	20	1.02E-03	9.99E-04	1.22	10.233	8.407
6	25	1.02E-03	1.24E-03	1.45	10.266	8.422
7	30	1.02E-03	1.47E-03	1.68	10.290	8.434
8	35	1.02E-03	1.71E-03	1.90	10.307	8.441
9	40	1.02E-03	1.94E-03	2.34	10.322	8.447
10	50	1.02E-03	2.38E-03	2.77	10.341	8.456
11	60	1.02E-03	2.82E-03	3.58	10.356	8.463
12	80	1.02E-03	3.64E-03	4.35	10.369	8.468
13	100	1.02E-03	4.42E-03	6.09	10.377	8.472
14	150	1.02E-03	6.19E-03	7.61	10.388	8.477
15	200	1.02E-03	7.74E-03	10.14	10.391	8.478
16	300	1.02E-03	1.03E-02	12.17	10.394	8.478
17	400	1.02E-03	1.24E-02	15.21	10.398	8.480
18	600	1.02E-03	1.55E-02	0.25	10.398	8.480

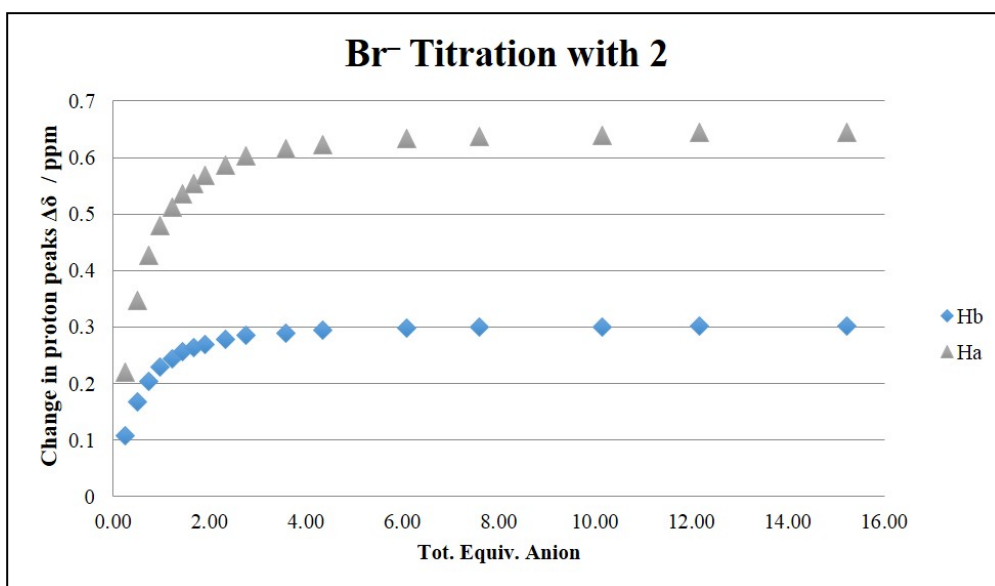


Figure 33: Binding isotherm for Br⁻ titration with 2.

Tetrabutylammonium iodide with 2. A concentrated solution of **2** (5.21 mg, [R]=5.17 mM) in 10% d_6 -DMSO/ $CDCl_3$ (2.00 mL) was prepared. A serial dilution was then performed with 576 μ L of 5.17 mM solution of **2** diluted to 3 mL to yield the stock solution of **2** ([R]=0.994 mM). This solution was used in the dilution of TBAI guest solution (26.11mg, [G]=31.21 mM). The remaining stock solution (0.600 mL) was used as the starting volume in the NMR tube.

