SYNTHESIS AND QUANTITATIVE TESTING OF ELECTROCHEMICAL
AND OPTICAL SENSORS IN SURFACE FORMATS

A Major Qualifying Project Report:
Submitted to the Faculty
of
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirements for the
Degree of Bachelors of Science
by

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Date: April 26, 2007

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Acknowledgements

I would sincerely like to thank Dr. W. Grant McGimpsey and Dr. Christopher Lambert for giving me the opportunity to begin working in their lab in the summer following my sophomore year and for all their patience, help and guidance especially early on when I had no idea how to conduct research on my own. I would also like to thank them for giving me a project that was very interesting and quite challenging which – being focused mainly on surface, supramolecular and electro-chemistry – forced me to become reasonably proficient in fields which are very large but not generally touched on in undergraduate studies. As such, I feel I have learned a tremendous amount over the past two years and for this I am very grateful.

I would also like to thank Dr. Ernesto Soto, Peter Driscoll and Eftim Milakani for teaching me the analytical methods and core theory associated with the surface and electrochemistry research conducted here and Nantanit Wanichacheva and Man Phewluangdee for providing invaluable organic synthetic aid. While my advisors, mentioned above, gave me the opportunity to conduct research it would have proven completely ineffective without the help and patience of these, then graduate students, who took time out of their own busy schedules to get me started and guide me along the way. For this I am, again, very grateful. Every academic award I have won, during my tenure at WPI I owe to and share with these people.
Abstract

The mechanisms governing the formation of self assembled monolayers of alkane thiols on gold have been investigated for the past several years and are believed to involve an initial rapid covalent binding followed by a much slower reorganization process which results a well-organized organic crystalline/semi-crystalline layer. What is less well understood is the process of self-assembly for SAM’s with bulky terminating groups which are known to form significantly less ordered and thus more defect laden organic layers. Many potential nano-technological applications (such as molecular recognition) of such layers would make use of such head groups whose associated imperfections can substantially influence charge-carrier characteristics of the layer leading to a large degree of irreproducibility in layer function. As such it is the goal of the author to obtain reproducible and defect-free layers from such compounds for use in micro-fluidic blood sensor formats.

A series of ionophore and fluoroionophore terminated compounds have been synthesized to be used in the formation of organic thin-films on gold electrodes via self-assembly. These SAMs’ bulk optical and electrochemical behavior has been shown to change in the presence of target analytes and calibration curves and thermodynamic binding parameters have been determined. The sensor response, however, has proven highly variable and recently a great deal of this work has focused on fully characterizing and correcting the sources of this variability.
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I. Introduction

1. Molecular Level Sensors

The work which is presented here, put simply, consists of: the synthesis and quantitative testing of nano-scale sensors for surface based device applications for use, ultimately in a micro-fluidic blood sensor format. This sensor will be highly accurate given two independent modes of detecting the same analyte and will be cheap and disposable which would make it appealing from both economic and sanitation standpoints. There are two essential components to molecular level sensors: a binding moiety which selectively attaches to an analyte of interest and a signaling moiety which provides a mode of detection of that binding event.\textsuperscript{1,2} Through the determination of calibration curves of sensor response to analyte concentrations it is possible to extract binding parameters such as association constants (i.e. iterative fittings to Langmuir and Langmuir-type models) which provide information on the dynamic range of the sensor response (the concentration range over which the sensor signal can be detected and used to indicate analyte concentration).\textsuperscript{3,4} In addition, by comparing association constants for different analytes it is possible to quantitate the sensor selectivity; that is, how effectively the sensor can detect the target analyte in the presence of interfering analytes (other molecules which will bind to it and provide some kind of sensor response).\textsuperscript{5,6} In this work, the binding moiety utilized has been the alkali metal ionophore: 18-crown-6 which is known to bind potassium with reasonable selectivity.\textsuperscript{3,7-10}

2. Modes of Detection Used in this Work

The first mode of detection used, in this work, was that of fluorescence. By attaching a fluorophore to an aza-crown ether, through the crown’s nitrogen, it is possible to create a fluoroionophore which can be used to optically detect ion concentrations. The mechanism by
which this type of sensor functions, involves the fact that a nitrogen lone pair, when near a fluorophore, effectively quenches the fluorescence of that fluorophore, in a process known as photoinduced electron transfer (PET). When a cation is complexed to the crown ether, the electron density at the nitrogen lone pair becomes occupied and is no longer able to quench the fluorescence of the fluorophore. Thus the magnitude of the fluorescence can be correlated with exposed ion concentration.

The second mode of detection utilized the fact that these fluoroionophore molecules were deposited as a thin-film onto a conductive substrate. By using the conductive substrates as one electrode and the studied ionic solution as another electrode it is possible to characterize these thin-layer films electrically. As organic coatings, in general act as insulators the solution/electrolyte-SAM-substrate/electrode system can be taken to act as a two plate capacitor. When cations bind to the crown ether portion of the layer they, in general, cause an increase in the dielectric constant and thus capacitance of the film. Thus the magnitude of the coating capacitance can also be correlated with exposed ion concentrations.

3. A Timeline of the Work Presented here

The initial goal of this work was form a fluoroionophore terminated self-assembled monolayer (SAM) on to a conducting and transparent substrate to make simultaneous fluorescent and electrochemical measurements the most feasible. The substrate used was indium tin-oxide (ITO) on glass and a multilayer approach to thin-film assembly was used wherein a first layer was attached which was terminated with an amino group which would in turn be reacted with an activated carboxylic acid group which would be tethered, via a long alkyl chain, to a fluoroionophore molecule (see Figure 7). This multilayer approach succeeded previous work on a monolayer one which had proven untenable on these substrates. Thus the first stage of
this work consisted of the eleven months of organic synthesis of an anthracene-crown-acid molecule (ACA).

Once this molecule was synthesized and first and second layer depositions were confirmed via surface hydrophobicity (i.e. water contact angle) and fluorescence measurements. Unfortunately, though fluorescent response to exposed alkali ions was obtained it was only obtainable when using non-polar salt solutions and proved highly variable and unquantifiable. What’s more electrochemistry on these substrates was shown to be nearly impossible most likely as a result of imperfections in the conducting ITO coating. At this point the transition was made to gold substrates/electrodes.

More surface characterization methods are available and better packed mono and multilayer SAM’s are known to form when using gold substrates. As such, first and second layer depositions were confirmed and characterized using contact angle, ellipsometric, cyclic voltammetric, grazing infrared and impedance spectroscopic measurements. Impedance spectroscopic titrations (detecting capacitance change) were conducted, over a period of several months, using this and two other analogous crown-ether terminated monolayers. The resulting curves were fit to Langmuir isotherms and association constants were extracted. At the same time, similar solution fluorescence calibration curves were determined in order to get an idea of the optical sensitivity and selectivity of these sensors. These were to be compared to gold surface fluorescence measurements conducted using a laser reflection mode of detection, at a later time.

Unfortunately, it was found (when conducting control experiments) that the perceived electrochemical response actually corresponded to a slow destruction of the layer. As such, all previous electrochemical measurement had to be thrown out and a month was spent trying to
figure out how to obtain SAM’s which were stable to electrochemical characterization. At the
eend of this period, however, it was found that the instrument had been applying a very large
potential across the sample layers for a millisecond preceding each measurement and so a new
instrument was ordered.

At this point, a series of general multilayer experiments were conducted with a series of
alkyl amine thiol, first layers and long alkyl chain carboxylic acid second layers. The purpose of
these experiments was to determine the optimal formation conditions (solvents, coupling
reagents, first and second layer materials, etc.) for obtaining multilayers with the best and most
reproducible characteristics. Characterization of the resulting layers was accomplished using all
methods other than impedance spectroscopy as the new instrument had not yet arrived yet. At
the same time construction of a series of analytical electrodes was begun so that, when the new
instrument did arrive, more quantitative capacitance measurements could be conducted allowing
more accurate determination of selectivity and sensitivity information with less measurements
(in titration/calibration experiments).

Unfortunately, even with the analytical electrodes, sensor layer characteristics and
response proved highly variable and so several months of work were devoted to identifying and
modulating various characteristics of the substrate, adsorbates and layer deposition process
which influenced this variability. At the end of this work an electrochemical tool box had been
developed which allowed the quantitative measurement and modulation of such things as
electrode cleanliness and roughness; and layer coverage, composition, crystallinity and ideality.
This should prove useful for future mechanistic work, which will be geared to evaluating the
reasons behind sensor layer characteristics and functioning, as a more complete understanding of
the systems under study will be obtainable.
II. Materials and Methods

A. Organic Synthesis

Most of the compounds here used were synthesized in the laboratory using anhydrous techniques. The specific synthetic procedures are detailed in the Synthesis section of the Results and Discussion. All reagents and solvents were used, without further purification, from Aldrich or Alfa Aesar. $^1$H-NMR and $^{13}$C-NMR spectra were obtained using a 400 MHz Bruker Avance NMR spectrometer, Mass spectra were performed by Synprep Corporation (Dublin, CA) or obtained using an electrospray Waters Micromass ZMD Mass spectrometer. Attenuated total reflection FT-IR spectra were acquired with a Nexus FT-IR model 670 spectrometer and UV-Vis absorption spectra were obtained using a Shimadzu UV2100U UV-Vis spectrophotometer.

B. Monolayer and Multilayer Preparation

Monolayers and Multilayers were prepared on either gold or indium tin oxide (ITO) substrates by immersing them in solutions of compounds featuring a long alkyl chain terminated at one end with a reactive head group known to bind to the substrate in question. ITO was initially used because of its optical transparency which, it was assumed, would make easier surface fluorescence-detection studies. Unfortunately the quality of the layers on ITO proved to be, in general, poor and quite variable such that electrochemistry (which would provide the other means of analyte detection for these sensing coatings) appeared to be completely unfeasible. As such the transition was made to gold substrates which provided significantly improved charge-transfer characteristics. As such another mode of fluorescence detection, involving reflecting lasers, was sought.

1. Monolayers and Multilayers on Gold
Clean gold provides a very active surface which is known to become very quickly passivated by the adsorption of many types of contaminants when left under ambient conditions. At the same time it is known to form a strong, covalent bond to sulfur which is quite stable and will readily displace and/or prevent the physisorption of other materials. When attached to an alkyl chain, sulfur-gold compounds have been shown to reorganize and assume a highly reproducible, pseudo-crystalline structure.\textsuperscript{17, 18} As such, self assembled monolayers on gold were formed by immersing the substrate/electrode in an alcoholic solvent containing a 1-5 mM concentration of a target thiol or disulfide compound. The specific reaction by which thiols bind to gold involves the oxidation of the gold surface and the reduction of either hydrogen or oxygen, depending on whether or not the SAM is formed in the presence of dissolved oxygen\textsuperscript{6}:

\begin{align*}
\text{Au} + \text{RSH} &\rightarrow \text{Au} - \text{SR} + \frac{1}{2} \text{H}_2 & (1) \\
\text{Au} + \text{RSH} + \frac{1}{4} \text{O}_2 &\rightarrow \text{Au} - \text{SR} + \frac{1}{2} \text{H}_2\text{O} & (2)
\end{align*}

Now, the mechanism of self-assembly for alkane thiols on gold involves first a very rapid adsorption step (reaching \textasciitilde 99\% coverage in minutes) followed by a much slower reorganization step which occurs on the order of hours typically but can take as long as a few days. This reorganization is made favorable by Van der Waals interactions between the long alkyl chains but can be impeded by the presence of a bulky terminating group at the other end of the chain.\textsuperscript{17-20} This complicates the reorganizing process and can lead to either longer reorganization times and/or less well packed layers depending on the size and nature of that functionalizing group.\textsuperscript{21} As very bulky head groups were used in these experiments, depositions times over 24 hours and longer tethering alkyl chains, than those used for similar work in the literature, were used (to maximize Van der Waals interactions and assure the formation of a well-packed, well-defined
coating). Multilayers were formed by first pre-functionalizing the gold surface with an amine-terminated thiol and immersing that surface in an activated, long-chain, carboxylic acid terminated compound. After being formed monolayers and multilayers were, typically, extensively rinsed with ethanol, deionized water, and dried with nitrogen before they were characterized. Preceding and following characterization they would be stored in their respective deposition solutions.

The two different types of gold substrates/electrodes which were utilized were large gold slides and analytical microelectrodes. The gold slides were purchased from Evaporated Metal Films (Ithaca, New York) and were composed of glass coated with 5 nm of titanium or chromium coated with 100 nm of gold. They were received 25 mm x 75 mm x 1 mm in size and were cut to different sizes depending on the experiment for which they were used. These experiments included contact angle, ellipsometry, External reflectance FT-IR and qualitative electrochemistry. These were chemically pretreated by immersing them in hot piranha solution (70% concentrated sulfuric acid, 30% hydrogen peroxide) for 5-10 minutes after which they were rinsed with deionized water and ethanol and dried with nitrogen. Immediately following drying they were placed in their deposition solutions to prevent contamination of the surface.

The analytical electrodes were made for quantitative electrochemical characterization using 2 mm and 0.5 mm diameter gold wires using the procedure of Maran. These electrodes were constructed by soldering a ~0.5 cm length of gold wire to 6-in length of copper wire which was placed within glass tubing and secured and sealed using Torrseal vacuum sealant. The gold surface was then revealed and brought to a mirror finish by polishing with successively higher grit silicon carbide papers (400, 1200, 4000) followed by polishing with successively smaller particle size diamond pastes on micro-cloth pads (6, 3, 1 and ¼ micron) all of which were
purchased from Streuers (Denmark). Between polishing steps the electrodes were briefly sonicated in isopropanol and at the end of the polishing sequence they were sonicated for 5 minutes in isopropanol with decolorizing carbon, briefly sonicated in acetone and stored in isopropanol. While mechanical polishing constituted the entire pretreatment procedure preceding SAM deposition, initially, electrochemical polishing and electrochemical cleaning were later added and were accomplished by cycling the working electrode between gold’s redox potentials in either sulfuric acid or phosphate buffer 200+ times for polishing and 25 times for cleaning immediately preceding placement in deposition solutions. More details on this procedure are given in the Electrochemical Polishing/Cleaning section.

**Figure 1.** Schematic of analytical electrode construction
2. Monolayers and Multilayers on ITO

Many different functional groups have been shown to adsorb to ITO including thiols, amines and carboxylic acids but the best and most reproducible results have been obtained using alkoxy and chloro-silanes. The mechanism which governs this adsorption is believed to involve the acid-catalyzed nucleophilic substitution of the alkoxy or chloride groups with hydroxyl groups which terminate the semiconducting ITO surface. Unfortunately, while high coverage silane SAM’s have been obtained on ITO, their quality has never proven comparable to those seen on gold, most likely due to the fact that the metal atoms in substrates such as gold are significantly more dynamic and mobile and thus can provide a further contribution to the reorganization/crystallization of an adsorbed film. When silanes attach to ITO or glass, on the other hand, they are more or less stuck, and so the formation of well packed and ordered 2D unit cells is significantly less likely.

ITO slides were obtained from Delta Technologies (Stillwater, MN) with dimensions 25 mm x 75 mm x 1.1 mm and were also cut according to experimental need. They were chemically pretreated by rinsing with ethanol, followed by sonication in 5% Hydrochloric acid for 15 minutes at which point they were rinsed with water and ethanol and dried with nitrogen. They were immediately placed in their respective depositions solutions which were, in general, 10% by volume triethoxysilane in alcoholic solvents. As with the gold substrates, they were rinsed with copious amounts of ethanol and water and dried with nitrogen immediately preceding characterization and they were stored either in their respective deposition solutions. Multilayers were also formed in an analogous manner using amine-terminated triethoxysilanes and activated carboxylic acid solutions.
C. Contact Angle Goniometry

Contact angle ($\theta$) measurements on a surface provide a quantitative measure of the wettability of that surface; that is, the favorability of interactions between the liquid and the surface where, angles around 0 are indicative of complete wetting and favorability, angles around 45 degree are indicative of partial wetting and favorability and contact angles over 90 degrees are indicative of no wetting and un-favorability. In practice, the angle formed between a liquid drop and a surface is measured at the contact line (circle) which defines the contact between the drop and the surface.

Partial Wetting ($\theta<90^\circ$)  
Partial Wetting ($\theta=90^\circ$)  
Complete Wetting ($\theta>90^\circ$)

Figure 2. The range of wetting behavior observed in this work.

At this line, there exists a three-phase equilibrium between the solid, the liquid and the vapor which is characterized by three interfaces: a solid-liquid interface, a solid-vapor interface and a vapor liquid interface each with its own free energy per unit area ($\gamma_{SL}, \gamma_{SV}$ and $\gamma_{VL}$, respectively). Now, it has been shown that the angle which forms between these three phases is solely dependant on these interfacial energies and that, at equilibrium, a differential shift in the line position should not have an effect on the energies of the overall system as, for instance, when the area increases between the surface and the vapor (+dx) and there is an accompanying decrease in the areas of the surface-liquid (-dx) and the liquid vapor interfaces (-cos(\theta)dx). Therefore, the total interfacial energy should remain the same such that:
\[ \gamma_{SV} dx - \gamma_{SL} dx - \cos \theta \gamma_{LV} dx = 0 \quad (3) \]

Or rather, dividing by \(dx\):

\[ \gamma_{SV} - \gamma_{SL} - \cos \theta \gamma_{LV} = 0 \quad (4) \]

As the liquid vapor energy is usually known (\(\gamma_{VL} \equiv \gamma\)) it is therefore possible to directly relate the equilibrium contact angle directly to the difference in the two surface interfacial energies:

\[ \theta = \cos^{-1} \frac{\gamma_{SV} - \gamma_{SL}}{\gamma} \quad (5) \]

Now the typical static contact angle (which is measured by simply applying a drop to the surface and measuring the contact angle) is often a macroscopic measure of a range of microscopic surface energies and in these cases constitutes a weighted average.\(^{23}\) By measuring, in addition, the dynamic advancing (measured as a drop is placed on the surface) and receding contact angles (measured as a drop is withdrawn from a surface) more information can be gleaned on the range of those surface energies.\(^{23}\) This can be explained using water as an example: when there exists hydrophilic and hydrophobic domains on the surface in question then as the drop advances hydrophobic domains will have the most pronounced effect as they will effectively pin the motion of the contact line increasing the contact angle. Hydrophilic regions, on the other hand, will have the most significant effect on the contact angle as the drop recedes, holding back the draining motion of the contact line, decreasing the observed contact angle. The difference between the advancing and receding contact angles is known as hysteresis and can be directly related to the non-ideality of the surface. It has been directly correlated with physical roughness (or for SAM’s, the roughness of the substrate) and heterogeneity of surface energies at the surface (which for SAM’s can translate to the presence of defects and/or the presence of conformational differences in the intended surface functionality (i.e. poor packing)). Additional
factors which can influence contact angles of SAM’s especially include, the temperature and pH which can be used to confirm the presence of such terminal groups as carboxylic acids and amines which are susceptible to protonation/deprotonation based on differences in between basic and acidic contact angles (e.g. a carboxylate will be more hydrophilic than a carboxylic acid).\textsuperscript{6, 23} As contact angle measurements only probe the outer most few angstroms of a surface essentially all of the information which will be gleaned from contact angle measurements on SAM’s will relate to the nature, orientation and state of the functional groups used to terminate the SAM.\textsuperscript{24-26}

Contact angle measurements were made using a Rame-Hart Model 100-00 Goniometer (Mountain Lakes, NJ). 1 \( \mu \)L drops of base (pH 12), deionized water (pH 5.5) and acid (pH 2) were deposited using a micropipette and the static contact angle was measured. The angle was measured using an attached protractor and microscope.

D. Ellipsometry

The polarization of an incident optical wave, upon reflection, changes in a manner dependant on the reflective and refractive properties of the bulk material and any coatings or effective coatings at the surface.\textsuperscript{27} This change can be resolved into two polarized components, one parallel to (p) and one perpendicular to (s) the surface and can be defined in terms of relative phase and amplitude changes between these two components.\textsuperscript{28} The two angular metrics of ellipsometry, are the relative retardation (\( \Delta \), the relative change in phase between the reflected and incident components) and the polarization dependant loss angle (\( \Psi \), or the anti-tangent of the ratio of the ratio of the reflected and incident component amplitudes) defined in the equations below where \( \beta \) and \( A \) are frequency and amplitude, respectively:\textsuperscript{27, 28}

\[
\Delta = (\beta_p - \beta_s)_{\text{refl}} - (\beta_p - \beta_s)_{\text{inc}} \quad (6)
\]
\[
\tan \Psi = \frac{(A_p / A_s)_{refl}}{(A_p / A_s)_{inc}}
\]  

(7)

These changes, in general, directly related to the optical properties of the surface but in addition, when used to characterize thin-layer films, are sensitive to the refractive index (n), extinction coefficient (k) and thickness of those layers. Using then, known or independently determined values for the refractive indices and extinction coefficients for the substrates and the layer material it is possible to, by examining the change in polarization of incident monochromic light, determine the thickness of those films un-invasively.\(^{27, 28}\) Such things as temperature and substrate roughness are known to influence ellipsometric measurements.

**Figure 3.** Schematic of a general manual null ellipsometer\(^6, 27\)

The roughness of a surface can be quantitated in terms of the average height and length of irregularities about an average surface plane. The ratio of these values to the wavelength of the probing light can be used to determine whether these defects to "macroscopic" or "microscopic" roughness of the surface. When the height and length values of the irregularities are significantly greater than the wavelength of the light, then the light is scattered and only the light which reflects off the portion of the defects parallel to the average plane are detected. While this will not affect the thickness values obtained it can significantly reduce the sensitivity of the
instrument, making it very difficult to obtain reliable measurements especially when coverage is poor.27

Figure 4. The two different effects of surface roughness on ellipsometric measurements.

