MODIFICATION, CHARACTERIZATION AND ELECTROCHEMICAL PROPERTIES OF REDOX ACTIVE AND BIOLOGICALLY RELEVANT THIN FILMS ON ELECTRODE SURFACES

by

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The function of materials often relies heavily on properties that derive from the interface. There are often specific developments of surface modifications within certain fields of study to alter these properties, but because thin films span such a vast array of applications, there is need for a concentrated effort on surface functionalization development. To this, we have developed cross coupling methods to construct 10 unique thin films on electrode materials, 1 known thin film for the first time on a carbon paper support and 2 new thin films that relied on well-developed surface chemistry primarily for energy storing devices and sensing applications.

In all 13 unique thin films were synthesized on carbon based and indium tin oxide (ITO) conducting surfaces. The 13 thin films can be segregated by the type of chemistry used to synthesize the thin film (chapter 2), the conductive support (chapter 3), and finally thin films for applied use (chapter 4 and 5).

Carbon paper electrodes (CPE) were capped with alkynes based on the electrochemical reduction of aryl diazonium salts. Briefly, triisopropylsilyl protected ethynyl benzene diazonium (4-TIPS-ethynyl-ph-N₂⁺) was electrodeposited on the substrate and subsequently treated with tetrabutylammonium fluoride (TBAF) to remove the TIPS group exposing a terminal alkyne monolayer. The grafted substrates were characterized by X-ray photoelectron spectroscopy (XPS) for the presence and absence of the silicon (from the TIPS group in 4-TIPS-ethynyl-ph-N₂⁺) before and after deprotection.

ABSTRACT
Sonogashira cross coupling and Glaser cross coupling chemistries were developed as a method to build molecular wires starting from the alkyne terminated CPE’s. Ferrocene appended reactants were used to determine the success of cross coupled products by observing the presence of iron by XPS and the ferrocene/ferrocenium redox couple by cyclic voltammetry. Glaser cross coupled electrodes varied from Sonogashira cross coupled electrodes by an additional alkynyl unit, which extends the π-conjugation within the film. We also performed copper catalyzed azide alkyne cycloaddition (CuAAC) for the construction of a third type of wire. This series of molecular wires on CPE’s are compared with respect to the impact on the ability to modulate charge transfer. We found the rate of electron transport is effected by molecular wire architecture with the surface Glaser cross coupled product producing remarkably fast kinetics.

Next, three additional carbon electrode platforms (isomolded graphite (IG), glassy carbon (GC) and diamond (DE)) were modified using identical conditions developed for the surface Sonogashira cross coupling for CPE’s. The native carbon substrates were extensively studied to obtain a clear picture of the chemical environment of the surface before attaching the alkyne. Surface oxides were determined by XPS. The distribution of these oxides within the material were examined by XPS depth profiling and electron dispersive X-ray spectroscopy. These techniques show that GC and DE have the highest amount of surface oxides, but they are confined to the top several nanometers of the material. IG on the other hand contains oxides that penetrate deep within the bulk of the film. Carbon paper contains negligible detectable oxides. Once the surfaces were cross coupled via Sonogashira cross coupling chemistry with 4-iodophenylferrocene, the electrode dynamics were
measured using the Laviron formalism. Comparisons were made between the electron kinetics through the wire and the parent electrode’s surface resistivity. The parent electrode’s resistivity’s were measured using a 4-point kelvin probe and their conductivities were measured using cyclic voltammetry in an electrolyte solution. It was found that the electron kinetics through the wire does not out compete with the native materials resistivity’s.

We next exchanged the redox active halogen reactant (4-iodophenylferrocene) with a maleimide appended halogen (4-iodophenylmaleimide) for surface Sonogashira cross coupling. The maleimide functionalized carbon paper was characterized by XPS for the presence of nitrogen from the maleimide group located at 400.7 eV. There was significant iodination of the carbon paper surface after Sonogashira reaction with 4-iodophenylmaleimide with atomic percentages in excess of 1%. This contrasts with the Sonogashira cross coupling with 4-iodophenylferrocene in which iodide on the surface was intermittently found, and always less than < 0.1%. We attempted to remove the iodide from the surface electrochemically, resulting in successful removal of the iodide but a compromised film. Hypothesizing that the iodide was the unwanted side product of the possible competing addition of a deprotonated maleimide backbone with the immobilized alkyne, (leaving the iodide apex on the wire) we replaced the maleimide with a methylated version. Sonogashira cross coupling with the methylated maleimide resulted in removal of the iodide contamination and preservation of the electronics of the molecular wire suggesting that the competing pathway was a source of the iodide fouling. We performed the Michael addition with the ferrocene appended cysteine on 4-iodophenylmaleimide and methylated 4-iodophenylmaleimde cross coupled product. We found that the methylated maleimide group slows the addition of
the cysteine, but we were able to achieve comparable surface coverage of ferrocene at 24 hr reaction time at room temperature.

We also performed a Michael addition with a thiol appended Ru (tris)bipyridine derivative. In the presence of a sacrificial reductant we observed a catalytic enhancement of current as well as photonic emission. Immobilization of the Ru (tris)bipyridine luminophore successfully produced an ECL device which can be used for sensing applications.

Finally, ITO electrodes were terminated with COOH groups, passivating the electrode. EDC/NHS coupling was used to couple and immobilize amine terminated DNA. After DNA was conjugated to the surface, the electrode regained conductance. The immobilized DNA was characterized by XPS, electrochemical impedance spectroscopy (EIS) and time-of-flight secondary mass spectrometry (TOF-SIMS); the last technique demonstrated a covalent bond between the ITO and the appended DNA. EIS was utilized to show to the reversibility of protein binding. This was done by showing an increased resistance to charge transfer after ITO-dsDNA was incubated with target protein. A decrease in the resistance to charge transfer was observed after the incubated IT-dsDNA electrodes were further soaked in a concentrated solution of ds-DNA. This result is evidence of the reversible binding of the protein to and from the ITO-dsDNA.
Chapter 1

INTRODUCTION

1.1 Thin Films in Everyday Life

Thin films are comprised of many different compounds for many different applications making them hard to segregate into a category on their own. While bulk materials are in the size range of what we interact with daily, (a thin piece of human hair’s diameter is about 10 µm, the social space between two interacting humans is about 2 m) a thin film is typically only nanometers. While this unilateral spatial domain of a thin film is very small, the change it can induce on a material’s function is in fact very large. Below are several examples from daily life in which a material’s function is altered by the presence of a thin film and illustrated in Figure 1.1.

Glass windows designed with a thin film introduce a new function in which windows are not only transparent, but heat resistant. A metallic film so thin (1-10 nm) it retains its transparency can be called a heat mirror. The heat mirror is sandwiched between two gas cavities between two glass panes, allowing the transmittance of visible light, but reflect longer wavelengths such as near IR.1-3 The thin film allows windows to maintain cool interior environments in warm climates. More recent technology includes the development of “smart” windows from thermochromic glazing. Here a thin film of vanadium (IV) oxide is coated onto the glass. The material undergoes a structural change at 68 °C from a transparent monoclinic phase to a rutile darkened metallic phase creating a “smart” window that reflects light and heat and
higher temperatures.\textsuperscript{4} Doping of the vanadium (IV) oxide with tungsten has led to a decrease in the transition temperature as low as 42 °C.\textsuperscript{5}

**Figure 1.1.** An amine thin film enhances properties of carbon composites (top left). A transparent metal thin film prevents heat transference in windows (top middle). A thin electro-catalytic film for CO\textsubscript{2} reduction (top right). A thin chromium film ad-layer helps with gold adsorption; A thin film of alkyl thiols provides a platform for fundamental studies (bottom left). A thin film of oxides protects the anodic graphite (anode, bottom right). A thin MgO film increases the lifetime of a lithium battery (cathode, bottom right).
Thin films can also strengthen structural reinforcements. Carbon fibers and epoxy resins mix to form a hardened lightweight material with excellent structural integrity making them ideal for high performance vehicles and aircraft. A thin film of reduced amine onto the fibers prior to curing resulted in a product with a larger resistance to shear stress.\textsuperscript{6}

Thin films aid the many studies of self-assembled monolayers on gold films by providing a platform for the gold layer to adhere. Gold tends to have poor adsorption properties, and without first depositing a thin layer of chromium prior to gold film preparation, the gold will delaminate and produce flakes.\textsuperscript{7} In addition, thin films of alkyl thiol self-assembled monolayers on gold have provided a platform for extensive fundamental studies nanotechnology and sensors.

The above examples show how thin films can alter optical, mechanical and physical properties of a bulk materials. Another property that can be adjusted by thin films (either intentionally or not) are the electronic properties of a material. Often a thin film’s purpose is to protect an electrode and not alter the electronic properties at all as is the case for a MgO coating of the cathode in a lithium ion battery.\textsuperscript{8} The MgO stabilizes the LiCoO\textsubscript{2} core structure by preventing dissolution of the Co, as well as diffusing into lithium vacant sites upon charging. Other times a thin film protects the material, but changes the electronic properties as well. An example in which the electronic properties are changed for the better from a surface modification is seen by the oxidation of edge planes of the graphitic anode in a lithium ion battery.\textsuperscript{9} The oxidation prevents exfoliation and enhances nanochannels available only to the Li\textsuperscript{+} ions increasing the lifetime and function of the anode.
Other thin film modifications include exploited electron transfer. One such example is a cobalt porphyrin that has been immobilized on an electrode which still displayed CO$_2$ electrochemical reduction to CO.$^{10}$ The electron dynamics are of key importance in this entire system, demonstrating the electrochemical power of thin film modifications on electrode assemblies.

In all cases the alteration of the materials’ properties are better understood with a deeper knowledge of the composition of the thin film. Below is discussed common methods for thin films synthesis and the challenges associated with the methods. The discussion is limited to thin films generated on conductive surfaces (as opposed to insulating surfaces as in the example of “smart” windows). Below is not an exhaustive list; but instead offers the most common methods that are relevant to the thin films we synthesize.

### 1.2 Common Methods to Synthesize Thin Films on Electrodes

Common methods to synthesize thin films on electrodes include chemical$^{11,12}$ and electrochemical methods (see Figure 1.2).$^{13}$ Chemical methods include the chemisorption of alkyl thiols on gold substrates$^{14,15}$ in which thiols or disulfides selectively adsorb onto gold surfaces with a bond strength around 40-50 kcal/mol.$^{16,17}$ Drawbacks for this electrode assembly include the conservative potential window the electrode can stably operate. With the oxidation of Au to Au(III) at a formal potential of 1.41 V vs. NHE$^{18}$ and the electrochemical desorption of the thiol at -0.8 V vs. NHE, the theoretical stable operating window spans only 2.21 V.$^{15}$ In fact, the experimental stable operating window span is reported as low as 1.2 V.$^{19}$ In addition to their electrochemical instability, alkyl thiols also undergo exchange reactions and
photooxidation\textsuperscript{15} adding to the list of challenges with working with alkyl thiols on gold electrodes.

**Common Methods to Synthesize Thin Films**

*Self-Assembled Monolayer Chemisorption on Gold*

![Diagram](image)

*Phosphonic Acid Hydrolysis on Metal Oxides*

![Diagram](image)

*Conductive Polymers Spincating on Electrodes*

![Diagram](image)

*Electrochemical Reduction of Aryl Diazonium Salts*

![Diagram](image)

Figure 1.2. Common methods to introduce thin films on electrode assemblies.
Another class of thin films include the condensation reaction of phosphonic acids and esters with metal oxide surfaces. Pendant hydroxy groups on a metal oxide can be displaced by phosphonic acid, producing water, or displaced by phosphonate esters producing the respective alcohol. Often these films require a subsequent heating step to ensure progress of the condensation reaction adding days to the preparation of the thin film. The thin films on some metal oxides, such as indium tin oxide (ITO) are also dependent on how well the native oxide is prepared, producing heterogeneous thin films on defected polycrystalline surfaces. While there are many techniques to characterize the success of the condensation reaction, it is hard to characterize the coordination of the phosphonate on the metal oxide. The phosphonate can coordinate with the metal oxide via one, two or three of its hydroxy groups contributing to 8 possible binding modes. If additional hydrogen bonding interactions from the metal oxide or adjacent phosphonic acids are also considered than the monolayer can have up to 12 binding modes. Because the phosphonate can coordinate in various ways and is dependent many factors (such as pH, temperature, crystallinity of native oxide) the resulting connectivity of the thin film is ill-defined.

Thin film deposition from conducting polymers has generated a substantial amount of interest because they show promise in the next generation of flexible electronics. Among typical conducting polymers used for thin film deposition is polyaniline. Polymeric thin films exhibit a high degree of complexity compared with electrodes modified with small molecules and thus present challenges in molecular characterization of the films. The interaction between the substrate and the polymeric thin film is sometimes physical (adsorption), and so the connectivity
between the electrode and thin film is undefined presenting a difficulty in understanding electron mobility throughout the films.

Finally, thin films can be generated electrochemically via the reduction of aryl diazonium salts. This method produces covalent bonds making these films very robust. Because the thin film is covalently bound, it can be viewed as an extension of the bulk material (if the underlying material is carbon). The drawbacks to this method and the research devoted to overcoming such drawbacks are detailed in section 1.3.3. Due to the ease in generating these films, and the robustness of them, we chose this method to further develop more complex thin films. The evolution of electrochemical reduction of aryl diazonium salts are discussed below, with an emphasis to the prospects and challenges that are relevant in my own research.

1.3 Electrochemical Method to Synthesize Thin Films Using Aryl-diazonium Salts

1.3.1 Mechanisms of Grafting

Aryl diazonium salts are synthesized from the aniline precursor with a nitrosonium ion (generated in situ from nitrate in an acidic solution, Scheme 1.1a) as indicated in Scheme 1.1b. Aryl diazonium salts are not particularly stable, although they are stable enough to be isolated and used immediately at room temperature for electrochemical grafting of electrodes. Isolated aryl diazonium salts can also be stored below 0 °C for several days before decomposing via a mechanism such as the Balz Schieman Reaction (if the counter ion is BF$_4$, Scheme 1.1c). Electrochemical grafting of the aryl diazonium salt can then form a base layer of phenyl rings on an electrode surface. An alkyl layer cannot be achieved by this method because the alkyl
diazonium precursor is unstable and immediately forms the carbocation. The stability for aryl diazonium salts is due to the resonance structure (see **Scheme 1.2**).

Scheme 1.1. Formation of nitrosonium ion in acid (A). Mechanism for the formation of aryl diazonium salt (B). Decomposition of aryl diazonium salt via the Balz-Schiemann Reaction (C).
Scheme 1.2. Stability of aryl diazonium salt through resonance structure (top). Immediate decomposition of an unstable alkyl diazonium (bottom).

**Aryl Diazonium**

The general accepted mechanism of electrochemical grafting of aryl diazonium salts onto electrodes proceed via the generation of an aryl radical and loss of molecular nitrogen. The aryl radical then attacks the electrode surface forming a covalent bond. This general mechanism is shown in Scheme 1.3.

Three important details should be acknowledged concerning the general accepted mechanism. The first is the generation of the aryl radical, which can either proceed via inner electron transfer or outer electron transfer. Efforts have been made to demonstrate this as an outer sphere mechanism. There have also been counter arguments that challenge outer-sphere electron transfer as a solitary pathway. Overall the mechanism is dependent on the factors such as the nature of the reducing agent and less dependent on the aryldiazonium itself. The influence of an inner sphere mechanism may become important in determining the source of the diazo linkages formed directly on a surface (see chapter 4).
Scheme 1.3. Mechanism of diazonium grafting. Growth of the film can be spontaneous or by electron transfer. The scheme shows possible pathways of the diazo linkage directly on the film (left side product).

The second detail is the heterolytic dediazonation to form the aryl cation. Electrodes can attack both an aryl cation forming the phenyl film as well as attack the diazonium forming the diazo linkage. This heterolytic dediazonation is studied in detail for the spontaneous grafting of aryl diazonium salts as this is the preferred route of thin film formation in the absence of an electron donor. In the case of electrochemical reduction, it is most certainly the aryl radical formed (as evidence form the current enhancement from the cyclic voltammogram), but it is important to realize the heterolytic dediazonation may still be happening.
The third detail is the multilayer film growth. The growth is generated through a radical chain reaction which forms multilayers, often thick enough to passivate the electrode surface. Also contributing to multilayer film growth is diazo linkages.

These details taken together with the general accepted mechanism of diazonium grafting on electrode surfaces give a more complete picture to the mechanism of diazo linkages and film growth.

1.3.2 Experimental Evidence of a Covalent Bond Between the Substrate and the Aryl ring

It is now accepted that the electrochemical reduction of aryl diazonium salts form covalent bonds with the electrode substrate. Before analytical evidence, this idea was formulated from the stability of the films after successive treatments in various solvents and sonication procedures. Aryl diazonium salts can be synthesized with specific meta, ortho, or para substituents that are tailored to a specific characterization technique. 4-nitrophenyl diazonium is a historic example in which following immobilization, the nitro group is detection by XPS. The presence of a well resolved nitrogen peak at 406 eV is observed and attributed to the nitrogen in nitrate. Nitrate was also detected for immobilized 4-nitrophenyl diazonium by polarization modulation IR reflection spectroscopy. After immobilization two vibrations are observed that match the nitro group. It must be noted that the evidence of the para substituent is not direct evidence of a covalent bond between the phenyl ring and the substrate, as the thin films could be physically bound. Crafty experiments have been devised to confirm the presence of the substrate-C bond. 4-nitrophenyl diazonium also contains a redox active handle: the nitro group. This reduction of NO$_2$ to NH$_2$ was observed for electrochemically deposited 4-nitrophenyl diazonium on carbon
substrates in electrolyte. The stability of the NO$_2$ on the films is clearer evidence of a covalent bond as a physisorbed film could delaminate at such reducing potentials.$^{44}$ It was also shown by auger spectroscopy the presence of the NO$_2$ group on HOPG after electrochemical deposition with 4-nitrophenyl diazonium. Again, the appearance of the para substituent, NO$_2$, is not enough evidence to conclude a covalent bond, but the stability of the peak past 700 °K under vacuum that could not be removed until temperatures of 1400 °C is stronger evidence of a strong interaction between the film and substrate.$^{43}$ Another account observed the C-C vibrational stretch of the immobilized phenyl-substrate by raman spectroscopy at 1240-1280 cm$^{-1}$. Highly oxidative potentials of 1.8 V vs. Ag/AgCl for carbon$^{46}$ and 2.2 V vs. Ag/AgCl for ITO$^{47}$ were recorded for the stripping of the phenyl film after deposition also signifying a strong bond. A peak was found using surface enhanced raman scattering at 412 cm$^{-1}$ corresponding to a Au-C bond. This Au-C band was also observed by HREELS and predicted from Gaussian calculations.$^{48}$ Direct evidence of the covalent nature of the aryl group and the substrate were found by XPS for iron$^{49}$ and copper oxide$^{50}$ electrodes.