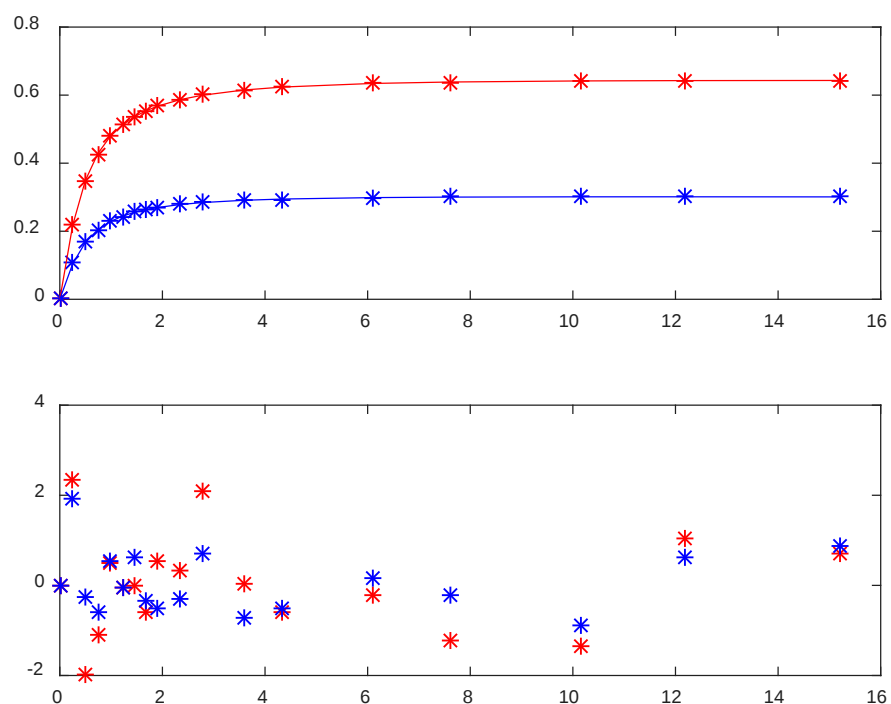


Figure 34: MatLab fit of binding isotherm for Br^- titration with **2**.

Table 8: Representative titration data for I^- with 2.

	Guest (μL)	[2] (M)	$[\text{I}^-]$ (M)	Equiv.	$\text{H}^a \delta$ (ppm)	$\text{H}^b \delta$ (ppm)
1	0	9.94E-04	0.00E+00	0.26	9.644	8.131
2	5	9.94E-04	2.58E-04	0.51	9.651	8.135
3	10	9.94E-04	5.12E-04	0.77	9.661	8.141
4	15	9.94E-04	7.61E-04	1.01	9.668	8.146
5	20	9.94E-04	1.01E-03	1.26	9.675	8.151
6	25	9.94E-04	1.25E-03	1.50	9.681	8.154
7	30	9.94E-04	1.49E-03	1.73	9.685	8.156
8	35	9.94E-04	1.72E-03	1.96	9.689	8.160
9	40	9.94E-04	1.95E-03	2.42	9.691	8.161
10	50	9.94E-04	2.40E-03	2.86	9.698	8.166
11	60	9.94E-04	2.84E-03	3.70	9.708	8.170
12	80	9.94E-04	3.67E-03	4.49	9.718	8.177
13	100	9.94E-04	4.46E-03	6.28	9.726	8.183
14	150	9.94E-04	6.24E-03	7.85	9.750	8.197
15	200	9.94E-04	7.80E-03	10.47	9.767	8.206
16	300	9.94E-04	1.04E-02	12.57	9.778	8.214
17	400	9.94E-04	1.25E-02	15.71	9.788	8.223
18	600	9.94E-04	1.56E-02	0.26	9.803	8.230

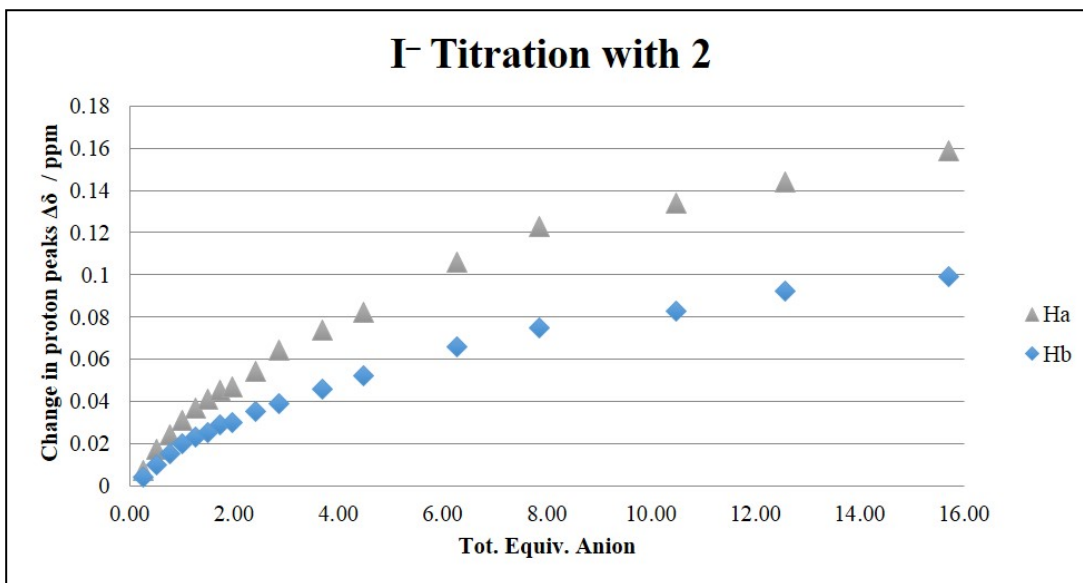


Figure 35: Binding isotherm for I^- titration with 2.