When the height and length of values are significantly smaller than the wavelength of the probing light, the light no longer interacts with the defects individually but treats them in bulk as if they constituted a distinct layer with a refractive index intermediate to that of the substrate and whatever medium filled the defect troughs (e.g. air). Given the volume fractions of the different media which constitute this film, it is possible to determine an effective refractive index for this film. For thin-films on metallic surfaces it is possible to use the average of the bulk material and the microscopically rough surface-air refractive indices to approximate what would
amount to an effective "real" substrate refractive index and obtain usable thickness values. Otherwise using either the bulk substrate material refractive index or the rough substrate surface refractive index can result in errors up to the order of the roughness height. An effective index of refraction can also be obtained for some incomplete thin-films (e.g. island formation) if surface coverage information is independently obtained in order to approximate actual (as opposed to average) film thicknesses.27

Ellipsometric measurements were made using a Manual Photoelectric Rudolf 439L633P ellipsometer (Rudolph Instruments, Fairfield NJ). The measurements were made using a 632.8 nm wavelength, HeNe laser at a 70° angle of incidence. This light initially passed through two optical elements called a polarizer and a compensator, which elliptically polarized the light, before it came into contact with the analyzed surface. The reflected light then passed through an other optical element called an analyzer before it finally reached a photo detector. The polarization of the light (before and after reflection) was then measure by determining the rotational azimuth angles for the polarizer, compensator and analyzer at which the laser light was completely extinguished. In general, two sets of polarizer and analyzer measurements were taken, one with the compensator set at +45° and the other at -45° and these values (A1, A2, P1 and P2) were then plugged into a software package (because of the complexity of the calculations involving \( \Psi \) and \( \Delta \)) which determined layer thickness values. Values used for the refractive index and the extinction coefficients of gold and the thin films studied were taken from the literature and were 3.3(n) and 0.2(k) and 1.47(n) and 0(k), respectively.6

E. External Reflectance FT-IR

When infrared light is passed through and organic sample, a frequency dependant reduction in intensity of that light is observed which is a result of interactions with sample bonds
with similar transition energies and from this IR spectra are obtained. In external reflectance IR this reduction of intensity is dependant on both the species adsorbed to a metal, reflective surface and the polarization of the light reflected. The electric field of the dipole moments of bonds oriented parallel to the metal substrate is effectively nullified by the presence of a countering, induced dipole moment in the metal substrate; as a result, s-polarized radiation (which would be oriented to interact w/ such dipoles creating absorbencies) is not adsorbed by thin film samples and only p-polarized radiation can be used to probe adsorbed species. This means that only dipoles and dipole components oriented perpendicular to the substrate surface can be observed in reflectance IR spectroscopy and this is known as the surface selection rule.\textsuperscript{29-33}

Judicious use of this selection rule has been used to determine the orientations of bonds at the electrode surface and thus obtain quantitative conformation and organizational information on self assembled monolayers. Different methods have been utilized but they all basically involve the comparison of expected and observed absorbance intensities, assuming that the total observed intensity corresponds to the purely perpendicular component and given that only one dimension (out of three) of the incident light actually probes the sample. Below is the equation that is used where $\theta$ is equal to the angle from the surface normal (the tilt angle) and $I$ is the absorption intensity.\textsuperscript{29, 34}

\[ I^\perp = I (1 - \cos^2 \theta) \]
\[
\cos \theta = \left( \frac{I_{\text{obs}}}{3I_{\text{calc}}} \right)
\]  

Generally, the expected values is obtained by separately determining the absorbance and reflective properties of the thin film substance and the substrate, independently and plugging these values into a mathematical model for the reflective thin film system.\textsuperscript{25, 29} Unfortunately, these models are never perfect as many simplifying assumptions are made but the results obtained are usually reasonable. Another method of establishing a base line, which avoids the use of models, involves the use of comparisons of ratios of intensities in transmission spectra to external reflectance spectra to obtain more generalized molecular orientation data. With alkane thiol SAM’s, the ratios of specifically methyl and methylene bands are most often used.\textsuperscript{30}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure6.png}
\caption{Tilt Angle and changes in dipole directions with film conformation.}
\end{figure}

The exact frequency at which expected spectral bands appear has also been utilized to obtain more qualitative information on the order, packing and relative orientation of the molecules which make up studied layers. For example, by comparing the methylene absorbance
positions to those of liquid and crystalline polyethylene one can evaluate the relative crystallinity (degree of well packing) in the layer. Lower frequencies around 2920/2850 cm\(^{-1}\) can be taken to represent the absorbances of well packed layers while higher frequencies around 2928/2856 cm\(^{-1}\) can be taken to represent the absorbencies of poorly packed layers.\(^{31}\) For the linking alkyl chain of a self assembled monolayer the presence or absence of internal defects (which affect packing) can also be inferred using the presence or absence of specific peaks. In the 1400-1500 cm\(^{-1}\) range a band at 1467 cm\(^{-1}\) can be taken to represent the methylene scissoring of an all-trans, well-packed chain while the presence of a band at 1460 cm\(^{-1}\) be indicative of scissoring near gauche defects. In the 1300-1400 cm\(^{-1}\) range bands at 1345, 1366/1306 and 1353 cm\(^{-1}\) can be taken as evidence for the presence of chain end gauche, internal kink and double gauche defects, respectively. Carbon-carbon stretch absorbencies can also be used where gauche C-C bonds can be found in the 1065-1080 cm\(^{-1}\) range.\(^{32}\)

FT-IR surface spectra were obtained using a Nexus FT-IR model 670 spectrometer equipped with a ThermoNicolet grazing angle accessory and a liquid-nitrogen cooled MCTA detector. The IR beam was incident at 75° to the gold substrates and its path, through the instrument, was purged for 30 minutes before and during experiments. A cleaned gold substrate was used as the spectral background and 64 scans at 4 cm\(^{-1}\) resolution were taken. The spectra obtained were automatically corrected for the presence of atmosphere, water and carbon dioxide in the sample.

F. Fluorescence Spectroscopy

As fluorescence modulation provided one of the two primary means of detection for this sensor project (as originally envisioned) fluorescence experiments on those molecules which were used to form self assembled monolayers and incorporated fluorophores were conducted on
optically transparent indium tin oxide (ITO) and in solution. Now, in essence, fluorescence occurs when an electronically excited species (excited using UV light) relaxes to its ground state in a manner which emits light of a slightly longer wavelength (and lower energy).\textsuperscript{11,35} Aromatic species with low-energy $\pi \rightarrow \pi^*$ transition levels typically fluoresce the most intensely and by attaching such an aromatic moiety to an aza-crown ether through a nitrogen in the crown it is possible to create a fluoroionophore (a molecule who’s binding of ions in one position influences the fluorescence of a moiety in another position) which can be used to optically detect ion concentrations. The mechanism by which this type of sensor functions, involves the fact that a nitrogen lone pair, when near a fluorophore, effectively quenches the fluorescence of that fluorophore, in a process known as photoinduced electron transfer (PET).\textsuperscript{4,11,36} The energetics of this process are presented in the frontier orbital diagram in Figure 7 where it is shown that the non-bonding electrons from the highest occupied molecular orbital (HOMO) of the nitrogen/azacrown are well position to immediately fill the ground state of the fluorophore following excitation of an electron from that state. As the excited electron cannot relax to the HOMO of the fluorophore any longer it instead relaxes to the HOMO of the crown ether and no fluorescence is observed. \textsuperscript{5,11}
On the other hand, when a cation is complexed to the crown ether that electron density at the nitrogen lone pair becomes occupied and the lone pair is no longer able to quench the fluorescence of the fluorophore. Energetically, the energy of the HOMO of the azacrown/nitrogen, in the complexed state, is no longer greater than the energy of the HOMO of the fluorophore and thus the PET effect is no longer thermodynamically favorable. The result, for an individual molecule, is an “off-on” switch where, when the crown ether is in an uncomplexed state no fluorescence occurs but when it is a complexed state near complete fluorescence occurs. In solution or on a surface the number of fluoroionophores in a bound state is dependant on the equilibrium constant of the ionophore/crown ether-cation binding reaction.
and the concentration of the binding cation. As such it is possible, if the concentration of sensor
is kept constant, to directly relate the concentration of the target cation in solution or exposed to
a surface directly the intensity of the fluorescence observed, via the construction of calibration
curves. The extraction of equilibrium constants to quantitatively define the sensor response
range is also possible by fitting Langmuir-type isotherms to these curves.\textsuperscript{1, 37}

Fluorescence measurements were made using a Perkin Elmer LS50B Luminescence
Spectrometer. Fluorescence on ITO was initially determined by securing the functionalized slide
to a quartz cuvette stir bar using parafilm and the orienting it within a cuvette, in the
spectrometer so that the angle of incidence was approximately 45\degree. This set-up, however
showed marked irreproducibility (due to the fact the angles were not precisely maintained
between experiments) and the cuvette was replaced with a specially constructed slide holder.

\textbf{Figure 8.} The two different setups used for obtaining surface fluorescence spectra
G. Electroanalytical Chemistry

1. The Electrode Interface

When a conductor is immersed in an electrolyte, a transfer of electrons takes place which creates an excess of charge at the conductor’s surface and a balancing build up of the opposite charge in the layers of solution immediately adjacent to it. The charged surface layer is caused by limiting mobilities of the ions in solution and consists of an inner layer and an outer layer within which the charge decreases with increasing distance from the electrode, linearly and exponentially, respectively. This layer of charge at the electrode surface is known as an electrical double layer and has a capacitance and potential associated with it.35,38

The two types of current which conduct the transfer of electrons across this interface are known as faradaic and nonfaradaic/capacitive currents. In the case of faradaic current, charge is conducted through the electrode/solution interface through redox reactions of a species in solution at the surface of the electrode which acts as either an oxidizing or reducing agent. The conductor and solution current are then coupled but in solution, current is only conducted through the mass transfer of a respective redox active species. The three processes which govern this solution current are convection, or the mechanical motion of the electrolyte as a result of stirring or flowing: migration, the movement of ions as a result of electrostatic attraction to an electrode of opposite charge and diffusion, the movement of species as a result of a concentration gradient. When faradaic current predominates the potential which develops over the interface is

Figure 9. The inner (d0- d1) and outer (d1- d2) layers of the electrical double layer.
determined by the identities and concentrations of the reagents and products in solution through the governing equilibrium constants.\textsuperscript{35}

When redox species are not available and the electrical current is no longer coupled with solution currents (through chemical reactions) the conduction of alternating currents is still possible. This is accomplished by switching the sign of the potential applied, each half-cycle, such that ionic species in solution move toward and away from the electrode in question, each half cycle. In two electrode cells, the electrodes, then, act as the two plates of a capacitor where a build up of negative charge occurs at one electrode and then discharges across the external circuit to the other, and vice versa. The current which results, in solution (and at the interface through the reorientation of dipoles, etc.), is known as capacitive or non-faradaic current.\textsuperscript{35}

2. The Electrochemical Cell

The reactions which occur at a single electrode are electronic half reactions of the sort:

\[
\text{Cu}^{2+} + 2e^- \leftrightarrow \text{Cu(s)} \quad (9)
\]

\[
\text{Zn}^{2+} + 2e^- \leftrightarrow \text{Zn(s)} \quad (10)
\]

When two electrode half-reaction, such as these, are coupled such that a net redox reaction takes place an electrochemical cell is the result where oxidation takes place at one electrode (anode) and reduction at the other (cathode).\textsuperscript{35}

\[
\text{Cu(s)} + \text{Zn}^{2+} \leftrightarrow \text{Cu}^{2+} + \text{Zn(s)} \quad (11)
\]

This can be accomplished by immersing, for example, a copper electrode in a copper sulfate solution and a connected zinc electrode in a zinc sulfate solution and then connecting the two solutions with a potassium chloride salt bridge. The role of the salt bridge is to conduct ionic current without allowing direct contact/reaction between the copper ions and the zinc electrode and vice versa. The direction the current flows through the cell is determined by the free energy
of the above reaction and thus the equilibrium constant and the concentration of reagents. Once
the complete cell reaches equilibrium no more current will flow and therefore the instantaneous
current and the potential of the cell is determined by how far it starts from equilibrium. 35

\[
\text{Anode} \quad \text{Zn(s)} \rightarrow \text{Zn}^{2+} + 2e^- \quad E^0 = +0.763 \text{ V (J/C)}
\]

\[
\text{Cathode} \quad \text{Cu}^{2+} + 2e^- \rightarrow \text{Cu(s)} \quad E^0 = +0.337 \text{ V (J/C)}
\]

**Figure 10.** An example of a galvanic electrochemical cell

The potential which develops at an electrode interface is determined by the activities of the
reagents in the electrolyte and not directly by their concentration. The relationship between the
two is defined below where \( \gamma_x \) is the activity coefficient of solute X. 35

\[
a_x = \gamma_x [X] \quad (12)
\]

For the above redox reaction, in equilibrium, the equilibrium constant is defined below as \( K \) and
a comparable quotient, \( Q \), is given for the cell’s initial state:

\[
K = \frac{a_{\text{Cu}^{2+}} \cdot a_{\text{Zn}(s)}}{a_{\text{Cu}(s)} \cdot a_{\text{Zn}^{2+}}} \quad (13)
\]

\[
Q = \frac{(a_{\text{Cu}^{2+}})_i \cdot (a_{\text{Zn}(s)})_i}{(a_{\text{Cu}(s)})_i \cdot (a_{\text{Zn}^{2+}})_i} \quad (14)
\]

Therefore, the free energy change (maximum work obtainable) from the resulting currents would
be defined below, where \( R \) is the gas constant and \( T \), the temperature:

\[\text{Free energy change} = -RT \ln Q\]
\[ \Delta G = RT \ln Q - RT \ln K \]  

(15)

This can be related to the overall potential of the cell \((E_{cell})\) by equation 16 where \(n\) equals the number of electrons and \(F\) the Faraday constant:

\[ \Delta G = -nF \cdot E_{cell} \]  

(16)

Combining these two equations and given the fact the activities of solid metals are unity we get the equation:

\[ E_{cell} = \frac{RT}{nF} \ln K - RT \ln \frac{(a_{Cu^{2+}})^{i} / (a_{Zn^{2+}})^{i}}{} \]  

(17)

\[ E^{0} = \frac{RT}{nF} \ln K \]  

(18)

The first term in equation 17 above is a constant known as the standard electrode potential for the cell \((E^{0}_{cell})\) and is defined as the cell potential when all reagents and products are at unit activity. This is a form of the Nernst equation which is one of the most used equations in electroanalytical chemistry. As aforementioned, the free energy change and thus the cell potential defined in the equation above determine the direction of current flow. If the cell is used to generate energy/electricity (equilibration of the cell driving electron flow through the circuit) then it is known as a galvanic cell. If, on the other hand, the opposite potential is applied (the same potential in the opposite direction) to the cell and it is used to drive forward the unfavored reaction, then energy will be consumed by the cell, this is known as an electrolytic cell. 35

3. Electrode Potentials

Questions regarding such things as current direction and potential signs are difficult at they are governed by generally accepted conventions and therefore can be easily confused as, quite often, “what’s what” is simply a question of perspective. As previously mentioned, an electrochemical cell is made up of at least two electrodes: an anode, where oxidation occurs and
a cathode, where reduction occurs. Now there is a potential associated with each electrode which is a measure of the driving force of the half-reaction which occurs at that electrode and these half-reactions and their associated standard potentials ($E^0$) by convention are written as reductions:

\[
\begin{align*}
\text{Cu}^{2+} + 2e^- & \leftrightarrow \text{Cu}(s) \quad E^0 = +0.337 \text{ V} \quad (19) \\
\text{Zn}^{2+} + 2e^- & \leftrightarrow \text{Zn}(s) \quad E^0 = -0.763 \text{ V} \quad (20)
\end{align*}
\]

Each of these half-reactions is governed by a Nernst equation just like the complete redox reaction above and as such, a positive potential is indicative of a more negative free energy change and thus a more spontaneous process. Therefore, the reduction of copper will be spontaneous but the reduction of zinc will not. Instead the oxidation of zinc would be spontaneous and the coupled reaction which would occur spontaneously would be:

\[
\text{Cu}^{2+} + \text{Zn}(s) \rightarrow \text{Cu}(s) + \text{Zn}^{2+} \quad E^0 = +1.100 \text{ V} \quad (21)
\]

Thus, in the above described electrochemical/galvanic cell the copper electrode would be the cathode and the zinc electrode would be the anode. The overall electrochemical cell standard potential is defined as the difference between the potential of the anode from the cathode:

\[
E_{cell} = E_{cathode} - E_{anode}
\]

(22)

Unfortunately as voltmeters can only measure differences in potential as it is impossible, at this point, to measure the absolute potential of a single electrode. As such, in order to determine the standard potential of a particular half-reaction it is necessary to have another well-defined and stable half-reaction at the counter electrode in a cell, which the potential of interest, can be measured relative to. This is known as a reference electrode and it is generally a self-contained, half-reaction vessel connected to the cell electrolyte through a salt bridge which allows the transport of ions/current. The universal standard reference electrode is the hydrogen
gas electrode which has a platinum electrode immersed in an H\(^+\) electrolyte, sealed in a glass tube and filled with hydrogen gas. As such the reference half reaction potential, here, is that of the H\(_2\)/H\(^+\) redox couple which is defined as 0V:

\[
2\text{H}^+ + 2e^- \leftrightarrow \text{H}_2(g) \quad E^0 = 0.000 \text{ V} \quad (23)
\]

Relative to this all electrode potentials are defined. In practice hydrogen electrodes are difficult to make and work with and as such Ag/AgCl and Hg/Hg\(_2\)Cl\(_2\) electrodes are more commonly used. In experiments the counter electrode, at which the redox reaction of interest occurs, is known as the working electrode and it is treated, by convention, as the cathode where the reference is the anode. Potentials which are applied to this cell are done so with respect to the working electrode/cathode as such positive potentials maintain this working-cathode, reference – anode relationship while negative potentials reverse their roles.\(^{35}\)

In the above discussion on electrode potentials the standard electrode potential has specifically been referred to instead of simply the “electrode potential.” This is because a specific “electrode potential” is dependant on both the equilibrium constant and the instantaneous activities/concentrations of the reagents and products in the electrolyte. The standard electrode potential, on the other hand, is a constant, defined by a particular redox reaction’s equilibrium constant, solely. As such, it is quite useful as a “physical constant which gives a quantitative description of the relative driving force for a half-cell reaction.\(^{35}\)” Further, by comparing standard electrode potentials, between different species, one can obtain information on the extant and direction of an electron-transfer reaction within an electrochemical cell as was demonstrated above with the zinc and copper half-reactions. The actual electrode
potential in different cell formats can easily be determined, when necessary, by plugging concentration/activity information into the Nernst equation:\(^{35}\)

\[
nX + 2e^- \rightarrow X(s) \quad (24)
\]

\[
E = E^0 - \frac{RT}{nF} \ln \frac{1}{a_X^n} \quad (25)
\]

H. Cyclic Voltammetry

1. The Three Electrode Setup

In voltammetry the potential applied to an electrochemical cell is swept over a certain range and the current response which results is recorded as a function of that applied potential. In general, these experiments are conducted using a three-electrode cell which has a working electrode, a reference electrode and a counter electrode. Here the applied potential is applied between the reference and the working, just as in two electrode set ups described above, but in three electrode set-up the resistance of the reference is kept so large that no current passes through it and thus the resulting current passes between the working and the counter. This splitting of the role of reference electrode between two electrodes has proven necessary as in two electrode set-ups it has to be assumed that the potential of the working electrode is simply the difference between the applied potential and the potential of the reference. This assumption is, however, invalid as in high resistance electrolytes there is an additional current dependant IR drop which hampers direct comparison of the applied potential and the resulting current. The result is distorted voltammograms. \( I \) is current and \( R \) is resistance below.

\[
E_{\text{applied}} = E_{\text{cathode}} - E_{\text{anode}} - IR \quad (26)
\]
In a three-electrode set up, on the other hand, the current and potential are determined between different electrodes and so this source of error is eliminated.\textsuperscript{35}

1. Electrolyte Preparation

The cell solution is generally purged of dissolved gases, such as oxygen, and contains the studied analyte with excess supporting, unreactive electrolyte. The purging is accomplished through bubbling of inert gases such as nitrogen or argon and is important to prevent spurious currents caused by redox reactions of contaminants. The reduction of dissolved oxygen is especially common:

\[
\begin{align*}
\text{O}_2 + 2\text{H}^+ + 2\text{e}^- &\leftrightarrow \text{H}_2\text{O}_2 & (27) \\
\text{O}_2 + 4\text{H}^+ + 4\text{e}^- &\leftrightarrow 2\text{H}_2\text{O} & (28)
\end{align*}
\]

The unreactive supporting electrolyte is kept in 50 to 100 fold excess of the analyzed species in order to wash out the effects of migration (the movement of ions as a result of electrostatic attraction to an electrode of opposite charge) on the analyte so that the fraction of total current

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{three-electrode-cell.png}
\caption{Schematic of a three electrode cell}
\end{figure}
carried by the analyte approaches zero and the migration of analyte become effectively independent of the applied potential.  

2. Raw Cyclic Voltammogram Data

The concentration of the redox species immediately adjacent to the electrode, in voltammetry, is determined by the Nernst equation, where $c_p^0$ is the concentration of products and $c_R^0$ the concentration of reactants:

$$E_{\text{applied}} = E^0 - \frac{RT}{nF} \ln \frac{c_p^0}{c_R^0}$$  

In cyclic voltammetry, the applied potential is swept from one end point of the potential range to the other and back. When the redox species in the electrolyte is reversible the profile of the current response is similar to that shown in Figure 12.

![Cyclic Voltammogram](image)

**Figure 12.** A Cyclic Voltammogram taken in the presence of a reversible redox species.
At the beginning of the cycle no significant current is observed until the potential becomes positive enough to begin the reduction of the species at the electrode interface. Here there is a surge of current which is made up of both diffusion controlled current and the initial current required to rapidly adjust the interfacial concentrations of the redox species. This latter current decays rapidly as the oxidized reagent is depleted around the electrode until the current is only made up of the former, diffusion controlled contribution. When the potential scan switches direction the current remains cathodic until the potential becomes significantly negative enough to now oxidize the accumulated reduced analyte at which point a similar current surge and decay are observed as the relative concentrations of redox species switch. 35

3. Setup and Instrumentation

All voltammetric experiments were conducted using either an EG&G Princeton Applied Research Potentiostat/Galvanostat Model 273 or a Gamry Instruments Potentiostat/Galvanostat Model 600. The former most was found, after several months of work, to apply a brief but exceedingly large and destructive potential across the cells studied immediately before taking measurements and was replaced with the latter. As a result the greater portion of the work done with the former proved unusable as what had, initially, appeared to be sensor response turned out instead to be the gradual destruction of the sensing sample. A three electrode set up was used with all instruments and consisted of a standard calomel reference electrode (SCE), a platinum wire counter electrode and a SAM coated gold working electrode. Three different types of working electrodes were used: approximately 1 cm² gold EMF slides for more qualitative measurements and 0.0315 cm² and 0.002 cm² analytical electrodes, constructed in house, for more quantitative measurements.
The electrochemical cell was constructed by immersing these three electrodes in an appropriate electrolyte within either an unsealed beaker or a sealed, nitrogen purged four-neck flask. All glassware was rinsed and sonicated in DI water immediately preceding and following use. The electrolyte, the potential range and the scan rates used in a given experiment depended on the application for which cyclic voltammetry was used. These applications included: characterization of the insulating behavior of SAM’s, determining the molecular coverage of SAM’s, polishing of analytical electrodes and determining the real area of analytical electrodes.

The well-packed-ness of SAM’s was qualitatively determined by examining the magnitude of faradaic current observed upon sweeping the applied potential range about the formal potential of a redox species in solution. When little to no current was observed it could be assumed that the SAM, at the working electrode, was relatively defect-free being thus able to block the redox reaction which otherwise would have occurred. The concentration of the redox species used was 1mM and the concentration of unreactive supporting electrolyte was 0.1 M. Ruthenium hexamine trichloride and potassium ferricyanide were used as positively charged and negatively charged redox couples, respectively. The supporting electrolyte and the potential range used were tetraethylammonium chloride and +0.3V_{SCE} to -0.5V_{SCE}, for the former, and potassium chloride and -0.5V_{SCE} to +0.7V_{SCE}, for the latter. Scan rates and increments for both were 50mV/s and 1mV, respectively. The ruthenium redox couple was most used as it’s positive charge could be used for ion-gating experiments (the number of alkali cations bound to the sensing SAM studies could modulate the magnitude of Ruthenium hexamine cation transfer/current through the film via electrostatic repulsion) and given the fact the potassium is one of the analytes of the films studied and so no real base line could be established with potassium ferricyanide.
I. Cyclic Voltammetric Experiments

1. Linear Stripping Voltammetry

Molecular coverage of the SAM’s was determined using a technique known as linear stripping voltammetry which sweeps the applied potential over the potential known to cleave the sulfur-gold bond by which the SAM’s are attached to the electrode.\textsuperscript{40, 41}

\[
\text{Au - SR} + e^- \rightarrow \text{Au} + \text{RS}^-
\]  

(30)

This is known as reductive desorption and by measuring the amount of current associated with this process and one can determine the surface coverage of the adsorbate at the electrode surface using equation 31, where \( \Gamma \) is the surface coverage in mol/cm\(^2\), \( Q \) is the amount of current which is determined by integrating the area of the voltammetric peak, \( n \) is the number of moles of electrons associated with the process, \( F \) is the faraday constant and \( A \) is the area of the electrode:

\[
\Gamma = \frac{Q}{nFA}
\]  

(31)

If the adsorbate completely dissolves in the electrolyte solution then this process is irreversible and a second scan, only, is needed to establish a baseline, capacitive current to subtract from the total current peak (and obtain only the faradaic current of the reduction process). This is not always the case, however, as some of the SAM can be re-adsorbed by the reverse scan and multiple scans (~5) are often needed. These experiments were conducted by a literature procedure in a nitrogen-purged, 0.1 M KOH electrolyte over a potential range of +0.1V\textsubscript{SCE} to -1.4V\textsubscript{SCE} using a scan rate of 20mV/s.\textsuperscript{39, 41} Analytical electrodes, only, were used for these experiments as exact knowledge of the surface area of the working electrode is needed (see equation 31 above).
2. Electrochemical Polishing/Cleaning

Electrochemical polishing/cleaning was accomplished by cycling a previously mechanically polished electrode between the redox potentials of gold itself. At applied potentials around $+1.0V_{SCE}$ an oxide layer is known to form at the gold surface and be removed around $+0.6V_{SCE}$. By successive cycling between these potentials, first surface contaminants can be oxidized and removed and second by repeated application and removal of the oxide layer a reorganization of the gold atoms at the electrode surface can be induced which is essentially equivalent to the use of a molecular scale grit polishing paper. In this manner an electrode can be cleaned immediately before being placed in a SAM deposition solution and the surface roughness of the electrode can be controlled. The electrolytes used for this procedure included 0.5 M sulfuric acid and pH 7.0, 0.1 M phosphate buffer and the potential range was, typically, $-0.1V_{SCE}$ to $+1.2V_{SCE}$. The scan rate was usually 50mV/s and the number of scans ranged from 25 to 200 for an individual experiment.  