1.3.3 **Drawbacks and Limitations**

Electrochemical film growth on substrates is dependent on the substitution on the aryl diazonium salt, sometimes causing multilayer formation which poisons the electrode.$^{39-3940,51-52}$ As such, targeting monolayer formation for new systems requires time by varying the electrochemical deposition parameters and characterizing the film thickness.$^{30}$ Skillfully synthesized diazoniums have been made that provoke monolayer formation through steric congestion. This has been accomplished with 3,5-di-$\text{-}tert$-butylaryldiazonium,$^{53}$ trimethylsiyl and triethylsiyl-ethynyl-aryldiazonium
salt\textsuperscript{54}, tri(isopropyl)silyl-ethynyl-aryl diazonium salt,\textsuperscript{55} and triisopropylsilyl-protected benzylid hydroxyl-aryl diazonium salt\textsuperscript{56} The siyl series has the advantage of incorporating functionalities that can be exposed after deprotection of the siyl group. The most relevant of these is the deprotection of triisopropylsilyl from the ethynyl-aryl diazonium to afford a monolayer of terminal alkynes. This is shown in Scheme 1.4 and is the platform for much of my work (chapters 2, 3 and 4).

Scheme 1.4. Electrochemical grafting of triisopropylsilyl protected diazonium; A monolayer of terminal alkynes are exposed after deprotection of the TIPS group.

1.3.4 Electrodes Typically Modified with Aryl Diazonium Salts

It seems that aryl diazonium salts are a ubiquitous class of compounds capable of forming thin films with any electrode it is reduced by. Glassy carbon, carbon fibers, carbon powder and HOPG were among the first that exhibited this chemistry.\textsuperscript{42} Since then, metals, nanoparticles, micro-electrode arrays, and semiconductors have joined
the ranks of materials that generate thin films from aryl diazonium salts under reducing conditions.

It is logical to take a step back and ponder the relevance of such a display of diazonium derived thin films. Of course, the substrate becomes important when the application is explicit, in contrast to fundamental research of the thin film’s properties where the identity of the bulk material plays less of a role. Below is a brief description of the electrode materials of interest for electrochemical reduction of diazonium salts. The importance in this section will be highlighted in chapter 3, in which four different carbon based electrodes are subject to diazonium electrochemistry.

After the discovery of the electrochemical reduction of aryl diazonium salts on carbon materials, much work was devoted to discovering the nature of the strong bond it formed with carbon (see section 1.3.2). It was realized that this chemistry applied to semi-conductors as well when Si(111) was modified with a film of phenyl rings from the aryl diazonium precursor.\(^{57}\) The implications of the discovered surface chemistry of H-terminated silicon substrates is important because the phenyl film can protect the silicon substrate from oxide growth and passivation, hence maintaining the conductivity of the material. Iron and steel alloys were next studied, a decade after the discovery of electrochemical aryl diazonium reduction chemistry on carbon.\(^{49,58}\) Modification on iron was an important piece that helped validate the existence of the covalent bond between the aryl group and the substrate, but the film also demonstrated anti-corrosive properties for steel alloys. Soon after it was realized that this chemistry could be extrapolated to many other metals, as was demonstrated with a series of coinage metals, Co, Ni, Cu, Zn, Pt and Au in one paper.\(^{59}\) Very quickly diazonium chemistry was demonstrated on UNCD,\(^{60}\) Si, Pd, GaAr\(^{61}\) and ITO\(^{62}\) as a proof of
concept. Concomitantly, diazonium electrochemistry was being applied on very specific electrode surfaces for precise applications. This included SWCNT, screen printed electrodes, micro electrode arrays, and boron doped diamond for biochemical sensing, high throughput screening, and most recently immobilization of electro-catalysts.63-66

Figure 1.3. Timeline of the evolution of diazonium grafting. The first 15 years contained efforts to expand the diazonium grafting on a variety of electrodes. The last decade has focused on diazonium grafting for specific applications.

Figure 1.3 shows a timeline of the explosion of diazonium grafting highlighting monumental strides. The number of materials used for diazonium chemistry increased from 4 to 18 from 1991 to 2005. The number of materials past
2005 have not been counted in part due to the growth of types of materials. For example, diamond has been studied as UNCD, nano-crystalline, MCD, powders and nanoparticles, all of which have been studied for the grafting aryl diazonium salts\textsuperscript{60,66-68} and a detailed inventory of material class and their subsets with respect to diazonium grafting is beyond the purpose of this section.

1.4 Subsequent Chemistry on an Electrode Surface: Promotion of Electrode Materials from a Non-Polarizable Electrode to an Applicable Device

Many more complex systems have been synthesized to make the electrode an applicable device as a result of the significance of thin films and the development of diazonium electro-grafting. Unfortunately, the plethora of new and more complicated devices that are built from the diazonium platform often are not subject to the same scrutiny as the research devoted to the existence of the substrate-C bond (see section 1.3.2). We have introduced 5 subsequent chemistries onto diazonium functionalized surfaces to create a total of 13 new and unique thin films, giving careful analysis along the way to ensure the molecular architectures are well understood. These 13 thin films are the fabric of this thesis. Of the 13 thin films, 12 are built from the alkyne monolayer shown in Scheme 1.4.

Two of the subsequent reactions (Sonogashira and Glaser cross couplings) have never been introduced onto a surface with a monolayer derived from diazonium electro-grafting. We have further constructed electrode assemblies following the cross coupling to introduce biologically relevant compounds as well as luminophores through sulfhydryl cross linker chemistry. Below is a description of the chemistries introduced to construct the unique thin films and outlined in Scheme 1.5. Figure 1.4
shows the deduced molecular structure of each thin film and the chapter in which their chemistry is described.
Figure 1.4. 13 unique thin films constructed form electrochemical deposition of aryl diazonium salts.
1.4.1 Sonogashira Cross Coupling Chemistry

Traditionally Sonogashira cross coupling chemistry conjoins an aryl halide with a terminal alkyne to afford the aryl alkyne in the presence of palladium and copper catalysts. By terminating an electrode with a layer of alkynes we could use the surface as the alkyne source. Ideally, when performing synthesis, one does so with pure starting material. Synthesis on a surface presents a unique challenge in that the immobilized reactant is never “pure” because of the tethered support. In the most ideal situation the support is completely inert and does not affect the intended chemistry between the immobilized reactant with the solvated molecule to produce the immobilized product. Surfaces are unfortunately (or fortunately, depending on the chemistry) known to be extremely reactive. This reactivity is derived from the discontinuity the atoms experience at the surface relative to the bulk. Only half of the atoms experience the bulk of the film while the other half is dangling with the external environment. It is slightly counterintuitive to think of a surface as a reactant in which one needs a catalyst to proceed; surfaces are classically viewed more often as catalysts themselves. With further understanding of surfaces and their characterization it has become important to develop the methods to construct thin films at the molecular lever. Developing this cross-coupling chemistry on alkyne appended surfaces provides a way in which a molecule can be tethered to a surface if the molecule has an appended aryl halide. A prior attempt at surface Sonogashira cross coupling had been made that involved reacting alkynes with immobilized iodide. The 4-iodophenyl diazonium precursor used was not sterically designed for monolayer formation and their film formation was determined to be 3.4 nm. It should be noted that their analysis of the success of the Sonogashira cross coupling chemistry begins on an ill-defined surface. In addition, by putting iodide on the surface first, any
unreacted iodide atoms now become a source of iodide contamination, which could be disadvantageous to specific kinds of chemistry.

1.4.2 Glaser Cross Coupling Chemistry

Glaser cross coupling chemistry connects two alkynes together in the presence of oxygen and copper by forming the alkynyl radical. Two alkynyl radicals react with each other to form the di-alkynyl product.\textsuperscript{72,73} In contrast to work that studies surfaces as the catalyst to facilitate Glaser cross coupling in ultra-high vacuum at 78 K,\textsuperscript{74} our chemistry using the surface as the reactant producing a di-alkynyl thin film product.

1.4.3 Copper Catalyzed Azide-Alkyne Cycloaddition (CuAAC)

CuAAC chemistry joins an alkyne (terminal or internal) with an azide to form a regioselective 1-4 triazole ring product.\textsuperscript{75,76} This chemistry can be performed in the absence of a copper catalyst, but with loss of the regioselectivity, forming the 1,4 and 1,5 triazole product.

Surface CuAAC was not a new concept at the time we began our work.\textsuperscript{55,77-83} It was still important for us to incorporate a method that has been known in the literature to make valuable comparison between the Sonogashira and Glaser surface cross couplings. Surface CuAAC chemistry has been achieved on carbon based electrodes by means of introducing an azide group on the surface from IN\textsubscript{3}\textsuperscript{78,81,83} but IN\textsubscript{3} is explosive and must be handled with caution, providing an obstacle for this method. Azide groups have been developed on surfaces by Hamers starting from a hydrogen terminated diamond surface requiring 4 steps that vary in complexity.\textsuperscript{84,85} This is an inconvenient method compared to the one step procedure for IN\textsubscript{3}. Monolayers of azides of course could be formed on gold substrates by means of an
alkyl thiol appended azides\textsuperscript{77} or on glass by means of an azido trimethoxysilane\textsuperscript{80} but monolayers of CuAAC products from diazonium electro-grafting wasn’t achieved until Hapiot’s demonstrated it from a monolayer of terminal alkynes.\textsuperscript{55} A prior report of diazonium grafting of alkynes using alkynyl-aryldiazonium was reported but no precaution was given to prevent multilayer growth through steric design.\textsuperscript{79} We used Hapiot’s work as our platform to synthesize thin film \textbf{CP5}, which only differs from Hapiot’s thin film by electrode type (Hapiot’s electrode was glassy carbon and PPF, our work uses carbon paper).

\subsection*{1.4.4 Thiol-based Cross Linker Chemistry}

Surface chemistry with thiols represent an avenue to introduce biological materials on surfaces. A cysteine is an amino acid with a free thiol group so that in understanding and demonstrating thiol cross linker chemistry on surfaces, we can exploit the same chemistry of a cysteine onto a surface. Immobilization of cysteines show that immobilization of more complex biomolecules is possible through cross linkage of the cysteines. Biologically relevant systems are important in sensors and thiols certainly provide a roadmap to successfully immobilize such molecules. Even though this is one demonstration of thiol-based cross-linker chemistry, thiol reactions can also participate in many other types of chemistry such as a thiol-yne reactions which produced surface \textbf{CP11}. We have also used thiol-based crosslinker chemistry to immobilized a ruthenium based luminophore to achieve an immobilized ECL device (surface \textbf{CP10} and \textbf{CP11}).
1.4.5 EDC/NHS Chemistry

EDC/NHS chemistry joins a carboxylate and amine to form an imide bond through an EDC/NHS intermediate. EDC/NHS coupling has been utilized so much to immobilize biomolecules onto surfaces or nanoparticles that it has been referred to as a “classical” bioconjugation method.\(^{86}\) Individual reports of surface functionalization using EDC/NHS chemistry are beyond the scope of this section and several reviews and textbooks have dedicated specific sections to this method.\(^{87-89}\)

The advantages to using EDC/NHS for surface immobilization include the ease in conjugation, water solubility of the EDC making it compatible with biological systems and the absence of additional functionalities between the two cross linked reagents (dubbing EDC/NHS as a “zero cross-linker”). Not only is EDC/NHS chemistry used often, but the overuse of this method has even been acknowledged as referenced to as a “shotgun” method. The emphasis of this phrase suggests that EDC/NHS chemistry is sometimes an accepted route to bioconjugation with less thought of the drawbacks that could potentially interfere with the surface chemistry that derive from the actual mechanism.\(^{90}\)

The first step in forming the imide bond is the formation of an \(o\)-acylisourea intermediate. The EDC is protonated at one of the diamines and forms a carbocation which is immediately attacked by the carboxylate to form \(o\)-acylisourea. The optimal pH for this step is between 4-6 as the pH should favor deprotonation of the carboxylic acid group and favor protonation of the EDC. The \(o\)-acylisourea is short lived and prone to hydrolysis to form the urea product and the carboxylic acid.\(^{91}\) To help prevent this fast hydrolysis, NHS can be added which is still prone to hydrolysis, but at a much slower rate than the \(o\)-acylisourea.\(^{92}\) Lastly, the amine is either added in a next step or is present in a one-step synthesis. The EDC/NHS coupling has been
successful either way, but the advantage to doing a 2-step synthesis is the ability to change the pH to the optimal 7-8 in which the amine exists as the free amine. In a one pot synthesis, in which the pH is between 4-6, the equilibrium favors the protonated conjugate acid over the free amine. While this manifest itself in a slowed final step it does has the advantage that the protonated amine is positively charged which can preconcentrate around the negatively charged carboxylate groups which has been noted as an important step in high yielding EDC/NHS surface coupling.⁸⁸
Scheme 1.5. Chemistries applied to diazonium modified substrates. $R_{\text{sur}}$ denotes the location of the surface with respect to the functional group and final immobilized product.

**Sonogashira Cross Coupling (Section 1.4.1)**

$$
\begin{align*}
R_1\text{-}X + \equiv R_{\text{sur}} & \xrightarrow{\text{Pd, Cu co-catalysts}} & R_1\equiv R_{\text{sur}}
\end{align*}
$$

**Glaser Cross Coupling (Section 1.4.2)**

$$
\begin{align*}
R_1\equiv & \quad \equiv R_{\text{sur}} & \xrightarrow{\text{Cu catalyst}} & \equiv R_{\text{sur}}
\end{align*}
$$

**CuAAC (Section 1.4.3)**

$$
\begin{align*}
N_3 R_1 \equiv R_{\text{sur}} & \xrightarrow{\text{Cu catalyst}} & \equiv R_{\text{sur}}
\end{align*}
$$

**Thiol Cross-linking (Section 1.4.4)**

$$
\begin{align*}
R_1\text{-}SH + \text{succinimidyl carbonate} & \xrightarrow{\text{EDC/NHS}} & \text{succinimidyl carbonate}
\end{align*}
$$

**EDC/NHS Cross-linking (Section 1.4.5)**

$$
\begin{align*}
R_{\text{sur}}\text{-}OH + R_1\text{-}N\equiv & \xrightarrow{\text{EDC/NHS}} & R_{\text{sur}}\text{-}N\equiv R_1
\end{align*}
$$
1.5 Conclusion

The following chapters will discuss the synthesis, characterization and applications of the thin films shown in Figure 1.4. The thin films are introduced on electrode assemblies through electrochemical reduction of aryl diazonium salts followed by various chemistries summarized in section 1.4.

Chapter 2 will compare varying molecular wires on carbon paper electrodes attained through different cross coupling chemistries. Electron mobility throughout the different molecular constructs are compared. Chapter 3 compares identical molecular linkers on 4 different electrode platforms and discusses at length the variability in the reactivity as a function of the environment of the surface. Chapter 4 extends the concept of surface Sonogashira cross coupling to introduce a new moiety, the maleimide functional group, which can further react with thiol derivatized compounds for specific applications. Finally, chapter 5 will discuss the immobilization of DNA and the ability to bind transcription factors. This is done with the intention to tag proteins with a luminophore and exploit electrochemiluminescence as a new sensing technique to screen for small molecule antagonists.
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Chapter 2

DEVELOPMENT OF CROSS COUPLING CHEMISTRY ON CARBON PAPER ELECTRODES TO INCLUDE SONOGASHIRA, GLASER AND CUAAC

2.1 Introduction

The ability to control charge transport between conducting substrates and discrete redox sites is of critical importance to the development of electrochemical energy storing architectures such as lithium batteries,\textsuperscript{1} solar water splitting assemblies\textsuperscript{2-5} and fuel cell devices.\textsuperscript{6,7} Furthermore, controlling the molecular topology of electrode–catalyst interfaces is a critical factor in engineering devices with specific electron transport kinetics and catalytic efficiencies. An indication of the importance of optimized interfacial charge transfer to a system’s energy conversion efficiency has been demonstrated by Kubiak and Mayer who have shown that the current density for the photoelectrochemical reduction of CO\textsubscript{2} at a styrene-modified p-Si electrode in the presence of a Re(I)bipyridine complex is enhanced roughly threefold compared to the same process at a hydrogen or hexane terminated p-Si electrode.\textsuperscript{8,9} Further indication that the properties of an electrode surface need to be addressed when developing artificial photosynthetic systems is illustrated by recent studies showing DSC photocurrent is sensitive to topological variations at the semiconductor interface.\textsuperscript{10-13} Interfacial charge transport is also fundamentally important to the development of hybrid biomolecule-electrode assemblies designed for energy conversion\textsuperscript{14-18} and sensing applications.\textsuperscript{19,20}
Various strategies have been employed to tailor the molecular topology and connectivity of electrode interfaces. A common method used to modify conducting substrates involves the formation of thiol-based self-assembled monolayers (SAMs) to which redox active moieties can be anchored.\textsuperscript{21} This approach is confronted by several practical limitations, however, as it is typically only applicable for modification of expensive gold substrates and produces modified electrodes with relatively low conductivities\textsuperscript{22} and poor thermal\textsuperscript{23} and oxidative stabilities.\textsuperscript{23-25}

Another popular method for electrode modification is the arylation of conducting surfaces via the electrochemical reduction of aryl diazonium salts.\textsuperscript{26,27} This approach can be used to modify a variety of conducting supports. Electrochemical generation of aryl radicals at a glassy carbon (GC) electrode leads to formation of strong C—C bonds between the aryl group and carbon surface to furnish robust modified electrodes. There are, however, limitations associated with this general approach that compromise its utility in producing functionally modified electrodes. For example, preparation of the requisite diazonium appended synthons requires strongly oxidizing and/or acidic conditions, which are generally incompatible with complex arenes and catalysts for molecular energy conversion applications. Moreover, the highly reactive aryl radicals formed upon reduction of the diazonium-appended catalyst can participate in undesirable side reactions and contribute to catalyst decomposition. Equally problematic is the fact that side reactions between the aryl radical intermediates and arenes already grafted onto the conducting substrate can lead to formation of a disordered tangle of arene polymers with ill-defined molecular topologies and electronic properties.\textsuperscript{28} The longer and more complex the diazonium derivative used to functionalize the surface, the more side reactions are possible and
the greater the likelihood of polylayer formation. Polylayers of this type are not ideal for controlling charge transfer at the modified electrode since the molecular connectivity and electron-transfer pathways are ill-defined.\textsuperscript{28} As such, the development of new methods for the production of robust functionalized electrodes with uniform/well-defined pathways for electron transfer to surface appended redox sites are needed.