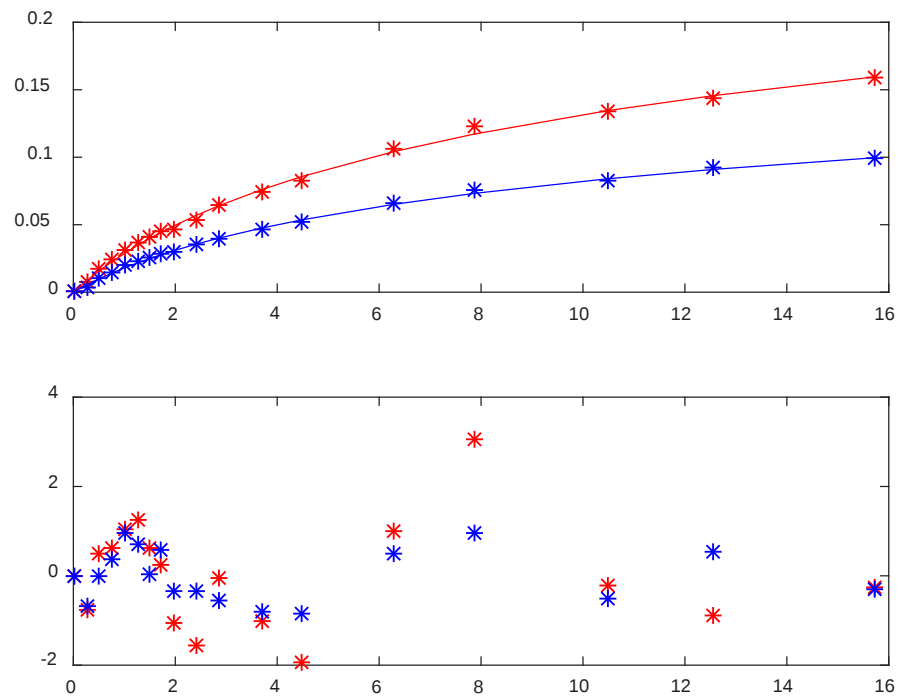


Figure 36: MatLab fit of binding isotherm for I^- titration with 2.

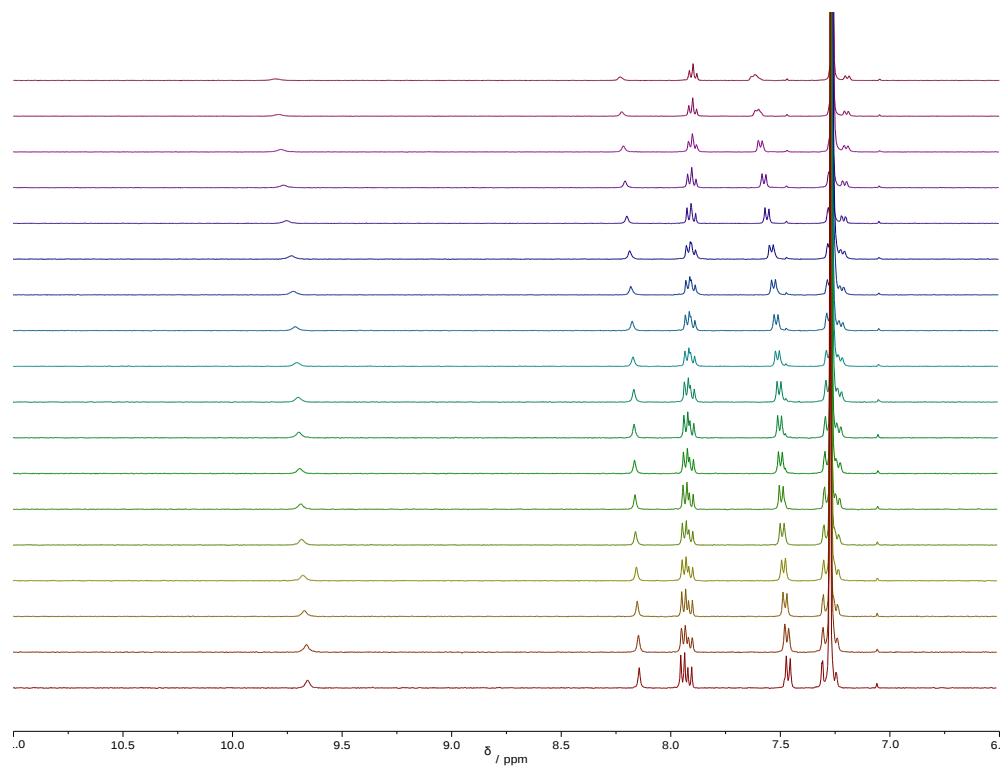


Figure 37: ^1H NMR spectra of I^- titration with **2**.

UV-Vis Titrations

General Conditions. UV-Vis titrations were carried out on an HP 8453 UV-Vis spectrometer. Water-saturated 10% DMSO/CHCl₃ was prepared in the same manner as for the ¹H NMR titrations. Association constants were determined by non-linear regression using Open Data Fit.¹⁹

Tetrabutylammonium chloride with 2. A concentrated solution of **2** (2.00 mg, [R]=0.199 mM) in 10% DMSO/CHCl₃ (20.00 mL) was prepared. A serial dilution was then performed with 50 µL of 0.199 mM solution of **2** diluted to 5 mL to yield the stock solution of **2** ([R]= 1.99 µM). A 2 mL solution of TBACl (2.53 mg, [G]=0.984 mM) was prepared by serial dilution with the stock solution of **2**. The starting volume in the cuvette was 2.0 mL.