3. Determining Electrode Roughness/Real Area

By a similar method the real area and roughness of an electrode could be characterized. Using a standardized literature procedure at specific scan rates and in specific electrolytes the real area of electrode can be determined by the amount of oxide formed at the gold electrode. The amount of charge needed to reduce the chemisorbed oxygen layer on polycrystalline gold has been determined to be approximately 390 $\mu$C/cm$^2$ in pH 7.0, 0.1 M phosphate buffer using a scan rate of 50 mV/s. As such by integrating the area of the voltammetric oxide reduction peak and dividing by 390 $\mu$C/cm$^2$ the real, electrochemical area of the electrode can be determined. Now the roughness of an electrode is defined by the ratio of the real over the geometric surface area.$^{39-43}$: 
\[
\text{roughness} = \frac{A_{\text{real}}}{A_{\text{geo}}}
\] (32)

One can obtain a measure of the microscopic roughness of an electrode by dividing the real area by the apparent area calculated using the dimensions of the electrode. These results can be checked using a qualitative roughness test as it is known that dark finishes tend to indicate roughness on the order of 50-200; satin finishes, 5-10 and mirror-polish finishes are typically indicative of a roughness ratio of 2-3.³⁹

J. Impedance Spectroscopy

1. Reactance versus Resistance

According to Ohm’s law, the resistance of a circuit is equal to the applied potential divided by the resulting current.

\[
R = \frac{E}{I}
\] (33)

Now while this is valid when using simple direct current it also carries many implicit assumptions including: for alternating current (with a characteristic frequency) that the input potential and output current will be in phase and that the resistance is independent of the frequency of the applied potential. Circuits which include such elements as capacitors or inductors are significantly more complex as these components modulate the output current in response to an input voltage in a manner best termed “reactance.” This behavior is specifically opposed to resistance as there is no power dissipation when current passes through these circuit elements but rather power storage in either an electric (capacitor) or magnetic field (inductor).⁴⁴

2. Deriving the Reactance of a Capacitor
For example, a capacitor in its simplest form is made up of two conducting plates separated by an insulating material known as the dielectric and it has the property, where $C$ is capacitance:

$$Q = CE$$

(34)

where at an applied potential $E$ the charged stored at one plate of the capacitor is $Q$ and $-Q$ at the opposite. If you take the derivative of this equation with respect to time then you get the expression, where $I$ is the current:

$$I = C \frac{dE}{dt}$$

(35)

It can be seen here that the current which results from a capacitor is directly proportional to the rate of change of the applied voltage, or rather, the rate of change of the voltage (when a capacitor is charging up) is proportional to the applied current. If you plug in a sinusoidal alternating current (defined by a sine wave): $E(t) = E_0 \sin(\omega t)$ into equation 35 and you differentiate you get the below expression for the current response:

$$I(t) = C \omega \cos(\omega t)$$

(36)

As such the response current of an ideal capacitor is 90 degrees/completely out of phase with the excitation potential (this is the reason there is no power dissipation). This is not the case with ideal resistors which only influence the magnitude not the phase of the current response.

3. Ohm’s Law Generalized for Linear Circuits

The ideal capacitor and inductor behave linearly in so far as the magnitude of the output increases exactly in proportion to the input. As such, the output of a circuit composed of linear components (resistors, capacitors and inductors) when a sine wave input potential is applied will itself be a sign wave, of the same frequency, with at most changes in signal amplitude and phase.
More generally the response of a linear system to a perturbation \( E(t) \) is determined by a differential equation of the \( n \)th order\(^{45} \):

\[
 b_0 \frac{d^n I(t)}{dt^n} + b_1 \frac{d^{n-1} I(t)}{dt^{n-1}} + \ldots + b_n I(t) = a_m \frac{d^m E(t)}{dt^m} + a_{m-1} \frac{d^{m-1} E(t)}{dt^{m-1}} + \ldots + a_1 \frac{d E(t)}{dt} + a_0 E(t) \quad (37)
\]

And given this if the input is the sine wave: \( E(t) = E_0 \sin(\omega t) \) then the response is also a sine wave with a phase shift \( \phi \):

\[
 I(t) = I_0 \sin(\omega t + \phi) 
\]

(38)

Using Euler’s relationship, \( e^{j\theta} = \cos \theta + j \sin \theta \) it is possible to express the potential and current as complex functions where the real portion actually represents the real behavior but the complex representation simplifies treatment of data when dealing with phase differences:

\[
 E(t) = E_0 e^{j\omega t} 
\]

(39)

\[
 I(t) = I_0 e^{j(\omega t + \phi)}
\]

(40)

With these expressions taking into account both the reactance (inductors and capacitors) and resistive (resistors) contributions of a circuit composed of linear elements it is possible to generalize Ohms law past resistance such that it can relate the input potential and output current in terms of a property known as impedance, \( Z \) (essentially: reactance + resistance)\(^{38, 45} \):

\[
 Z = \frac{E}{I} = \frac{E_0 e^{j\omega t}}{I_0 e^{j(\omega t - \phi)}} = \frac{E_0}{I_0} e^{j\phi} = Z_0 e^{j\phi} 
\]

(41)

Again using Euler’s relationship it is possible to break up this impedance into real and imaginary components that can be represented in Cartesian coordinates (in a complex plane):

\[
 Z_0 e^{j\phi} = Z_0 \cos \phi + Z_0 j \sin \phi = \text{Re } Z + \text{Im } Z 
\]

(42)

If you plug the complex expression 41 in for voltage in the capacitor current equation above and differentiate with respect to time again you get\(^{44} \):
\[ I(t) = (j\omega C)E_0 e^{j\omega t} = \frac{E_0}{1/j\omega C} = \frac{E}{1/j\omega C} \quad (43) \]

Now this has the same form of the Ohm’s law generalized if you rearrange for current:

\[ I = \frac{E}{Z} \quad (44) \]

Therefore the impedance/reactance associated with an ideal capacitor is frequency dependant and represented by a complex number (the reactance nature is modeled by \( j \))\textsuperscript{38, 44, 45}:

\[ Z_c = \frac{1}{j\omega C} \quad (45) \]

The table below summarizes impedance data for resistors, capacitors and inductors:

<table>
<thead>
<tr>
<th>Element</th>
<th>Current vs. Potential</th>
<th>Impedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistor</td>
<td>( E = IR )</td>
<td>( Z = R )</td>
</tr>
<tr>
<td>Capacitor</td>
<td>( I = C \frac{dE}{dt} )</td>
<td>( Z = 1/j\omega C )</td>
</tr>
<tr>
<td>Inductor</td>
<td>( E = L \frac{dl}{dt} )</td>
<td>( Z = j\omega L )</td>
</tr>
</tbody>
</table>

The total impedance of a system is divided between a real part representative of resistances and an imaginary part representative of reactances and the frequency dependence is controlled by the reactant components.
In Impedance spectroscopy, a small AC potential (1-10 mV/s) is applied to the system under study in order to assure pseudo-linearity (as real systems are never linear). This AC potential is applied over a frequency range and the current response at each frequency is used to determine the complex impedance as a function of frequency. The impedance spectra can then be examined as a complex plot, known as Nyquist plot, and based on its shape an appropriate equivalent circuit can be chosen and fitted to the data. The values then obtained, upon fitting, for the different elements of the circuit can then be used to obtain real physical data (e.g. resistance being related to reaction rates, etc.) on the system.\textsuperscript{38, 45, 46}

For the Randles circuit pictured above the complex plot (where \( \text{Re} \, Z = Z_0 \cos \phi \) and \( \text{Im} \, Z = Z_0 j \sin \phi \)) is always a semicircle.\textsuperscript{38, 45, 46} Over the complete frequency range the impedances of the resistors do not change (by definition \( Z_R = R \)) and so the only thing that changes as you move along the plot is the impedance of the capacitor and the magnitude of its effect on the behavior of the overall circuit is directly related to the magnitude of the imaginary
component. As can be seen in the plot below at the higher frequencies the impedance of the capacitor becomes negligible:

\[ \lim_{\omega \to \infty} Z_C = \lim_{\omega \to \infty} \frac{1}{j\omega C} = 0 \]  

(46)

![Figure 14. Circuit behavior at high frequencies](image)

and so the capacitor acts like a short and the path of least resistance for the current involves just the first resistor \( R_1 \) which dominates the impedance of the circuit. At lower frequencies, on the other hand, the impedance of the capacitor approaches infinity:

\[ \lim_{\omega \to 0} Z_C = \lim_{\omega \to 0} \frac{1}{j\omega C} = \infty \]  

(47)

![Figure 15. Circuit behavior at low frequencies](image)

As such the capacitor acts like an effective break in the circuit, at lower frequencies, and the path of least resistance, which dominates the circuit response, is the sum of the two resistances \( R_1 \) and \( R_2 \).
5. Circuit Elements used to Model SAM’s

In the three-electrode electrochemical cells which are used in this work (with a self assembled, sensing coating on the working electrode) there are a specific set of circuit elements which characterize the physical behavior of this system which include: the electrolyte resistance, the SAM coating capacitance, the SAM charge transfer resistance, the SAM polarization resistance, the Warburg impedance and the constant phase element.

**Electrolyte resistance** between the counter and the reference is compensated for in a three-electrode cell but the solution resistance between the reference and the working is not and must be taking into account. This value is dependant on the ionic concentration, the type of ions, the temperature (which affect the solutions resistively, \( p \)), the area of the working electrode \((A)\) and the length of solution carrying uniform current \((l)\)\(^{38,45}\):

\[
R = p \frac{l}{A} \quad (48)
\]

The previously discussed double layer acts as an effective capacitor at a metal electrode interface and can prove important in establishing a base line for the uncoated electrode with which to compare a coated electrode. What’s more, depending on the integrity of the coating and/or the type of defects, the double layer can make a significant contribution acting in parallel with more intact elements of the coating, in places where bare electrode is exposed. Now as the double layer charge is immediately adjacent to the electrode charge and given equation 49 for capacitance (where \( \varepsilon_0 \) is the permittivity of vacuum, \( \varepsilon_r \) is dielectric constant, \( A \) is the area and \( d \) is the distance between the “plates”):

\[
C = \frac{\varepsilon_0 \varepsilon_r A}{d} \quad (49)
\]
The distance between the effective plates is very small (on the order of angstroms) as such the capacitance per unit area of bare metal is quite high, usually on the order of ~30uF/cm$^2$. This value is however dependant on such things as, the type of ions, the applied potential, the temperature, etc.$^{38, 44, 45}$

The charge-transfer resistance is usually associated with a kinetically controlled electrochemical reaction where the reaction is slower than diffusion from the bulk solution to the interface. These reactions have specific speeds which depend on the kind of reaction, the temperature, the concentration of reagents and the applied potential. For SAM coated electrodes the speed of charge transfer is also modulated by the interfacial transfer of the redox species across the coating which has the effect of effectively reducing the rate constant and increasing the resistance. $^{38}$

When the potential of an electrode is forced away from its value at the open circuit capacitive current will flow and this also can be modulated by the presence of a coating. With this polarization resistance, also, the amount of current is also controlled by the transfer of ions across the coating when it is significantly slower than diffusion from the bulk solution and this is known.$^{38}$

When current is in part diffusion controlled a new type of impedance behavior is observed which id frequency dependant and known as a Warburg element. At high AC frequencies when ions don’t have to move very far before they switch direction and thus diffusion (and the Warburg element) make a negligible contribution to the impedance of the circuit. At low frequencies, however, when the reagents have to diffuse further diffusion makes a significant contribution and its phase behavior is intermediate to that of a resistor and capacitor.
such that in the Nyquist plot it manifests itself as a 45 degree line. When added to a Randles circuit it manifests itself only at lower frequencies.\textsuperscript{38,45}

The SAM coating also acts as a capacitor where the coating is the insulator/dielectric and the electrolyte solution and the electrode act as the two plates. Given again the above equation (49) for capacitance, the capacitance of the coating is determined by the dielectric of the coating (which is related to the permittivity of the material and thus also optical constants such as the refractive index), the area of the electrode and the thickness of the coating.\textsuperscript{38,45} In order to compare results with different electrodes of different areas capacitance values for SAM’s will hereafter be consistently referred to per unit area.

6. Models used to fit Electrochemical Cells with SAM modified Working Electrodes

![Figure 16. The Helmholtz Circuit and Impedance Behavior](image)

Perfect SAM’s which manifest no charge transfer or polarization resistance are usually fitted using a Helmholtz circuit model which is composed of a solution resistance and a coating capacitance. These Nyquist plots appear as a straight vertical line (given the pure capacitive behavior the phase angle is 90 degrees throughout the frequency range ) which begins as the solution resistance.\textsuperscript{38}
For imperfect SAM’s manifesting some leakage current the circuit of choice is the Randles circuit which is composed of a solution resistance in series with a coating capacitance and a charge transfer or polarization resistance in parallel to each other. This circuit’s impedance behavior has been described in more detail previously.\textsuperscript{38}

When you have an imperfect SAM wherein the coating transfer kinetics are fast and thus the current is diffusion controlled the the circuit of choice is a Randles circuit where the polarization or charge transfer resistance is coupled with a Warburg impedance. This circuit’s impedance behavior has also been described in more detail previously.\textsuperscript{38}
Figure 19. The Degraded Coating Circuit and Impedance Behavior

Sometimes, especially as a result of degradation of a coating, a second electrolyte/electrode interface develops within the SAM which has its own associated capacitance and resistance. The circuit used to model this behavior is pictured below. Here $C_m$ is the capacitance of the intact coating, $R_m$ is the ion/water migration resistance in the pores/defects of the SAM, $R_d$ describes the ease of moving ions from the interface to the SAM and $C_d$ reflects the ability of the SAM to store ions.\(^{38, 47}\)

Figure 20. Ideal (red) and non-ideal (blue) capacitive behavior in EIS measurements and models

7. The Constant Phase Element

As extensive use has been made of equivalent circuits involving a constant phase element (in place of an ideal capacitor) in the below interpretation of electrical impedance spectroscopic (EIS) data it was felt to be worth while to, as best as possible, describe what exactly that element represents. In general, impedance spectroscopy studies
conducted using solid electrodes capacitances exhibit at least some degree of non-ideality manifesting itself as a frequency dependant capacitance. This manifests itself in EIS complex plots as a non vertical line and an arc/depressed semicircle in the case of Helmholtz and Randle’s type systems, respectively, and can be represented by a constant phase element (CPE) which is a complex impedance with a phase angle (\(\Phi\)) between 90° (ideal capacitor) and 0° (ideal resistor).

The CPE impedance is defined by equation 50 where \(Y_0\) represents the capacitance-type quantity (CPE) which is determined by model fitting, \(\alpha\) is a fractional term related to the phase angle by equation 51 where a value of 1 yields completely capacitive behavior and a value of 0 yields completely resistive behavior.\(^{46, 48-51}\)

\[
Z_{\text{CPE}} = \frac{1}{Y_0 (j\omega)\alpha} = \frac{1}{(C_{\alpha}j\omega)\alpha} \quad (50)
\]

\[
\Phi = 90 \times \alpha \quad (51)
\]

Now this fractional exponent dependence on frequency of the \(Z_{\text{CPE}}\) arises from the inability of ordinary differential equations to describe the studied system in terms of bulk (ideal) constant parameters and as such partial differential equations are used to model as system with distributed capacitive values (the \(\alpha\) giving a measure of that dispersion). Further, systems exhibiting this dispersive behavior cannot be ideally polarizable and as such capacitive charging/discharging must occur irreversibly and dissipatively; the use of a CPE element in EIS fittings would reflect such energy dissipation.\(^{38, 46, 52}\)

The value of \(\alpha\) has been shown, unambiguously, to be directly related to the degree of atomic scale irregularity/roughness of the electrode with smoother and more homogenous electrodes exhibiting much closer to ideal capacitive behavior (\(\alpha \approx 1\)). This electrode roughness can cause current-density inhomogeneities making the overall impedance of the layer a complex function of the local resistances and interfacial capacitances which inhibits separation of real and
imaginary impedance components and can manifest itself as a frequency dependence of capacitance.\textsuperscript{49, 51} In addition, interfacial processes appear to contribute significantly to observed dispersive behavior as energetically heterogeneous/rough surfaces can effect a distribution of activations energies which leads to an exponentially greater dispersal of rate and time constants. In fact, it appears to be the coupling of both surface and interfacial characteristics which results in CPE-type behavior as specific attenuation of either of the above factors has been shown to result in near-ideal capacitive behavior.\textsuperscript{46, 48, 51}

Several dissipative interfacial processes have been implicated here, the most common being that of anion (commonly halides) adsorption. At applied potentials where anion adsorption or phase transitions of anionic layers are promoted a marked reduction in the $\alpha$ factor has been observed. At potential ranges too negative to allow anion adsorption, on the other hand, near ideal capacitive behavior has been approached. A different model for anion adsorption has been developed, however, which incorporates additional diffusion and capacitance elements and does not require the use of a CPE to for adsorption data. Within its framework, the degree of semicircle depression is indicative of the adsorbate diffusion kinetics instead of specific capacitive distributions.\textsuperscript{46, 53}

Reorientation of solvent dipoles at specific potentials on an electrode surface has also been shown to bring about CPE-type behavior but again ideal behavior was obtainable at alternate applied potentials. Finally, it has been shown that increasing the concentration of a non-adsorbing electrolyte can have the effect of reducing capacitance dispersion through the reduction of the volume of the dissipative diffuse layer.

In spite of a degree of ambiguity regarding the physical meaning of the CPE (especially with regard to anion adsorption) within the simple formalism that the CPE represents a distribution of
capacitances across an electrode surface and the resulting dispersion of activation energies
influences kinetic and charge transfer processes at the interface equations 52 and 53 have been
proposed to yield the “apparent interfacial capacitances” from the CPE circuit parameters for the
CPE-Helmholtz and CPE-Randles circuits, respectively\textsuperscript{46, 49, 50}:

\[
C = \sqrt{\frac{Y_0}{R_{so \ln^{-1}}}} \quad (52)
\]

\[
C = \sqrt{\frac{Y_0}{R_{CT^{-1}} + R_{so \ln^{-1}}}} \quad (53)
\]
III. Results and Discussion

A. Anthryl Crown Acid Multilayers

1. Anthryl Crown Acid (ACA) Synthesis

7-Anthracen-9-ylmethyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadecane (1). 1,10-diaza-18-crown-6 (3.07 g, 11.68 mmol), cesium carbonate (7.62 g, 23.37 mmol) and cesium iodide (160 mg, 0.62 mmol) were combined in anhydrous THF (175 mL) and heated to reflux, at which point 9-(chloromethyl)-anthracene (2.82 g, 12.45 mmol) in THF (450 mL) was added by addition funnel over a period of 4-5 hours and left to reflux overnight under nitrogen. The intense orange product solution was then filtered through celite and the solvent removed in vacuo. The powdery-orange-oil residue was then dissolved in slight excess hot ethanol and the white-yellow powdery di-substituted product removed by hot gravity filtration followed by cooling in the fridge and suction filtration. The filtrate was concentrated to a clear orange-red oil in vacuo and dissolved in a minimum of hot ethyl acetate to crystallize remaining crown-6 as a white powder which was immediately removed by suction filtration. The mother-liquor was concentrated under vacuum and purified by flash column chromatography (8:2, DCM: MeOH(doped: 25:1, MeOH: Et₃N)) 1.4 g crude/column. 2.09 g of the anthryl-crown was then collected from the
product fractions by extensive vacuuming as slightly opaque, orange, flower-type crystals in 39.5% yield (pale-orange needles could be collected following 2-week recrystallization of product from chloroform). $^1$H-NMR: $\delta$ 8.58 (d, $J = 8.8$ Hz, 2H), 8.39 (s, 1H), 7.95 (d, $J = 8.2$ Hz, 2H), 7.52-7.43 (m, 4H), 4.61 (s, 2H), 3.63-3.55 (m, 16H), 2.90 (t, $J = 5.5$ Hz, 4H), 2.82 (t, $J = 4.62$ Hz, 4H); $^{13}$C-NMR: $\delta$

![Chemical structure of ethyl-12-bromododecanoate](image)

**Ethyl-12-bromododecanoate (2).** Concentrated sulfuric acid (0.3 mL) was added drop-wise to a solution of 12-bromododecanoic acid (5.00 g, 17.9 mmol) in anhydrous ethanol (25 mL) which was refluxed overnight. The solvent was then removed *in vacuo* and the cloudy-brown oil crude dissolved in 30 mL hexanes and washed with 10% aqueous sodium bicarbonate (15 mL), DI water (10 mL), brine (15 mL) and dried over magnesium sulfate. The mother liquid was filtered through celite and concentrated in *vacuo* isolating 4.56 g of ethyl-12-bromododecanoate as a clear, pale-yellow, light oil in 80.2% yield. $^1$H-NMR: $\delta$ 4.08 (q, $J = 7.1$ Hz, 2H), 3.36 (t, $J = 6.8$ Hz, 2H), 2.24 (t, $J = 7.5$, 2H), 1.81 (quin, $J = 7.3$ Hz, 2H), 1.57 (quin, $J = 7.1$ Hz, 2H), 1.57 (m, 20H).
Ethyl-12-(7-Anthracen-9-ylmethyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadecanyl)-dodecanotate (3). Anthryl-crown (1) (1.0 g, 2.36 mmol), cesium carbonate (3.95 g, 11.75 mmol) and cesium iodide (0.03 g, 0.12 mmol) were combined in anhydrous THF (36 mL) and heated to reflux, at which point ethyl-12-bromododecanoate (2) (2.46 g, 7.77 mmol) was added drop-wise and the combination refluxed overnight. The intense yellow product solution was filtered through celite and the solvent removed in vacuo. The thick orange oil crude was loaded onto a flash column through which the remaining ethyl-bromo-ester (2) was washed with EtoAc (500 mL). The column was run (1:9, Et$_3$N:EtoAc) and the product collected as a clear, thick, yellow (green-tinged), oil in 60% of theoretical yield upon extensive vacuuming. $^1$H-NMR ($CDCl_3$) $\delta$ 8.55 (d, $J$ = 8.6 Hz, 2H), 8.40 (s, 1H), 7.98 (d, $J$ = 8.1 Hz, 2H), 7.51-7.43 (m, 4H), 4.60 (s, 2H), 4.12 (q, $J$ = 7.1 Hz, 2H), 3.62-3.55 (m, 17H), 2.90 (t, $J$ = 5.8 Hz, 4H), 2.77 (t, $J$ = 5.8 Hz, 4H), 2.47 (t, $J$ = 7.5 Hz, 2H), 2.28 (t, $J$ = 7.5, 2H), 1.38-1.23 (m, 25H); $^{13}$C-NMR
12-(7-Anthracen-9-ylmethyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadecanyl)-dodecanoic Acid (4). The anthryl-crown ester (3) (100 mg, 1.45 mmol) was refluxed for 3 hours in a saturated solution of cesium hydroxide in anhydrous ethanol. The resulting orange crude solution was concentrated in vacuo and to the red oil residue was added 1 M aqueous acetic acid (10 mL). The product was then extracted by DCM (2×10 mL) and dried over magnesium sulfate and filtered. The solvent was removed in vacuo yielding the anthryl-crown-acid as a thick red-orange oil in quantitative yield. $^1$H-NMR (CDCl$_3$): $\delta$ 8.53 (d, $J = 8.8$ Hz, 2H), 8.40 (s, 1H), 7.99 (d, $J = 8.2$ Hz, 2H), 7.52-7.43 (m, 4H), 4.59 (s, 2H), 3.76-3.53 (m, 18H), 2.90 (t, $J = 5.7$ Hz, 4H), 3.10 (t, $J = 5.0$ Hz, 4H), 2.75 (t, $J = 8.0$ Hz, 2H), 2.27 (t, $J = 6.5$, 2H), 1.61-1.25 (m, 20H);
¹³C-NMR (CDCl₃, adjusted): δ 22.3, 24.7, 25.6, 26.6, 28.1, 28.3, 28.5, 28.6, 28.8, 28.9, 35.6, 52.2, 53.8, 54.2, 54.7, 67.0, 70.5, 70.9, 125.2, 125.5, 126.0, 127.9, 129.4, 130.6, 131.8, 131.8, 176.6; MS m/z (fragment): 191.1 (methyl-anthracene), 459.5 (crown-chain acid), 651.3 (M+), 659.9 (?); UV: 350(5050), 369(7927), 389(7563).