It has demonstrated that aryl diazoniums with properly positioned steric bulk can be cleanly grafted onto carbon or copper electrodes to generate precise surface appended monolayers.\textsuperscript{29} For example, electrochemical reduction of 3,5-di-\textit{tert}-butylphenyl diazonium at carbon does not generate polyarenes, as the \textit{tert}-butyl substituents protect the initially grafted layer from subsequent arylation.\textsuperscript{30} Similarly, Hapiot and coworkers have demonstrated the electrodeposition of a monolayer of a TIPS-protected ethynyl benzene diazonium onto carbon. In this case, the large footprint of the triisopropylsilyl substituent precludes formation of polyarene tangles, as observed for less sterically demanding systems. Moreover, it was shown that these monolayers could be synthetically modified via a copper catalyzed azide alkyne cycloaddition (CuAAC),\textsuperscript{31-33} following removal of the terminal TIPS groups. Although only this reactivity has been demonstrated for this system, we rationalized that this general method for presentation of a terminal alkyne monolayer on conducting surfaces, could provide a versatile platform for several cross-coupling chemistries and allow for the modular construction of tailorable electrode surfaces with robust molecular wires (MWs) that exhibit well-defined molecular topologies, conductivities and morphologies.\textsuperscript{34}
In efforts to develop modular and efficient methods for the preparation of such architectures we have sought to establish a modular on-surface metal mediated cross coupling chemistry to synthetically install arrays of MWs onto inexpensive conducting supports. To this end, we have developed on-surface variations of Sonogashira\textsuperscript{35,36} and Glaser\textsuperscript{37,38} cross couplings reactions for modification of conducting substrates. Herein we describe the development and utility of these methods for fabrication of novel surface modified assemblies and demonstrate that these systems have notable electronic properties that are distinguished from more commonly employed surface modification chemistries such as (CuAAC) reactions and non-specific small molecule adsorption.

2.2 Materials and Methods

2.2.1 General Considerations

Reactions were performed in oven-dried round-bottomed flasks unless otherwise noted. Reactions that required an inert atmosphere were conducted under a positive pressure of N\textsubscript{2} using flasks fitted with Suba-Seal rubber septa or in a nitrogen filled glove box. Solvents for synthesis were of reagent grade or better and were dried by passage through activated alumina and then stored over 4 Å molecular sieves prior to use. Surface deposition and surface cross-coupling reactions (except for Glaser coupling) were carried out in a nitrogen-filled glovebox. All glassware was oven dried for a minimum of two hours or flame dried under vacuum prior to use. Reagents including 4-((triisopropylsilyl)ethynyl)aniline,\textsuperscript{39} 4-((triisopropylsilyl)ethynyl)arylazonium tetrafluoroborate\textsuperscript{40} (2.1), and 4-iodophenylferrocene\textsuperscript{41} (2.2) were synthesized as reported in the literature.
Tetrabutylammonium hexafluorophosphate (TBAPF₆) was purchased from TCI. TBAPF₆ was recrystallized from hot ethanol and dried in vacuum overnight prior to use. All other substances and reagents were obtained from commercial sources and used as received. Carbon paper was purchased from Fuel Cell Earth LLC and stored under air. Before modification, the paper was cut into 15mm x 25mm rectangles and transferred into a nitrogen-filled glovebox, unless otherwise noted. “Double manifold” refers to a standard Schlenk-line gas manifold equipped with nitrogen and vacuum (ca. 100 mtorr).

2.2.2 Synthesis of Ferrocene Homologs

Ferrocene homologs were synthesized and characterized by Dr. Amber Gietter-Burch. Her synthetic protocol are as follows:

Sonogashira Homolog Fc1 - A hot 1-dram vial equipped with a magnetic stir bar and a Teflon-lined septum cap was attached to a double manifold using a syringe needle and cooled under vacuum. The vial was backfilled with nitrogen, the cap was removed, and copper iodide (1.8 mg, 9.7 μmol), tetrakis(triphenylphosphine)palladium (11.2 mg, 9.7 μmol), and 4-iodophenylferrocene (2) (47 mg, 121 μmol) were added. The septum was replaced, the vial was reattached to the double manifold, and evacuated and backfilled with nitrogen three times. Dry, degassed triethylamine (51 μL), anhydrous degassed dimethylformamide (2.4 mL), and phenylacetylene (27 μL, 242 μmol) were added via syringe. The reaction was heated in an oil bath to 80°C for 15 h. The reaction was cooled to rt, diluted with dichloromethane (10 mL), and extracted twice with saturated aqueous NaCl (10 mL). The organic layer was dried with magnesium sulfate and concentrated in vacuo. The crude reaction was purified by column chromatography (1% EtOAc: hexanes) to afford Fc1 (37 mg, 84%) as an
orange-red solid: 1H NMR (400 MHz, CDCl3) δ 7.56 - 7.52 (m, 2H), 7.45 (s, 4H), 7.38 - 7.32 (m, 3H), 4.67 (t, J = 1.9 Hz, 2H), 4.36 (t, J = 1.9 Hz, 2H), 4.05 (s, 5H); 13C NMR (101 MHz, CDCl3) δ 140.0, 131.8, 131.7, 128.5, 128.3, 126.0, 123.6, 120.5, 89.9, 89.5, 84.4, 69.9, 69.5, 66.7; FTIR (cm-1) 3080, 1595, 1526, 843, 820, 755, 688; APCI 362.1 (M)+; HRMS (EI+) m/z, calculated for [C24H18Fe]+: 362.0758; found: 362.0763.

Glaser Homolog Fe2 - This compound was prepared by an amended literature procedure. To a 10 mL round bottom flask equipped with a magnetic stir bar was added copper chloride (4.5 mg, 45 μmol), alkynyl ferrocene 3 (28.5 mg, 0.100 mmol), pyridine (4 mL), and phenyl acetylene (22 μL, 0.200 mmol). The reaction was heated in an oil bath to 60 °C for 3 h. After cooling to rt, the reaction was concentrated in vacuo. A saturated solution of NH4Cl (7 mL) was added and the aqueous layer was extracted twice with CH2Cl2 (7 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The crude reaction was purified by column chromatography (1% CH2Cl2: hexanes) to afford Fe2 (18.9mg, 49%) as an orange solid: 1H NMR (400 MHz, CDCl3) δ 7.54 (dd, J = 7.8, 1.7 Hz, 2H), 7.44 (d, J = 1.2 Hz, 2H), 7.39 - 7.31 (m, 3H), 4.66 (t, J = 1.9 Hz, 2H), 4.37 (t, J = 1.8 Hz, 2H), 4.04 (s, 5H); 13C NMR (101 MHz, CDCl3) δ 141.3, 132.7, 132.6, 129.3, 128.6, 126.0, 122.1, 118.8, 84.0, 82.3, 81.7, 74.3, 74.0, 69.9, 69.7, 66.8, 58.5; FTIR (cm-1): 3091, 2919, 2213, 1523, 1486, 822, 814, 756, 691; APCI 386.1 (M)+; HRMS (EI+) m/z, calculated for [C26H18Fe]+: 386.0758; found: 386.0752.

Copper Catalyzed Azide Alkyne Cycloaddition (CuAAC) Homolog Fe3 - A hot 25 mL Schlenk flask equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. The flask was backfilled with
nitrogen, the septum was removed, and copper sulfate pentahydrate (4.0 mg, 15.8 μmol), ascorbic acid (4.2 mg, 23.7 μmol), and azide 4 (50.0 mg, 158 μmol) were added. The septum was replaced, the flask was reattached to the double manifold, and evacuated and backfilled with nitrogen three times. Anhydrous dimethylformamide (3.16 mL) and phenylacetylene (87.0 μL, 0.788 mmol) were added and the reaction was stirred at rt for 12 h. The septum was removed and CH2Cl2 (5 mL) was added. The reaction was washed twice with brine (10 mL) and the organic layer was dried over magnesium sulfate and concentrated in vacuo to afford Fc3 (61.9 mg, 93%) as an orange solid: 1H NMR (400 MHz, CDCl3) δ 7.82 (d, J = 7.2 Hz, 2H), 7.70 (s, 1H), 7.48 (d, J = 8.2 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.36 - 7.29 (m, 1H), 7.23 (d, J = 8.2 Hz, 2H), 5.55 (s, 2H), 4.63 (t, J = 1.8 Hz, 2H), 4.33 (t, J = 1.8 Hz, 2H), 4.04 (s, 5H); 13C NMR (101 MHz, CDCl3) δ 148.4, 140.4, 132.1, 130.7, 129.0, 128.3, 126.8, 125.8, 119.6, 84.4, 69.8, 69.4, 66.7, 54.2; FTIR (cm⁻¹): 3118, 3091, 294, 1527, 1466, 817, 786, 694; APCI: 419.1 (M)+, 275.0 (M-C8H6N3); HRMS (EI+) m/z, calculated for [C25H21FeN3]+: 419.1085; found: 419.1078.

2.2.3 Synthesis of Ferrocene Appended Cross Coupling Reagents

Ferrocene cross coupling reagents and their precursors were synthesized and characterized by Dr. Amber Gietter-Burch. Her synthetic protocol are as follows

*TMS protected alkylnylphenylferrocene 2.3a* - A hot 50 mL Schlenk flask equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. The flask was backfilled with nitrogen, the septum was removed, and iodophenylferrocene (1.00 g, 2.58 mmol), copper (I) iodide (6.4 mg, 33.6 μmol), and bis(triphenylphosphine) palladium(II) dichloride (18.2 mg, 25.8 μmol) were added. The septum was replaced, the flask was reattached to the
double manifold, and evacuated and backfilled with nitrogen three times. Dry, degassed triethylamine (26 mL) and ethynyltrimethylsilane (1.10 mL, 7.73 mmol) were sequentially added using standard syringe technique. The reaction was heated to 55°C in an oil bath for 12 h. After the allotted time, the reaction was cooled to rt and opened to air. The reaction was diluted with dichloromethane (25 mL), transferred to a separatory funnel and washed twice with water (50 mL). The organic layer was dried with magnesium sulfate, filtered, and concentrated in vacuo. The crude reaction mixture was purified by column chromatography (10% CH2Cl2 in hexanes) to afford 1.3a (94%, 867 mg) as an orange solid: 1H NMR (400 MHz, CDCl3) δ 7.39 (apparent s, 4H), 4.65 (t, J = 1.8 Hz, 2H), 4.34 (t, J = 1.8 Hz, 2H), 4.01 (s, 5H), 0.26 (s, 9H); 13C NMR (101 MHz, CDCl3) δ 140.2, 132.1, 125.8, 120.3, 105.6, 94.2, 84.2, 69.9, 69.5, 66.6, 0.2; FTIR (cm⁻¹): 3085, 2958, 2678, 2153, 1524, 1250, 868, 843; GC/MS (Cl) 358.2 (M)+, 343.2 (M-Me)+; HRMS (EI+) m/z, calculated for [C21H22FeSi]+: 358.0840; found: 358.0832.

Alkynylphenylferrocene 2.3 - To a 200 mL round bottom flask equipped with a magnetic stir bar was added TMS alkyne 2.3a (867 mg, 2.42 mmol), dichloromethane (7.8 mL), methanol (89 mL), and [1M] KOH (30 mL). The reaction was sealed with a polyethylene cap and stirred at rt for 18 h. Upon completion, the cap was removed, and the reaction was diluted with dichloromethane (30 mL). The reaction was washed once with water (25 mL) and the aqueous layer was extracted three times with dichloromethane (20 mL) until no orange color remained. The organic layer was dried with magnesium sulfate and concentrated in vacuo to afford alkyne 1.3 (99%, 691 mg) as a dark orange solid: 1H NMR (400 MHz, CDCl3) δ 7.41 (apparent s, 4H), 4.65 (t, J = 1.9 Hz, 2H), 4.35 (t, J = 1.9 Hz, 2H), 4.03 (s, 5H), 3.11 (s, 1H); 13C NMR (101
Benzoic acid 4-ferrocenyl-, ethyl ester 1.4a- A hot 200 mL Schlenk flask equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. The flask was backfilled with nitrogen, the septum was removed, and palladium (II) acetate (29.3 mg, 131 μmol), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos, 107 mg, 261 μmol), K3PO4 • H2O (6.01 g, 26.1 mmol), and ferrocene boronic acid (3.06 g, 13.3 mmol) were added. The septum was replaced, the flask was reattached to the double manifold, and evacuated and backfilled with nitrogen three times. Anhydrous toluene (89 mL) was added via syringe and the reaction was heated to 100°C in an oil bath. Once the reaction reached 100°C, ethyl 4-bromobenzoate (2.13 mL, 13.1 mmol) was added via syringe. The reaction remained at 100°C for 24 h. Once complete, the septum was removed and the reaction was cooled to rt and diethyl ether (50 mL) was added. The reaction was flushed through a silica gel plug to remove solids and concentrated in vacuo. The crude reaction mixture was purified by column chromatography (10% diethyl ether in hexanes) to afford ester 1.4a (86%, 3.75 g) as an orange solid: 1H NMR (400 MHz, CDCl3) δ 7.96 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 4.71 (t, J = 1.8 Hz, 2H), 4.41 – 4.34 (m, 4H), 4.03 (s, 5H), 1.40 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 166.8, 145.1, 129.8, 127.8, 125.7, 83.5, 70.0, 69.9, 67.0, 61.0, 14.6; FTIR (cm-1): 3106, 2979, 1704, 1608, 1278, 832, 814; GC/MS (CI) 334.1 (M)+, 306.1 (M-C2H5)+; HRMS (EI+) m/z, calculated for [C19H18FeO2]+: 334.0656; found: 334.0650.
**4-ferrocenylbenzyl alcohol 2.4b** - A hot, dry 200 mL round bottom flask equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. The flask was backfilled with nitrogen, the septum was removed, and ester 2.4a (3.70 g, 11.1 mmol) was added. The septum was replaced, the flask was reattached to the double manifold, and evacuated and backfilled with nitrogen three times. Anhydrous toluene (111 mL) was added via syringe and the reaction was cooled to –78°C. Once cool, DIBAL (1.2M in toluene, 36.9 mL) was slowly added. The reaction was allowed to slowly warm to rt over 6 h. The reaction was re-cooled to 0°C and sodium sulfate decahydrate (2.00 g) was added and the slurry was allowed to stir for 1 h after which the reaction was filtered and concentrated in vacuo to afford alcohol 2.4b (2.82 g, 87%) as an orange solid: 1H NMR (400 MHz, CDCl3) δ 7.48 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.2 Hz, 2H), 4.67 (d, J = 5.9 Hz, 2H), 4.64 (t, J = 1.9 Hz, 2H), 4.32 (t, J = 1.9 Hz, 2H), 4.04 (s, 5H), 1.63 (t, J = 5.9 Hz, 1H); 13C NMR (101 MHz, CDCl3) δ 139.0, 138.5, 127.4, 126.4, 85.2, 69.7, 69.1, 66.6, 65.5; FTIR (cm⁻¹): 3314, 2936, 2872, 1527, 1424; APCI: 292.0 (M)+, 275.0 (M-OH)+; HRMS (EI+) m/z, calculated for [C17H16FeO]+: 292.0551; found: 292.0558

**4-ferrocenylbenzyl chloride 2.4c** - A hot, dry 25 mL round bottom flask equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. The flask was backfilled with nitrogen, the septum was removed, and alcohol 2.4b (500 mg, 1.71 mmol) was added. The septum was replaced, the flask was reattached to the double manifold, and evacuated and backfilled with nitrogen three times. Anhydrous CH2Cl2 (10 mL) was added via syringe and the reaction was cooled in an ice/NaCl bath to -10°C. Dry, degassed
triethylamine (523 μL, 3.75 mmol) and methanesulfonyl chloride (291 μL, 3.75 mmol) were added via syringe. The reaction was allowed to warm to rt and remain at rt for 12 h. The septum was removed and crude reaction was concentrated in vacuo and purified by column chromatography (1:1 CH2Cl2: hexanes) to afford chloride 1.4c (405 mg, 76%) as an orange solid: 1H NMR (400 MHz, CDCl3) δ 7.46 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 4.64 (t, J = 1.8 Hz, 2H), 4.59 (s, 2H), 4.33 (t, J = 1.8 Hz, 2H), 4.04 (s, 5H); 13C NMR (101 MHz, CDCl3) δ 139.9, 135.0, 128.8, 126.5, 84.8, 69.8, 69.3, 66.7, 46.5; FTIR (cm⁻¹): 3094, 1914, 1608, 1525, 1266, 1105, 842, 819; APCI 310.0 (M)+, 275 (M-Cl)+, 306 (M-Cl+OMe)+; HRMS (EI+) m/z, calculated for [C17H15FeCl]+: 310.0213; found: 310.0205.