Table 9: Representative titration data for Cl⁻ with **2**.

	Guest (µL)	[2] (M)	[Cl ⁻] (M)	Equiv.
1	0	1.99E-06	0.00E+00	0.00
2	5	1.99E-06	2.45E-06	1.23
3	10	1.99E-06	4.89E-06	2.46
4	15	1.99E-06	9.74E-06	4.90
5	20	1.99E-06	1.93E-05	9.71
6	25	1.99E-06	2.87E-05	14.42
7	30	1.99E-06	3.78E-05	19.05
8	40	1.99E-06	4.68E-05	23.58
9	50	1.99E-06	5.79E-05	29.13
10	60	1.99E-06	6.86E-05	34.55
11	70	1.99E-06	8.94E-05	45.02
12	80	1.99E-06	1.09E-04	55.03
13	100	1.99E-06	1.28E-04	64.60
14	120	1.99E-06	1.47E-04	73.76
15	140	1.99E-06	1.64E-04	82.54
16	180	1.99E-06	1.97E-04	99.05
17	220	1.99E-06	2.27E-04	114.28
18	300	1.99E-06	2.55E-04	128.39
19	400	1.99E-06	3.05E-04	153.69
20	600	1.99E-06	3.49E-04	175.73
21	800	1.99E-06	4.22E-04	212.24

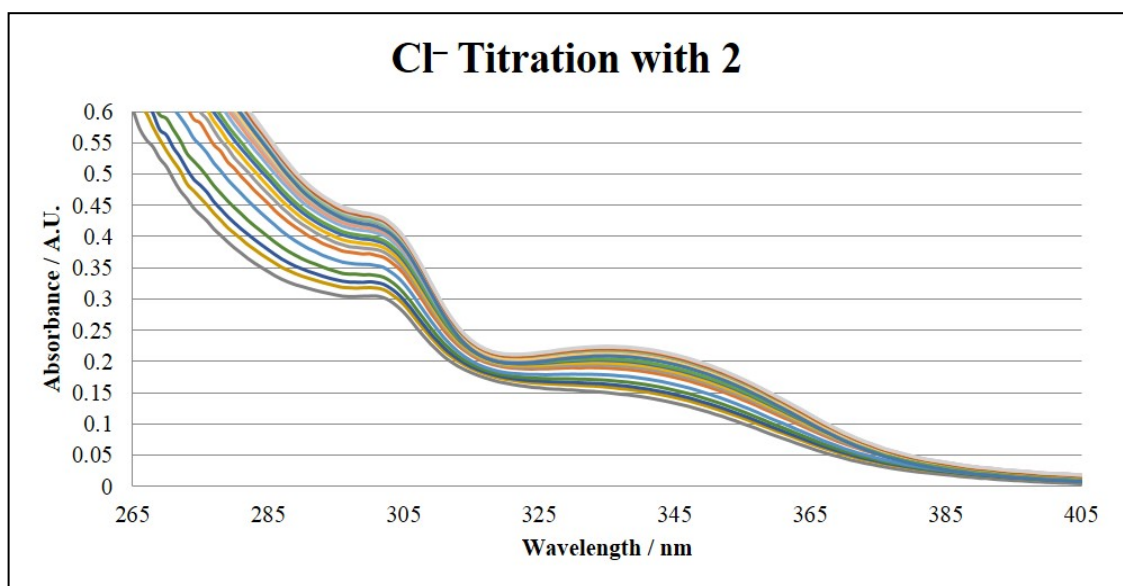


Figure 38: UV-Vis spectra of 2 titrated with Cl⁻.