3-aminopropyltriethoxysilane self-assembled monolayer on ITO (5). Twelve 1×2.5cm ITO plates (DI contact angle: 61.4° (± 9.2)) were cut, rinsed with EtOH and sonicated in aqueous 5% HCl (50 mL) for 15 minutes. The slides were then washed with DI water and EtOH and dried with nitrogen (DI contact angle: 24.3° (± 4.3)). They were then placed in an anhydrous 3% (by volume) 3-aminopropyltriethoxysilane/EtOH solution for 8 hours after which they were washed with EtOH and dried with nitrogen (DI contact angle: 47.3° (± 4.69))

12-(7-Anthracen-9-ylmethyl-1,4,10,13-tetraoxa-7,16-diaza-cyclooctadecanyl)-dodecanoic Amide self-assembled monolayer on ITO (6). A solution of Anthryl-crown acid(4) (100mg) in
anhydrous THF (4 mL) was cooled, with stirring, in an ice bath before excess (a few pipette drops in addition to a stoichiometric amount) triethylamine and isobutylchloroformate were added. The white precipitate which formed was then micro-filtered off and four freshly prepared 3-aminopropyltriethoxysilane-SAM/ITO plates (5) were placed in the resulting clear, red-orange solution and allowed to stand overnight. The slides were then washed with THF and EtOH and dried with nitrogen manifesting a DI contact angle: 59.8° (± 5.18) and the Fluorescence-spectra profile of anthracene.

2. Solution Binding Experiments

In order to confirm analyte binding to the sensor and obtain a rough idea of sensor selectivity and sensitivity a series of binding experiments were conducted in solution. It was believed that this solution data could then be used as a basis with which to compare sensitivity and selectivity on a surface, in an electrochemical cell format. A more qualitative NMR titration (results shown in Figure 21 below) was conducted in which the chemical shifts of the azacrown’s N-C-H’s was used to evaluate the degree of ion binding of potassium and cesium (it has been shown in the literature that their degree of shielding is influenced by the electron withdrawing nature of bound cations54) and quantitative fluorescence (PET) titrations were conducted in a series of different solvents using different alkali ions (potassium, sodium and cesium). The titrations curves were then fit to Langmuir isotherms which assumed 1:1 complexation and from these fittings values were obtained for respective maximum signal (fluorescence) responses and association constants.
The first real solution fluorescence titration conducted were modeled after Katherine Dennen’s original Anthryl-crown titrations in which a 50:50 dichloromethane : methanol solvent system was used (pure DCM would not dissolve KC$_2$H$_3$O$_2$) and the solution was treated with an equivalent of benzyl trimethyl ammonium hydroxide (BTMAH) in order to assure deprotonation of the PET-active amine. Therefore a 10$^{-5}$ M solution of the anthracene-crown acid sensor was made and titrated with a 5×10$^{-3}$ M stock solution of KC$_2$H$_3$O$_2$ using the same solvent. This titration was repeated, varying the ratio of methanol to dichloromethane. The results were reproducible and are summarized in Figure 22.
The above data was fitted iteratively to equation 54 and values for the binding constants and maximum fluorescence intensity of the sensor were obtained:\textsuperscript{37, 55, 56}

\[
\Delta F L = \frac{[M+] \times \Delta F L_{\text{MAX}}^2 \times K_a}{(\Delta F L_{\text{MAX}} + [\text{Sensor}] \times K_a + [M+] \times \Delta F L_{\text{MAX}} \times K_a)} \approx \frac{[M+] \times \Delta F L_{\text{MAX}} \times K_a}{(1 + [M+] \times K_a)} \tag{54}
\]

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\Delta F L_{\text{MAX}}$</th>
<th>$K_a$ (M$^{-1}$)</th>
<th>$\Delta F L_{\text{MAX}}$</th>
<th>$K_a$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Methanol</td>
<td>3699</td>
<td>149500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% Methanol</td>
<td>2412</td>
<td>121900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Methanol</td>
<td>1087</td>
<td>13500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Methanol</td>
<td>1535</td>
<td>6708</td>
<td>549.6</td>
<td>1242</td>
</tr>
</tbody>
</table>

In order to determine the direct effects of the solvent system on the anthracene’s fluorescence a $10^{-5}$ M ACA + $4 \times 10^{-4}$ M BTMAH DCM solution was next titrated with a $10^{-5}$ M ACA + $4 \times 10^{-4}$ M BTMAH MeOH solution (Figure 23).
Figure 23. Fluorescence Intensity of the Anthracene Crown Acid as a function of MeOH fraction in DCM (Ex: 365 nm Ex slit: 5 nm Em slit: 2.5 nm)

Table 3. Initial solvent system Fluorescence area and magnitude Fluorescence change factor for DCM-MeOH Titrations

<table>
<thead>
<tr>
<th>Initial FL area</th>
<th>$K^+ \Delta FL_{\text{MAX}}$</th>
<th>Magnitude binding Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Methanol</td>
<td>2365</td>
<td>3699</td>
</tr>
<tr>
<td>50% Methanol</td>
<td>877</td>
<td>2412</td>
</tr>
<tr>
<td>95% Methanol</td>
<td>111</td>
<td>1087</td>
</tr>
<tr>
<td>100% Methanol</td>
<td>181</td>
<td>1535</td>
</tr>
</tbody>
</table>

An aqueous potassium titration of the anthracene crown acid, was attempted, but the sensor proved insoluble in water until an equivalent of base (BTMAH) was added. When a titration of this sample, was attempted little to no response was observed without the use of saturated solutions. Therefore in order to evaluate the effect of the benzyl trimethyl ammonium hydroxide, in as close to an aqueous solution as possible, a $10^{-5}$ M ACA solution in 25% methanol/water by volume was made and aliquots of BTMAH (Figure 24) added. When the
fluorescence decrease plateaued (presumably as a result of completely deprotonating the crown) aliquots of KC$_2$H$_3$O$_2$ (Figure 25) were added.

At this point, as it seemed that the majority of the potential fluorescence response to metal ions could well be masked by the effects of protonation it was decided that the maximum plateau concentration of BTMAH (Figure 24: $\sim$4×10$^{-4}$ M) would be used in all further aqueous titration experiments. Therefore I next carried out a series of potassium and sodium titrations using different MeOH/H$_2$O solvent systems. The data obtained from these plots is outlined in Tables 4 and 5.
Figure 26. 0%, 25%, 50%, 75%, 100% H2O/MeOH (4e-4 BTMAH) K+ ACA Titration (Ex: 365nm Ex Slit: 5nm Em lit: 2.5nm)

Table 4. H2O-MeOH Solvent System Titration Results

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>K⁺ ΔFL_MAX</th>
<th>K⁺ K_a (M⁻¹)</th>
<th>Na⁺ ΔFL_MAX</th>
<th>Na⁺ K_a (M⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% H₂O</td>
<td>1534.6</td>
<td>6708</td>
<td>549.6</td>
<td>1242</td>
</tr>
<tr>
<td>0% H₂O-BTMAH</td>
<td>494.7</td>
<td>17787</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25% H₂O-BTMAH</td>
<td>462.7</td>
<td>864</td>
<td>151.8</td>
<td>175</td>
</tr>
<tr>
<td>50% H₂O-BTMAH</td>
<td>182.8</td>
<td>1614</td>
<td>69.8</td>
<td>805</td>
</tr>
<tr>
<td>75% H₂O-BTMAH</td>
<td>122.4</td>
<td>292</td>
<td>59.7</td>
<td>96</td>
</tr>
<tr>
<td>100% H₂O-BTMAH</td>
<td>61.5</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. H2O-MeOH Fluorescence Increase Magnitudes

<table>
<thead>
<tr>
<th>Initial FL area</th>
<th>K⁺ ΔFL_MAX</th>
<th>Na⁺ ΔFL_MAX</th>
<th>K⁺ response</th>
<th>Na⁺ response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% H₂O</td>
<td>181</td>
<td>1534.6</td>
<td>549.6</td>
<td>9.5x</td>
</tr>
<tr>
<td>0% H₂O-BTMAH</td>
<td>391</td>
<td>494.7</td>
<td></td>
<td>2.3x</td>
</tr>
<tr>
<td>25% H₂O-BTMAH</td>
<td>134</td>
<td>462.7</td>
<td>151.8</td>
<td>4.5x</td>
</tr>
<tr>
<td>50% H₂O-BTMAH</td>
<td>86</td>
<td>182.8</td>
<td>69.8</td>
<td>3.1x</td>
</tr>
<tr>
<td>75% H₂O-BTMAH</td>
<td>71</td>
<td>122.4</td>
<td>59.7</td>
<td>2.7x</td>
</tr>
<tr>
<td>100% H₂O-BTMAH</td>
<td>50</td>
<td>61.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The completely aqueous-BTMAH titration above was repeated but this time, in order to keep the ACA concentration constant, a titrating metal ion solution which included $10^{-5}$ M of the sensor (to make sure the titration curves were not prematurely plateauing due to dilution of the sensor accompanying addition of metal ion) was used. This experiment was conducted using both sodium and cesium as well. The results are summarized in Figure 27 below.

![Figure 27. 100% H2O (4E-4M BTMAH 1E-5M ACA) K+, Na+, Cs+ ACA Titration](Image)

As can be seen above the binding curve for sodium and cesium appeared to have changed markedly from a Langmuir-type to an almost parabolic type curve which could not be fit using the 1:1 binding model used previously (Equation 54). In all previous experiments involving methanol and methanol-water titration solutions sodium’s titration curve had consistently paralleled potassium’s in profile even though it always manifest significantly reduced association constants and maximum fluorescence responses (e.g. Figure 28).
The potassium and sodium titrations shown in Figure 27 were repeated to much higher concentrations (Figure 29).
Now, this new titration behavior of sodium and cesium in aqueous solution cannot be fit using the old Langmuir-type model which supposed 1:1 binding and as such must be indicative of a new binding mechanism. Indeed, in general, the sigmoidal profile of the binding curve can be taken to indicate cooperative binding of some sort. Now the specific stoichiometry involved here (e.g. 2:1 or 2:1 (crown:ion)) can perhaps be determined by evaluating the applicability of other models supposing other ligand to analyte ratios.

Assuming an association constant of the form for the binding of N ions:

\[ K_N = \frac{[\text{Complex}]}{[\text{Crown}][\text{Ion}]^N} \]  \hspace{1cm} (55)

and given the fraction of bound sites (\(\theta\)) would equal:

\[ \theta = \frac{[\text{Complex}]}{[\text{Crown}]+[\text{Complex}]} \]  \hspace{1cm} (56)

Then rearranging equation 55:

\[ [\text{Complex}] = K_N[\text{Crown}][\text{Ion}]^N \]  \hspace{1cm} (57)

and plugging this into equation 56:

\[ \theta = \frac{K_N[\text{Crown}][\text{Ion}]^N}{[\text{Crown}]+K_N[\text{Crown}][\text{Ion}]^N} = \frac{K_N[\text{Ion}]^N}{1+K_N[\text{Ion}]^N} \]  \hspace{1cm} (58)

Now, assuming \(\Delta F_L = \theta \times \Delta F_{\text{MAX}}\) the following model was obtained:

\[ \Delta F_L = \frac{\Delta F_{\text{MAX}}K_N[\text{Ion}]^N}{1+K_N[\text{Ion}]^N} \]  \hspace{1cm} (59)

Trying to fit this to the sodium titration data in Figure 30 the best fit was obtained using a\( N \) value of 3 (Figure 30) which apparently would indicate a sensor to analyte stoichiometry of 1:3.
Comparing previous potassium aqueous titrations (with decreasing sensor concentration) with current titrations (with constant sensor concentration), it does seem that the dilution of the sensor compound led to premature plateauing of the titration curve and thus underestimation of the \( \Delta F_{\text{MAX}} \) (61 vs. 109) and overestimation of \( K_a \) (29 vs. 18). Also, extending the titrations to more obviously plateaued regions appears to lend to a better model estimate of \( \Delta F_{\text{MAX}} \) (194) and \( K_a \) (6). The former error can perhaps be corrected by scaling up all titration data by the same factor the sensor concentration has been reduced at any given point in the titration. Errors of the latter sort can probably only be corrected for by extending all titrations to very well plateaued regions.

Nevertheless from the above data certain clear trends are evident. First, in general, association constants decrease as one goes from less to more polar (dichloromethane to methanol to water) solvents which is exactly what would be expected given corresponding increasing solvation energies which would make the complex less thermodynamically favorable relative to the uncomplexed crown and ion. Secondly, in virtually all solvent systems the anthracene
crown acid exhibits significantly larger binding constants associated with potassium than with sodium (Tables 2 and 4). This also is expected and for several of the methanol and methanol-water solvent systems the selectivity coefficient \( K_{ij} \) can be determined from potassium and sodium’s association constants \( K_i \) and \( K_j \), respectively. Defining selectivity by equation 60.5:

\[
K_{ij} = \frac{K_i}{K_j} \quad (60)
\]

**Table 6.** Selectivity of Anthracene-Crown-Acid for K\(^+\) over Na\(^+\)

<table>
<thead>
<tr>
<th></th>
<th>( K_K )</th>
<th>( K_{Na} )</th>
<th>( K_{K,Na} )</th>
<th>( \log(K_{K,Na}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% H(_2)O</td>
<td>6708</td>
<td>1242</td>
<td>5.4</td>
<td>0.73</td>
</tr>
<tr>
<td>0% H(_2)O-BTMAH</td>
<td>17787</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25% H(_2)O-BTMAH</td>
<td>864</td>
<td>175</td>
<td>4.9</td>
<td>0.69</td>
</tr>
<tr>
<td>50% H(_2)O-BTMAH</td>
<td>1614</td>
<td>805</td>
<td>2.0</td>
<td>0.30</td>
</tr>
<tr>
<td>75% H(_2)O-BTMAH</td>
<td>292</td>
<td>96</td>
<td>3.0</td>
<td>0.48</td>
</tr>
<tr>
<td>100% H(_2)O-BTMAH</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As can be seen above, the selectivity of this sensor is rather marginal which would also perhaps be expected given reference in the literature to reduction in alkali ion selectivity of aza-crowns with the substitution of soft-base nitrogens for hard base oxygens.\(^{58}\) A third observation that can be made from the above data (Table 3) is that with increasing polarity of the solvent in the DCM-MeOH solution titrations the final and initial fluorescence both decrease but the factor by which the initial fluorescence is increased actually increases. This can perhaps be explained by considering Figure 23 which describes the effect of methanol/dichloromethane fraction on initial fluorescence. According to this data very small fractions of methanol have the effect of actually significantly increasing the magnitude of the anthracene’s fluorescence but this effect peaks at around 10% at which point larger fractions of methanol have the opposite effect, significantly reducing the anthracene’s fluorescence. This first effect can possibly be explained by small concentrations of methanol (where it acts more as a solute) initially hydrogen bonding with the aza-crown and thus attenuating the PET quenching effect. Once the methanol concentration
reaches a certain point, however, this effect no doubt begins to be overshadowed by solvent effects of the methanol wherein the oxygen lone-pairs become more readily available to quench the anthracene’s fluorescence themselves. The second effect (the increasing fluorescence increase factor) may be explicable through the more polar solvent stabilizing charge separation within the crown which could lend to more electron density localized about the heteroatoms, including the PET-active nitrogen. Such an effect could presumably increase the probability of PET-quenching (as there would be more nitrogen electron density available) and thus increase the effect of PET-reduction/fluorescence increase.

A fourth observation which could be made is that while again increasing polarity in the MeOH-H$_2$O solvent systems decreases the initial and final fluorescence of the sensor (no doubt for reasons similar to those discussed above) the magnitude of the fluorescence increase factor, this time, appears to peak at the 25% MeOH/H$_2$O solvent system. This may have something to do with protonation proving a more significant factor in the other solvent systems with a higher fraction of water (given the same amount of BTMAH was used in all systems). This would doubtless artificially inflate the magnitude of the initial (no metal ion) fluorescence and reduce the magnitude of the final fluorescence increase.

A final observation that could be made is that the methanolic fluorescent response (Table 5), at least, the initial fluorescence appears to increase (181 to 391) while the final fluorescence change appears to decrease (1535 to 495) following addition of base (BTMAH). This increase in initial fluorescence intensity (with addition of BTMAH) also appears to be born out in dichloromethane-methanol solvents if one compares values in Figure 23 and Table 3. Therefore the BTMAH must interact with the anthracene-crown system in some manner which attenuates the fluorescent response magnitude in non aqueous solvents, at least. This, in conjunction with
discussion arising from the fourth observation, could possibly explain the seeming local maximum of fluorescent response and local minimum in association constant for the 25% H2O/MeOH anthracene-crown-acid system.

3. Surface/ITO Fluorescence Experiments

Following deposition of the second layer anthracene crown, initial fluorescence measurements off the indium tin oxide (ITO) substrate were taken using the sample set up pictured in Figure 8. The ITO slide were rinsed thoroughly with THF and EtOH and dried with nitrogen. They were then secured into a cuvette stir bar using a small amount of parafilm and placed within a quartz cuvette. Spectra obtained with different solvents filling the cuvette can be seen pictured in Figure 31. In spite of a great deal of clear variability in the spectra obtained from a single sample (doubtless as a result of slight changes in position of the ITO slide) there

Figure 31. Fluorescence Spectra of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5
nevertheless appears to be a clear trend in solvent effects which do not (at least directly) seem to correspond to changes in polarity, specifically, of the solvent.

The initial binding experiment (Figure 32) consisted of a brief Li\(^+\) titration of an ACA-ITO surface immersed in ethanol. Spectra were first obtained from the sample in air, then immersed in 3 mL of ethanol. Aliquots were then added using a 1.0 mL micropipette and the solution mixed by repeated removal and addition of solvent using the same micropipette. Pipette tips were changed between aliquot additions.

Observing no change in the fluorescence intensity following addition of the first two aliquots and an actual decrease in fluorescence intensity following addition of the last aliquot a titration in water was attempted using 1 M KCl, 1 M LiC\(_2\)H\(_3\)O\(_2\) and a 10\(^{-4}\) M CsOH aqueous solutions. Believing that Cs\(^+\) would not bind the 18-crown-6 on a surface, the SAM was first

---

**Figure 32.** Ethanolic Li\(^+\) Titration of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5

![Graph showing fluorescence intensity against wavelength for different solvent conditions.](image)
treated with a bit with CsOH in the hopes of deprotonating any protonated PET-active amines which would, conceivably attenuate detection of metal ion by prematurely preventing quenching of the anthryl fluorescence (BTMAH was not used as it has been known to destroy alkoxy silane SAM’s\textsuperscript{16}). Unfortunately, the SAM fluorescence actually increased slightly as a result. Two aliquots of potassium were then added and both showed rather more marked (if still slight) fluorescence increases but, taking multiple measurements for a single concentration, showed the magnitude of this increase was subject to some variation. The SAM fluorescence showed no response to another addition of base and actually decreased completely to its starting value when the solution was completely exchanged with a 1M KCl or 10\textsuperscript{-4} M CsOH solution.
Figure 33. Aqueous K$^+$ Titration of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5

Figure 34. Aqueous Li$^+$ Titration of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5
Another aqueous lithium binding experiment was attempted, the results of which are shown in Figure 34, above. Thinking the lack of substantial or reproducible fluorescence response on the SAM’s above may be as a result of protonation of the PET-active amine the effect of adding acid and base to the SAM was next explored.

**Figure 35.** Two Aqueous HCl Titrations of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5
Adding acid appeared to have either no discernible effect (within the variation between individual measurements) past possibly a slight reduction in the surface fluorescence. If the acid was, in fact, protonating the amine then it would be expected to increase the fluorescence by occupying the lone-pair which would otherwise quench excited anthracene.\textsuperscript{4, 10, 11, 59, 60} The acidity was then adjusted of an ethanol solution within which an ACA SAM was immersed:

**Figure 36.** Ethanolic HCl and CsOH Titrations of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5

Yet again the SAM, within the solution, showed no fluorescence response to changing immersion environment which was significantly different from the variability of fluorescence response observed between replicate measurements. Indeed the angle of the SAM appears to heavily influence the profile and intensity of the spectra obtained perhaps either as a result of more profound ITO interference (e.g. the peak at about 490 nm see Figure 36) or more substantial self-absorbance of the SAM from the angle from which the fluorescence is collected.
Another potassium binding experiment was conducted, this time using dichloromethane as my immersed solvent (Figure 37) in the hope that the crown-metal complex would be more favorable in a less polar environment, it was reasoned, if the above anomalous results were simply the result of surface-crown binding not being favorable changing the solvent might indicate this. Unfortunately, no significant change in fluorescence intensity was observed until the solvent was removed and this was accompanied by a significant profile change. Therefore, it proved impossible distinguish how much of this fluorescence increase was as a result of the shifting ITO plate and how much was a result of ion binding. In the next set of experiments fluorescence measurements were, in general, taken out of solution rather than in solution. It was reasoned that the fluorescence of the starting SAM had consistently been greatest out of solution so perhaps the fluorescence response would be greatest out of solution as well. An anthracene-crown SAM was then dipped in basic (CsOH) and acidic (HCl) solutions (Figure 38).
Figure 37. Dichloromethane K+ Titrations of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em:

- Air
- 2.5 mL DCM
- 2.5 mL DCM + 0.5 mL sat. K+/DCM
- sat. K+/DCM
- Air (K+ sat DCM removed)

Figure 38. HCl, CsOH Dippings of Anthracene-Crown on ITO Ex: 355 Slits: ex: 2.5 em: 5

- Air
- Dipped in 10^4 M HCl
- Dipped in 10^3 M CsOH
- Dipped in 10^4 M HCl
- Dipped in 10^3 M CsOH
- Dipped in 10^2 M HCl
Unfortunately, aside from a consistent but small increase in fluorescence (all of the large increases being accompanied by major profile changes which call into question their validity of signally binding events) upon dipping in both acid (perhaps as a result of crown protonation) and base (perhaps as a result of cesium binding) no other specific acidic or basic effect was observable.

In order to try and establish a base line for these experiments the excitation and emission spectra of anthracene, the anthracene-crown-acid, ITO and the anthracene-crown acid on ITO were determined (at this point substantially less ACA was used in the second layer coupling reaction given the fact anthracene is known to stick to glass and using as high a concentration as had been used was suggested to possibly result in such physisorption):

**Figure 39.** Fluorescence Excitation and Emission Spectra of Anthracene Slits: 5 nm
**Figure 40.** Excitation and Emission Spectrum of the Anthracene-Crown-Acid Slits: 5 nm

**Figure 41.** Excitation and Emission Spectrum of the Indium Tin Oxide Slits: 5 nm
As can be seen in Figure 41 the ITO emission profile showed a wide range of variation with each emission spectrum being obtained in duplicate at one of two slightly different angles within the apparatus pictured in Figure 8. Unfortunately the Anthracene-Crown-Acid SAM on ITO showed emission and excitation spectra indistinguishable from ITO’s above. The ITO interference spectra were subtracted from these but still no anthracene-type profile was observed.