4-ferrocenylbenzyl azide 2.4 - To a 50 mL round bottom flask equipped with a magnetic stir bar and fitted with a reflux condenser was added chloride 2.4c (405 mg, 1.30 mmol), sodium azide (211 mg, 3.25 mmol), and dimethylformamide (13 mL). The reaction was heated to 70°C for 15 h in an oil bath. Once complete, the reaction was cooled to rt and dichloromethane (15 mL) was added. The solution was extracted three times with brine (15 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude reaction was purified by column chromatography (2:1 hexanes: CH2Cl2) to afford azide 1.4 (371 mg, 90%) as a yellow solid: 1H NMR (400 MHz, CDCl3) δ 7.49 (d, J = .3 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 4.65 (t, J = 1.9 Hz, 2H), 4.34 – 4.30 (m, 4H), 4.04 (s, 5H); 13C NMR (101 MHz, CDCl3) δ 139.8, 132.9, 128.5, 126.6, 84.7, 69.8, 69.2, 66.7, 54.9; FTIR (cm⁻¹): 3083, 2924, 2850, 2189, 2097, 1652, 1559, 818; APCI: 317.2 (M)+; HRMS (EI+) m/z, calculated for [C17H15FeN3]+: 317.0615; found: 317.0609
2.2.4 Instrumentation

2.2.4.1 Electrochemistry

All electrochemistry was performed using either a CHI-620D potentiostat/galvanostat, a CHI-760D bipotentiostat, or a BAS CV-50 potentiostat. Cyclic voltammetry was performed in a N₂ or Ar filled glove box using a standard three-electrode configuration. Cyclic voltammograms for Fc₁ – Fc₃ were recorded for quiescent solutions using a glassy carbon working disk electrode (3.0 mm diameter), a platinum wire auxiliary electrode and a Ag/AgNO₃ reference electrode. Unless otherwise noted, CV scans were recorded for quiescent solutions using a modified carbon paper, a platinum gauge auxiliary electrode and a Ag/AgNO₃ reference electrode. All CV experiments were performed using dry acetonitrile containing 0.1 M TBAPF₆ as the supporting electrolyte.

2.2.4.1.1 Laviron Analysis

CV analysis of modified carbon substrates were initially carried out using a scan rate of 50 mV/s with a sensitivity of 100 μA/V. Laviron plots were constructed by measuring the variation in anodic (Eₐ) and cathodic (Eₖ) peak potential as a function of scan rate. Scan rates were varied as follows: 8, 10, 15, 20, 30, 50, 80, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, and 4000 mV/s. Laviron’s formalism was applied by plotting $E_c - E_{1/2}$ and $E_a - E_{1/2}$, where $E_{1/2} = (E_a + E_c)/2$, as a function of log of scan rate.⁴² The electron transfer rate constant (ETRC) was determined for CP₃ – CP₅ by fitting these data when $E_a - E_c \geq 200$ mV.
2.2.4.1.2 Surface Area Analysis

The active surface area of the carbon paper substrates was assessed by chronocoulometry conducted using a 1.0 mM solution of ferricyanide \( (D = 6.3 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}) \) in Millipore water containing 0.1 M KCl.\(^{43}\)

2.2.4.1.3 Surface Coverage Calculation

The surface coverage, \( \Gamma \), was calculated by using the following equation:

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

where \( Q \) is the total charge passed under the voltammetric peak, \( n \) is the number of electrons transferred (for the reduction of diazonium, \( n=1 \)), \( F \) is faraday’s constant and \( A \) is the geometrical surface area of the electrode.

2.2.4.2 X-Ray Photoelectron Spectroscopy

All XPS spectra were acquired using a VG ESCALAB 220I-XL spectrometer. The X-Rays used were monochromatic Al K\( \alpha \) X-Rays (1486.7eV) with a power of 105W (15 kv, 7 mA). The operating pressure in the main chamber was less than \( 1 \times 10^{-8} \text{ torr} \). The X-Ray spot size was elliptical in shape with a semi-major axis of roughly 400\( \mu \text{m} \).

Initial XPS survey scans were collected at a pass energy of 80 eV using a step size of 0.25 eV. All atomic percentages \( (\chi_i) \) were calculated from the surveys and were determined using the equation:

\[
\chi_i = \frac{A_i}{\Sigma_i A_i/S_i}
\]
where \( A_i \) is the area calculated with a Shirley-type baseline, and \( S_i \) is the relative sensitivity factor. Atomic percentages do not include hydrogen in XPS. The atomic percentages reported are the compiled averages taken from a minimum of five separate spots per each individual carbon substrate sampled, and typically 8 to 10 individual carbon substrates. High-resolution XPS spectra were collected at a pass energy of 20 eV using a step size of 0.1 eV. Reported values for average atomic surface composition are the compiled averages of at least five surface spots from at least eight individual samples (\( n \geq 40 \)).

### 2.2.5 Generation of Alkynyl Modified Carbon Paper Substrates (CP1 and CP2)

Electrochemical grafting and deprotection of diazonium 1.1 on bare carbon paper substrates was carried out by modifying a previously published procedure. Within a nitrogen filled glove box, a piece of carbon paper (~1 × 2 cm) working electrode was clipped to a platinum wire and immersed in 10 mL of a 0.1 M solution of TBAPF₆ in acetonitrile containing 18.6 mg (50 μmol) of diazonium 1.1. A platinum gauze auxiliary and silver wire pseudo reference electrode were also placed within the vial. The electrodeposition was carried out by varying the applied potential between 0.5 and −0.3 V with a scan rate of 50 mV/s. The electrochemical grafting to generate CP1 was generally complete after roughly 5–7 cycles between these potentials. Following the electrochemical grafting, CP1 was rinsed with 8 mL of dry acetonitrile and then soaked in 4 mL of dry acetonitrile for 10 min. This cleaning process was repeated two additional times and the CP1 substrate was then allowed to dry under N₂.

The TIPS protecting groups of CP1 were removed by immersing the modified carbon substrate in 10 mL of acetonitrile within N₂ filled glove box. To the suspended CP1 substrate was added TBAF (13.0 mg, 50 μmol), and the resulting solution was
allowed to stand at room temperature for 2 hr. Following removal of the modified carbon paper with forceps, the alkyne terminated carbon paper (CP2) was rinsed with 8 mL of dry acetonitrile and then soaked in 4 mL of dry acetonitrile for 10 min. This cleaning process was repeated two additional times and the CP2 substrate was then allowed to dry under N₂.

2.2.6 Procedure for on Surface Cross Coupling to Generate CP3

In a nitrogen-filled glove box copper iodide (2.4 mg, 12.6 μmol) tetrakis(triphenylphosphine)palladium(0) (13.8 mg, 12.0 μmol), iodophenylferrocene, 2.2 (58 mg, 150 μmol), triethylamine (63.6 μL, 45.0 μmol), and anhydrous dimethylformamide (3 mL) were added to a 20 mL vial. CP2 with approximate dimensions of 1.0 × 0.5 cm was then added to this solution using forceps. The vial was capped with a polyethylene-lined cap and heated in an aluminum block to 80 °C for 15 min. The reaction solution was then allowed to cool to room temperature and the modified carbon substrate was removed from solution using forceps. The modified substrate was cleaned by pipetting 4 mL of dry acetonitrile onto both faces of the paper in order to remove excess reagents. The washed substrate was then placed in a clean vial and soaked in 8 mL of dry dimethylformamide for 1 hr. Following removal from the DMF with forceps, the carbon paper was washed again with 4 mL of dry acetonitrile and then soaked for an additional our in 8 mL of acetonitrile. Following one final rinse with 4 mL of acetonitrile, the modified CP3 substrate was dried by evaporation under nitrogen for several minutes.
2.2.7 Procedure for on Glaser Cross Coupling to Generate CP4

Copper bromide (2.6 mg, 18.0 μmol), ferrocene derivative 2.3 (21.5 mg, 75 μmol), and pyridine (3 mL) were combined in a 20 mL vial under air. A piece of alkynyl modified carbon paper (CP2) with approximate dimensions of 1.0 × 0.5 cm was then added to this solution using forceps. The vial was loosely sealed with a polyethylene-lined cap and heated in an aluminum block at 60 °C for 3 hrs. After the reaction was cooled to room temperature, the carbon substrate was removed from vial using forceps and was transferred to a nitrogen-filled glovebox. The modified substrate was cleaned by pipetting 4 mL of dry acetonitrile onto both faces of the paper in order to remove excess reagents. The washed substrate was then placed in a clean vial and soaked in 8 mL of dry dimethylformamide for 1 hr. Following removal from the DMF with forceps, the carbon paper was washed again with 4 mL of dry acetonitrile and then soaked for an additional hour in 8 mL of acetonitrile. Following one final rinse with 4 mL of acetonitrile, the modified CP4 substrate was dried by evaporation under nitrogen for several minutes.

2.2.8 Procedure for on Glaser Cross Coupling to Generate CP5

In a nitrogen-filled glovebox, copper sulfate pentahydrate (2.5 mg, 10.0 μmol), ascorbic acid (2.6 mg, 15.0 μmol), ferrocene derivative 2.4 (31.7 mg, 0.100 mmol) and dry dimethylformamide (2 mL) were combined in a 20 mL vial. A piece of alkynyl modified carbon paper (CP2) with approximate dimensions of 1.0 × 0.5 cm was then added to this solution using forceps and the vial was sealed with a polyethylene-lined cap. The reaction mixture was allowed to stand in the glovebox at room temperature. After 2 hrs, the modified carbon paper was removed from solution using forceps. The modified substrate was cleaned by pipetting 4 mL of dry acetonitrile onto both faces
of the paper in order to remove excess reagents. The washed substrate was then placed in a clean vial and soaked in 8 mL of dry dimethylformamide for 1 hr. Following removal from the DMF with forceps, the carbon paper was washed again with 4 mL of dry acetonitrile and then soaked for an additional hour in 8 mL of acetonitrile. Following one final rinse with 4 mL of acetonitrile, the modified CP5 substrate was dried by evaporation under nitrogen for several minutes.

2.3 Results and Discussion

2.3.1 Electrode Choice – Carbon Paper

We selected fuel-cell grade carbon paper as our electrode-substrate since it is inexpensive, highly conducting, and of commercial interest for the construction of electrochemical energy conversion devices. It was also important to recognize the high number of surface reactions that would be generated in order to maximize screening of appropriate surface reaction conditions. Carbon paper provided us numerous electrodes that could be disposed of cheaply. Carbon paper’s brittleness also served as an advantage. Since carbon paper can be easily broken, we could lacerate small electrode areas for analysis without compromising the entire electrode.

2.3.2 Diazonium Electrochemical Deposition and TIPS Deprotection to Afford a Terminal Alkyne Electrode: CP2

Hapiot and coworkers have demonstrated that a monolayer of sterically bulky aryl diazonium units can be electrochemically grafted onto carbon electrodes. Building on this work we carried out cyclic voltammetry (CV) using a piece of carbon paper as a working electrode for an acetonitrile solution of triisopropylsilyl (TIPS) protected aryl diazonium (1.1) containing 0.1 M TBAPF6. Upon sweeping to negative
potentials, we observed a large, irreversible cathodic current. This response was suppressed upon subsequent CV passes, with stable CV traces emerging after approximately six cycles (Figure 2.1). This CV response is consistent with diazonium reduction and aryl grafting to the carbon paper substrate to generate CP1. The suppression of cathodic current as the number of CV sweeps increases indicates passivation of the carbon paper surface as the bulky TIPS groups form an insulating monolayer that impedes subsequent diazonium reduction processes. Treatment of CP1 with tetrabutylammonium fluoride (TBAF) in dry acetonitrile efficiently removed the silicon protecting groups from the modified carbon substrate, to reveal alkyne-terminated surface CP2 (Figure 2.2). Survey and high resolution XPS analyses were consistent with these proposed surface structures. The XPS data of the TIPS-modified surface of CP1 shows a strong peak attributable to silicon (2p and 2s) at 101 and 152 eV. These features were almost completely absent in alkyne-terminated surface CP2 (Figure 2.2).
Figure 2.1. Repeated CV scans using a carbon paper working electrode in a solution of 1.0 mM diazonium 2.1, containing 0.1 M TBAPF$_6$ (scan rate = 50 mV/s). First sweep shown in black; last sweep shown in red.
Figure 2.2. Survey (left) and high resolution (right) XPS spectra for the silicon 2p region of unmodified carbon paper (black), CP1 (red) and CP2 (blue). The survey spectrum parameters for unmodified carbon paper are 100 eV pass energy and 1.0 eV step size. The survey spectrum parameters for CP1 and CP2 are 80 eV pass energy and 0.25 eV step size.
2.3.3 On-Surface Sonogashira Cross Coupling

2.3.3.1 Homogeneous Model Reactions

Scheme 2.1. Homogeneous reaction conditions for Sonogashira cross coupling (top). Reaction conditions for surface Sonogashira cross coupling on the electrode

With an inexpensive acetylene-terminated electrode substrate in hand, we turned to the use of transition metal-catalyzed cross-coupling reactions for surface modification. We began by examining the on-surface Sonogashira cross-coupling\textsuperscript{44-47} of 4-iodophenylferrocene (1.2) (Scheme 2.1). A ferrocene derivative was selected as a coupling partner, since it provides a convenient handle for both electrochemical and XPS analysis. To carry out the coupling and avoid surface fouling by precipitates, we first identified conditions under which all molecular reagents and catalysts were soluble. Model reactions between phenyl acetylene and 1.2 revealed that the use of catalytic Pd(PPh\textsubscript{3})\textsubscript{4} and CuI in DMF at 80 °C with Et\textsubscript{3}N as base resulted in
homogenous reactions that afforded rapid C–C bond formation. These reaction conditions afforded Fc1 in near quantitative yield as a crystalline orange material. Crystals of Fc1 suitable for X-ray diffraction analysis were grown by slow evaporation of concentrated CH2Cl2 solutions. The solid-state structure of this compound is shown in Figure 2.12 and relevant crystallographic data are summarized in Table 2.2.

2.3.3.2 XPS Analysis of CP3

Using the optimized methods for preparation of Fc1, we found that the CP2 could be easily modified to give the alkyne-bridged surface CP3 as shown in Scheme 2.1. Several independent experiments were conducted to determine optimal reaction time and temperature for this surface modification. XPS analysis showed the highest surface-bound iron content when the reactions were conducted at 80 °C for 15 min. XPS spectra recorded for the modified carbon substrate revealed the presence of surface-bound iron atoms (Figure 2.3). The peak shape and position for the Fe 2p doublet at 708.1 and 720.8 eV are consistent with previously reported ferrocene XPS data.48 Significantly, only trace amounts of copper, palladium, and iodine were observed on the modified surfaces by high resolution XPS (Figure 2.3). In all cases, these elements were detected at significantly lower levels than the signal for iron (Table 2.1), which strongly supports a successful on-surface cross-coupling reaction. Moreover, control reactions in which the Pd and Cu co-catalysts were omitted or using unfunctionalized carbon paper substrates, showed no quantifiable grafting of iron by XPS (Figure 2.4).
Figure 2.3. XPS high resolution spectra for Fe, Cu, I and Pd for CP3 electrodes.

Figure 2.4. XPS High resolution spectra for the Fe 2p$_{3/2}$ peak for CP3 (top in green), CP2 exposed to catalyst free Sonogashira reaction conditions (middle in light gray) and unmodified carbon paper subject to Sonogashira reaction conditions (bottom in black).
Table 2.1. Atomic percentages for CP3 elements

<table>
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<tr>
<th>Element</th>
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</thead>
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<tr>
<td>C 1s</td>
<td>94.3 ± 1.3</td>
</tr>
<tr>
<td>Fe 2p</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td>Cu 2p</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>I 3d</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Pd 3d</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

2.3.3.3 Electrochemistry of CP3

CV analysis carried out for CP3 showed a reversible redox wave attributable to electron transfer from the tethered ferrocene units (Figure 2.5). This feature is absent in both the unmodified carbon paper, as well as on both alkynyl modified substrates lacking a ferrocene moiety (CP1 and CP2). These potentials were measured to be $E_{1/2} \text{CP3} = 0.197 \text{ V vs. Ag/AgNO}_3$. Further evidence that the ferrocenyl moieties of CP3 are chemically linked to the carbon surface is garnered, by noting that these redox potentials are significantly perturbed from those recorded for the analogous small molecule analogues (Fc1). While the Sonogashira modified carbon substrate, CP3 displays a redox wave centered at 0.197 V, the homologous ferrocene monomer has an oxidation potential of $E_{1/2} \text{Fc1} = 0.123 \text{ V versus Ag/AgNO}_3$. Anchoring of the ferrocenyl adduct to the carbon support shifted the associated $E_{1/2}$ values to higher potentials by slightly more than 70 mV.
CV experiments have also allowed us to establish how the surface modification topology described above attenuate electronic communication between the ferrocene moiety and conducting support. Unlike electron transfer at an electrode-solution interface under diffusion controlled conditions, the anodic and cathodic peak potentials ($E_a$ and $E_c$, respectively) for redox events involving electro-active species anchored to an electrode surface diverge as scan rate ($\nu$) is increased (Figure 2.6). By applying a formalism developed by Laviron, in which $E_c - E_{1/2}$ and $E_a - E_{1/2}$ are plotted as a function of log ($\nu$), we have determined the electron-transfer rate constants.
(ETRCs) for CP3. Laviron plots for each of these systems were constructed (Figure 2.6) and ETRCs were extracted by fitting the portions of these plots in which \((E_a - E_c) \geq 200\) mV. This analysis afforded ETRC values for CP3 of \(36 \pm 6\) s\(^{-1}\).

![Laviron plot showing ΔE for the anodic waves (red squares) and the cathodic waves (black squares). The line is fit to the data whose conditions satisfy \(E_a - E_c \geq 200\) mV (left). Cyclic voltammograms taken at increasing scan rates for CP3 (right). Scan rates vary from 0.15 V/s (black) to 1 V/s (green).](image)

**Figure 2.6.** Laviron plot showing ΔE for the anodic waves (red squares) and the cathodic waves (black squares). The line is fit to the data whose conditions satisfy \(E_a - E_c \geq 200\) mV (left). Cyclic voltammograms taken at increasing scan rates for CP3 (right). Scan rates vary from 0.15 V/s (black) to 1 V/s (green).