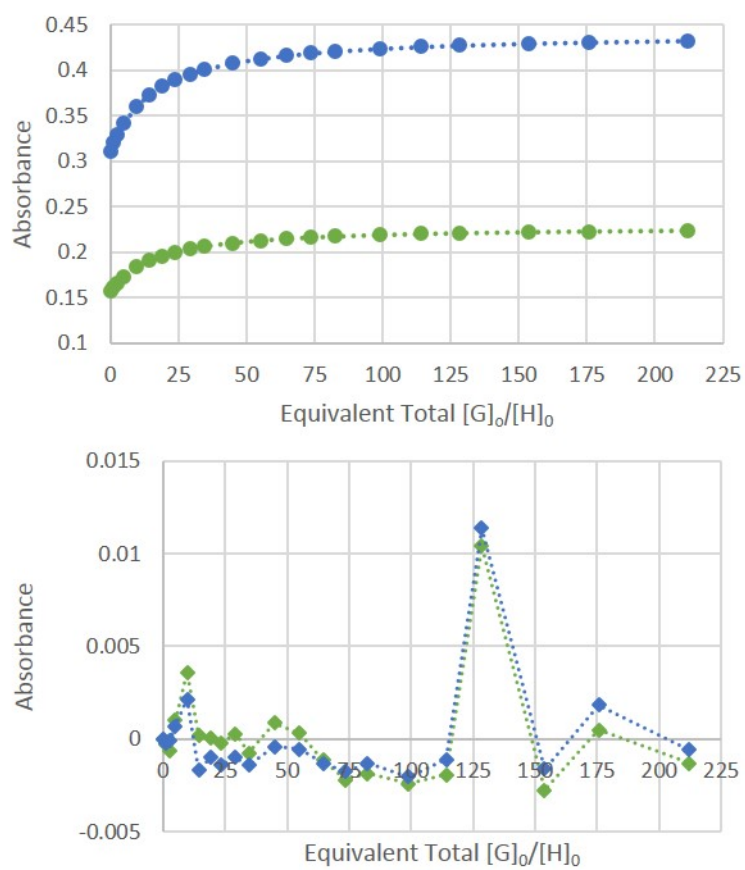


Figure 39: Open Data Fit fit of binding isotherm for Cl⁻ titration with 2.

Tetrabutylammonium Perchlorate with 3

A concentrated solution of **3** (2.12 mg, [R]=0.262 mM) in CHCl_3 (10.00 mL) was prepared. A serial dilution was then performed with 625 μL of 0.2615 mM solution of **2** diluted to 3 mL to yield the stock solution of **3** ([R]= 0.0327 mM). This solution was used in the dilution of $\text{TBA}\cdot\text{ClO}_4$ guest solution (3.99 mg, [G]= 5.85 mM). The starting volume in the cuvette was 2.0 mL.

Table 10: Representative titration data for ClO_4^- with **3**.

	Guest (μL)	[3] (M)	$[\text{Cl}^-]$ (M)	Equiv.
1	0	3.28E-05	0.00E+00	0.44
2	5	3.28E-05	1.46E-05	0.89
3	10	3.28E-05	2.90E-05	1.76
4	15	3.28E-05	5.78E-05	3.49
5	20	3.28E-05	1.14E-04	5.18
6	25	3.28E-05	1.70E-04	6.84
7	30	3.28E-05	2.24E-04	8.47
8	40	3.28E-05	2.78E-04	10.47
9	50	3.28E-05	3.43E-04	12.41
10	60	3.28E-05	4.07E-04	16.17
11	70	3.28E-05	5.30E-04	19.77
12	80	3.28E-05	6.48E-04	23.21
13	100	3.28E-05	7.61E-04	26.50
14	120	3.28E-05	8.69E-04	29.65
15	140	3.28E-05	9.72E-04	35.58
16	180	3.28E-05	1.17E-03	41.06
17	220	3.28E-05	1.35E-03	46.12
18	300	3.28E-05	1.51E-03	55.21
19	400	3.28E-05	1.81E-03	63.13
20	600	3.28E-05	2.07E-03	76.25
21	800	3.28E-05	2.50E-03	0.44

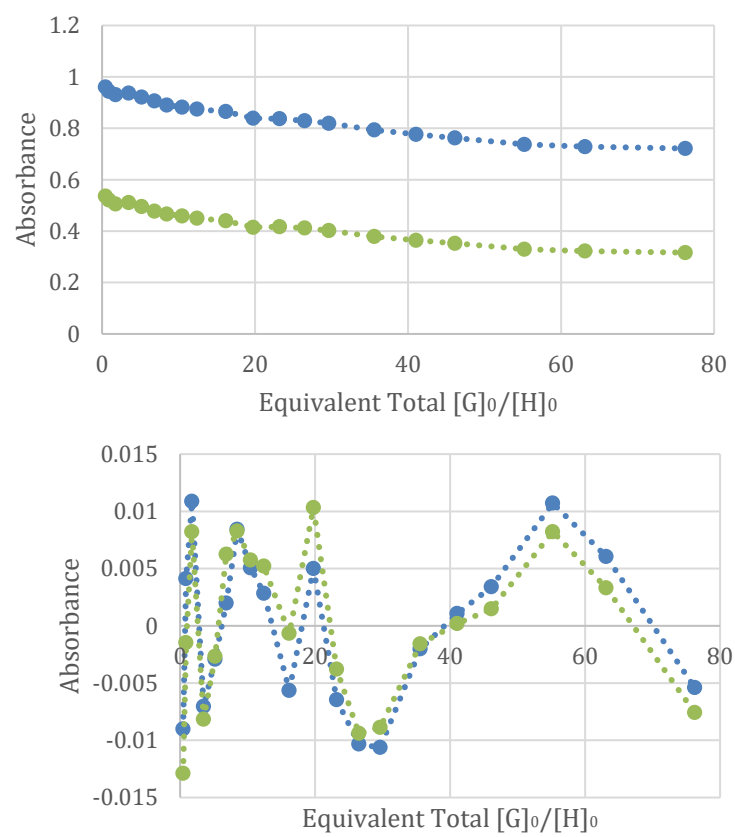


Figure 40: Open Data Fit fit of binding isotherm for ClO_4^- titration with **3**.