**Figure 42.** Fluorescence Spectra of the Anthracene Crown SAM Slits: 5 nm

Presuming that this inability to obtain fluorescence off the ITO SAM was a result of atmospheric water having the effect of quenching the anthracene’s fluorescence spectra were next obtained using a SAM which had been washed in EtOH, MeOH and immersed in anhydrous DCM and, at this point, the anthryl emission profile was again observed. Having obtained a new specially made ITO holder meant to maintain the an angle and position of slides within a
fluorometer spectra were then taken from a new slide in the new holder and in the anhydrous DCM/cuvette set up just mentioned (Figure 42).

Being unable to obtain anthracene fluorescence off these SAM’s using the previous excitation parameters (Ex: ~350 nm Slits: ~5nm) but knowing that the new slide holder was needed to maintain a constant incident angle between experiments (in order to avoid the wide variability in spectral intensities and profiles previously observed) the parameters used by Christopher Cooper for similar experiments on quartz and were used (excitation wavelength of 266 nm and slit bandwidths of 10 nm). With these parameters spectra were obtained of crystalline anthracene, anthracene in dichloromethane and anthracene off the surface in order in confirm and compare the presence of anthracene fluorescence from the SAM (Figure 43).

**Figure 43.** Fluorescence Spectra of the solid, solution and surface bound Anthracene Ex: 266 nm Slits: 10 nm
Now, in the binding experiments which followed the first showed the best initial response (following immersion in lithium saturated dichloromethane) but unfortunately this did not appear reversible as all subsequent measurements (taken after rinsing, applying base, and applying other ions) showed only insignificant changes which could well have been attributable to slight changes in the ITO incidence angle (resulting from removing and the slide in the holder between measurements). The second experiment did appear to demonstrate a significant change but only, again, when a saturated solution of lithium ion in dichloromethane was used. Following these experiments a series of fluorescence spectra from the SAM were taken using different excitation wavelengths to determine if there was some optimal wavelength (using slits bandwidths of 10 nm) for which the best spectra would be obtainable (Figure 45). Unfortunately using these slit sizes some kind of massive wavelength dependant “emission” emerged, doubtless originating from the ITO scattering (cf. Figure 41), which provided significant interference at several wavelengths.
Figure 44. First and Second Anthryl-Crown binding Experiment using parameters: Ex: 266 nm Slits: 10 nm
Figure 45. Fluorescence Spectra of Anthracene-Crown SAM at different Excitation wavelengths Slits: 10 nm

Figure 46. Last Binding Experiment taken without removing SAM from holder Ex: 260 nm Slits: 10 nm
4. Electrochemical Experiments on ITO and Gold

Impedance spectroscopy and cyclic voltammetry (using a ferricyanide redox probe) were attempted on ITO slides functionalized with the anthracene crown multilayers here considered. Unfortunately the cyclic voltammetry experiments showed no redox current at all on either the bare substrate, the amine terminated first layer or the anthryl-crown second layer. The Nyquist plots obtained in impedance experiments proved extremely variable and noisy and quite often uninterpretable. All this information (but most especially the fact that oxidation/reduction of the redox species in solution was not observed when just the bare ITO was used as the working electrode) together led us to the conclusion that the ITO substrates used were simply unsuitable for electrochemical characterization. It was reasoned that the conducting layer of ITO was somehow discontinuous such that the working electrode showed variable to no conductivity across the length of the plate which would result in either significantly more complex cell behavior or an incomplete cell and thus no observable behavior past noise. Nevertheless, some useable impedance spectra were obtained and were fitted using the Randles-CPE circuit. A undecanoic acid second layer was also attached to the first layer and characterized to provide a base line with which to compare anthryl-crown second layer results. The capacitance values obtained from these spectra are summarized below.

<table>
<thead>
<tr>
<th>Contact angle</th>
<th>Capacitance (DC: -0.4V)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amine:</td>
<td>47.3° (±4.69)</td>
<td>32.9 ± 3.8 μF/cm² (0.1 M Et₄NCl)</td>
</tr>
<tr>
<td>Anthryl-Crown acid:</td>
<td>59.8° (±5.18)</td>
<td>22.5 ± 4.8 μF/cm² (0.1 M Et₄NCl)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.3 ± 5.6 μF/cm² (0.1 M KCl)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.2 ± 3.0 μF/cm² (0.1 M LiCl)</td>
</tr>
<tr>
<td>Undecanoic acid:</td>
<td>66.8° (±6.18)</td>
<td>44.8 μF/cm² (0.1 M Et₄NCl)</td>
</tr>
</tbody>
</table>

From the significant increase in DI water contact angle and decrease in capacitance it is clear that the second layer did deposit in the case of the anthracene crown acid (given the reduced
hydrophilicity of a terminating crown ether relative to that of a terminating amine and the increased thickness (cf. equation 49) of the insulating layer associated with deposition of a long chain second layer). The high capacitance values, however, are rather more comparable to those of a bare conducting substrate (~25-50 μF/cm²) than those of a well ordered self-assembled monolayer (~1-5 μF/cm²) and this could be indicative of a very poorly packed/low coverage SAM such that, in the overall behavior of the electrode, the double layer capacitance of the bare electrode makes a significant contribution. What’s more, the relatively low contact angle of what should be a methyl terminated multilayer (and thus highly hydrophobic) could also be indicative of a poorly packed first layer (assuming the second layer reaction goes to completion) or poor yields in the second layer reaction. Either way, it is clear from all of these results that these ITO monolayers are of very poor quality and this can perhaps explain the very small and insignificant capacitive response observed in response to potassium and lithium ions. These impedance measurements were taken at -0.5 V_SCE and were comparable to results obtained at 0 and +0.4 V_SCE.

At this point, in order to obtain better quality films with more reproducible conducting behavior the transition was made to gold substrates and the above impedance and cyclic voltammetric measurements repeated. This was accomplished by functionalizing the gold substrate with an amine terminated monolayer, specifically cystamine dihydrogen chloride, and using the same second layer coupling reaction used for amino functionalized ITO slides above.

The 1×2.5cm gold plates were cut, and immersed in Piranha solution for 5-10 min. The slides were then washed with DI water and EtOH and dried with nitrogen. They were then placed in a 10 mM solution of cystamine dihydrogen chloride in DI water overnight after which they were washed with DI water, EtOH and dried with nitrogen. The impedance spectra
obtained were also fitted with the Randles-CPE circuit and treated with equations 52 and 53 and to extract real capacitance values and the results are summarized in Table 7.

<table>
<thead>
<tr>
<th>Contact angle</th>
<th>Capacitance (DC: -0.5V_{SCE})</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amine:</td>
<td>32.7° (± 5.65)</td>
<td>12.9 ± 3.4 μF/cm² (0.1 M Et₄NCl)</td>
</tr>
<tr>
<td>Anthryl-Crown acid:</td>
<td>55.6° (± 6.15)</td>
<td>6.5 ± 2.2 μF/cm² (0.1 M Et₄NCl)</td>
</tr>
<tr>
<td>Undecanoic acid:</td>
<td>65.4° (± 6.79)</td>
<td>3.5 μF/cm² (0.1 M Et₄NCl)</td>
</tr>
</tbody>
</table>

The capacitance values for these coating are low enough that it can be seen that a significantly more complete monolayer and multilayers were obtained here than with ITO. At the same time, however, the multilayer capacitances are still relatively high compared to those of alkane thiol SAM’s on gold of similar thicknesses.21, 47, 62 This is to be expected if the second layer coupling reaction did not proceed to completion (due to steric hindrance of a surface reaction?) and bulky terminating groups which both can be expected to contribute to poorer packing of the overall layer.21, 63 The former can perhaps find support in the relatively low contact angle measurements obtained for the methyl terminated and crown-ether terminated second layers as while they certainly demonstrate deposition of the more hydrophobic terminated layers the change in hydrophilic is not significant enough to actually make the surface hydrophobic/unwetted.50, 64 The presence of unreacted, short-chain amino groups acting in parallel to the long chain, reacted multilayers on the surface can possibly explain these seeming intermediate wettabilities and capacitances.

At this point a large number of impedance/capacitive titrations were conducted over a period of several months using these multilayers and other sensing monolayers which will be discussed later. Unfortunately what was believed to be sensor response (capacitive increase) to increasing analyte concentration, turned out to be, at least in part, the slow destruction of the sensing layer as a result of the Potentiostat applying a very high voltage (~5V_{SCE}) across the
electrochemical cell for a millisecond before each measurement. When impedance measurements were repeated using three other instruments, it was found that the only the first measurement obtained using the dysfunctional instrument was valid, in general, and all other subsequent measurements (taken on the same sample (in the course of a titration, for example)) had to be thrown out. As such, virtually no usable ion-binding and sensor response data was obtained for these multilayer SAM’s as most subsequent titrations were conducted using much simpler, but analogous, sensing monolayers.

5. General Multilayers Experiments

**Figure 47.** First layer Cystamine Disulfide SAM Deposition

In order to determine the best method to form amide multilayers on top of amine monolayers a set of undecanoic amide coupled SAM’s were made and fully characterized. Clean, 12 1×2.5 cm, gold slides were immersed in a 10 mM aqueous solution of cystamine disulfide dihydrochloride (Figure 47). These SAM’s were characterized by contact angle, grazing IR, CV and Ellipsometry and four separate second layer coupling reactions (differing in coupling reagent and solvent used) were used (see Table 8 and Figure 48).

**Table 8.** Summary of Initial Four Coupling Reaction Conditions

<table>
<thead>
<tr>
<th>Reaction #1</th>
<th>Reaction #2</th>
<th>Reaction #3</th>
<th>Reaction #4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>THF</td>
<td>THF</td>
<td>EtOH</td>
</tr>
<tr>
<td>Coupling Reagent</td>
<td>IBCF, Et₃N</td>
<td>DCC</td>
<td>DCC</td>
</tr>
</tbody>
</table>
The resulting multilayered SAM’s were next characterized by contact angle, CV and Ellipsometry. The results are summarized below (including Anthryl-Crown-undecanoic-Acid multilayer data obtained):

Table 9. Cystamine Disulfide Multilayer Experimental Results Summary

<table>
<thead>
<tr>
<th></th>
<th>Contact Angle</th>
<th>Ellipsometry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI H₂O (pH 6)</td>
<td>Acid (pH 2)</td>
</tr>
<tr>
<td>Cystamine</td>
<td>44.5 ± 4.2°</td>
<td>43.6 ± 4.2°</td>
</tr>
<tr>
<td>ACA #1</td>
<td>62.6 ± 4.2°</td>
<td>64.0 ± 4.1°</td>
</tr>
<tr>
<td>Undecanoic #1</td>
<td>53.8 ± 3.3°</td>
<td>58.0 ± 1.4°</td>
</tr>
<tr>
<td>Undecanoic #2</td>
<td>50.7 ± 0.7°</td>
<td>54.5 ± 0.7°</td>
</tr>
<tr>
<td>Undecanoic #3</td>
<td>61.7 ± 2.6°</td>
<td><strong>60.8 ± 3.2°</strong></td>
</tr>
<tr>
<td>Undecanoic #4</td>
<td>60.6 ± 2.1°</td>
<td>56.4 ± 1.7°</td>
</tr>
</tbody>
</table>

As can be seen above based solely on hydrophobicity of the SAM’s Reaction scheme #3 seems to be the most effective as it manifests the largest contact angles and thus presumably the most/best packed alkyl chains (with undecane thiol SAM’s manifesting contact angles ~100° ).

The fact that the contact angles show significant pH dependence may indicate the presence of a
large number of unreacted amines left on the surface. Looking at the CV’s of the different SAM’s (Figure 49) reaction scheme #3 and #4 again seem to provide the best/most in insulating SAM’s though the difference is not very significant.

**Figure 49.** Second Layer Undecanoic Acid Coupling Scheme

Unfortunately, the fact that none of the undecanoic amide SAM’s demonstrate contact angles as high as the Antracene Crown amide SAM is difficult to explain as a terminal methyl group *should* be more hydrophobic than a terminal crown ether. What’s more the bulky anthryl-crown *should* sterically inhibit well packing of the second layer. A possible explanation can perhaps be found in the fact that undecan**dio**ic acid may have been used instead of undecanoic acid in the above coupling reactions. Adjusting the Figure 48 reaction scheme accordingly (Figure 50) the expected SAM would probably feature a mixture of terminal carboxylic acids and bridging methylene’s:
A majority of bridged coupling with some unbridged intermixed could quite well account for the general if smaller than expected (being attenuated by free carboxylic acids) contact angle increase. Therefore, the fact that reaction schemes #1 and #2 resulted in contact angles significantly smaller than those observed from #3 and #4 can be explained by a larger fraction of un-bridged dicarboxylic acids making up these second layers. This could also account for significant pH dependence of contact angle measurements observed for almost all multilayered SAM. The fact that reaction scheme #3 results in the most hydrophobic and insulating surface with the lowest pH dependence would seem to indicate that this reaction goes the furthest to completion (more carboxylic acid’s reacting resulting in more bridging). As such, this scheme was used in all subsequent multilayer depositions.
The identity of the first layer amine-SAM was next varied in order to study the role of the first layer in colvalent multilayer assembly. Three amine terminated SAM’s were formed using cystamine disulfide dihydrochloride (\(\text{SC}_2\text{NH}_3\)\(_2\)), cystamine thiol hydrochloride (\(\text{HSC}_2\text{NH}_3\)), and a 11-carbon chain cystamine thiol hydrochloride (\(\text{HSC}_{11}\text{NH}_3\)) (Figure 51). It was supposed that a better packed first layer could result from the use of the thiol given the fact the disulfide deposits its amines in pairs (and thus would less apt to fill in defects given a greater surface area requirement per molecule deposition). In addition a longer spacer chain length could result in a better packed layer given a significant increase in Van der Waals which would drive SAM formation and organization. These new monolayers were fully characterized and along with their corresponding undecanoic amide multilayers in order to determine how, in fact, these effects (if apparent) might influence multilayer formation. Deposition of the two amine-thiols was conducted using 1 mM ethanolic solutions while the disulfide was again deposited using a 10 mM aqueous solution. An 11-carbon hydroxyl terminated SAM was accidentally made but data from it is included data for comparison. Initial characterization was conducted using contact angle, ellipsometry and external-reflectance FTIR.

Unfortunately, most of the spectra obtained from these SAM’s were relatively poor with no clear peaks being distinguishable aside from (perhaps) those associated with aliphatic C-H stretch.
Table 10. Three Amine Monolayers Experimental Results Summary

<table>
<thead>
<tr>
<th></th>
<th>Contact Angle</th>
<th>Ellipsometry (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SC₂NH₃)₂</td>
<td>44.5 ± 4.2°</td>
<td>0.3/0.4</td>
</tr>
<tr>
<td>HSC₂NH₃</td>
<td>40.5 ± 5.8</td>
<td>39.1 ± 1.1</td>
</tr>
<tr>
<td>HSC₁₁NH₃</td>
<td>62.7 ± 4.5</td>
<td>63.2 ± 3.8</td>
</tr>
<tr>
<td>HSC₁₁OH</td>
<td>32.1 ± 2.5</td>
<td>35.4 ± 7.4</td>
</tr>
</tbody>
</table>

This can be made clear by overlaying the C-H stretch regions of all the above spectra (Figure 53) and comparing their profiles to the strong asymmetric and symmetric C-H stretch absorbencies observed for the 11 carbon amine SAM (Figure 52). The frequencies of these peaks are summarized in Table 11.

Table 11. Amine Monolayer CH₂ stretch Frequency Summary

<table>
<thead>
<tr>
<th></th>
<th>(SC₂NH₃)₂ (cm⁻¹)</th>
<th>HSC₂NH₃ (cm⁻¹)</th>
<th>HSC₁₁NH₃ (cm⁻¹)</th>
<th>HSC₁₁OH (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>υₐsCH₂</td>
<td>2931</td>
<td>2929</td>
<td>2928</td>
<td>2920</td>
</tr>
<tr>
<td>υₛCH₂</td>
<td>2858</td>
<td>2855</td>
<td>2854</td>
<td>2851</td>
</tr>
</tbody>
</table>

Figure 52. IR Spectrum of C₁₁-Cystamine Thiol SAM
Now, hydrocarbon stretching absorbancies can be used to evaluate the amount of order in and to a certain extent the conformation of the SAM by comparing their frequencies with the C-H stretching frequencies of crystalline and liquid polyethylene. Given asymmetric/symmetric frequencies of 2918/2851 cm\(^{-1}\) are characteristic of solid and 2926/2855 cm\(^{-1}\) are characteristic of liquid polyethylene the crystallinity of the amine terminated alkyl chains increases in the order: \((\text{SC}_2\text{NH}_3)_2 < \text{HSC}_2\text{NH}_3 < \text{HSC}_{11}\text{NH}_3 < \text{HSC}_{11}\text{OH}\). \(^{31}\) Now this order of crystallinity/good packing agrees with expectation given first disulfide steric effects inhibiting complete film adsorption, short alkyl chains providing minimal intra-chain Van der Waals stabilization and the charged head group electrostatically destabilizing close-chain packing.

The contact angle results above (Table 10) are rather difficult to interpret given the marked difference between the three amine-terminated layers and the fact that these supposedly charged
monolayers (at acidic pH’s) exhibit greater hydrophobicities than an uncharged analogue. Now the disulfide contact angle’s agree very well with literature values obtained for the non-HCl analogue cysteamine thiol (−45° pH < 11.5, −30° pH > 11.5). Unfortunately this specific relation does not really make sense as at higher pH’s the layer should be deprotonated and thus uncharged and more hydrophobic. And indeed the trend which one would expect was born out in the case of the cystamine thiol hydrochloride (Table 10). Why these two completely analogous layers would exhibit completely different pH dependencies is hard to explain but might have something to do with the different deposition solutions used (water and ethanol).

Regarding the differences in general hydrophobicities of the different SAM’s (SC2NH2: ~45°, HSC2NH3: ~40°, HSC11NH3: ~60°) these may be explicable through differences in the conformations of the upper most layer as contact angle measurements only really probe the outer most few angstroms of a SAM.24 The all trans conformations pictured in Figure 51 probably do not well reflect the equilibrium state of the monolayers given such conformations are heavily favored only in well packed pseudo-crystalline SAM’s which these SAM’s have been shown specifically not to be. As such more guacche, conformations such as those pictured in Figure 54 below may better reflect the terminal states of these SAM’s especially in the case of the HSC11NH3 monolayer even though it was shown (according to grazing IR) to be the best packed on average.65
Cyclic Voltammetry measurements were also taken of the first layer SAM’s using ferricyanide and ruthenium hexamine as redox probes:

Figure 55. Ferricyanide Cyclic Voltamagrams of Amine-terminated Monolayers (0.1M KCL + 1 mM K₃Fe(CN)₆)

Figure 56. Ruthenium Cyclic Voltamagrams of Amine-terminated Monolayers (0.1M KCl + 1 mM Ru(NH₃)₆Cl₃)
The fact that the 11-mercaptoundecanol SAM showed the best insulating properties in response to ferricyanide supports the idea that it formed the highest quality SAM while the fact that the 11-mercaptoundecylamine SAM showed the most insulating behavior in response to ruthenium hexamine can be explained through a combination of its forming the best packed amine-terminated SAM and the ion-gating effects of that protonated amine which would have the effect of repelling the positively charged ruthenium redox probe (most effectively attenuating the observed current).

Using coupling scheme #3, a second layer of undecanDiolic acid was attached to each amine SAM and the same measurements were taken.

<table>
<thead>
<tr>
<th></th>
<th>DI H₂O (pH 6)</th>
<th>Acid (pH 2)</th>
<th>Base (pH 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SC₂NH₃)₂</td>
<td>61.7 ± 2.6</td>
<td>60.8 ± 3.2</td>
<td>56.8 ± 3.4</td>
</tr>
<tr>
<td>HSC₂NH₃</td>
<td>67.8 ± 2.5</td>
<td>67.3 ± 3.8</td>
<td>66.7 ± 2.3</td>
</tr>
<tr>
<td>HSC₁₁NH₃</td>
<td>41.3 ± 0.6</td>
<td>47</td>
<td>44.0 ± 1.4</td>
</tr>
</tbody>
</table>

Table 12. Three Amide-Undecandioico Multilayers Contact Angle Summary
Table 13. Undecandioic Amide Multilayer IR Absorption and Assignment Summary

<table>
<thead>
<tr>
<th></th>
<th>2972</th>
<th>2934</th>
<th>2860</th>
<th>1631</th>
<th>1580</th>
<th>1467</th>
<th>1111</th>
<th>1064</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSC2NH3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSC11NH3</td>
<td>2928</td>
<td>2854</td>
<td>1630</td>
<td>1552</td>
<td>1466</td>
<td>1292</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assignment:26,64</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>υ&lt;sub&gt;as&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>υ&lt;sub&gt;s&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC=O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-CO CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>(wag)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>υ&lt;sub&gt;C-C&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

First of all, based on the ferricyanide results (Figure 55), it would appear that only the thiol amine 1<sup>st</sup> layers reacted well enough with the undecanoic acid to form a significantly insulating second layer. This is supported by grazing IR data (Table 13) which was only obtainable for those SAM’s and provided unambiguous evidence of amide-bond formation through the presence of characteristic amide I and amide II absorbencies. 26, 64 Electrochemical support can also be found in the attenuation of the ammonium gating effect observed for the 11-carbon thiol following second layer deposition (Figure 61b) and by comparing the capacitances of the cystamine disulfide multilayer (9.8 ΜF/cm<sup>2</sup>) and the cystamine thiol multilayer (3.0 ΜF/cm<sup>2</sup>). Information regarding the quality of these two insulating SAM’s can be obtained by comparing the C-H stretching absorbencies of their respective first and second layers. In the case of the cystamine-thiol SAM second layer deposition was accompanied by a shift of these peaks to higher frequencies (cystamine: 2929/2855  undecylamide: 2934/2860) which, according to the first IR discussion above, would be indicative of a reduction in the ordering/crystallinity of the overall layer. On the other hand, in the case of the 11-mercaptopoundecylamine SAM, second layer deposition was accompanied by no change in frequencies of these peaks which would indicate that the average ordering of the layer also did not change. If this is taken to mean that the second layer is comparable to the first in ordering then it might be reasonable to assume that the second layer bonding is comparably trans and thus fully extended with carboxylic acid groups terminating the multilayer. Contact angle measurement (Table 12) support to this idea as they indicate a marked hydrophilicity of this 2<sup>nd</sup> layer relative to the first layer. Contact angle
measurements made on the cystamine disulfide and thiol multilayers, alternatively, indicate marked hydrophobicity of these layers (relative to their respective monolayer and the 11-carbon amine multilayer). This may point to more bridging amide formation for these multilayers (Figure 50) and/or more bridging type conformations involving more gauche bonding through the chain (which the previously mentioned reduction in crystallinity of the cystamine thiol multilayer may also indicate). Therefore it seems that the cystamine thiol affected better second layer formation than the disulphide (perhaps as a result loose spacing in the first layer translating to loose spacing in the second) and that both cystamines exhibited some kind of amine-bridging conformation/bonding which resulted in an overall more hydrophobic but less ordered layer. The 11-mercaptoundecylamine multilayer, on the other hand, appears comparably ordered to the first, indicating perhaps, a more all-trans layer manifesting significantly less amine-bridging. What is rather odd, however, is the fact that there was no evidence of carboxylic acid peaks in any of the IR spectra obtained. This may be explainable through significant hydrogen bonding making these peaks less resolvable especially using external reflectance infrared spectroscopy.