### 2.3.4 On-Surface Glaser Cross Coupling

#### 2.3.4.1 Homogeneous Model Reactions

Having demonstrated that Sonogashira reactions can be used to modify conducting carbon supports, we next determined if other types of transition metal-catalyzed C–C bond forming reactions could also be used to similar effect. In particular, we envisioned that the Glaser reaction, which joins two terminal alkynes to
form a new C\text{sp}–C\text{sp} bond would offer a powerful way to modify electrode surfaces by allowing moieties of interest to be tethered via a strongly conductive diyne linker.

As before, we began by identifying homogenous conditions for the model reaction of ferrocene-linked alkyne 1.2 and phenyl acetylene (Scheme 2.2). We found that use of catalytic CuCl in pyridine at 60 \degree C under air led to the formation of Fc2 in 49\% yield after 3 hours, along with homo-coupled byproducts. Crystals of Fc2 suitable for X-ray diffraction analysis were easily obtained, and the solid-state structure of this compound is reproduced in Figure 2.12 and relevant crystallographic data are summarized in Table 2.2.

The reaction conditions used for preparation of Fc2 translated well to on-surface cross-coupling by substituting CuBr for CuCl. Treatment of the CP2 with alkynyl ferrocene derivative 1.3, resulted in the formation of the diacetylene modified surface CP4 (Scheme 2.2).
Scheme 2.2. Homogeneous reaction conditions for Glaser cross coupling (top). Reaction conditions for surface Glaser cross coupling on the electrode.

2.3.4.2 XPS Analysis of CP4

Linkage of the ferrocene unit to the acetylene modified carbon paper was once again demonstrated by XPS, which showed the presence of significant surface-bound ferrocene and only trace amounts of copper or bromine (Figure 2.7). As with the case for the Sonogashira coupling, control experiments in which the copper catalyst was omitted, and independent runs employing nonfunctionalized carbon substrates did not result in surface-bound ferrocene as judged by XPS (Figure 2.8). Integrating the area under the Fe 2p peak by XPS shows that the atomic percent is only 0.12 ± 0.05, a three-fold decrease from the atomic percentage obtained by on-surface Sonogashira cross coupling.
Figure 2.7. XPS high resolution spectra of Fe 2p, Br 3d and Cu 2p regions for CP4.

Figure 2.8. XPS high resolution spectra for Fe 2p\textsubscript{3/2} peak for CP4 (top in orange), CP2 exposed to catalyst free Glaser reaction conditions (middle in light gray) and unmodified carbon paper subject to Glaser reaction conditions (bottom in black).
2.3.4.3 Electrochemical Analysis of CP4

Electrochemical analysis shows a reversible wave corresponding to the immobilized ferrocene unit shifted by 70 mV to its homogeneous counterpart (Figure 2.9). Integration of the cyclic voltammetric peak also resulted in a three-fold decrease in coulombic charge when compared to CP3 in agreement with the XPS results. The decrease in iron coverage for CP4 is attributed to the homocoupling of the alkynes. A percentage of starting material is going to dimerize by nature of the Glaser chemistry. In addition, the surface alkynes are also susceptible to dimerization, although strain of the dienyl bridged product likely makes this pathway less energetically favorable. The Laviron formalism was applied CP4 and values for the ETRC were obtained. The ETRC measured was $44 \pm 10 \text{ s}^{-1}$. These results are consistent with a successful on-surface Glaser cross-coupling.
Figure 2.9. Cyclic voltammogram of Fc2 in 100 mM TBAPF$_6$ in acetonitrile. Scan rate is 50 mV/s (top). Cyclic voltammogram of CP3 (green) and unmodified carbon paper (black) in 100 mM TBAPF$_6$ in acetonitrile. Scan rate is 10 mV/s (bottom).

2.3.5 On-Surface Copper Catalyzed Azide Alkyne Cycloaddition Cross Coupling

2.3.5.1 Homogeneous Model Reactions

Finally, in order to make a direct comparison with the triazole bridged electrode assemblies that have been reported by Hapiot and others, we prepared ferrocene azide (Scheme 2.3). Treatment of this compound with phenyl acetylene,
CuSO₄ and ascorbic acid in DMF at room temperature, cleanly afforded Fc3. These cycloaddition conditions, which are completely homogenous, were easily translated to the analogous on-surface coupling reaction allowing CP2 to be transformed to the triazole-linked ferrocene-modified surface (CP5) as shown in the bottom portion of Scheme 2.3. Crystals of Fc3 suitable for X-ray diffraction analysis were easily obtained, and the solid-state structure of this compound is reproduced in Figure 2.12 and relevant crystallographic data are summarized in Table 2.2.

Scheme 2.3. Homogeneous reaction conditions for CuAAC coupling (top). Reaction conditions for surface CuAAC cross coupling on the electrode
2.3.5.2 XPS Analysis of CP5

Similar to the Sonogashira and Glaser reactions, XPS analysis of the CuAAC-modified carbon paper (CP5), showed the presence of bound ferrocene with a strong signal corresponding to iron at 708.0 and 720.8 eV (Figure 2.10, left). The presence of nitrogen on the modified surface was also evident from the high-resolution XPS spectrum, which showed a strong feature at 400 eV (Figure 2.10, middle). This signal corresponds to the nitrogen atoms that comprise the triazole moiety linking the ferrocene unit and carbon surface. This peak cannot simply be attributed to unreacted azide starting material adsorbed onto the carbon paper, which is characterized by a sharp XPS feature at 404.8 eV (Figure 2.10). The CuAAC-modified substrate (CP5) also showed low levels of copper and sulfate deposition, although copper contamination was slightly more problematic than for the other on-surface couplings described above (see section 2.3.5.3).

Figure 2.10. XPS high resolution of Fe 2p region for CP5 (left), N 1s high resolution for Fc3 starting material showing the presence of N 1s located at 404.8 eV from the positively charge N in the azide (middle) and the N 1s region for CP5 showing no residual starting azide on the surface (right).
2.3.5.3 Electrochemical Analysis of CP5

Electrochemistry experiments have also revealed the contrasting surface properties of the triazole-containing modified surfaces (CP5) compared to the Sonogashira (CP3) and Glaser (CP4) modified electrodes. Initial XPS analysis of CP5 substrates showed slightly elevated levels of residual copper relative to CP3 or CP4. CV analysis of freshly prepared CP5 electrodes that had been rigorously washed to remove residual reagents and noncovalently linked species revealed an irreversible redox wave at 0.3 V versus Ag/AgNO3, which obscured the ferrocene couple. Notably, this irreversible wave only appears during the first few CV sweeps; in subsequent anodic cycles this feature does not appear, thereby allowing the ferrocene couple at 0.189 V to be resolved (figure 2.11, left).

Figure 2.11. Cyclic voltammogram of CP5. First sweep showing a large contribution to the oxidative current. By the 5th sweep, the symmetry of the FeII/FeIII redox feature returns (left). XPS high resolution of the Cu 2p3/2 and the Fe 2p3/2 regions before (red) and after (black) electrochemical oxidation of the ligated Cu(I) to Cu(II).
XPS spectra recorded for CP5 after CV analysis show that the amount of residual copper on the surface was dramatically reduced, while the level of iron was virtually unchanged within experimental error (Figure 2.11, right). Given that triazole-based complexes of copper are known, these results are consistent with enhanced binding of Cu(I) to the surface-anchored triazoles of CP5. Oxidation of the bound Cu(I) ions results in decreased affinity and loss of Cu(II) to the electrolyte solution. These results suggest that molecular wire formed via CuAAC reactions at a conducting surface may generate modified electrodes that display a coordination chemistry that is not innocent. This realization is important given the popularity of CuAAC methods for electrode modification chemistry.\textsuperscript{49-56}

Laviron formalism was also applied and the corresponding ETRC values measured were 35 ± 3 s\(^{-1}\).

2.3.6 Comparison of CP3, CP4 and CP5

While the Sonogashira, Glaser and CuAAC chemistries translated on the surface of alkyne modified carbon papers, there are significant comparisons to be made. First, in all cases, anchoring of the ferrocenyl adduct to the carbon support shifted the associated E\(_{1/2}\) values to higher potentials by slightly more than 70 mV.

Second, the ETRC values for each assembly should be discussed. At first glance, the fact that these values are so similar would appear to suggest that the three MW topologies we have probed do not significantly differ in their ability to modulate charge transport between the ferrocene moiety and carbon surface. A closer inspection of these modified electrodes, however, shows that the three MWs studied are of different lengths. Based on the crystallographic structures of Fc1–Fc3, we can estimate the distal separation between the ferrocene center and aryl carbon grafted to
the electrode surface to be 12.59 Å for CP3, 15.01 Å for CP4 and 12.62 Å for CP5 (see Figure 2.12). Given that the rate of electron transport between two centers decreases as the distance between them is augmented, one would expect CP4 to display a smaller ETRC compared to CP3 and CP5 if each topology was equally proficient in modulating charge transport. The fact that the measured ETRC for CP4 is larger than for the other shorter systems studied suggests that the diyne linker may be a more conductive molecular architecture than the others we have probed.

Figure 2.12. Crystal structure for Fc1, Fc2 and Fc3 homologs.
Table 2.2. Crystallographic data and structure refinement parameters for Fc1, Fc2 and Fc3.

<table>
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<th></th>
<th>Fc1</th>
<th>Fc2</th>
<th>Fc3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>C\textsubscript{24}H\textsubscript{18}Fe</td>
<td>C\textsubscript{26}H\textsubscript{18}Fe</td>
<td>C\textsubscript{25}H\textsubscript{23}FeN\textsubscript{3}</td>
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<td>200(2) K</td>
<td>200(2) K</td>
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<td><strong>Unit cell dimensions</strong></td>
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<td>\begin{align*} a &amp;= 7.1257(6) \text{ Å} \ b &amp;= 9.7812(9) \text{ Å} \ \beta &amp;= 81.2310(10)° \ c &amp;= 14.6087(13) \text{ Å} \end{align*}</td>
<td>\begin{align*} a &amp;= 11.3194(6) \text{ Å} \ b &amp;= 5.7682(3) \text{ Å} \ \beta &amp;= 94.364(4)° \ c &amp;= 14.8959(8) \text{ Å} \end{align*}</td>
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Goodness-of-fit on $F^2$

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<td>0.289 and -0.342 e/Å$^{-3}$</td>
<td>0.273 and -0.272 e/Å$^{-3}$</td>
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</tr>
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Third, the on-surface Sonogashira and Glaser reactions we have developed are distinguished from the CuAAC on-surface cross coupling, since they do not introduce ancillary metal ion binding sites onto the modified conducting substrates.

### 2.4 Conclusion

The development of a modular method to prepare electrically conducting substrates with tailored morphologies at the electrode surface were performed. In carrying out this work, metal-mediated cross-coupling chemistry have been adapted to synthetically modify carbon-paper substrates. These on-surface modification methods have been applied to build carbon-supported MWs with terminal ferrocene units. The current study has focused on the construction of MW-electrode assemblies with three distinct molecular compositions and topologies. Using the methodology described herein, we have prepared well-defined carbon-paper substrates appended with alkyne (CP3), diyne (CP4) and triazole (CP5) based units. We have also demonstrated that each different MW topology gives rise to distinct electronic properties, as evidenced by the disparate $E_{1/2}$ values recorded for CP3–CP5. Notably, these systems also display variations in the kinetics of electron transfer between the ferrocene units and electrode surface, with the Glaser-based electrode (CP4) displaying a larger ETRC than the other systems we have studied.
The observation that the diyne MWs of CP4 afford a modified electrode with enhanced electron transport kinetics, even though this linker is nearly 2.5 Å longer than the alkyne (CP3) or triazole (CP5) containing MWs indicates that the diyne linker is a more efficient bridge for electron transport. This result is consistent with prior work that has demonstrated polyacetylenic linkers to be superior mediators of electronic communication in donor–acceptor assemblies.578596061 Similarly, polyacetylenic linked porphyrin-fullerene dyads have been shown to undergo extremely efficient and rapid photoinduced electron transfer, with associated attenuation factors that are exceptionally small (β ≈ 0.06 Å⁻¹).62 When taken together, these prior studies reinforce our observation that electron transport between small molecules anchored at an electrode surface and the bulk material is facilitated by a diyne bridge. Building on this work, we would expect electrode substrates grafted with longer polyacetylenic based MWs to also display relatively large ETRCs and efficient charge transport kinetics. Future work from our laboratories will take advantage of the on-surface coupling methods we have developed to enable the construction and physical interrogation of such systems.
REFERENCES


Chapter 3

IMPLEMENTATION OF SONOGASHIRA CROSS COUPLING SURFACE CHEMISTRY ON OTHER CARBON BASED ELECTRODES TO INCLUDE GLASSY CARBON, ISOMOLDED GRAPHITE AND DIAMOND

3.1 Introduction

Covalent modification of electrodes has become a crucial area of research. Its value has been established through multiple reviews involving biochemical sensing\textsuperscript{1,2} molecular electronics,\textsuperscript{3} dye sensitized solar cells,\textsuperscript{4,5} and catalysis\textsuperscript{6-9} to name a few. As important as the utility of modified electrodes, is the fundamental understanding of the connectivity and electronic properties of the modification. Every chemical alteration distorts the behavior of the electrode, either enhancing or attenuating some property (such as absorbance or conductivity) that is observable or infinitesimal. Detailing the properties associated with specific modifications, or more precisely how these properties change by substituting one component of the modification gives us the insight to tailor our construction of covalently modified electrodes to best suit a specific application. For example, recently the Crabtree lab has discovered that by exchanging the widely accepted carboxylate or phosphonate anchoring groups with hydroxamate on TiO\textsubscript{2}, they could achieve higher sunlight-to-electricity efficiency of their dye-sensitized solar cell.\textsuperscript{10} Another well studied covalent modification of an electrode is the electrochemical reduction of aryl diazonium salts, first developed in 1992 when glassy carbon, HOPG, carbon fiber and carbon powder were electrochemically modified via the reduction of aryl diazonium salts using an applied electrochemical potential.\textsuperscript{11} The reduction of diazonium salts onto carbon based
surfaces provided a means to access a new interfacial carbon-carbon bond as its first point of connectivity.\textsuperscript{12-15} A drawback to the robust covalent carbon-carbon bonds formed, are the ill-defined, insulating multilayer of arenes that are grown as the reaction propagates.\textsuperscript{16-17} In comparison to the well-studied self-assembled monolayers (SAMs) on gold surfaces, the carbon based electrodes offer more stability through its carbon-carbon bond, but less control in forming well defined monolayers.\textsuperscript{18} The exception to multilayer formation when using diazonium grafting occurs when sterically bulky groups are placed at the meta positions of the aryl diazonium.\textsuperscript{19-20} These large groups prevent multilayer formation by creating destabilizing steric interactions with approaching aryl radicals. In 2010, Hapiot showed a monolayer can also be accessed when electrochemical deposition is performed with a triisopropylsilyl (TIPS) protected acetylene derivative (3.1).\textsuperscript{21-22} The TIPS protecting group can easily be removed by subjecting the modified electrode surface to a solution of tetrabutylammonium fluoride (TBAF), which affords a monolayer of free alkynes. Using this approach our lab has shown that in addition to creating a well-defined monolayer, we can apply a variety of cross coupling methods on a carbon paper electrodes (CE) to further modify the molecular linker (see chapter 2).\textsuperscript{23}

CE’s porosity has made this material ideal for gas diffusion electrode assemblies.\textsuperscript{24} With the success of cross-coupling methods onto CE, one can imagine covalent modification of gas diffusion electrodes supporting more favorable gas-interface associations. Yet, CE is not a representative carbon based electrode substrate for other types of applications. Because of this, it was of interest to demonstrate the cross-coupling chemistry to other types of carbon electrodes. We chose diamond (DE), glassy carbon (GE) and isomolded graphite (IE) as the additional carbon based
electrode substrates. Despite all substrates being carbon based, the substrates are in fact very different. For example, the molecular connectivity for diamond is sp$^3$, while glassy carbon and graphite is sp$^2$ hybridized. The materials have varying physical properties such as resistivity and conductivity which is partly due to their differing molecular environments (as indicated by their amount of native oxides, see section 3.3.1). These reasons contribute to the variation in price, and electrochemical performance for each electrode. DE is oxidatively robust, more so than GE, CE and IE. GE is commercially available and widely used in electrochemical disciplines due to its inertness at extreme potentials. Demonstrating the success and limitations of the cross coupling Sonogashira reaction onto these additional carbon-based surfaces allows us to use Sonogashira cross coupling chemistry as an indispensable tool for surface modification.

3.2 Materials and Methods

3.2.1 General Considerations

Reagents including 4-((triisopropylsilyl)ethynyl)aniline, (triisopropylsilyl)ethynyl)aryldiazonium tetrafluoroborate (3.1) and 4-iodophenylferrocene (3.2) were synthesized as reported in the literature. Tetrabutylammonium hexafluorophosphate (TBAPF$_6$) was recrystallized from hot ethanol before use. All other chemicals were purchased from a major chemical supplier and used as received. All solvents were of reagent grade or better and were dried by passage through activated alumina and then stored over 4 Å molecular sieves. All reactions were carried out in a nitrogen filled glovebox. 1 x 1 x 0.2 cm GE were purchased from SpiSupples. GE's were polished in 0.05 μm alumina powder and
sonicated in 18 MΩ Millipore water for 5 minutes, ethanol for 15 minutes and finally acetone for 15 minutes before dried under a stream of N₂ and stored in the oven prior to use. DE was purchased from element6. DE's were used as received. IE was purchased from Fuel Cell Earth and cut into 2 x 1 cm² pieces. IE was sonicated in ethanol for 15 minutes and then acetone for 15 minutes and stored in the oven prior to use.

3.2.2 Generations of Alkynyl Modified Carbon-based Electrodes (CP1 and CP2; IE1 and IE2; GE1 and GE2; DE1 and DE2)

In a nitrogen filled glovebox, a 5 mM solution of 3.1 in acetonitrile was grafted onto substrates using the following cyclic voltammetric parameters. CP1: A starting potential of 0.5 V was applied and swept until -0.3 V at a scan rate of 0.1 V/s for a total of 6 cycles. IE1: A starting potential of 0.4 V was applied and swept until -0.4 V at a scan rate of 0.1 V/s for a total of 6 cycles. GE1: A starting potential of 0.5 V was applied and swept until -0.3 V at a scan rate of 0.1 V/s for a total of 6 cycles. DE1: A starting potential of 0.0 V was applied and swept until -0.7 V at a scan rate of 0.1 V/s for a total of 6 cycles (See Figure 3.5). Following electrochemical deposition, substrates were soaked in acetonitrile for 5 min three times.