Job's Plot Analysis

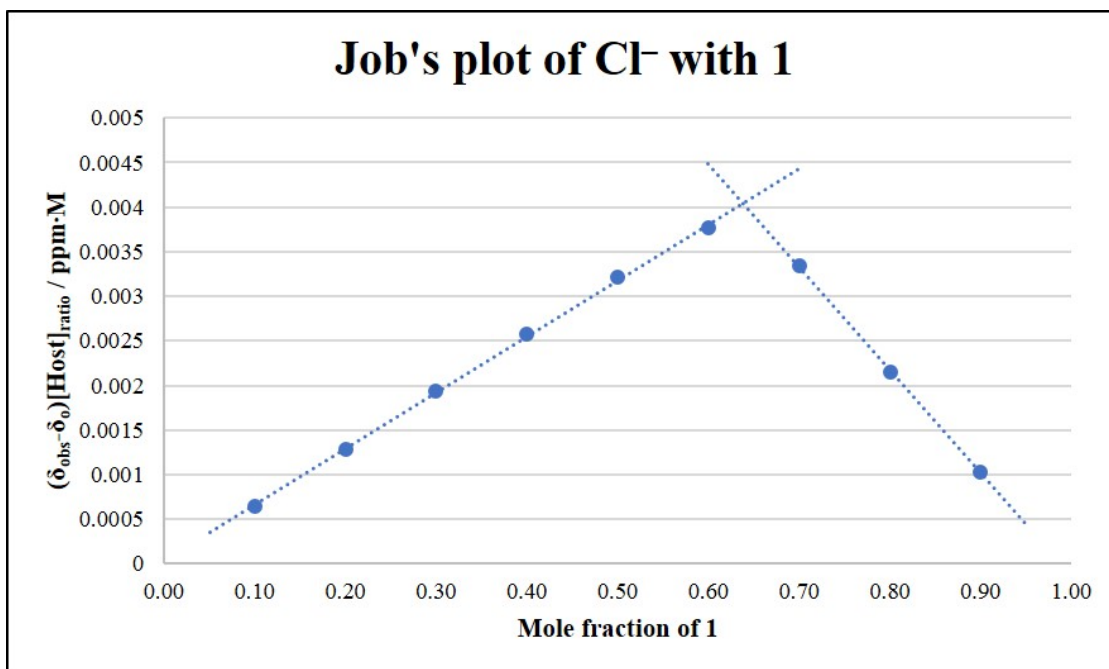


Figure 40: Job's plot analysis for Cl⁻ titration with 1.

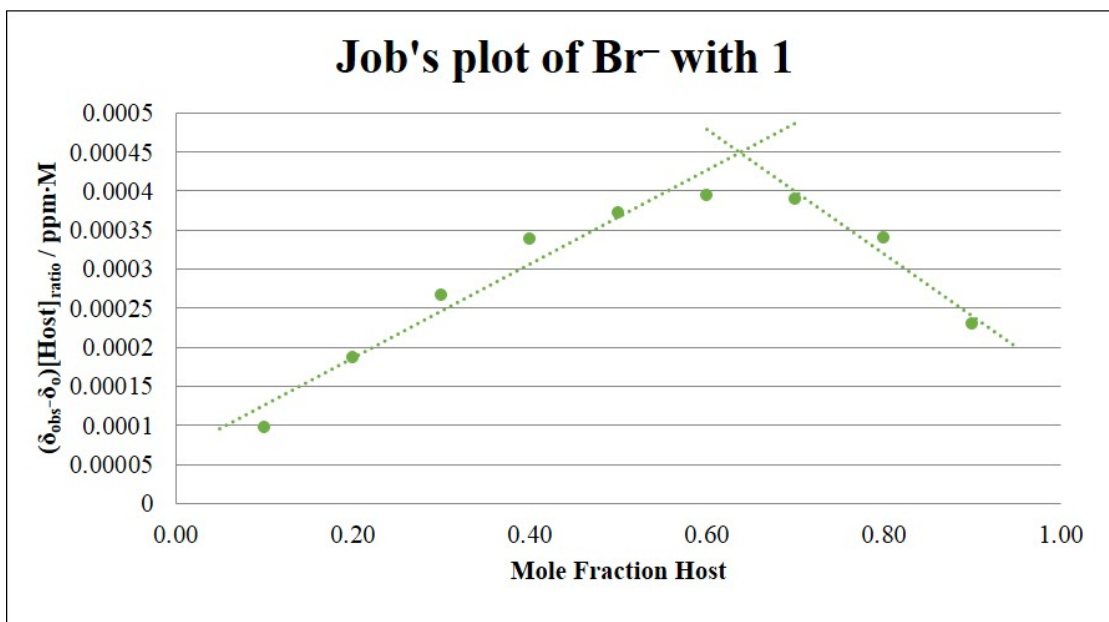


Figure 41: Job's plot analysis for Br⁻ titration with 1.