**Figure 57.** Ferricyanide Cyclic Voltamagrams of Undecanoidic Amide Multilayers

(0.1M KCL + 1 mM K₃Fe(CN)₆)
Figure 58. Ruthenium Cyclic Voltamagrams of Undecanoic Amide Multilayers

(0.1M KCl + 1 mM Ru(NH$_3$)$_6$Cl$_3$)

Figure 59. Cyclic Voltamagrams of Cystamine Disulfide Multilayer Series

(0.1M KCl + 1 mM K$_3$Fe(CN)$_6$)
Figure 60. Cyclic Voltamgrams of Cystamine Thiol Multilayer Series
A: 0.1M KCL + 1 mM K$_3$Fe(CN)$_6$; B: 0.1M KCl + 1 mM Ru(NH$_3$)$_6$Cl$_3$
Figure 61. Cyclic Voltamograms of C11-Cystamine Thiol Multilayer Series
A: 0.1M KCl + 1 mM K$_3$Fe(CN)$_6$  B: 0.1M KCl + 1 mM Ru(NH$_3$)$_6$Cl$_3$
Again, using coupling scheme #3, undecanoic acid was attached to each of the considered amine terminated monolayers and characterized by the same techniques.

**Table 14.** Three Amide-Undecanoic Multilayers Experimental Results Summary

<table>
<thead>
<tr>
<th></th>
<th>Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI H2O (pH 6)</td>
</tr>
<tr>
<td>(SC2NHOC10)2</td>
<td>71.0 ± 7.5</td>
</tr>
<tr>
<td>HSC2NHOC10</td>
<td>79.0 ± 3.6</td>
</tr>
<tr>
<td>HSC11NHOC10</td>
<td>78.3 ± 2.1</td>
</tr>
</tbody>
</table>

**Table 15.** Undecanoic Amide Multilayer IR Absorption and Assignment Summary

<table>
<thead>
<tr>
<th></th>
<th>Assignment:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>υasCH2</td>
</tr>
<tr>
<td>(SC2NH3)2</td>
<td>2928</td>
</tr>
<tr>
<td>HSC2NH3</td>
<td>2928</td>
</tr>
<tr>
<td>HSC11NH3</td>
<td>2927</td>
</tr>
</tbody>
</table>

Again, based only on contact angle measurements (Table 14) and it would appear that the 2 and 11 carbon amine-thiol monolayers provide better templates for a second layer deposition than the 2-carbon disulfide given greater hydrophobicity is expected for a better packed alkyl chain SAM. Hydrocarbon stretching frequencies (Table 15) agree with this as the order of multilayer packing indicated by them, form best to worst is: HSC11NH3 > HSC2NH3 > (SC2NH3)2. Interestingly enough, the average packing of each individual SAM seems to have improved following deposition of the second layer, in all cases (which was not the case with the undecanodioic acid). Now, while this might be expected given the much longer 2nd layer alkyl chains which would presumably have significantly greater intra-chain Van der Waals interactions the fact remains the first layer sets up limits on
the number of second layer chains which can attach and how exactly they can space themselves. One possibly explanation lies in the fact that formation of the amide bond may force a trans organization of the first layer as the nitrogen would need to be available to attack the sterically hindered DCC-activated acid or the interactions of the alkyl chains following the reaction could twist the cystamine layer into a new orientation more favorable to intra-chain interaction. Also there could exist additional acid chains in the layer stabilized by non-specific Van der Waals and/or hydrogen bonding interactions which would additionally explain the presence of two new bands observed in these second layer spectra as free (~1735) and hydrogen bound (~1710) acid-carbonyl absorbencies. The fact that the amide I peaks (1627) are shifted to slightly lower frequencies in than those observed for the undecandioic systems (1630) might be explicable through hydrogen bonding, in the layer, between amide groups and possibly involving carboxylic acids as well.

<table>
<thead>
<tr>
<th></th>
<th>Amine (SC&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Anthryl-Crown</th>
<th>Undecandioic</th>
<th>Undecanoic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SC&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>12.9</td>
<td>6.5</td>
<td>9.8</td>
<td>3.5</td>
</tr>
<tr>
<td>HSC&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>19.8</td>
<td>3.3</td>
<td>1.4</td>
<td>0.4</td>
</tr>
<tr>
<td>HSC&lt;sub&gt;11&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6.9</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Amine (SC&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Anthryl-Crown</th>
<th>Undecadionioic</th>
<th>Undecanoic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SC&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>9.1</td>
<td>17.5</td>
<td>14.2</td>
<td>26.8</td>
</tr>
<tr>
<td>HSC&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>8.3</td>
<td>15.7</td>
<td>22.7</td>
<td>198</td>
</tr>
<tr>
<td>HSC&lt;sub&gt;11&lt;/sub&gt;NH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>7.6</td>
<td>14.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Simple Azacrown Ether Monolayers

A series of much simpler crown ethyl terminated thiol’s of various chain lengths were next synthesized in order to, initially, evaluate the effects of linking chain length on ionophore terminated SAM sensing behavior. These compounds were used for significantly more, later on, as they proved valuable as simplified models of our multilayer electrochemical sensors. What’s
more, as these were monolayers, it was expected that better packed SAM’s would result which should lend to more reproducible charge-transfer behavior and thus more reproducible sensor response. As such they could provide a point of reference with which to evaluate the feasibility of multilayer sensing.

1. Synthesis

\[ \text{16-mercapto-N,N-(1, 4, 7, 10, 13-Pentaoxa-16-azacyclooctadecyl)-hexadecanamide (ArwC11).} \]

16-mercaptohexadecanoic acid (0.50 g, 1.73 mmol), 1-aza-18-crown-6 (0.46 g, 1.73 mmol) and DMAP (0.02 g, 0.17 mmol) were combined in anhydrous DCM (10 ml) and stirred together until completely dissolved. DCC (0.36 g, 1.73 mmol) in DCM (3 ml) was added and the reaction flask capped with a calcium chloride drying tube and allowed to stir overnight at room temperature. The precipitated urea (a white solid) was removed by micro-suction filtration and the solvent removed in \textit{vacuo} yielding 1.12 g of a thick, powdery oil. This pale yellow crude was purified by silica gel column chromatography (50:1 DCM:MeOH) and 0.53 g of the product
was obtained as circular white crystals in 53.7% of theoretical yield. $^1$H-NMR (CDCl$_3$) 3.63-3.60 (m, 26H), 2.50 (q, $J = 7.3$ Hz, 2H), 2.34 (t, $J = 7.6$ Hz, 2H), ~1.9 (m, 1H), 1.28-1.25 (m, 29H) MS $m/z$ (fragment): 534.6 (M+1), 556.6 (M+Na), 572.6 (M+K), 1088.1 (2M+Na)

16-mercapto N,N-1-aza-18-crown-6 hexadecanamide monolayer on Gold. 1×2.5cm gold plates were cut, and immersed in Piranha solution for 5-10 min. The slides were then washed with DI water and EtOH and dried with nitrogen. They were then placed in a 1 mM solution of 16-mercapto N,N-1-aza-18-crown-6 hexadecanamide in EtOH overnight after which they were washed with EtOH and dried with nitrogen. DI H$_2$O contact angle: 53.8 (±3.6); Ellipsometry: 1.40 ± 0.79 nm

11-mercapto-N,N-(1, 4, 7, 10, 13-Pentaoxa-16-azacyclooctadecyl)-undecanamide (ArwC16). 11-mercaptoundecanoic acid (1.66 g, 7.60 mmol), monoaza-16-crown-6 (2.00 g, 7.60 mmol) and DMAP (0.09 g, 0.76 mmol) were combined in anhydrous DCM (25 ml) and stirred together until completely dissolved. DCC (1.53 g, 7.60 mmol) in DCM (15 ml) was added and the reaction flask capped with a calcium chloride drying tube and allowed to stir overnight at room
temperature. The precipitated urea (a white solid) was removed by micro-suction filtration and
the solvent removed in vacuo yielding thick oil. This pale yellow crude was purified by silica
gel column chromatography (50:1 DCM:MeOH) and 1.43 g of the product was obtained as
circular white crystals in 41.9% of theoretical yield. \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) 3.65 (m, 24H,
Crown), 2.51 (m, 2H, CH\(_2\)S), 2.34 (t, 2H, COCH\(_2\)) 1.62 (m, 4H, CH\(_2\)), 1.33-1.27 (m, 12H, CH\(_2\))
; \(^{13}\)C-NMR (CDCl): \(\delta\) 25.1 (CH\(_2\)S), 25.7, 28.8, 29.5, 29.8, 33.5 (CH\(_2\)), 34.5 (COCH\(_2\)), 47.2,
49.3 (CNCH\(_2\)), 69.9-71.2 (CH\(_2\)Crown), 173.9 (CO); MS \(m/z\) (fragment): 464.5 (M+1), 486.5
(M+Na), 502.5 (M+K), 947.9 (2M+Na)

11- mercapto N,N-1-aza-18-crown-6 hexadecanaminde monolayer on Gold. 1\(\times\)2.5cm gold
plates were cut, and immersed in Piranha solution for 5-10 min. The slides were then washed
with DI water and EtOH and dried with nitrogen. They were then placed in a 1 mM solution of
11- mercapto N,N-1-aza-18-crown-6 undecanaminde in EtOH overnight after which they were
washed with EtOH and dried with nitrogen. DI H\(_2\)O contact angle: 51.9 (\(\pm1.9\)); Ellipsometry:
1.36 \(\pm\) 0.68 nm

2. Projected Electrochemical Response

The change in capacitance which results from binding of analyte to sensing self assembled
monolayers can be treated according to the below form of equation
49 and has been assumed to result from a change in dielectric
constant and/or thickness of the material.\(^3\)

\[
C_{\text{SAM}} = \frac{\varepsilon_0 \varepsilon_r}{d} \quad \Delta C_{\text{SAM}} = \frac{\varepsilon_0 \times \Delta \varepsilon_r}{d}
\]

Figure 63. SAM used to estimate 18-crown-6 Capacitance
With crown ether terminated SAM’s used for detection of metal ions, the thickness is a constant and thus the sensor response should be dictated solely be the change in the dielectric constant/relative permittivity of the film. As the binding occurs only within the crown ether, the origin of this response can be traced specifically to a change in dielectric constant of the crown ether itself and thus when quantitatively treating the magnitude of capacitance response of such SAM’s the capacitance is divided between the variable crown capacitance \( C_{\text{crown}} \) and the surface-linking, invariable, chain capacitance \( C_{\text{chain}} \) the capacitance of the monolayer then being the sum of these capacitances in series:\(^7\)

\[
\frac{1}{C_{\text{SAM}}} = \frac{1}{C_{\text{crown}}} + \frac{1}{C_{\text{chain}}} \tag{60}
\]

Now, using literature\(^7\) impedance spectroscopic results with the short chain 18-crown-6 SAM, to the left, to approximate the \( C_{\text{crown}} \) for 18-crown-6 SAM’s, in general (given the minimal invariable chain contribution), \( C_{\text{chain}} \) and the dielectric constants for the three sensing SAM’s pictured below, were estimated from their measured capacitances:

![Figure 64. Summary of Sensors Considered in this](image)
The invariable chain capacitance associated with each SAM, was determined by rearranging equation 60 to 61 and using 6.8 μF/cm² for $C_{crown}$ and experimentally determined capacitances for the overall SAM’s (see Table 18). For example, for ArwC16:

$$C_{ARWC16} = \frac{C_{crown} \times C_{chain}}{C_{crown} + C_{chain}} = 2 \times \frac{6.8 \times C_{chain}}{6.8 + C_{chain}} \quad (61)$$

$$C_{chain} = 2.833 \mu F/cm^2$$

Then, using approximate lengths of the crown and the chains and assuming a 30° tilt angle of the SAM the dielectric constants of each chain was estimated:

$$C_{chain \_ARWC16} = \frac{\varepsilon_0 \varepsilon_r}{d} = \frac{2.83 \times 8.85 \times 10^{-8} \times \varepsilon_r}{17.6 \times 10^{-8} \times \sin(60)}$$

$$\varepsilon_r = 4.87$$

**Table 18** Estimated Dielectric Constants of the Invariable Chain Capacitance

<table>
<thead>
<tr>
<th>SAM</th>
<th>$C_{SAM}$ (μF/cm²)</th>
<th>$C_{crown}$ (μF/cm²)</th>
<th>$C_{chain}$ (μF/cm²)</th>
<th>d (Å)</th>
<th>$\varepsilon_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArwC3</td>
<td>6.5</td>
<td>6.8</td>
<td>147.33</td>
<td>3.3</td>
<td>47.58</td>
</tr>
<tr>
<td>ArwC11</td>
<td>3.0</td>
<td>6.8</td>
<td>5.37</td>
<td>12.6</td>
<td>6.62</td>
</tr>
<tr>
<td>ArwC16</td>
<td>2.0</td>
<td>6.8</td>
<td>2.83</td>
<td>17.6</td>
<td>4.87</td>
</tr>
</tbody>
</table>

The dielectric constant of the potassium bound and unbound crowns was calculated, again using literature estimates of 6.8 μF/cm² for unbound $C_{crown}$ and 8.6 μF/cm² for bound $C_{crown}$:

$$C_{crown} = \frac{\varepsilon_0 \varepsilon_r}{d} = 6.8 = \frac{8.85 \times 10^{-8} \times \varepsilon_r}{7.83 \times 10^{-8} \times \sin(60)}$$

$$\varepsilon_r = 5.21$$

$$C_{K-crown} = \frac{\varepsilon_0 \varepsilon_r}{d} = 8.6 = \frac{8.85 \times 10^{-8} \times \varepsilon_r}{7.83 \times 10^{-8} \times \sin(60)}$$

$$\varepsilon_r = 6.59$$

Using the above chain and potassium-bound and unbound crown capacitance values the maximum capacitance response to potassium was approximated, using the equation 64:
Table 19. Estimated Potassium Binding Response of the ArwC Sensors

<table>
<thead>
<tr>
<th></th>
<th>$C_{SAM}$ (μF/cm²)</th>
<th>$C_{SAM+K}$ (μF/cm²)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArwC3</td>
<td>6.5</td>
<td>8.13</td>
<td>20%</td>
</tr>
<tr>
<td>ArwC11</td>
<td>3.0</td>
<td>3.31</td>
<td>10%</td>
</tr>
<tr>
<td>ArwC16</td>
<td>2.0</td>
<td>2.13</td>
<td>7%</td>
</tr>
</tbody>
</table>

Now these projected responses are almost certainly underestimations given the fact the SAM considered to be the solely the “crown” in fact did involve an invariable chain linker which would at least partially attenuate the magnitude of the actual crown-capacitive response. What’s more, poor packing, of the bulky crown could also be anticipated to reduce the magnitude of the capacitance change given by magnitude of fractional coverage (given the parallel capacitance of gold)⁶¹:

$$C_{SURFACE} = \theta C_{SAM} + (1 - \theta)C_{AU} \quad (62)$$

This indeed is born out through experimental potassium binding results which have, in general, demonstrated a capacitive response on the order of 0.2-0.4 μF/cm². Unfortunately, attempts at obtaining titration curves have been frustrated by a relatively high degree baseline variation. Now this most recent sensor work has been conducted on 0.002 cm² analytical electrodes and repeated impedance measurements conducted immediately preceding those titrations have yielded a measure of that noise with the standard deviation of that baseline ranging from $\pm 2 \times 10^{-5} \mu F$ to $\pm 9 \times 10^{-4} \mu F$ which converts to $\pm 0.01 \mu F/cm²$ to $\pm 0.05 \mu F/cm²$. Thus, the maximum signal to noise ratio appears to be, using this set up, approximately one order of magnitude.

3. Projected SPR Response

The same binding event/coating dielectric change can also be detected optically through the use of surface plasmon resonance (SPR). The sensing portion of a SPR device consists of an optically transparent substrate coated with a thin metal film. When light, originating from the
substrate side, is reflected off the substrate-metal interface particular wavelengths of that light couple with an evanescent surface plasmon wave which results in a sharp reduction in intensity of that light. Now, the wavelength at which this minimum occurs is very sensitive to the refractive index of the medium immediately above the gold and changes in that medium, as a result, define SPR’s response, through a shift in that SPR wavelength\textsuperscript{66, 67}.

In the case of detecting adsorption of self-assembled monolayers to the metal surface, the SPR response would be determined by the final effective refractive index ($\eta_{\text{eff}}$) of the adlayer-deposition solution system which would be defined by an appropriately, weighted average of the bulk refractive indices of the adlayer material ($\eta_a$) and the deposition solution ($\eta_{\text{soln}}$). Now as light is the probe of refractive index it is natural to assume that the correct weighting factor at each point above the metal surface would be proportional to the intensity of that light. Given the electromagnetic field decays exponentially into the adlayer-solvent system with a characteristic decay length, $I_d$, and the intensity of light is the field strength squared it would appear that the refractive index at height $z$ from the metal surface should be weighted by the square of the field intensity at that height. The average refractive index of the SAM-solvent system would then be determined by the below integral\textsuperscript{66}:

$$\eta_{\text{eff}} = (2 / I_d) \int_0^\infty (\eta(z) \exp(-2z / I_d)) \exp(-z / I_d) dz$$

Assuming that the SAM has a constant height ($d$) and refractive index ($\eta_a$) then this equation reduces to:

$$\eta_{\text{eff}} = \eta_{\text{soln}} + (\eta_a - \eta_{\text{soln}})(1 - \exp(-2d / I_d))$$

and the effective SPR response $R$, following adsorption becomes (m being a sensor calibration factor):

$$R = m(\eta_{\text{eff}} - \eta_{\text{soln}}) = m(\eta_a - \eta_{\text{soln}})(1 - \exp(-2d / I_d))$$
Rearranging this equation one obtains an expression for average thickness of the adlayer from the SPR response\(^{66}\):

\[
d = \left(\frac{I_d}{2}\right) \ln(1 - \frac{R}{R_{\text{max}}}) \tag{66}
\]

\[
R_{\text{max}} = m(\eta_1 - \eta_{\text{soln}}) \tag{67}
\]

And this is immediately convertible to coverage \((\theta, \text{molecules/cm}^2)\) from SPR response by multiplying by the bulk density of the adsorbate \((N, \text{molecules/cm}^3)\)

\[
\theta = \left(\frac{I_d}{2}\right) N \ln(1 - \frac{R}{R_{\text{max}}}) \tag{68}
\]

When the thickness of the film is small compared to \(I_d\) (~300 nm) this equation can be reduced further to\(^{66}\):

\[
\theta = \left(\frac{I_d}{2}\right) N \left(\frac{R}{R_{\text{max}}}\right) \tag{69}
\]

and this type of behavior (when the coverage of the film is directly proportional to the SPR response) is what is known as the “linear response regime”\(^{66}\).

**Figure 65.** SPR/Capacitive model

Now in the specific case of crown-ether terminated SAM’s, the seeming best way to treat these would be as a multilayer system, as in the capacitive treatment above, where the crown represents a portion of the layer which is variable, in terms of permittivity, in series with the surface bound chain representing an invariable portion of the layer. In this case if \(d_b\) is taken as the height of the chain; \(d_a\), the height of the
crown and a constant refractive index assumed through out the crown ($\eta_{crown}$) and chain ($\eta_{chain}$) then the above depth integral for the effective refractive index would reduce to:

$$\eta_{eff} = \eta_{chain} (1 - \exp(-\frac{2d_a}{I_d})) + \eta_{crown} (\exp(-\frac{2d_a}{I_d}) - \exp(-\frac{2d_b}{I_d})) + \eta_{so_{in}} \exp(-\frac{2(d_a + d_b)}{I_d})$$

Substituting this equation into the equation for SPR response and simplifying it to give the portion of the SPR response due only to the crown one obtains the following expression:

$$R = m(\eta_{crown} - \eta_{so_{in}}) (1 - \exp(-\frac{2d_a}{I_d})) \exp(-\frac{2d_b}{I_d})$$

(70)

which is identical to the single layer equation with an additional scaling factor $\exp(-2d_b/I_d)$. Additionally when $d_b$ is small compared to $I_d$ that scaling factor approaches 1 and the equation can be well approximated by:

$$R = m(\eta_{crown} - \eta_{so_{in}}) (1 - \exp(-\frac{2d_a}{I_d}))$$

(71)

Thus, the difference in response, between the second layer and the first being nearly indistinguishable on the thickness scale of these SAM’s (~2 nm) it is certainly possible to treat the SPR adsorption response of the entire molecule by using a volume weighted average of its different components. Using then the experimental density and molecular weights of the corresponding alkane thiol and 18-crown-6 approximate bulk densities (N) for each of the ArwC sensor SAMs’ components should be determinable (Table 20).

<table>
<thead>
<tr>
<th>Mol. Wt. (g/mol)</th>
<th>density (g/cm³)</th>
<th>Bulk density ($\times 10^{21}$ molecules/cm³)</th>
<th>Vol. Fraction (%) in Comparable SAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-crown-6</td>
<td>264.32</td>
<td>1.237</td>
<td>2.818</td>
</tr>
<tr>
<td>propane-thiol</td>
<td>76.16</td>
<td>0.844</td>
<td>1.978</td>
</tr>
<tr>
<td>undecane thiol</td>
<td>188.37</td>
<td>0.8432</td>
<td>1.378</td>
</tr>
<tr>
<td>hexadecane thiol</td>
<td>258.51</td>
<td>0.8556</td>
<td>1.167</td>
</tr>
</tbody>
</table>

Table 20. Bulk Densities and Volume Fractions of Sensor Constituents
Now, the refractive index of a material is determined by the relative permeability ($\mu_r$) and relative permittivity/dielectric constant ($\varepsilon_r$) of that material according to the following equation:

$$\eta = \sqrt{\mu_r\varepsilon_r} \quad (72)$$

For non-magnetic materials where $\mu_r$ is very close to 1 the refractive index can be approximated by:

$$\eta = \sqrt{\varepsilon_r} \quad (73)$$

Given then, the dielectric constants estimated from impedance spectroscopy measurements of the sensor SAM’s in the previous section (Table 18), refractive index of the constituent portions of each SAM should be determinable along with an expected adlayer sensor response (given the volume fractions just determined):

<table>
<thead>
<tr>
<th>Table 21. Estimated Bulk Refractive Indices of Sensor Constituents and Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoaza-18-Crown-6</strong></td>
</tr>
<tr>
<td>ArwC3 chain</td>
</tr>
<tr>
<td>ArwC11 chain</td>
</tr>
<tr>
<td>ArwC16 chain</td>
</tr>
</tbody>
</table>

Using the equation 65 then, and assuming an $I_d$ of 300 nm and the modeling thicknesses used in the previous section, estimates of the SPR response are obtainable:

<table>
<thead>
<tr>
<th>Table 22. Estimated SPR response to Adsorption of Sensors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ArwC3 chain</strong></td>
</tr>
<tr>
<td>ArwC11 chain</td>
</tr>
<tr>
<td>ArwC16 chain</td>
</tr>
</tbody>
</table>

Unfortunately these estimates are almost certainly inflated given the fact the dielectric constants of the SAM’s were determined in an aqueous electrochemical format and thus, doubtless, had water within the layer which artificially increase the determined dielectric constant ($\varepsilon_r =$
80\textsuperscript{71}) and most certainly doesn’t fit with the $\eta = \sqrt{\varepsilon}$ estimation above (water, $\eta = 1.33\textsuperscript{71}$). Therefore a more accurate estimate of the above SAMs’ adsorption SPR response could be obtained using literature refractive indices of the crown’s and chains (all of which are about 1.46 and thus the averages for the SAM’s are $\sim 1.46\textsuperscript{72-74}$):

**Table 23. Revised Estimated SPR responses to Adsorption of Sensors**

<table>
<thead>
<tr>
<th>SAM</th>
<th>Estimated $\eta_{SAM}$</th>
<th>Estimated $d$ (nm)</th>
<th>$\eta_{EtOH}$</th>
<th>Estimated $\Delta\eta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArwC3 chain</td>
<td>1.46</td>
<td>0.964</td>
<td>1.36</td>
<td>0.0006</td>
</tr>
<tr>
<td>ArwC11 chain</td>
<td>1.46</td>
<td>1.769</td>
<td>1.36</td>
<td>0.0012</td>
</tr>
<tr>
<td>ArwC16 chain</td>
<td>1.46</td>
<td>2.202</td>
<td>1.36</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

Now given the detection limit of our machine is approximately $3 \times 10^{-4}$ refractive index units\textsuperscript{67} (RIU) if we plug this to the equation 69:

$$\theta_{\text{min}} = \left(\frac{I_d N_{SAM}}{2}\right)\left(\frac{R_{\text{min}}}{R_{\text{max}}}\right) = \left(\frac{I_d N_{SAM}}{2}\right)\left(\frac{m\Delta\eta_{min}}{m(\eta_{SAM} - \eta_{EtOH})}\right)$$

$$\theta_{\text{min}} = \left(\frac{I_d N_{SAM}}{2}\right)\left(\frac{\Delta\eta_{min}}{\Delta\eta_{max}}\right) \quad (74)$$

we can obtain the following estimates of detection limits for adsorption of each SAM, using the bulk densities ($N$) of the constituent portions (Table 20) of the SAM to estimate the bulk density of the entire SAM ($N_{SAM}$):

**Table 24. Estimated Layer Coverage Detection Limits**

<table>
<thead>
<tr>
<th>$N_{SAM}$ ($\times 10^{31}$ molecules/cm$^3$)</th>
<th>$I_d$ ($\times 10^{-7}$ cm)</th>
<th>$\Delta\eta_{min}$</th>
<th>$\eta_{SAM}$</th>
<th>$\eta_{EtOH}$</th>
<th>$\theta_{min}$ ($\times 10^{11}$ molecules/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArwC3 chain</td>
<td>1.162</td>
<td>300</td>
<td>$3 \times 10^{-6}$</td>
<td>0.1</td>
<td>5.23</td>
</tr>
<tr>
<td>ArwC11 chain</td>
<td>0.925</td>
<td>300</td>
<td>$3 \times 10^{-6}$</td>
<td>0.1</td>
<td>4.16</td>
</tr>
<tr>
<td>ArwC16 chain</td>
<td>0.825</td>
<td>300</td>
<td>$3 \times 10^{-6}$</td>
<td>0.1</td>
<td>3.71</td>
</tr>
</tbody>
</table>

Now, assuming the contribution of water to the dielectric constant of the SAM does not change upon potassium binding of the crown ethers and therefore that the estimated change in
dielectric constant is only a characteristic of the SAM and also that the \( \eta = \sqrt{\varepsilon} \) estimate holds for crown ethers then the change in crown refractive index and projected SPR response and detection limits for potassium binding of the above SAM’s can be determined.