Electrodes were then soaked in a 5 mM solution of TBAF for 2 hrs to afford CP2, IE2, GE2 and DE2. Following deprotection of the TIPS groups, substrates were soaked in acetonitrile for 5 min three times.

3.2.3 Procedure for Sonogashira Surface Cross Coupling to Generate CP3, IE3, GE3 and DE3

In a nitrogen-filled glove box copper iodide (2.4 mg, 12.6 μmol) tetrakis(triphenylphosphine)palladium(0) (13.8 mg, 12.0 μmol), iodophenylferrocene,
3.2 (58 mg, 150 μmol), triethylamine (63.6 μL, 45.0 μmol), and anhydrous dimethylformamide (3 mL) were added to a 20 mL vial. A piece of alkynyl modified carbon electrode (CP2, GE2, IE2, or DE2) was then added to this solution using forceps. The vial was capped with a polyethylene-lined cap and heated in an aluminum block to 80 °C for 15 min. The reaction solution was then allowed to cool to room temperature and the modified carbon electrode was removed from solution using forceps. The modified substrate was cleaned by rinsing with dry acetonitrile to remove excess reagents. The washed substrate was then placed in a clean vial and soaked in 8 mL of dry dimethylformamide for 1 hr. Following removal from the DMF with forceps, the carbon paper was rinsed again with 4 mL of dry acetonitrile and then soaked for an additional hour in 8 mL of acetonitrile. Following one final rinse with 4 mL of acetonitrile, the modified carbon electrode (CP3, GE3, IE3, or DE3) was dried by evaporation under nitrogen for several minutes.

3.2.4 Instrumentation

3.2.4.1 Electrochemistry

All electrochemistry was performed using either a CHI-620D potentiostat, or CHI-760D bipotentiostat in a N₂ filled glove box. A standard 3-electrode cell was used for all electrochemical measurements with the substrate (CE, GE, DE or IE) as the working electrode, Pt gauze as the auxiliary electrode and a freshly made Ag/AgNO₃ reference electrode (Ag wire was used as a pseudo reference for the electrochemical deposition of I). The supporting electrolyte was 0.1 M TBAPF₆ in acetonitrile.
3.2.4.2 XPS Analysis

All XPS spectra were acquired using a VG ESCALAB 220I-XL spectrometer. With the exception of the DE or unless otherwise noted, the X-Rays used were monochromatic Al Kα X-Rays (1486.7 eV) with a power of 105 W (15 kv, 7 mA). The operating pressure in the main chamber was less than 1 x 10⁻⁸ torr. The X-Ray spot size was elliptical in shape with a semi-major axis of roughly 400 μm. Initial XPS survey scans were collected at a pass energy of 80 eV using a step size of 0.25 eV. Atomic percentages determined by XPS do not include hydrogen. High-resolution XPS spectra were collected at a pass energy of 20 eV using a step size of 0.1 eV. Reported values for average atomic surface composition were taken by integration of the high-resolution spectra and are the average of a minimum of 3 samples. For the DE, the X-Rays used were non-monochromatic Al Kα X-Rays (1486.7eV) with a power of 300 W (15 kv, 20 mA).

The native carbon substrates were analyzed using a Thermo Scientific K-alpha+ spectrometer equipped with a monochromatic x-ray source and a 128 channel CCD detector plate. The depth profiling was conducted using a MAGCIS dual beam etch source utilizing an Ar+ small cluster source at an energy of 8 keV at an angle of 30°. Spectra were acquired before, and in-between 8 cycles of 45 second etching.

3.2.4.3 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX)

Scanning electron microscopy images and energy dispersive X-ray (EDX) spectra were acquired with a JEOL JSM 7400F Scanning Electron Microscope (SEM).
3.2.4.4 4-point Kelvin Probe Resistivity Measurement

Resistivity measurements were made by an Alessi spring loaded 4 point probe. Current is passed through the outermost probes and the voltage is read on the interior probes. Resistivity is solved according the following equation:

\[ \rho = \frac{V}{I \left( \frac{1}{s_1} + \frac{1}{s_3} + \frac{1}{s_1 + s_2} + \frac{1}{s_2 + s_3} \right) ^2} \]

where \( \rho \) is the resistivity, \( V \) is the voltage read on the interior probes, \( I \) is the current passed on the exterior probes, \( s_1 \) is the distance between probe 1 and 2, \( s_2 \) is the distance between probe 2 and 3 and \( s_3 \) is the distance between probe 3 and 4.

3.3 Results and Discussion

3.3.1 Evaluation of the Bare Carbon Electrode Substrates

Surface chemistry occurs at the termination of bulk material, defined by the interface. The abrupt termination of the bulk material is usually more reactive, therefore often times the study of the interface is restricted to the ability to prepare and maintain samples in very protected environments (i.e. inert atmosphere, ultra-high vacuum conditions, etc.); often, much technology and preparation go into preparing samples. With intentions to apply the Sonogashira cross coupling chemistry onto four types of carbon substrates with excellent reproducibility, it was important to show if this chemistry could translate onto substrates with minimal preparation. Each carbon substrate was either used as received or prepared by a simple treatment of polishing with alumina powder followed by a sequential 15-minute sonication in acetone, ethanol, and water, then dried in an oven.
With no rigorous pretreatment, each substrate is vulnerable to reactions with air and moisture. These reactions produce a variety of oxygenated functional groups on the surface, generally referred to as "surface oxides". Identifying the types of functional groups present for each type of carbon material are beyond the scope of this analysis, but the relative quantities of oxygen found at the surface do provide an idea of the environment in which the molecular linker is constructed. We evaluated each carbon material based on oxide/oxygen content on the surface, thickness of the oxide layer, surface resistivity, electrochemical double layer capacitance and roughness.
3.3.1.1 Elemental Oxygen Distribution in Carbon Electrodes

Figure 3.1. High resolution C 1s (left) and O 1s (right) before etching (black), after etching for 45 seconds with an Ar\(^+\) cluster source at an energy of 8 keV (dark gray) and after etching for 360 seconds (light gray). The carbon electrodes from top to bottom are PE, IE, GE and DE.
Using a cluster Ar$^+$ dual beam ion source, each carbon material was etched to determine if oxygen persisted within the bulk of the material. **Figure 3.1** shows the C 1$s$ and O 1$s$ spectra of the native material before and after sputtering. The breadth of the large peak (FWHM) for the DE C 1$s$ spectra is larger than that of PE, IE and GE. This is due to the presence of sp$^3$ carbons in addition to adventitious carbon. sp$^3$ carbons are shifted to a slightly higher binding energy, which broadens the peak. IE has noticeable additional oxidized functional groups at 290 eV and 286 eV which do not seem to diminish post sputtering as compared with the other three substrates.

The oxygen and carbon content is quantified and the ratio is displayed in **Figure 3.2**. Using EDX, the oxygen content in the bulk of the material was quantified.
The figure shows that DE and GE have the most surface oxides. The steep slope for GE indicate that the oxides are not uniformly distributed between the surface and the bulk material; most of the oxygen is allocated to the upper most layer of the material. On the other hand, the oxygen content for IE and CE do not differ much from surface to bulk material, carbon paper noticeably having the least amount of oxygen incorporated. The slope for diamond would not represent the same thickness as the other carbon substrates, as diamond is the hardest material, and the sputter rates for diamond are expected to be much lower (the Ar\(^+\) sputtering conditions was identical for all 4 substrates).\(^\text{31}\n\)

### 3.3.1.2 Conductivity of Carbon Electrodes

With respect to the carbon material's electrochemistry it was important to examine the conductivity of each electrode. The capacitance of each electrode was measured in a solution of 0.1 M TBAPF\(_6\). The cyclic voltammetric parameters are the same as that used for electrochemical deposition and are shown in Figure 3.3. Table 3.1 shows the capacitance of each carbon substrate. The noticeably higher double-layer capacitance for IE is due to the greater amount of oxides in the material in agreement with the EDX data. The correlation between a greater double layer capacitance and a relatively large amount of surface/bulk oxides for IE agrees with the literature: the capacitance for edge plane graphite (the plane perpendicular to the hexagonal lattice created by sp\(^2\) type carbons in which there are greater amount of oxygenated functional groups) was 50-100 μF/cm\(^2\) and the capacitance for basal plane graphite (the plane parallel to the hexagonal lattice created by sp\(^2\) type carbons) was measured as low as 3-4 μF/cm\(^2\).\(^\text{32-33}\) IE is analogous to edge plane graphite by means of the amount of oxides present. The greater double layer capacitance is attributed to
the increased amount of oxides which create a larger domain of dangling bonds, which have been shown to increase the DOS.\textsuperscript{34} In addition to the large double layer capacitance for IE, the profile for DE, IE and GE take on a slanted cyclic voltammetry profile (\textbf{Figure 3.3}). A true capacitor as measured through CV, should be rectangular, as is the case for PE. If faradic processes contribute to the signal, the shape of the capacitor changes, and a sloped current vs. potential is observed.\textsuperscript{35} The slant can be attributed to the charging and reduction/oxidation of oxides, in which DE, IE and GE contain a measurable amount (\textbf{Figure 3.2}) while PE contain relatively no oxides, therefore displaying a more rectangular double layer capacitance.

\begin{table}
\centering
\caption{Capacitance Values Measured for Each Carbon Electrode}
\begin{tabular}{ l r }
\hline
\textbf{Capacitance (\textmu F/cm\textsuperscript{2})} \\
\hline
\textbf{Paper} & 140 \\
\textbf{Diamond} & 15 \\
\textbf{Isomolded Graphite} & 780 \\
\textbf{Glassy Carbon} & 33 \\
\hline
\end{tabular}
\end{table}
Figure 3.3. Capacitance measurements for carbon substrates. Top to bottom: PE, IE, GE and DE
3.3.1.3 Resistivity of Carbon Electrodes

A 4-point kelvin probe was used to measure the sheet resistance of each carbon electrode. The values obtained for the sheet resistance are in units of Ω per “square”. This special unit is converted to resistivity by multiplying it by the thickness of the material. The sheet resistance, thickness of the material and corresponding resistivity of the material are listed in Table 3.2.

Table 3.2. Resistivity of Carbon Electrodes

<table>
<thead>
<tr>
<th>Carbon Electrode</th>
<th>Sheet Resistance (Ω per “square”)</th>
<th>Thickness (cm)</th>
<th>Resistivity (Ω • cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>0.2</td>
<td>0.018</td>
<td>0.0036</td>
</tr>
<tr>
<td>IE</td>
<td>0.012</td>
<td>0.282</td>
<td>0.0034</td>
</tr>
<tr>
<td>GE</td>
<td>0.05</td>
<td>0.272</td>
<td>0.014</td>
</tr>
<tr>
<td>DE</td>
<td>1.4</td>
<td>0.038</td>
<td>0.053</td>
</tr>
</tbody>
</table>

3.3.1.4 Evaluation of Roughness for Carbon Electrode

In addition to the capacitance and surface resistivity, we sought out to measure the roughness of each electrode. A known method for measuring the roughness of the electrode is through chronocoulometry using the Anson equation shown below. Briefly the electrochemical surface area was determined by monitoring the decay rate of ferrocene after an applied pulse potential of 0.8 V. Plotting this charge decay as a function of the square root of time yields a straight line. The area is solved from the slope of this line according to the following equation.

\[ Q = 2nFAc_oD^{1/2}t^{1/2}π^{-1/2} \]

where \( n \) is the number of electrons involved in the oxidation of ferrocene to ferrocnium, \( F \) is faraday’s constant, \( c_o \) is the concentration in the bulk of the solution, and \( D \) is the diffusion coefficient for ferrocene in acetonitrile taken to be \( 2.25 \times 10^{-5} \).
cm² s⁻¹. The roughness factors are determined by dividing the area solved from equation X by the geometric surface area of the electrode. The roughness values are listed in Table 3.3 with IE having the most roughness, also contributing to its higher double layer capacitance value. The roughness value as determined by the Anson equation does not however explain the high double layer capacitance value for CE as CE roughness factor is 1.13 ± 0.03. CE however does have a visible macroscopic texture from the fibers that make up the paper as seen in the SEM image in Figure 3.4. The texture observed in the SEM images are on the order of microns, while the roughness listed in Table 3.3 is simply a geometrical roughness factor and is much more complicated. The surface roughness can actually be divided in two categories: microscopic roughness (fractal anomalies < 10 nm) and macroscopic roughness (features > 10 nm). In addition, porosity further complicates the analysis of roughness adding through pores (pathways) and blind pores (cavities) whose pore size are portioned in three categories: micropores (pore size < 2 nm), mesopores (pores between 2 – 50 nm) and macropores (pore size > 50 nm). The measurement of the surface roughness listed in Table 3.3 does not completely depict these features. This become important because how the roughness effects the double layer capacitance is dependent on which dimensional category the roughness falls. So while the CE has a measured roughness factor of 1.13 ± 0.03, indicating weak microscopic roughness, it is CE’s macroscopic roughness (i.e. the texture and porous network as seen in the SEM image), that likely contributes to its higher capacitance.

Table 3.3. Roughness Factor for Each Carbon Electrode

<table>
<thead>
<tr>
<th>Roughness Factor</th>
<th>Carbon Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.13 ± 0.03</td>
</tr>
<tr>
<td>Material</td>
<td>Value</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>Glassy Carbon</td>
<td>1.25 ± 0.17</td>
</tr>
<tr>
<td>Graphite</td>
<td>1.81 ± 0.18</td>
</tr>
<tr>
<td>Diamond</td>
<td>1.11 ± 0.22</td>
</tr>
</tbody>
</table>

Figure 3.4.  Scanning Electron Microscopy for PE (top left)  IE (top right) GE (bottom left) and DE (bottom right) at magnification of 500 x’s and 5000x’s for the insert.

3.3.2  Diazonium Electrochemical Deposition and TIPS Deprotections to Afford Terminal Alkyne Electrodes: CP2, IE2, GE2 and DE2

As shown in Figure 3.5, the electrochemical deposition of 3.1 onto each of the carbon substrates shows a single irreversible reduction peak corresponding the reduction of the aryl diazonium salt to an aryl radical via the loss of nitrogen. It is
worth noting the more negative potential associated with the reduction of the aryl diazonium onto DE. This may be due to sp$^3$ hybridization of carbon for DE, while CE, GE, and IE are primarily sp$^2$ hybridized. The carbon electrodes represented in Scheme 3.1 symbolizes localized regions of graphene or diamond. The actual carbon electrode surfaces are not equivalent, as their morphology (see Figure 3.4), surface oxides and capacitance (vide supra) differ for each carbon substrate. The silicon was quantified by XPS and shown in Table 3.4.

Scheme 3.1. Electrochemical deposition of 3.1 onto carbon substrates.
Figure 3.5. Deposition of 3.1 onto PE (left), IE (second to left), GE (second to right), and DE (right) to afford CP1, IE1, GE1 and DE1. Scan rates are 0.1 V/s for a total of 6 cycles.

Table 3.4. Silicon Atomic Percent Before and After TBAF Deprotection

<table>
<thead>
<tr>
<th></th>
<th>TIPS</th>
<th>TBAF</th>
<th>% Silicon Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>3.2 ± 0.8</td>
<td>0.4 ± 0.9</td>
<td>86</td>
</tr>
<tr>
<td>IE</td>
<td>1.6 ± 0.5</td>
<td>0.3 ± 0.2</td>
<td>81</td>
</tr>
<tr>
<td>GE</td>
<td>2.4 ± 1.2</td>
<td>0.6 ± 0.4</td>
<td>75</td>
</tr>
<tr>
<td>DE</td>
<td>3.2 ± 1.6</td>
<td>0.7 ± 0.6</td>
<td>78</td>
</tr>
</tbody>
</table>
There is no correlation between the total charge passed upon deposition (area under the cyclic voltammetric curve) and the percent of silicon on the surface relative to carbon (Figure 3.6). The amount of silicon would correlate linearly with the charge passed if each phenyl radical that is produced upon reduction forms a bond with the electrode surface. What is more likely is that some reduced phenyl radicals bond with the surface of the film, while other phenyl radicals diffuse away and react with other species in solution\textsuperscript{14,41} as shown in Scheme 3.1. There is a strong inverse correlation when the percent silicon on the electrode is overlaid with the amount of oxygen in the bulk of the film (Figure 3.6) which suggests two things: 1) the state of the electrode during deposition (i.e. reducing conditions) is represented best by the data acquired by EDX, in which most to all the surface oxides are reduced and 2) the more oxides present in the bulk structure of the film acts as inhibitors for film formation. The latter suggestion is significant, as it opposes a claim in the literature that the amount of...
oxides constitute reactivity, and hence would suggest more film coverage. It is important to make the distinction that the carbon type that this argument is made for is graphene and highly ordered pyrolytic graphite, in which edge plane (whose oxygen content is greater) is more reactive than basal plane.\textsuperscript{12,42-43} This greater reactivity is attributed to an increased density of states for edge plane\textsuperscript{44} as well as greater inherent disorder which induces strain in the graphene, allowing the hybridization from sp\textsuperscript{2} to sp\textsuperscript{3} to facilitate more easily\textsuperscript{45-47} as it invokes a displacement from the graphitic plane by 0.7 Å (as illustrated in Scheme 3.1).\textsuperscript{48} The carbon electrode substrates used in this study, while three of them are graphitic based, are \textit{not} best represented by a long range ordered structure such as HOPG.

Following the electrochemical deposition of 3.1, all four substrates were analyzed by XPS. The binding energy for silicon in the TIPS group is around 100 eV. This is where we find the peak for silicon in CE1, DE1 and GE1 after functionalization. For IE1, the peak is located at 101 eV, shifted by about 1 eV suggesting that the oxides in IE contribute to the oxidation of 3.1.