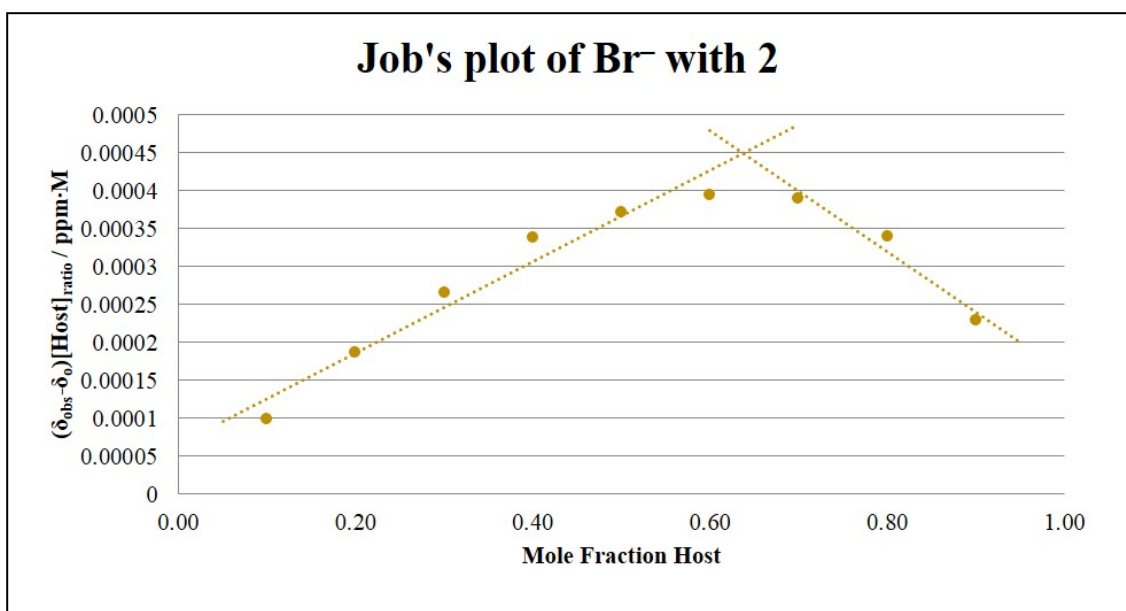


Figure 42: Job's plot analysis for Cl⁻ titration with 2.

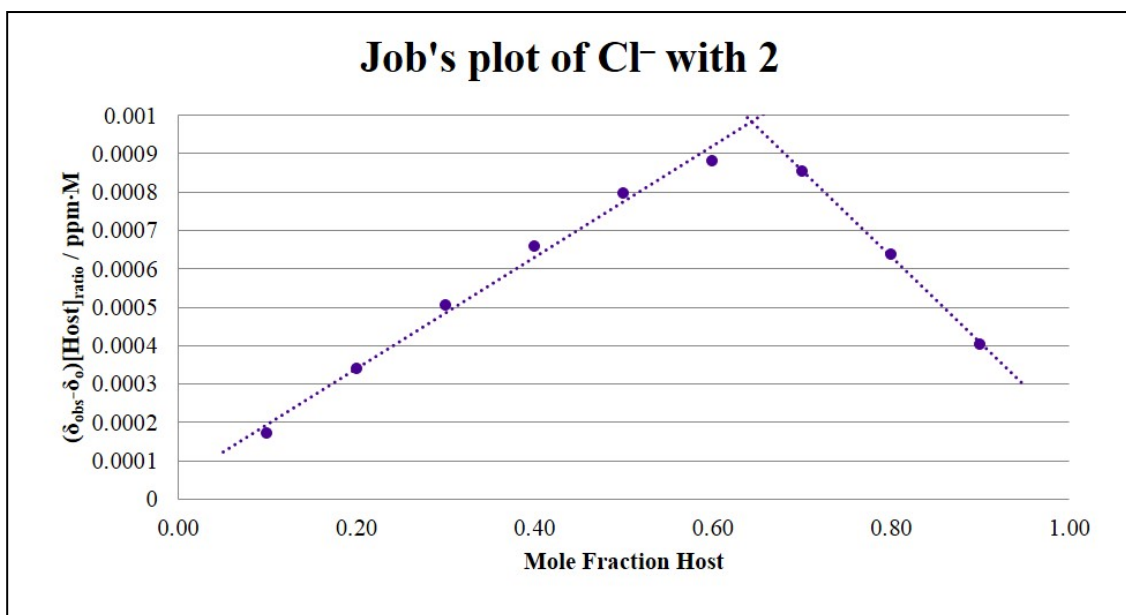


Figure 43: Job's plot analysis for Br⁻ titration with 2.

Plate Reader Screening

Screening was performed using a Tecan Spark 20M plate reader with a Hellma-Analytics quartz glass 96-well plate. All receptors were excited at 365nm.