<table>
<thead>
<tr>
<th>Table 25. Estimated Bulk-Crown Refractive Index Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>18-crown-6</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>18-crown-6</td>
</tr>
<tr>
<td>18-crown-6-K+ complex</td>
</tr>
</tbody>
</table>

Taking the difference between bound and unbound equivalents of equation 70 the SPR refractive index response for a change in refractive index of the crown layer should be:

\[
\Delta \eta = (\eta_{\text{crown} + K^+} - \eta_{\text{crown}})(1 - \exp(-\frac{2d_b}{l_d})) \exp(-\frac{2d_b}{l_d}) \tag{75}
\]

\[
\Delta \eta_{\text{ARWC16}} = (0.285)(1 - \exp(-\frac{2 \times 7.83 \times \sin(60)}{300})) \exp(-\frac{2 \times 17.6 \times \sin(60)}{300}) = 0.0114
\]

Rearranging this to get it in the same form as equation 65 we can again obtain an expression for the instrumental detection limits and estimate a per molecule response (this time for the crown-binding):

\[
\exp\left(\frac{2d_b}{l_d}\right) \times \Delta \eta_{\min} = (\eta_{\text{crown} + K^+} - \eta_{\text{crown}})(1 - \exp(-\frac{2d_b}{l_d})) \tag{76}
\]

\[
\theta_{\min} = \left(\frac{I_d N_{\text{crown}}}{2}\right) \frac{\Delta \eta_{\min} \times \exp(2d_b / l_d)}{\eta_{\text{max}}} \tag{77}
\]

\[
\theta_{\min} = \left(\frac{300 \times 10^{-7} \times 2.818 \times 10^{21}}{2}\right) \left(\frac{3 \times 10^{-8} \times \exp(2 \times 17.6 \times \sin(60) / 300)}{0.285}\right) = 4.93 \times 10^{11}
\]

<table>
<thead>
<tr>
<th>Table 26. Estimated Number of Binding Sites Detection Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N_{\text{CROWN}}</strong> (( \times 10^{21} ) molecules/cm(^3))</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>ArwC3 chain</td>
</tr>
<tr>
<td>ArwC11 chain</td>
</tr>
<tr>
<td>ArwC16 chain</td>
</tr>
</tbody>
</table>
In summary, for each of the above sensor compounds, the projected maximum response, detection limits and ratio of response over noise follow:

<table>
<thead>
<tr>
<th>Table 27. Summary of Projections for SPR Response to Sensor Adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>adsorption response</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>ArwC3 chain</td>
</tr>
<tr>
<td>ArwC11 chain</td>
</tr>
<tr>
<td>ArwC16 chain</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 28. Summary of Projections for SPR Response to Potassium Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>binding response</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>ArwC3 chain</td>
</tr>
<tr>
<td>ArwC11 chain</td>
</tr>
<tr>
<td>ArwC16 chain</td>
</tr>
</tbody>
</table>

The binding response is almost certainly inflated due to the overly large change in bulk refractive index of unbound to potassium bound crown (0.283). I suspect that the $\eta = \sqrt{\varepsilon_r}$ approximation is not valid for the crown and that knowledge of the crown’s relative permeability ($\mu_r$) is needed and would result in a substantial reduction of the magnitude of the projected response (by the factor $\sqrt{\mu_r}$) perhaps by about an order of magnitude (as is the case with water: $\eta = 1.33$, $\varepsilon_r = 80^{71}$).

The validity of the projected SAM adsorptive response, on the other hand, finds support in the fact the projected maximum coverage (can be found by multiplying the maximum signal to noise ratio by the coverage detection limit) for all the SAM’s considered ranges from 1-1.7 $\times$ 10\text{14} molecules/cm\text{2} which is inline with coverage estimates of well packed SAM’s obtained via other methods in the literature.\textsuperscript{17}
4. Qualitative Electrochemistry: Plate work

Before the construction of analytical electrodes all electrochemical experiments were conducted using a SAM-coated, 1 × 2.5 cm, gold slide with approximately 1 cm² immersed in the electrolyte of an unsealed beaker, three-electrode cell. While this set up was useful for getting an idea for layer capacitances, resistances and ideality, it could not be used for quantitative measurements given the fact that the electrode area was not controlled (cf. equation 49). That said, some preliminary impedance characterization data was obtained and is presented below and in previously presented results.

Table 29. Preliminary Plate-Electrode Characterization Data

<table>
<thead>
<tr>
<th></th>
<th>C16SH</th>
<th>ArwC16</th>
<th>ArwC11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitance:</td>
<td>1.07 ± 0.17</td>
<td>2.00 ± 0.48</td>
<td>3.02 ± 0.43</td>
</tr>
<tr>
<td>CPE alpha:</td>
<td>0.97 ± 0.02</td>
<td>0.91 ± 0.02</td>
<td>0.92 ± 0.02</td>
</tr>
</tbody>
</table>

That the two crown ether molecules manifest similar coating heterogeneity/non-ideality (seen in the alpha factor deviation from unity) is not surprising as they are very similar molecules. Using a hexadecane thiol SAM, of similar thickness, as a reference – which was deposited using the exact same procedure and the same substrate batch – it seems clear that this capacitive dispersion is directly related to the presence of a bulky, terminating crown ether. The capacitive values obtained are inline with expectations given poorer packing associated with bulky head groups and the different sensor length lengths which translate to different layer thicknesses (cf. equation 49).

Some qualitative binding experiments were also conducted using three ArwC16 plates where the supporting electrolyte was changed from 0.1 M Et₄NCl to 0.1 M KCl.
Table 30. Three ArwC16 Plate Titration Experiments

<table>
<thead>
<tr>
<th></th>
<th>Plate #1</th>
<th>Plate #2</th>
<th>Plate #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 M KCl</td>
<td>2.43 ± 0.21</td>
<td>1.55 ± 0.01</td>
<td>1.52 ± 0.03</td>
</tr>
<tr>
<td>0.1 M KCl</td>
<td>2.53 ± 0.02</td>
<td>1.65 ± 0.01</td>
<td>1.91 ± 0.05</td>
</tr>
</tbody>
</table>

Another mode of detection which has been used with SAM’s of this type takes advantage of the fact that when cations are bound to sensor molecules they effectively charge the surface coating causing it to electrostatically repel other positively charged species. When a positively charged redox species is present within the electrolyte then, the amount of current observed/resistance of the layer can be effectively modulated by the amount of analyte in solution, this is known as the ion gating effect. Cyclic voltammetry can be used to monitor ion-gating changes in layer resistance by measuring the reduction in redox current following addition of analyte ions. Impedance spectroscopy can be used to monitor the same by taking repeated measurements at the formal potential of the redox species. At this point in time, due to previously described instrumental difficulties, impedance spectroscopy was not possible with these plate electrodes and only cyclic voltammetric experiments were conducted. These only showed a small change this change was consistent and reproducible.
5. Quantitative Electrochemistry: Analytical Electrode work

In order to be able to quantitate electrochemical data it is necessary to know the exact area of the working electrode immersed in the electrolyte as characteristics such as electrode/SAM capacitance and resistance are heavily dependant on that value (cf. equations 48 and 49). As such, a series of 0.031 cm$^2$ and 0.002 cm$^2$ electrodes were constructed, cleaned and functionalized with the ArwC16 and ArwC11 monolayers. The 0.031 cm$^2$ electrodes were labeled 1-6 and the 0.002 cm$^2$ electrodes were labeled A-G. Initially, the full pretreatment procedure, used preceding SAM deposition, consisted only of mechanical polishing followed by sonication in isopropanol and acetone. This, however, led to significant variability in both the characteristics of the bare electrode and the coating SAM’s and for these reasons this section is mainly concerned with determining the pretreatment procedures necessary to obtain reproducible, working electrode behavior.

When the electrodes were first constructed and cleaned they where characterized using impedance spectroscopy and the results are summarized in Table 31. From these it is clear that there existed significant contamination of the electrode surfaces given the fact the capacitance values were significantly lower than expected for an electrode double layer. What’s more, the alpha factors’ deviation from unity also lent support to this though, at this point, they were only believed to reflect the roughness of the metallic electrode.$^{21,47}$

<table>
<thead>
<tr>
<th>Electrode 0.002cm$^2$:</th>
<th>CPE alpha</th>
<th>Capacitance (μF/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.90</td>
<td>6.36</td>
</tr>
<tr>
<td>B</td>
<td>0.87</td>
<td>4.60</td>
</tr>
<tr>
<td>C</td>
<td>0.87</td>
<td>4.01</td>
</tr>
<tr>
<td>D</td>
<td>0.88</td>
<td>7.35</td>
</tr>
<tr>
<td>E</td>
<td>0.89</td>
<td>5.79</td>
</tr>
<tr>
<td>F</td>
<td>0.89</td>
<td>6.95</td>
</tr>
<tr>
<td>G</td>
<td>0.89</td>
<td>5.93</td>
</tr>
</tbody>
</table>
Believing that any contamination at the electrode surface would be taken care of by exchange\(^6\) with more energetically favorable gold sulfur bonds these electrodes were immersed in an ArwC16 solution for three days. The resulting SAM’s were characterized by impedance spectroscopy at an applied potential of -0.156 V\(_{SCE}\) (a literature value for the formal potential the ruthenium hexamine redox couple\(^3\)) in a 0.1 M Et\(_4\)NCl supporting electrolyte using a ruthenium hexamine redox probe (1 mM) in an attempt to be able to resolve charge transfer resistance data.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.031cm(^2):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.67</td>
<td>1.26</td>
</tr>
<tr>
<td>2</td>
<td>0.84</td>
<td>7.26</td>
</tr>
<tr>
<td>3</td>
<td>0.84</td>
<td>3.60</td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>7.71</td>
</tr>
<tr>
<td>5</td>
<td>0.84</td>
<td>2.69</td>
</tr>
<tr>
<td>6</td>
<td>0.90</td>
<td>6.87</td>
</tr>
</tbody>
</table>

Table 32. Initial Analytical Electrode ArwC16 SAM Characterization Data

<table>
<thead>
<tr>
<th>Electrode</th>
<th>CPE alpha</th>
<th>Capacitance ((\mu)F/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.89</td>
<td>4.28</td>
</tr>
<tr>
<td>E</td>
<td>0.90</td>
<td>2.71</td>
</tr>
<tr>
<td>F</td>
<td>0.87</td>
<td>2.76</td>
</tr>
<tr>
<td>G</td>
<td>0.90</td>
<td>1.87</td>
</tr>
</tbody>
</table>

ArwC16 SAM’s were deposited on the other 0.002 cm\(^2\) electrodes but their impedance spectra showed an Warburg behavior at low frequencies and had to be fit using a Randle-CPE-Warburg circuit. Unfortunately no method is now known to extract comparable average capacitance values from the CPE values of such a circuit as with the simple Randles and Helmholtz circuits.

Titrations were attempted on all of these electrodes using potassium, sodium and cesium. Unfortunately significant charge-transfer resistance and capacitance results were only obtained from electrode B. The charge transfer response was initially linear and then plateaued as is inline with the literature\(^3\) and the capacitive response followed 1:1 langmuir binding behavior
and as such maximum capacitance change and association constant values were extractable from it.

**Figure 67. ArwC16 Rct Analytical Titration**

(0.1M Et4NCl + 1mM Ru(NH3)6Cl3, Vapp = -0.156 Vsce)

**Figure 68. ArwC16 Capacitance Analytical Titration**

(0.1M Et4NCl + 1mM Ru(NH3)6Cl3, Vapp = -0.156 Vsce)

\[ y = \frac{m2 \cdot m0 \cdot m1}{1 + m2 \cdot m0} \]

<table>
<thead>
<tr>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>0.17669</td>
</tr>
<tr>
<td>m2</td>
<td>211.86</td>
</tr>
<tr>
<td>Chisq</td>
<td>0.0022283</td>
</tr>
<tr>
<td>R</td>
<td>0.95929</td>
</tr>
</tbody>
</table>
All other titrations resulted in little to no capacitive sensor response at all.

6. Sources of Irreproducibility in Thin-Film Electrochemical Behavior

Given the above significant variability of both initial layer characterization data and sensor response the focus of this research shifted towards achieving reproducibility in the former (the idea being that a consistent/reproducible base line would be a prerequisite to reproducible sensor functioning). Now, the most likely explanation for this irreproducibility between samples would be the presence of defects within the pseudo-crystalline structure of the SAM’s. These could result in a distribution of capacitance and resistance values over the surface of the electrode significantly altering the charge-transfer/electrochemical characteristics of the bulk layer. This idea finds support in the fact that, in nearly all impedance spectra fitting used in this work a CPE element was used instead of an idea capacitor and, as previously described, the deviation from capacitive ideality associated with this circuit element is also directly related to surface heterogeneities, of some sort, at the electrode surface. Indeed some groups have specifically used the CPE’s alpha factor deviation from unity as a direct indicator of the type and frequency of defects present within self assembled monolayers. As can be seen below, a wide variety of defects have been detected in SAM’s which can be linked to characteristics of both the

Figure 69. A Summary of the Different types of Defects Observed in SAM’s
substrate (e.g. roughness, grain boundaries) and the adsorbate (e.g. purity, head group size).

7. Electrochemical Cleaning and Polishing

Knowing that initial electrode cleanliness was an issue and assuming substrate roughness to be the major source of capacitive dispersion/CPE-behavior/electrochemical irreproducibility\cite{17, 41, 42, 75}, electrochemical pretreatment (described in the Materials and Methods) of the electrodes was attempted and the results characterized by impedance spectroscopy. The electrolyte used at this stage for electrochemical cycling (between gold’s redox potentials) was 0.5 M sulfuric acid\cite{39} and impedance measurements were taken immediately preceding and following 25 cycles using a DC applied potential of -0.5 $V_{SCE}$. The changes in the capacitance and CPE alpha factors are summarized in Figures 70-71.

**Figure 70.** Analytical Electrode Capacitances before and after Electrochemical Cycling in 0.5M H$_2$SO$_4$
From the fact that the capacitance changed to near literature values for a clean, bare, metallic electrode (~20-25 $\mu$F/cm$^2$)\textsuperscript{38} it seemed clear that cleaning had taken place and from the increase in the CPE alpha factor it seemed possible that some electrochemical polishing had taken place. In the case of the latter effect, however, this reduction in “interfacial heterogeneity” could not be directly related to the roughness of the metal electrode itself as the initial surface contamination doubtless made some, undistinguishable, contribution to that heterogeneity. As such a standard literature procedure which used similar cycling in phosphate buffer was used to characterize the real area (by measuring the charge associated with the removal of an oxide layer and in conjunction with an an independently determined oxide/cm$^2$(real area) factor) and thus the roughness (defined as the ratio of real area over geometric area) of the electrode.\textsuperscript{41} This was done, along with impedance spectroscopy, before and after cycling in sulfuric acid. The results
are summarized below and in this set of experiments six electrodes were cycled 25 times (3 with a scan rate of 100 mV/s and 3 with a scan rate of 10 mV/s).

**Figure 72.** Changes in Electrode Cleanliness on Cycling

These capacitive results appear odd but rather indicate that the different electrodes are at different stages in the electrochemical cleaning process. The net decrease in capacitance which, is here observed for some electrodes, could not be indicative of electrode cleaning (as the net increase indicated in the previous experiments) but rather perhaps a reduction in the real area (cf. equation 49) and thus a polishing effect. Unfortunately, with these types of measurements, the cleaning and polishing effects are indistinguishable and thus it is impossible to determine from a single measurements the specific stage of cleaning/polishing an electrode is in (which could be modulated by its initial level of surface contamination and its initial surface roughness). The “before and after” roughness measurements also so similar variability but what was soon realized
was that the initial “real area” measurement could not be reliable given the known presence of contaminants (preceding extensive cycling) which would, doubtless, attenuate the oxide application and removal charge reflecting a significantly reduced area/roughness. What’s more, the fact that the electrolyte solution needed to be changed from a cleaning/polishing sulfuric acid solution to a phosphate buffer solution (for real area measurements to be taken), a rather time-consuming process, provides the opportunity for the further contamination of the gold surface (which occurs very rapidly) which could influence the observed “after” roughness as well.

Figure 73. Changes in Electrode "Heterogeniety on Cycling"
As such, in order to most fully characterize what occurred over the course of the electrochemical polishing process impedance and real area measurements were conducted simultaneously over the course of three 200 cycle electrochemical polishing experiments. This was accomplished by taking advantage of the fact that the real area determination and electrode polishing procedures use the same gold-oxide formation and removal mechanism such that the same electrolyte and CV parameters were used for both measurements with each polishing scan being, at the same time, a real area measurement. Every 5 scans an impedance measurement was also taken in order to characterize the capacitive behavior at each stage.
Figure 75. Electrochemical Polishing Plots

Figure 76. Surface Electrochemical Heterogeneity vs Cycle #
According to Figure 75 in the electrochemical polishing process appears to be divided into three different stages: an initial apparent increase, a rapid decrease in and a rapid decrease and a
subsequent plateauing of the substrate roughness. The initial (apparent) increase in the real area of the electrode almost certainly corresponds, rather, to the removal of contaminants from the electrode surface such that as more physiadsorbed material is removed, more of an oxide layer can be formed and removed and thus more of the real area is detected. The peak real area/roughness, on this plot, can be taken to represent the point at which the substrate is completely clean after which, only electrochemical polishing will take place. This finds support in impedance measurements which show that both the CPE alpha factor and the capacitance per unit real area peak at this same point and following that point plateau indicating that the character/cleanliness of the electrode surface does not change over the rest of the polishing procedure (only the roughness changes).

Electrode D was stored in isopropanol overnight and then resubject to electrochemical polishing twice. The results, summarized in Figure 79, demonstrate the ease with which clean gold electrodes become contaminated (as indicated by apparent roughness increase at each of these points (~250 and ~350 cycles)) even when not kept under ambient conditions. Because of this surface cleaning immediately preceding placement in SAM depositions solutions may be necessary.
8. ArwC11 SAM’s on Polished/Cleaned Electrodes

Two electrochemically polished electrodes (A and B) which had been stored in isopropanol were cleaned electrochemically (by cycling in phosphate buffer 25 time) and immediately placed in ArwC11 deposition solutions for 24 hours or more. Electrode A following cleaning/polishing was determined to have a roughness factor of 4.6 and when coated with a ArwC11 SAM exhibited a capacitance of 2.0 μF/cm². This capacitance was found to be stable over a period of 3 hours when kept in the 50 mL, 0.1 M Et₄NCl electrolyte of the electrochemical cell with a standard deviation of ± 0.1 μF/cm². After those three hours a 0.5 mL aliquot of 0.1 M KCl was added (the same molar concentration was used to maintain a constant ionic strength in the supporting electrolyte) with stirring and while subsequent impedance measurements demonstrated a capacitive change this change was slow and took around two hours to realize. The final capacitance value measured was 3.6 μF/cm² and at this point the SAM was sonicated for a few minutes in deionized water and replaced in its depositions solution.
The next day the same experiment was repeated with the same electrode, but this time smaller initial aliquots were used. The initial capacitance of the layer was somewhat higher 2.2 ± 0.2 μF/cm² and the response to aliquot addition was almost immediate and quite stable. As such, a nice titration curve (the highest capacitance measured being 3.5 μF/cm²) was obtained and following that titration the SAM capacitance response was shown to be at least partially reversible as after extensive sonication in deionized water it was shown to return to 3.0 μF/cm².

Again, following sonication the electrode was replaced in its deposition solution and the next day another titration was attempted with it. This time, however, the initial capacitance was 2.3 ± 0.1 μF/cm² and the highest capacitance obtained was 2.4 μF/cm² and that was only after the first 0.1 mL aliquot. With subsequent addition of KCl no further change was observed.

The same titration (using the same volumes and concentrations) was attempted on the ArwC11 SAM deposited onto electrode B (which had a substrate roughness of 3.0). Its initial
capacitance in the 0.1 M Et4NCl supporting electrolyte was 2.3 μF/cm² but this was not stable and slowly, over the course of several hours, changed to 2.8 μF/cm². At this point several aliquots of KCl were added but no significant capacitance change was observed. The SAM was extensively sonicated in deionized water, replaced in its deposition solution overnight and the titration repeated again with similar results.

Though perhaps the best titrations results thus far, were obtained here (with a SAM featuring immediate and stable capacitance changes with each aliquot addition), they were obtained from a sample which had shown (the day before) a very slow and large change in capacitance when exposed to the analyte and later showed (the day after) little to no capacitive response at all. A possible explanation for this behavior may be gleaned if the first long and slow capacitance change is assumed to have been more, the result of a reordering of the thin film. That is, this change started when the SAM was exposed to potassium and so maybe the presence of this cation slowly forced conformational changes in the crown ethers (e.g. if they had previously been buried within the SAM and not as available to the electrolyte) which would make them more susceptible to ion binding the next day. If this is true, then it could be thought of as a template-based preorganization of the sensor layer which optimized sensor functioning and there is indeed precedent in the literature for this type of thing in the literature.\(^8\) The lack of capacitive response the following day is rather more difficult to explain but may have something to do with the fact that there was still probably a significant amount of bound potassium in the layer (following sonication the capacitance went down to 3.0 μF/cm² \textit{not} 2.2 μF/cm²) and it presumably could have caused some other type of reorganization (e.g. due to electrostatic repulsion). The capacitance of the ArwC11 SAM on electrode B probably changed (over the course of a few hours, without the addition of potassium), similarly, due to some kind of
reorganization of its thin-layer structure.\textsuperscript{47} In this case however, it would seem to have been induced by simply placing the SAM into aqueous solution from an alcoholic one. This would indicate that the SAM was not well-packed/crystalline and probably exhibited a large number of defects making it more susceptible to influence from the immersing solution. Given the above continuing variability in layer character and function, another source of irreproducibility between SAM’s: the layer deposition/reaction time, was studied.