After electrochemical deposition of 3.1, all four substrates were soaked in TBAF for 2 hours to remove the TIPS group and expose a monolayer of terminal alkynes. The substrates were analyzed by XPS to verify the loss of signal in the Si 2\textit{p} high-resolution spectra, due to removal of the TIPS group (see Figure 3.7). As shown in Table 3.4, more than 70\% of the TIPS head group was removed for all substrates. DE as received contained a silicon oxide contaminant present at 103 eV (shown in green) which was not affected by the electrochemical deposition nor the deprotection.
Figure 3.7. Si 2p high resolution spectra of CP1, IE1, GE1, and DE1 (black) and CP2, IE2, GE2, and DE2 (red). Native silicon contamination on DE is shown in green.

3.3.3 Analysis of the Sonogashira Cross Coupled Carbon Electrodes

3.3.3.1 XPS Analysis

After exposure of the free alkyne, each carbon electrode was subjected to optimized Sonogashira cross-coupling conditions to afford a ferrocene terminated molecular wire (Scheme 3.2). Figure 3.8 shows the high-resolution XPS analysis for the Fe 2p region after Sonogashira surface cross coupling. For all four substrates, the
Fe 2p$_{3/2}$ peak is positioned at 708.9 eV, which is indicative of iron in the +2 oxidation state, consistent with ferrocene rather than iron oxide.$^{49}$

Scheme 3.2. Surface Sonogashira cross coupling reaction

While the binding energy position of the iron peak is indicative of Fe$^{2+}$, it was important to determine we were observing covalently bound ferrocene rather than unreacted starting material coating the electrode surface. To determine this, we compared the ratio of iron to iodide on the electrode surface. The ratio of Fe:I in the starting material is 1:1. If the starting material is being physisorbed onto the electrode surface then this ratio is expected. As shown in Table 3.5, the relative atomic percentages of Fe:I are always greater than 1:1 indicating at least partial success of the surface Sonogashira cross coupling. To further confirm the formation of a new covalent bond at the electrode interface, Sonogashira reactions were performed on unmodified electrodes. As shown in Figure 3.8, little to no iron is present on the electrode surfaces that do not have the terminal alkyne reactant. This further supports the molecular wire is being covalently bonded and not just physisorbed.
The chemistry of iodide on each electrode is not as straightforward as iron. For CP3 and DE3 minimum to no iodide is measured on the electrodes after the Sonogashira reaction for both native electrode and CP2/DE2. For IE3 and GE3 there is measurable iodide. This amount of iodide on the surface is even greater for the control reaction performed with native IE and GE surfaces. The amount of bulk oxides in the material (as measured by EDX in Figure 3.2) can serve as an explanation; the oxides
in the materials can exchange with the iodide to form phenols. Such a reaction is analogous to the homogeneous hydroxylation of aryl halides to form phenols in the presence of CuI.50

Table 3.5. Atomic Percentages of Fe and I for CP3, IE3, GE3 and DE3 and the Control Reactions with CE, IE, GE and DE.

<table>
<thead>
<tr>
<th></th>
<th>Fe Atomic %</th>
<th>I Atomic %</th>
<th>Fe Atomic % (Control)</th>
<th>I Atomic % (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP3</td>
<td>0.29 ± 0.08</td>
<td>&lt; 0.1</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IE3</td>
<td>0.69 ± 0.25</td>
<td>0.58 ± 0.20</td>
<td>0.16 ± 0.03</td>
<td>0.74 ± 0.24</td>
</tr>
<tr>
<td>GE3</td>
<td>0.60 ± 0.19</td>
<td>0.25 ± 0.09</td>
<td>0.10 ± 0.05</td>
<td>0.38 ± 0.11</td>
</tr>
<tr>
<td>DE3</td>
<td>0.11 ± 0.02</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Figure 3.9. 13d high resolution XPS spectra for CP3, IE3, GE3, and DE3 for an alkyne terminated carbon electrode (black) and the native carbon electrode (red).
3.3.3.2 Electrochemical Analysis

Figure 3.10. Laviron formalism (left), overlay of CV’s at scan rates increasing from 1.0 V/s to 13 V/s (middle), and insert of CV at 0.1 V/s (right) for GE3 (top), IE3 (middle) and DE3 (bottom).

Cyclic voltammetry was employed to probe the linker's ability to facilitate charge transfer. Figure 3.10 shows the redox couple for ferrocene at varying scan rates for GE3, DE3, IE3 (for CE, see section 2.3.3.3 in chapter 2). The electron
transfer rate constant (ETRC) was determined by constructing a Laviron plot. A Laviron plot shows the change in anodic and cathodic peak potential as a function of the log of scan rate (Figure 3.10, left). The ETRC’s are $329 \pm 69 \text{ s}^{-1}$, $219 \pm 78 \text{ s}^{-1}$, $36 \pm 6 \text{ s}^{-1}$ and $8 \pm 7 \text{ s}^{-1}$ for DE3, GE3, CP3, and IE3 respectively. The ETRC is extracted from the slope of the linear portion of the Laviron plot. The point of linearity begins when the separation between cathode and anode is greater than 200 mV. The scan rate in which there is greater than a 200 mV separation is indicative of how fast or slow the ETRC will be. This is evident for IE3 whose slowest rate constant has the largest separation between anode and cathode peaks at 0.1 V/s.

The varying ETRC’s are somewhat perplexing, given that we are measuring the transport of an electron through four identical molecular wires. Interestingly, we do see a correlation between the ETRC measured and the resistivity of the native material. Figure 3.11 shows ETRC plotted as a function of the natural log of the resistivity (see section 3.3.1.3). This result suggests that the molecular wires charge transport does not out compete with the conductivities of the underlying material.
3.4 Conclusions

We have applied the Sonogashira cross coupling reaction to four different of carbon electrodes. While the electrode did not prevent the cross coupling, there was a large effect on both the reduction potential of 3.1 as well as the rate of electron transport for each material. The four electrode materials, which differ in oxide content, morphology and conductivity, demonstrate how significant these differences are in conducting surface chemistry, especially the oxide content. We observed a correlation between minimal bulk oxide content and efficiency for electrochemical deposition. We also observed that the surfaces with minimal oxides do not contribute to iodide fouling of the surface during the Sonogashira cross coupling. Given these results, its
ideal to conduct Sonogashira cross coupling surface chemistry on carbon electrode materials that have minimum oxides. Although, oxides do not inhibit the reaction, nor impede in the ability for the cross coupled electrodes to facilitate charge transfer, the oxides do contribute significantly to side reactions. Finally, we showed that all four materials could efficiently exhibit charge transport through the wires, although the kinetics of the ETRC are a function of the native material’s resistivity.
REFERENCES


Chapter 4

APPLYING SURFACE SONOGASHIRA CROSS COUPLING TO INTRODUCE MALEIMIDE FUNCTIONALITY FOR BIOLOGICAL AND SENSING APPLICATIONS

4.1 Introduction

Immobilization of biomolecules encompasses a huge field of research and plays a critical role in applications such as sensing\(^1\),\(^2\) and biocatalysis.\(^3\) The most notorious and useful example of immobilized biomolecules are glucose oxidase enzyme electrodes that can monitor blood glucose levels. To put this in perspective, 29.1 million Americans (or 9.3% of the population) in 2012 did or would (28% would not be diagnosed until after 2012) benefit from this type of technology.\(^4\) The methods in which glucose oxidase is immobilized has evolved\(^5\)-\(^7\) and continues to be improved. Methods to immobilized glucose oxidase have even included electrochemical reduction of aryl diazonium salts.\(^8\) Biocatalysis also illustrates another application of immobilized biomolecules. Enzymes are the ideal catalysts, taking millions of years to evolve into a specific architecture to facilitate specific chemical transformations. Industrial and pharmaceutical processes incorporate biocatalysts for more efficient and specific synthesis.\(^9,10\) An advantage of immobilizing the biocatalyst is that separation between biocatalysts and product are easier (as the biomolecule is immobilized). Immobilizing biocatalyst onto electrode surfaces adds the additional feature of controlling rate with potential and eliminating the need for reducing cofactors by supplying electrons through the working electrode.\(^11\)-\(^13\) These two applications
illustrate the usefulness in developing methods to introduce biomolecules on electrode surfaces.

With the success of the Sonogashira cross coupling translated to carbon paper surfaces (see chapters 2 and 3), we envisioned immobilizing a functional group which has distinct reactivity towards biologically relevant material. The functional group we targeted was a maleimide which reacts specifically with a thiol group. The thiol reactivity is important because it allows us to target specific proteins. In biochemistry proteins are made of amino acids, but only 2 contain a sulfur atom. One of the two sulfur containing amino acids, named cysteine, has a free thiol (SH), providing a cite specific handle for the maleimide to react. Proteins that do not contain a cysteine can also be target for immobilization by tagging it with a cysteine.

In addition to demonstrating the compatibility with molecules of biological function such as the cysteine, we also applied the sulfur-maleimide coupling to another compound with a different function, a ruthenium based luminophore. Taking advantage of the sulfur-maleimide chemistry we immobilized a thiolated luminophore that was active for electrochemiluminescence. ECL has been incorporated into devices for commercial detection of multiple immunoassays,\textsuperscript{14-16} but it involves a sandwich technique in which the analyte is sandwiched between an immobilized compound and a lumiphore tagged compound. In contrast to this approach, we directly immobilized a luminophore on the electrode surface demonstrating an actual understanding of the molecular connectivity of the luminophore and electrode.
4.2 Materials and Methods

4.2.1 General Considerations

Solvents for synthesis were of reagent grade or better and were dried by passage through activated alumina and then stored over 4 Å molecular sieves prior to use. Surface cross-coupling and thiol addition reactions were carried out in a nitrogen-filled glovebox. All glassware was oven dried for a minimum of two hours or flame dried under vacuum prior to use. Reagents including 4-((triisopropylsilyl)ethynyl)aniline,\textsuperscript{17} 4-((triisopropylsilyl)ethynyl)aryl diazonium tetrafluoroborate,\textsuperscript{18} (4.1), and 2,2’-bipyridine, 4-[3-(2-chloroethoxy)propyl]-4’-methly\textsuperscript{19} (4.5a) were synthesized as reported in the literature. Tetrabutylammonium hexafluorophosphate (TBAPF\textsubscript{6}) was purchased from TCI. TBAPF\textsubscript{6} was recrystallized from hot ethanol and dried in vacuum overnight prior to use. All other substances and reagents were obtained from commercial sources and used as received. Carbon paper was purchased from Fuel Cell Earth LLC and stored under air. Before modification, the paper was cut into 15 mm x 25 mm rectangles and transferred into a nitrogen-filled glovebox, unless otherwise noted.

4.2.2 Synthesis of Maleimides (structures 4.2 and 4.3)

Maleimides were synthesized by Dr. Amber Gietter-Burch. Her synthetic protocol are as follows.

\textit{1-(4-iodophenyl)pyrrole-2,5-dione 4.2 -} To a 100 mL round bottom flask equipped with a magnetic stir bar was added 4-iodoaniline (6.57 g, 30.0 mmol), 2-methylmaleic anhydride (2.70 mL, 30.0 mmol) and diethyl ether (45 mL). The reaction was capped with a polypropylene cap and stirred at rt for 1 h. The reaction was cooled to 0 °C and the solid was collected by vacuum filtration and dried on a frit.
The crude solid was transferred to a 50 mL round bottom flask equipped with a magnetic stir bar and sodium acetate (984 mg, 12.0 mmol) and acetic anhydride (10 mL) were added. The flask was equipped with a magnetic stir bar and heated to reflux for 30 minutes. The flask was then cool to rt and the solution was poured onto cold water. The solid was filtered using vacuum filtration, dried on a frit and purified using flash silica gel chromatography to afford 4.2 (2.29 g, 24%) as a yellow solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.80 - 7.75 (m, 2H), 7.16 - 7.09 (m, 2H), 6.49 (q, $J = 1.8$ Hz, 1H), 2.18 (d, $J = 1.8$ Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 170.4, 169.3, 146.4, 138.3, 131.5, 127.7, 127.5, 92.8, 11.4; FTIR (cm$^{-1}$): 3100, 1710, 1648, 1489, 1391, 873; GC/MS (CI): 313.0 (M-e$^-$)+; HRMS mp = 108-109 °C.

1-(4-iodophenyl)-3-methylpyrrole-2,5-dione 4.3 - To a 100 mL round bottom flask equipped with a magnetic stir bar was added 4-iodoaniline (6.57 g, 30.0 mmol), 2-methylmaleic anhydride (2.70 mL, 30.0 mmol) and diethyl ether (45 mL). The reaction was capped with a polypropylene cap and stirred at rt for 1 h. The reaction was cooled to 0 °C and the solid was collected by vacuum filtration and dried on a frit. The crude solid was transferred to a 50 mL round bottom flask equipped with a magnetic stir bar and sodium acetate (984 mg, 12.0 mmol) and acetic anhydride (10 mL) were added. The flask was equipped with a magnetic stir bar and heated to reflux for 30 minutes. The flask was then cool to rt and the solution was poured onto cold water. The solid was filtered using vacuum filtration, dried on a frit and purified using flash silica gel chromatography to afford 4.3 (2.29 g, 24%) as a yellow solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.80 - 7.75 (m, 2H), 7.16 - 7.09 (m, 2H), 6.49 (q, $J = 1.8$ Hz, 1H), 2.18 (d, $J = 1.8$ Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 170.4, 169.3, 146.4,
138.3, 131.5, 127.7, 127.5, 92.8, 11.4; FTIR (cm\(^{-1}\)): 3100, 1710, 1648, 1489, 1391, 873; GC/MS (CI): 313.0 (M-\(\text{e}^+\)); HRMS mp = 108-109 °C.

4.2.3 **Synthesis of Cysteine Appended Ferrocene (structure 4.4)**

A cysteine appended ferrocene 4.4 and its precursors, the deprotected ferrocene linked cysteine dimer 4.4b and the trityl protected cysteine appended ferrocene 4.4a were synthesized by Dr. Amber Gietter-Burch. Her synthetic protocol are as follows.

*Trityl protected cysteine appended ferrocene 4.4a* - A hot 200 mL round bottom flask equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. Once cool, the flask was backfilled with \(\text{N}_2\), the septum was removed and L-Cys(Trt)-OEt (6.08 g, 15.0 mmol), ferrocene carboxylic acid (4.14 g, 18.0 mmol), HBTU (7.96 g, 21.0 mmol), and EDC·HCl (4.03 g, 21 mmol) were added. The septum was replaced, the flask was reattached to a manifold and evacuated and backfilled with \(\text{N}_2\) three times. DMF (60 mL) was added via syringe and the reaction was stirred at rt for 15 h. Once complete, the reaction was diluted with \(\text{CH}_2\text{Cl}_2\) (40 mL) and extracted 5x with brine (30 mL). The organic layer was dried with magnesium sulfate, filtered, and concentrated *in vacuo*. The crude reaction was purified by flash silica gel chromatography (65:35 hexanes : ethyl acetate) to afford 4.4a (3.01 g, 33%) as an orange solid: \(^1\text{H NMR} (400 MHz, \text{CDCl}_3) \delta 7.45 - 7.37 \text{ (m, 6H)}, 7.32 - 7.25 \text{ (m, 6H)}, 7.25 - 7.18 \text{ (m, 3H)}, 6.27 \text{ (d, } J = 7.9 \text{ Hz, 1H)}, 4.79 \text{ (dt, } J = 7.9, 5.2 \text{ Hz, 1H)}, 4.73 - 4.65 \text{ (m, 2H)}, 4.36 \text{ (s, 2H)}, 4.31 - 4.14 \text{ (m, 7H)}, 2.77 - 2.61 \text{ (m, 2H)}, 1.29 \text{ (t, } J = 7.1 \text{ Hz, 3H}); \(^{13}\text{C NMR} (101 MHz, \text{CDCl}_3) \delta 170.9, 170.1, 144.4, 129.6, 128.2, 127.0, 75.1, 70.8, 70.8, 70.1, 68.5, 68.4, 66.9, 62.1, 51.0,
34.4, 14.3; FTIR (cm⁻¹): 3315, 3056, 2982, 1739, 1636, 1533, 1187, 742, 701; GC/MS (ESI): 616.2 (M+Na)⁺; HRMS mp: 76-77 °C.

**Deprotected ferrocene linked cystine dimer 4.4b** - To a 50 mL round bottom flask was added 4.4a (1.9 g, 3.15 mmol), dichloromethane (9.0 mL) and trifluoroacetic anhydride (9.0 mL). The reaction was capped with a polypropylene cap and stirred at rt for 30 minutes after which the cap was removed triethylsilane (674 µL, 4.22 mmol) was added dropwise. The reaction was recapped with a polypropylene cap and stirred at rt for 15 h. The reaction was concentrated in vacuo and the solid was filtered and washed with TFA. The organic layer was reconcentrated in vacuo and purified by flash silica gel chromatography (600 mg, 53%) as an orange solid: ¹H NMR (400 MHz, CDCl₃) δ 6.61 (d, J = 6.9 Hz, 2H), 5.01 (dt, J = 7.6, 4.1 Hz, 2H), 4.74 (ddt, J = 11.7, 2.5, 1.3 Hz, 4H), 4.43 - 4.37 (m, 4H), 4.33 - 4.22 (m, 14H), 3.11 (dt, J = 8.9, 3.8 Hz, 4H), 1.34 (t, J = 7.2 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 170.6, 170.5, 75.0, 71.0, 70.9, 70.0, 68.5, 68.4, 62.3, 53.4, 27.3, 14.4; FTIR (cm⁻¹): 3306, 3095, 2981, 1739, 1635, 1533, 1189, 485; GC/MS (ESI): 743.3 (M+Na)⁺; HRMS mp: 76-77 °C.