Concentrated solutions of **3-13** (2.15-2.30mg, [R]=~0.0275 mM) in CHCl₃ (10.00 mL) was prepared. A serial dilution was then performed with 200-310μL of ~2.7x10⁻⁴ M solution of **3-13** diluted to 2 mL to yield the solution of **3** ([R]= 0.0325 mM). Concentrated solutions of TBA·ClO₄, TBA·NO₃, TBA·H₂PO₄, TBA·Br, TBA·I, and TBA·HSO₄ were prepared (2.08-2.17 mg, [G]= ~0.0012 mM). A serial dilution was then performed with 160-260μL of ~1.7x10⁻⁴M solution of **3** diluted to 2 mL to yield the solution of **3** ([R]= ~3.25x10⁻⁵M). Each well had a total volume of 200 μL. For the host wells, this included 100 μL of host, 60 μL of anion, and 40 μL of additional chloroform. For the blank anion wells, this included 60 μL of anion and 140 μL of additional chloroform. For the blank host wells, this included 100 μL of host and 100 μL of additional chloroform. The blank solvent well included only 200 μL of chloroform.

Glossary

Algal Bloom: A buildup of algae in a body of water. These outbreaks can lead to low oxygen environments that kills off aquatic life.

Alkyne: Carbon containing molecules that contain carbon-carbon triple bonds.



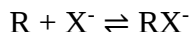
Anion: A negatively charged **ion**. An anion can be an atom or molecule (e.g. Cl^- , NO_3^-).

Arene: A six membered ring of carbons with alternating double bonds.



Aryl Unit: A functional group consisting of an aromatic ring (**arene**).

Association Constant: (K_a) Equilibrium constant associated with the binding reaction of a receptor molecule (also known as a binding constant).

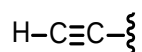


Conjugative Communication: Overlap of K_a electron orbitals so that electrons can be delocalized; this induces a fluorescence response.

Dimer: Two identical molecules linked together.

Electron orbital: A region of high probability for finding an electron.

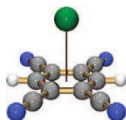
Ethynyl: Triple bonded carbon group



Fluorescence: The property of absorbing light of a short wavelength and emitting light of a longer wavelength.

Ion: A charged atom or molecule that forms an atom when it gains or loses one or more electrons.²⁰

Ion- π : Interaction between ions such as an anion and π electron orbitals.

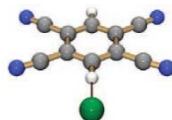


Job's plot analysis: Analytical technique used to determine the stoichiometry of a binding event.

Receptor: The molecule used to study the properties of other molecules or structures.

Heat Map: 2-dimensional representation of data that uses colors to represent individual values.

Hydrogen bonding: An attraction between two groups when a hydrogen is covalently bound to a highly electronegative atom.



Lambda Max (λ_{max}): The wavelength of maximum absorbance in an absorbance spectra.

Mole: An SI unit that measures the number of particles in a substance. A mole is equal to 6.022×10^{23} molecules or other elementary units (i.e. atoms, electrons, ions).

Mole Fraction: Another way of describing the concentration of a solution. Refers to the moles of one component divided by the total moles in the solution.

Monomer: One separate molecule that can bind chemically or supramolecularly to other molecules.

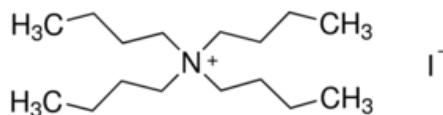
Proton: A positively charged hydrogen ion.

Quenching (fluorescence): The process of decreasing the fluorescence intensity of a substance.

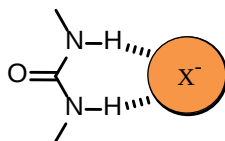
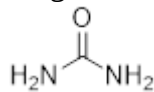
Stoichiometry: The relative quantities of reactants and products in chemical reactions. This research utilizes the term stoichiometry to represent the relative quantities of receptors and anions involved in a binding event.

Supramolecular Chemistry: The idea of two or more molecules or structures interacting or binding without forming strong irreversible bonds.

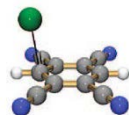
Tetrabutylammonium salt: A chemical compound consisting of an anion paired with a tetrabutylammonium cation. The tetrabutylammonium counter ion ensures dissociation in organic solvents while also avoiding cation effects in the binding cavity due to its large size.



Urea: Organic compound with the formula $\text{CO}(\text{NH}_2)_2$. This research incorporates urea groups within the binding cavity to hydrogen bond with the anion.



Weak- σ : An attractive interaction between an anion and σ electron orbital.



X-Ray Crystallography: A technique used for determining the molecular structure of a species in crystal form. This is done through measuring the diffraction of incident X-rays caused by the crystalline atoms present. This research attempts to isolate a single crystal of receptor bound with anion so we can perform X-ray crystallography and study the binding interactions occurring at a molecular level.

X-Ray Crystal Structure: Provides information about the connectivity of atoms and the binding interactions taking place.

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