9. ArwC11 SAM Deposition vs Time Studies

The mechanism of self-assembly of alkane thiols on gold involves first a very rapid adsorption step (reaching ~99% coverage in minutes) followed by a much slower reorganization step which occurs on the order of hours typically but can take as long as a few days. This reorganization is made favorable by Van der Waals interactions between the long alkyl chains but can be impeded by the presence of a bulky terminating group at the other end of the chain. This complicates the reorganizing process and can lead to either longer reorganization times and/or less well packed layers depending on the size and nature of that functionalizing group.\textsuperscript{17, 18, 21, 40, 76-79} As very bulky head groups were used in these experiments impedance measurements were taken over the course of a SAM deposition in order to determine the optimal time needed to obtain reproducible SAM behavior. Over the course of time the capacitance and resistances of these SAM’s showed the expected trend (lower capacitances and higher resistances) but also seemed to oscillate through this trend which may lend to either a more complex mechanism of assembly or rather to the idea these type of SAM’s are more dynamic structures.
It was suggested, given capacitance and resistance values are dependant on temperature, that these seeming oscillations may simply be the result of temperature changes over the course of a day. While this is certainly a possibility (and, in fact, the temperature dependence
of layer functioning is another study which will be conducted in the future) one would expect the resistance of the electrolyte solution to be effected in the same way. Over all the time periods
where curved peaks or troughs characterize the changing of SAM elements only linear changes are observed in the case of this bulk, electrolyte element.

These experiments were conducted by taking a SAM out of its deposition solution, rinsing it, placing it in a 0.1 M Et4NCl electrochemical cell, taking a measurement, rinsing it and replacing it in the deposition solution every hour or so over the course of several days. This time consuming and overly laborious method will be with one wherein automated impedance measurements will be taken during deposition and within the deposition solution, at regular time intervals. This will be accomplishing through the use of organic supporting electrolytes (within the electrochemical cell) and scripting. Surface plasmon resonance studies of the ArwC11 deposition process were also attempted, to be used in conjunction with the above, but these were hampered by instrumental difficulties, relating to, in large part, an inability to account for temperature drift.73, 80-85 Unfortunately, the only immediately useful information obtained from this data is the fact that the capacitance takes at least two days to plateau and even then it may change, cyclically with time.

10. Mixed Monolayers

As previously mentioned, SAM’s with bulky head groups are known to form significantly more disordered and defect-laden thin films. A possible explanation for this is that the head group forces the spacing out of the individual alkyl chains such that the Van der Waals interactions (which play the greatest role in SAM organization/crystallization) are minimized. By including filler molecules it should be possible then to fill in the gaps and perhaps obtain more reproducible layer behavior.6, 79, 86 Mixed deposition solutions made up of various ratios of sensor and filler molecules were made and the resulting SAM’s characterized.
Figure 85. The Sensor–Filler Mixed monolayer system used here

I. Decanethiol Reference SAM’s

The mixed monolayer system which was chosen for these experiments utilized ArwC11 sensor molecules and decanethiol filler molecules. It was reasoned that using a filler alkane thiol just shorter than the tethering chain of the sensor molecule would have the effect of maximizing methylene Van der Waals interactions without burying the active, terminal crown ether (which could impede sensor function). As such, in order to establish a baseline with which to compare the final mixed monolayers, a set of decanethiol SAM’s were formed on the 5 electrodes which were to be ultimately used for the mixed monolayer experiments.

Multiple Impedance measurements were conducted over the first few days of SAM deposition and the results, in terms of layer capacitances and CPE heterogeneities are summarized in Tables 33 and 34. As expected the layer capacitances did not reach their final, lowest values after the first day in solution. These final values were, however, significantly
higher than those obtained in the literature for similar SAM’s which may be due to impurities in the alkane thiol stock, roughness of gold substrate on a scale/of a sort which would lead to a greater frequency of defects or roughness of the gold substrate on a scale which would increase the effective area of the coated electrode. A greater frequency of defects is probably the most likely explanation if one looks at the CPE heterogeneity factor here (~0.93) which is significantly lower than that obtained for previous alkane thiol SAM’s on plate substrates (~0.99). What’s interesting is that while the alpha factor is here low, it reached its final value, after just the first day (unlike the capacitance). This is not easy to explain, but would seem to indicate that, somehow, that the electrolyte-SAM interfacial organization/ordering was established before the complete ordering of the SAM itself. That is, perhaps terminal group configuration was more quickly determined or perhaps rather terminating methylenes of an unorganized layer well correspond to terminating methyls of a organized layer.

**Table 33. The Capacitance of Reference Decanethiol SAM’s Over Time**

<table>
<thead>
<tr>
<th>Time</th>
<th>Electrode A</th>
<th>Electrode B</th>
<th>Electrode D</th>
<th>Electrode E</th>
<th>Electrode G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>2.4</td>
<td>2.3</td>
<td>2.5</td>
<td>2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>3 days</td>
<td>2.2</td>
<td>2.1</td>
<td>2.4</td>
<td>2.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Table 34. The CPE alpha of Reference Decanethiol SAM’s Over Time**

<table>
<thead>
<tr>
<th>Time</th>
<th>Electrode A</th>
<th>Electrode B</th>
<th>Electrode D</th>
<th>Electrode E</th>
<th>Electrode G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>0.94</td>
<td>0.94</td>
<td>0.92</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>3 days</td>
<td>0.94</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
</tr>
</tbody>
</table>

As alluded to in the last paragraph, the surface roughness of an electrode can have two distinct effects on the characteristics of a SAM coating behavior depending on the scale. Roughness with characteristic dimensions (see the section on Ellipsometry in the Materials and Methods) much greater than the atomic scale (which will be termed macroscopic roughness) will in general simply translate to a larger effective area of the electrode and the resulting SAM
coating. This would simply result in larger capacitance, charge transfer resistance and solution resistance values than expected (cf. equation 48 and 49). On the other hand, roughness with characteristic dimensions around the atomic scale (which will be termed microscopic roughness) can significantly effect the orientation and packing of long chain thiol molecules (e.g. with small pits or bumps creating a wide range of alkyl chain tilt angles with respect to the average surface normal). This can result in the formation of an assortment of different structural defects in the SAM which, in turn, can cause non-idealities and irreproducibilities in SAM behavior. In this case, however, little to no increase in the effective area of the SAM-coated electrode (and associated capacitances and resistances) is expected due as this scale roughness will not translate through the SAM due to interfacial organization of the more mobile groups. Thus the presence of a coating, has the effect of attenuating roughness of this size. Unfortunately neither effect has been shown to predominate, in general, for the work presented here. That is, neither small differences in fitted element values (the first effect) nor CPE surface heterogeneity (the second effect) have been effectively correlated with the measured roughness of the substrate preceding SAM deposition.

\[ \text{Roughness}_{\text{MEASURED}} = \text{Roughness}_{\text{MACRO}} + \text{Roughness}_{\text{MICRO}} \] (78)

Thus the surface roughness here measured seems to have a combined effect which can perhaps be explored, for each electrode, by examining general trends between surface roughness and layer un-ideality and circuit element values. Below is a summary of the data obtained for the electrodes and SAM’s used in this decanethiol SAM study.

<table>
<thead>
<tr>
<th></th>
<th>Electrode A</th>
<th>Electrode B</th>
<th>Electrode D</th>
<th>Electrode E</th>
<th>Electrode G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitance</td>
<td>2.2</td>
<td>2.1</td>
<td>2.4</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>CPE alpha:</td>
<td>0.94</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>Roughness:</td>
<td>2.4</td>
<td>2.9</td>
<td>3.3</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Coverage:</td>
<td>14×10^{14}</td>
<td>12×10^{14}</td>
<td>12×10^{14}</td>
<td>8×10^{14}</td>
<td></td>
</tr>
</tbody>
</table>
II. *ArwC11 Reference SAM’s*

The above decanethiol SAM’s were electrochemically desorbed (and from this coverage information was obtained in Table 35), the electrodes cleaned by cycling in phosphate buffer and immediately rinsed with water, ethanol and placed in ethanolic ArwC11 deposition solutions. Electrochemical data taken over the next two days was recorded and is summarized in Tables 36 and 37. Yet again the expected trend of capacitance vs. time was observed and the CPE interface heterogeneity term showed no significant change after the first day. The latter is not inline with previous impedance versus deposition time experiments but may be a result of the oscillating nature of the trend which could not be accounted for with this few measurements.

**Table 36. The Capacitance of Reference ArwC11 SAM’s Over Time**

<table>
<thead>
<tr>
<th>Time</th>
<th>Electrode A</th>
<th>Electrode B</th>
<th>Electrode D</th>
<th>Electrode E</th>
<th>Electrode G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>3.8</td>
<td>4.2</td>
<td>4.7</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>2 days</td>
<td>3.5</td>
<td>3.8</td>
<td>4.2</td>
<td>3.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**Table 37. The CPE alpha of Reference ArwC11 SAM’s Over Time**

<table>
<thead>
<tr>
<th>Time</th>
<th>Electrode A</th>
<th>Electrode B</th>
<th>Electrode D</th>
<th>Electrode E</th>
<th>Electrode G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>0.94</td>
<td>0.93</td>
<td>0.93</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td>2 days</td>
<td>0.95</td>
<td>0.93</td>
<td>0.93</td>
<td>0.92</td>
<td>0.90</td>
</tr>
</tbody>
</table>

A few electrode based trends can also be observed in these experiments and compared with previous and future deposited SAM’s. First of all, Electrode D, here, in previous alkane thiol and in the future mixed monolayer experiments consistently manifests the highest capacitance value. This is probably not a result of having a more poorly packed/defect laden SAM as the CPE alpha factor consistently indicates that the electrode D SAM’s exhibit medium ideality within each SAM set. As such, this is almost certainly an effect of a greater effective surface area of this electrode and thus greater macroscopic surface roughness. Looking at the
general trends in capacitance, SAM resistance and solution resistances for each SAM set presented in this mixed monolayer sequence it is possible to come up with the following general trend in macroscopic roughness for Electrodes A, B, D, E and G:

$$D > B, D > A, G$$

And this trend agrees rather well with the measured surface roughness trends which were determined for each SAM set. That this trend would be maintained through electrochemical cleaning and polishing procedures would be expected as electrochemical polishing is only expected to attenuate microscopic scale roughness (as it corresponds to a molecular level grit polishing). That the measured roughness values do not consist only of this macroscopic roughness component was confirmed by dividing all the “capacitance per geometric area,” values presented in this work, by their respective electrodes’ roughness factors (real area/geometric area) which would yield capacitance per real area values. These values showed even greater variation between samples than did capacitance per geometric area values indicating that microscopic roughness here makes a very significant contribution.

**Table 38.** Summary of Final Characteristics of Reference ArwC11 SAM’s on 0.002 cm$^2$ electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Capacitance</th>
<th>CPE alpha</th>
<th>Roughness</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.5</td>
<td>0.95</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>B</td>
<td>3.8</td>
<td>0.93</td>
<td>3.1</td>
<td>3.8</td>
</tr>
<tr>
<td>D</td>
<td>4.2</td>
<td>0.93</td>
<td>3.8</td>
<td>3.1</td>
</tr>
<tr>
<td>E</td>
<td>3.4</td>
<td>0.92</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>G</td>
<td>2.6</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Another per electrode trend, of note, is the order of ideality of SAM capacitive behavior (CPE alpha factor) on these electrodes which seems to be:

$$\text{Electrode A} > \text{Electrode B} > \text{Electrode D} > \text{Electrode E} > \text{Electrode G}$$

This trend is immediately apparent in the mixed monolayer and ArwC11 SAM sets and rather apparent in the alkane thiol set. This may be, in part, due to the amount and type of microscopic
roughness present on the gold substrates but, again, no direct comparison between the CPE alpha factor and the here used real area to geometric area ratio has proven, generally feasible.

| Table 39. Summary of Final Characteristics of Reference ArwC11 SAM’s on 0.03 cm² electrodes |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Electrode        | Capacitance      | CPE alpha        | Roughness        | Coverage         |
| Electrode 2      | 2.9              | 0.85             | 4.6              | -                |
| Electrode 3      | 2.5              | 0.89             | 3.6              | -                |
| Electrode 4      | 2.7              | 0.90             | 4.0              | -                |
| Electrode 5      | 2.4              | 0.87             | 4.5              | -                |
| Electrode 6      |                  |                  |                  |                  |

That said, another set of ArwC11 SAM’s were deposited onto a set of un-electrochemically polished electrodes which as a result manifest roughness factors on the order of 4-5 (as opposed to 2-4). If one assumes that electrochemical polishing only affects microscopic scale surface irregularities, then it would seem likely that these electrodes would exhibit many more of those type of microscopic substrate defects which are believed to influence overlaying thin-film ideality (CPE alpha factor). And indeed, as can be seen from Tables 38 and 39, un-electrochemically polished electrode demonstrate SAM alpha factors within the range 0.85-0.90 while electrochemically polished electrodes demonstrate SAM alpha factors within the range 0.9-0.95. What’s more, trends in the measured roughness factors, with the un-electrochemically polished electrodes, can be directly related to trends in respective SAM’s alpha factors with the roughest surfaces demonstrating the most marked SAM capacitive deviation from ideality in the order:

Electrode 2 > Electrode 5 > Electrode 4, Electrode 3

Therefore, it would seem that microscopic level irregularities make the greatest contribution to the measured roughness of electrodes which have not been electrochemically polished (given, here the comparability of CPE alpha factor and measured roughness trends) and that macroscopic level irregularities make the greatest contribution to the measured roughness of
electrodes which have been electrochemically polished (given, here, the comparability of capacitance/resistance and measured roughness trends). At the same time, however, the absolute roughness factor is most certainly not directly relatable, at any point, to any equivalent circuit element values given the indistinguishability (at least in this work) between the microscopic and macroscopic contributions.

While these roughened electrode SAM’s exhibit significant layer un-ideality and thus, presumably, a high frequency of defects, they – at the same time – exhibit markedly lower capacitance values (2.4-2.9 μF/cm²) than their polished counterparts (2.6-4.2 μF/cm²). A possible explanation for this may lie in the fact that a significantly roughened electrode may provide more (if irregular) area for binding of thiol groups which has been known to result in more dense packing. This type of system may actually be appealing as, having more active molecules on a surface, a greater magnitude of bulk layer response may be observable per unit area. This idea would find support in the fact that the best titration results obtained, thus far, were attained using an ArwC11 SAM on a significantly roughened electrode (4.6), manifesting a markedly low capacitance value (~2.3 μF/cm²), see the Anthryl Crown Acid section on Electrochemistry on gold.

III. Mixed ArwC11-Decanethiol SAM’s

Mixed monolayers were formed by immersing electrochemically cleaned and polished electrodes in mixed deposition solutions of varying ratios of decanethiol and ArwC11 (which are summarized in tables 40-41 below). Impedance measurements were again taken over the course of deposition time and are summarized in the two tables below. While these again manifest, in general, the expected trends what set these results apart from those above is the fact that even
after 4 days the mixed SAM’s (as opposed to the one unmixed SAM on electrode G) showed significant changes in both capacitance and layer ideality.

**Table 40.** The Capacitance of Mixed Monolayer SAM’s Over Time (Decanethiol:ArwC11)

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>2.4</td>
<td>2.4</td>
<td>2.6</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>4 days</td>
<td>2.3</td>
<td>2.4</td>
<td>2.7</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>8 days</td>
<td>2.1</td>
<td>2.2</td>
<td>2.4</td>
<td>2.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>

**Table 41.** The CPE factor of Mixed Monolayer (Decanethiol:ArwC11) SAM’s Over Time

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>0.97</td>
<td>0.95</td>
<td>0.94</td>
<td>0.92</td>
<td>.88</td>
</tr>
<tr>
<td>4 days</td>
<td>0.96</td>
<td>0.96</td>
<td>0.95</td>
<td>0.94</td>
<td>.88</td>
</tr>
<tr>
<td>8 days</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
<td>0.92</td>
<td>.88</td>
</tr>
</tbody>
</table>

The fact that, in addition, the capacitances values of each electrode, over this long period of time, approach their respective pure decanethiol values, may indicate that the composition of the SAM’s changes over time. That is, exchange between species in solution and on a surface is known to occur for alkane thiols on gold and constitute part of the SAM reorganization/crystallization mechanism and therefore perhaps, here, crown ether terminated molecules, over extended deposition periods, are slowly exchanged with decanethiol molecules. This would make sense from an energetic perspective given the fact that (while in the initial stages of molecular adsorption, all thiols would indiscriminately adsorb to the surface in ratios reflecting those of the deposition solutions) monolayers composed only of alkane thiols are known to be the most stable, given their maximal Van der Waals interactions, and thus, perhaps, the thermodynamic product of a mixed alkane thiol-sensor deposition solution is a pure alkane thiol monolayer. It may be possible to track the composition of these SAM’s over time using equation 79 (where \( \theta \) represents the fraction of sensor molecule) which assumes parallel,
fractional, capacitive contributions from both the sensor and the filler molecules but, in the early stages at least, the un-organization of the SAM would introduce another capacitive factor which would be difficult to account for.79, 86

\[ C_{\text{SAM}} = \theta \cdot C_{\text{ARBW}11} + (1 - \theta) \cdot C_{\text{C10SH}} \quad (79) \]

Table 42. Summary of Final Characteristics of First Mixed Monolayer SAM’s

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Capacitance</th>
<th>CPE alpha:</th>
<th>Roughness:</th>
<th>0.1 M K⁺ Capacitance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (1:9)</td>
<td>2.1</td>
<td>0.95</td>
<td>2.8</td>
<td>2.0</td>
</tr>
<tr>
<td>B (3:7)</td>
<td>2.2</td>
<td>0.94</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>D (5:5)</td>
<td>2.4</td>
<td>0.94</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>E (7:3)</td>
<td>2.0</td>
<td>0.92</td>
<td>3.0</td>
<td>3.6</td>
</tr>
<tr>
<td>G (10:0)</td>
<td>2.2</td>
<td>0.88</td>
<td>3.6</td>
<td>2.2</td>
</tr>
</tbody>
</table>

This in addition may explain why titration experiments which were conducted using all of these monolayers did not given any capacitive response, whatsoever (see Table 42).

These SAM’s were reductively desorbed (no coverage information was obtained as the reduction currents were inordinately large, probably as a result of insufficient nitrogen purging of the electrolyte used), the electrodes electrochemically cleaned and replaced in their respective deposition solutions for one and a half weeks. The electrodes were then rinsed and three potentiometric potassium titrations conducted with final concentrations around 0.05 M but this form of detection proved too noisy to provide useable data. Impedance measurements were taken before and after each titration and are summarized in Table 43. Here, interestingly enough, both Electrode G and Electrode D showed significant capacitive responses. The only real difference between either of these electrodes/SAM’s here and in the previous mixed monolayer experiments is the fact that the CPE alpha factor is further from unity for both electrodes and the roughness of electrode G is significantly higher.

Table 43. Summary of Final Characteristics of Second Mixed Monolayer SAM’s

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Capacitance</th>
<th>CPE alpha:</th>
<th>Roughness:</th>
<th>0.1 M K⁺ Capacitance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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Here, yet again, the largest capacitance response was realized with the roughest electrode exhibiting a very low initial capacitance.

**IV. Reference ArwC11 Titration**

One final experiment conducted with these electrodes which deserves note is an attempt at a titration with a reference ArwC11 SAM on Electrode D. Here, a baseline was established, two aliquots of potassium were added and a sequential capacitive decrease observed. Then the electrode was washed, the capacitance returned to its original value, two aliquots of potassium added and a sequential capacitive increase observed. These experiments are summarized in Table 44. The fact that the expected sensor response was only obtained after the opposite response was obtained (and shown to be reversible) may lend further evidence to the previously presented idea that pre-organization the crown either layer (by exposing it to template binding ions) may be necessary for reproducible sensor response to be obtained. What’s more, the fact that the magnitude of the capacitive decrease showed a concentration dependence and was reversible may indicate that the amount of conformation change (if that is what it is) associated with this pre-ordering is quantifiable. In any case, what is clear is that more work in regard to the effects of pre-exposing a SAM to the target analyte need to be studied in more detail.
<table>
<thead>
<tr>
<th>[KCl] (M)</th>
<th>SAM capacitance (μF/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>3.77</td>
</tr>
<tr>
<td>0.002</td>
<td>2.81</td>
</tr>
<tr>
<td>0.006</td>
<td>2.74</td>
</tr>
</tbody>
</table>

WASH (extensive sonication in deionized water)

<table>
<thead>
<tr>
<th>[KCl] (M)</th>
<th>SAM capacitance (μF/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>3.85</td>
</tr>
<tr>
<td>0.002</td>
<td>3.95</td>
</tr>
<tr>
<td>0.040</td>
<td>4.10</td>
</tr>
</tbody>
</table>
IV. Conclusion

An electrochemical toolbox was developed to facilitate a more complete understanding of the complex thin-layer systems here under study. This will prove useful for future and continuing work which will focus on both obtaining reproducible SAM behavior/sensor response and determining the mechanisms underlying that response. In addition, some tentative conclusions have been obtained from the great quantity of preliminary work, above, regarding what parameters are most important for obtaining reproducible sensor functioning in self-assembled monolayer and multilayer formats (e.g. regarding lower layer capacitances and rougher substrate roughnesses). There is, however, a great deal of work which needs to be done to confirm these hypothesis before such mechanistic studies (which are the ultimate goal of this work) can be carried out.

Regarding substrate/electrode roughness, more alkane thiol SAM data needs to be obtained from substrates exhibiting a wider range of roughness factors such that the effects of roughness on SAM formation and behavior, in general, can be quantitatively understood. What’s more the two types of surface roughness (microscopic and macroscopic) need to be directly quantitated on each substrate (preceding layer deposition) so that they can be more directly related to their electrochemical manifestations (layer ideality and circuit element values, respectively). This can perhaps be accomplished through the use of atomic force microscopy.

The dynamic “bulky-head-group layer deposition process” also needs to be better understood and characterized so that optimal deposition times can be determined. Therefore, more complete deposition versus time studies need to be conducted using surface plasmon resonance and by conducting impedance spectroscopy within a deposition solution. Other deposition time experiments involving other modes of characterization (e.g. hydrophobicity, IR
spectroscopy, etc.) can also be conducted. By coupling all these techniques a complete understanding of the self-assembly process of these adsorbates should be obtainable.

The composition of SAM’s at various stages of mixed monolayer formation also needs to be determined and distinguished from the effects of initial adsorbate reorganization so that it can be controlled (given the above determined necessity of kinetic control). This can perhaps be accomplished by conducting independent filler and sensor deposition versus time experiments. If, then, a form of equation 79 was used which used constituent capacitive values which were a function of time then one should be able to more accurately track the mixed monolayer fractional composition over time.

$$C_{SAM} = \theta \cdot C(t)_{ARWC11} + (1 - \theta) \cdot C(t)_{C10SIH}$$  \hspace{1cm} (80)

Given that data then, obtaining well-organized mixed layers of a particular composition would simply be a question of carefully varying the deposition time and solution composition.

Though a great deal of work has been done and data collected we are still very far from obtaining a detailed mechanistic picture of how sensing self-assembled monolayers assemble and function. That said, great progress has been made in elucidating methods of characterizing that response and the factors which underlie it and as such, the authors have become significantly better equipped. Thus we should now have the means to conduct better controlled and more elegant studies which should provide more meaningful results. Thus, at the very least, we are now on the right road to obtaining a complete understanding of these sensing surfaces.
V. References