**Cysteine modified ferrocene 4.4** - A hot 25 mL round bottom equipped with a magnetic stir bar and a rubber septum was attached to a double manifold and cooled under vacuum. Once cooled, the flask was backfilled with N₂, the septum was removed and 4.4a (300 mg, 417 µmol) was added. The septum was replaced, the flask was reattached to a double manifold and evacuated and backfilled with N₂ three times. Argon sparged, dry THF (4.17 mL) and "Bu₃P (156 µL, 625 µmol) was added via syringe. The reaction was stirred at rt for 1 h after which argon sparged H₂O (1.6 mL) was added via syringe. The reaction was stirred for an additional 2.5 h at rt. The reaction was removed from the double manifold, the septum was removed, and
CH₂Cl₂ (5 mL) was added. The solution was extracted 1x with H₂O (10 mL) and 1x with brine (10 mL). The organic layer was dried with magnesium sulfate, filtered, and concentrated in vacuo to afford 4.4 (61.6 mg, 41%) as a orange solid: NOTE: Sparring with argon was recommended to prevent reformation of the disulfide bond. This reaction was never conducted with N₂ sparged solvents: ¹H NMR (400 MHz, CD₂Cl₂) δ 6.54 (d, J = 8.4 Hz, 1H), 4.94 (dt, J = 8.0, 4.3 Hz, 1H), 4.71 (dt, J = 4.6, 1.9 Hz, 2H), 4.42 - 4.36 (m, 2H), 4.32 - 4.18 (m, 7H), 3.15 - 3.00 (m, 2H), 1.48 (t, J = 8.9 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H); ¹³C NMR (101 MHz, CD₂Cl₂) δ 170.3, 169.7, 70.7, 70.6, 69.8, 68.2, 68.2, 61.9, 27.0, 14.0; FTIR (cm⁻¹): 3305, 2982, 1738, 1640, 1530, 1187, 700; GC/MS (ESI): 362.1 (M+H)⁺; HRMS mp: 109-110 °C

4.2.4 Synthesis of Thiol Appended Ru tris(bipyridine) Luminophore (structure 4.5)

A thiol appended Ru(bpy)₃ 4.5 and its precursors, the Ru(bpy)₃ thioacetate 4.5c, bipyridine thioacetate 4.5b and bipyridine chloride 4.5a were synthesized by Alize Marangoz. Her synthetic protocol for compound 4.5c, 4.5b and 4.5 are as follows.

*Bipyridine-Thioacetate 4.5b.* This compound was prepared using a slightly modified literature procedure.²⁰ Potassium thioacetate (29.5 mg, 0.26 mmol) was dissolved in anhydrous N,N-Dimethylformamide (4 ml) and 4.5a (50.2 mg, 0.17 mmol) was added to the solution. The reaction was stirred at 80 °C under a nitrogen atmosphere. After 16 h, the resulting mixture is cooled to room temperature, and the solid was filtered and washed with N,N-Dimethylformamide and dichloromethane. The filtrate was concentrated under reduced pressure and dissolved in dichloromethane (25 mL), transferred to a separatory funnel and washed three times
with water. The combined aqueous layers were extracted with additional dichloromethane. Organic layers were combined and dried over sodium sulfate, filtered and concentrated under reduced pressure to yield 54.8 mg (96%) of the desired product. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.52 (dd, J = 12.5, 4.9 Hz, 2H), 8.21 (d, J = 10.0 Hz, 2H), 7.20 – 7.05 (m, 2H), 3.52 (t, J = 6.3 Hz, 2H), 3.44 (t, J = 6.2 Hz, 2H), 3.07 (t, J = 6.3 Hz, 2H), 2.76 (t, J = 7.7 Hz, 2H), 2.41 (s, 3H), 2.32 (s, 3H), 2.00 – 1.88 (m, 2H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 195.79 , 156.32, 156.11, 152.11, 149.19, 149.06, 148.29, 124.81, 124.15, 122.14, 121.46, 69.90, 69.41, 32.00, 30.74, 30.30, 29.19, 21.35.

$\text{Ru(bpy)}_3$ thioacetate 4.5c. This compound was prepared using a slightly modified literature procedure. $^{20}$ Ru(bpy)$_2$Cl$_2$ (52.2 mg, 0.10 mmol) was added to a solution of 4.5b (49.8 mg, 0.15 mmol) in ethanol (6 mL). The reaction was refluxed for 18 h. After cooling down to room temperature, the resulting mixture was concentrated under reduced pressure and re-dissolved in water. The solution was transferred to a separatory funnel and extracted five times with dichloromethane. Ammonium hexafluorophosphate (146.7 mg, 0.90 mmol) was added to the concentrated aqueous layer (2 ml). The mixture was stirred for 15 minutes. Orange precipitate was collected by vacuum filtration and washed with water to afford 87.8 mg of the product (84%). $^1$H NMR (400 MHz, CD$_3$CN) δ 8.49 (d, J = 8.2 Hz, 4H), 8.38 (d, J = 15.1 Hz, 2H), 8.04 (t, J = 8.0 Hz, 4H), 7.73 (d, J = 5.4 Hz, 4H), 7.54 (dd, J = 9.9, 5.8 Hz, 2H), 7.39 (q, J = 6.9 Hz, 4H), 7.24 (t, J = 6.8 Hz, 2H), 3.46 (q, J = 6.1 Hz, 4H), 2.96 (t, J = 6.4 Hz, 2H), 2.86 (t, J = 7.6 Hz, 2H), 2.53 (s, 3H), 2.27 (s, 3H), 1.90 (d, J = 7.4 Hz, 2H). $^{13}$C NMR (151 MHz, CD$_3$CN) δ 196.20 , 158.05 , 157.53,
Thiol appended Ru(bpy)$_3$ 4.5. The synthesis of this compound was adapted from a literature procedure. Sodium thiomethoxide (7.0 mg, 0.10 mmol) was dissolved in anhydrous methanol (0.7 ml) and this solution was added to 4.5c (51.5 mg, 0.05 mmol) in schlenk flask. The reaction was stirred at 50°C under a nitrogen atmosphere. After 2 h, aqueous HCl (1 ml, 0.1 M) was added to the resulting mixture and a precipitate was formed. The mixture was transferred to a separatory funnel and extracted three times with dichloromethane (10 mL). Organic layers were combined and washed with brine, dried over sodium sulfate and concentrated under reduced pressure to yield 46.6 mg of the product (94 %).

$^1$H NMR (600 MHz, CD$_3$CN) $\delta$ 8.50 (d, $J = 8.3$ Hz, 4H), 8.38 (dd, $J = 18.5$, 1.8 Hz, 2H), 8.05 (tdd, $J = 8.0$, 3.4, 1.8 Hz, 4H), 7.73 (q, $J = 5.3$ Hz, 4H), 7.55 (dd, $J = 10.7$, 5.8 Hz, 2H), 7.40 (dddd, $J = 13.1$, 7.3, 5.7, 1.3 Hz, 4H), 7.24 (ddd, $J = 8.5$, 6.0, 1.4 Hz, 2H), 3.61 (t, $J = 6.2$ Hz, 2H), 3.46 (t, $J = 6.1$ Hz, 2H), 2.92 – 2.79 (m, 4H), 2.53 (s, 3H), 2.16 (s, 3H), 1.92 (m, 2H).

$^{13}$C NMR (151 MHz, CD$_3$CN) $\delta$ 158.10, 157.57, 155.21, 152.70, 152.51, 151.89, 151.76, 151.47, 138.62, 129.31, 128.72, 128.53, 126.03, 125.36, 125.21, 70.27, 69.47, 39.60, 32.51, 30.60, 21.28.

4.2.5 Generation of Alkynyl Modified Carbon Paper Substrates CP1 and CP2

Electrochemical grafting and deprotection of diazonium 4.1 on bare carbon paper substrates was carried out according to our previous procedure. Within a nitrogen filled glove box, a piece of carbon paper (~1 x 2 cm) working electrode was clipped to a platinum wire and immersed in 10 mL of a 0.1 M solution of TBAPF$_6$ in acetonitrile containing 18.6 mg (50 μmol) of diazonium 4.1. A platinum gauze
auxiliary and silver wire pseudo reference electrode were also placed within the vial. The electrodeposition was carried out by varying the applied potential between 0.5 and –0.3 V with a scan rate of 50 mV/s. The electrochemical grafting to generate CP1 was generally complete after roughly 5–7 cycles between these potentials. Following the electrochemical grafting, CP1 was rinsed with 8 mL of dry acetonitrile and then soaked in 4 mL of dry acetonitrile for 10 min. This cleaning process was repeated two additional times and the CP1 substrate was then allowed to dry under N₂.

4.2.6 Procedure for on Surface Sonogashira Cross Coupling to Generate CP6 and CP7

**Maleimide capped carbon paper CP6** - In a nitrogen-filled glove box copper iodide (5.0 mg, 26.3 μmol) tetrakis(triphenylphosphine)palladium(0) (30.0 mg, 26.0 μmol), 4.2 (97 mg, 324 μmol), triethylamine (45 μL, 323 μmol), and anhydrous dioxane (6.5 mL) were added to a 20 mL vial. CP2 with approximate dimensions of 1.0 × 0.5 cm was then added to this solution using forceps. The vial was capped with a polyethylene-lined cap and heated in an aluminum block to 90 °C for 15 min. The reaction solution was then allowed to cool to room temperature and the modified carbon substrate was removed from solution using forceps. The modified substrate was cleaned by pipetting 4 mL of dry dimethylformamide onto both faces of the paper to remove excess reagents. The washed substrate was then placed in a clean vial and soaked in 3 mL of dry dimethylformamide for 15 min. Following removal from the DMF with forceps, the carbon paper was washed again with 4 mL of dry acetonitrile and then soaked for an additional 15 min in 3 mL of acetonitrile. The last acetonitrile rinse and soak was repeated an additional three times. Following one final rinse with 4
mL of acetonitrile, the modified CP6 substrate was dried by evaporation under nitrogen for several minutes.

Methylated maleimide capped carbon paper CP7 – This procedure is identical to CP6’s except for the following detail: compound 4.3 (102 mg, 324 μmol) was added to the vial in lieu of compound 4.2.

4.2.7 Procedure for on Surface Michael Addition to Generate CP8, CP9, CP10 and CP11

Cysteine appended ferrocene conjugated maleimide CP8 - In a nitrogen-filled glove box 4.4 (5.7 mg, 16 μmol) was dissolved in 2 mL of dry acetonitrile. CP6 was then added to this solution using forceps. The vial was capped with a polyethylene-lined cap and allowed to react for either 1 or 24 hr at rt to afford CP8. CP8 was cleaned by pipetting 4 mL of dry dimethylformamide onto both faces of the paper in order to remove excess reagents. The washed substrate was rinsed with 2 mL of dry acetonitrile then placed in a clean vial and soaked in 3 mL of dry acetonitrile for 15 min. This step was repeated an additional three times. Following one final rinse with 4 mL of acetonitrile, the modified CP8 substrate was dried by evaporation under nitrogen for several minutes

Cysteine appended ferrocene conjugated methylated maleimide CP9 – This procedure is identical to CP8’s except for the following detail: electrode CP7 was added to the solution containing 4.4 in lieu of electrode CP6.

Thiol appended ruthenium tris(bipyridine) conjugated methylated maleimide CP10 – This procedure is identical to CP8’s except for the following details: compound 4.5 (15.9 mg, 16 μmol) is dissolved in 2 mL of dry acetonitrile (in lieu of
compound 4.4) and CP7 was added to the solution containing 4.5 (in lieu of electrode CP6).

4.2.8 Instrumentation

4.2.8.1 Electrochemistry

All electrochemistry was performed using either a CHI-620D potentiostat, or CHI-760D bipotentiostat. A standard 3-electrode cell was used for all electrochemical measurements with carbon paper, or a modified carbon paper (CPX, where x =1, 2, 6, 7, 8, 9 or 10) as the working electrode and Pt gauze as the auxiliary electrode. Electrochemistry for CP8 or CP9 was done with a freshly made Ag/AgNO3 reference electrode and performed inside a glove box under an inert atmosphere of N2 in acetonitrile with 0.1 M TBAPF6 as the supporting electrolyte. Electrochemistry for CP10 was done with a freshly made Ag/AgCl reference electrode in 0.08 M phosphate buffer and 0.2 M NaNO3 supporting electrolyte (pH adjusted to 7.0).

Cyclic voltammetry parameters for CP8 and CP9 began at -0.2 V and swept anodically with a switching potentials at 0.6 V for 4 sweeps at a scan rate of 0.1 V/s.

Cyclic voltammetry parameters for the electrochemiluminescence for CP10 began at 0.0 V and swept anodically with switching potentials of 1.5 V and -1.2 V for 5 sweeps at a scan rate of 0.05 V/s. Bulk electrolysis was conducted at 1.35 V vs Ag/AgCl for 15 minutes.

4.2.8.2 XPS Analysis

The carbon substrates were analyzed using a Thermo Scientific K-alpha+ spectrometer equipped with a monochromatic x-ray source and a 128 channel CCD detector plate. the X-Rays used were monochromatic Al Kα X-Rays (1486.7 eV) with
a power of 72 W (12 kv, 6 mA). The operating pressure in the main chamber was less than 1 x 10⁻⁸ torr. The X-Ray spot size was elliptical in shape with a semi-major axis of roughly 400 μm.

4.2.8.3 Chemiluminescence Detection

Spectra were recorded on an automated Photon Technology International (PTI) Photon Technology International fluorimeter equipped with a 150 W arc lamp, a LPS-220B lamp power supply and a model 810 photon counting photomultiplier detector. During the cyclic voltammogram, emission was monitored at 595 nm in 1 sec increments. During the bulk electrolysis emission was detected from 520 nm to 700 nm in 2 nm increments with an integration time of 10 seconds.

4.2.8.4 Computations.

All density functional calculations were performed using the Gaussian 09 (G09) program package,²² with the Becke three-parameter hybrid exchange and Lee-Yang-Parr correlation functional (B3LYP).²³⁻²⁵ The 6-31G* basis set was used for C, N, H and I atoms. For calculations in solution, the SMD universal continuum model²⁶ was used with acetonitrile as the solvent (ε = 36.64). For instructions on how to calculate the redox potential of a molecule please refer to appendix B.

4.3 Results and Discussion

4.3.1 Electrochemically Formed Diazo Linkages from Diazonium Salts

An important moiety that is formed (and often overlooked) during electrochemical grafting of aryl diazonium salts is the diazo linkage (see Scheme 4.1). The presence of a nitrogen peak observed by XPS at 400 eV was attributed to this
linkage by Bélanger in 1997,\textsuperscript{27} six years after the discovery of electrochemical diazonium deposition on carbon electrodes.\textsuperscript{28} Since then several studies have been dedicated solely to understanding the nature and the mechanism of the diazo linkage.\textsuperscript{29-32} In chapters 2 and 3, we utilized XPS and electrochemistry together to justify the success of the cross coupling between \textbf{CP2} and \textbf{2.2}, but the these techniques were adventitious mainly because of the nature of the immobilized ferrocene molecule we were probing (Fe 2p’s well resolved XPS spectrum, and ferrocene’s redox activity). Substituting the ferrocene appended aryl halide reactant for the maleimide appended aryl halide, 4.2, complicates the XPS analysis and negates the usefulness of potentiometry as a detection tool for cross coupling success.

Scheme 4.1. Electrochemical deposition of diazonium salts showing the diazo linkage formed

The binding energy for the N 1s diazo linkage is centered at 400.0 eV\textsuperscript{31} and we do observe the presence of a peak in both \textbf{CP1} and \textbf{CP2} at 399.9 eV. Maleimide functionalized materials have shown the N 1s peak come in slightly above 400 eV,\textsuperscript{33} with another report indicating the binding energy was 400.3 eV.\textsuperscript{34} These reported values gave little hope in the ability to resolve the two nitrogens (immobilized maleimide and immobilized diazo) from one another as evidence for successful
surface Sonogashira cross coupling. Despite the reported values, compound 4.2 was analyzed by XPS spectroscopy as a powder and found that the peak position of the N 1s peak is centered at 400.6 eV which corresponds to a 0.7 eV separation from the diazo linkage of CP1 and CP2. The ratio for N to O and I for the 4.2 compound were also calculated (from the atomic percentages) and listed in Table 4.1.

4.3.2 Surface Sonogashira Cross Coupling of Electrodes to Introduce Maleimide Functional Groups (Generation of CP6 and CP7)

CP2 was subjected to Sonogashira reaction conditions that were optimized by Dr. Peter Gildner for the homologous synthesis of 1-[4(phenylethynyl)phenyl]pyrrole-2,5-dione.35 Further screening of the reaction with CP2 found that the reaction time was complete in 15 min (Scheme 4.2).
Scheme 4.2. Homogeneous reaction conditions for Sonogashira cross coupling with 4.2 and phenyl acetylene (top). Reaction conditions for surface Sonogashira cross coupling with 4.2 and CP2.

XPS analysis of CP6 showed the presence of nitrogen at 400.6 eV, consistent with the N 1s binding energy of the 4.2 powder. Figure 4.1 shows the N 1s high resolution spectrum for both CP2 and CP6 overlaid as well as the error associated with our measurement. The number of trials for CP2 is 5 and the number of trials for CP6 is 8, demonstrating that this binding energy shift is reproducible.
Figure 4.1. N 1s high resolution spectra of CP2 (green) and CP6 (yellow) showing the resolution between the diazo nitrogen and the maleimide nitrogen (left). The binding energy, error associated with the measurements and the number of trials are shown in the histogram (right).

XPS analysis of CP6 also showed the presence of significant amounts of iodide, suggesting fouling of the surface of some kind. The source of this iodination was hypothesized to be either persistent residual starting material or the product of an unfavorable side reaction to afford CP6a which could facilitate just in the presence of base (no catalyst, see Scheme 4.3). To understand the source of the contamination and further demonstrate successful Sonogashira cross coupling we attempted two different post treatments of CP6.
Scheme 4.3. Unfavorable side products that could contribute to iodide fouling of the surface.

The first treatment (referred to as T1) involved subsequent soakings in DMF and MeCN as a medium to desorb any unreacted starting material.

The second treatment (referred to as T2) involved electrochemically reducing CP6 in electrolyte. Electrochemistry of 4.2 in acetonitrile demonstrated a large cathodic current at -1.13 V vs. Ag/AgCl (see Figure 4.2, left). DFT calculations identified a reduction potential for 4.2 at -1.48 V vs. Ag/AgCl corresponding to the injection of an electron into the dione ring. Subjecting CP6 to negative potentials resulted in a peak around -1.1 V present only during the first sweep (see Figure 4.2, right). We attributed this peak to the reduction of maleimide. This method provides a more vigorous way to desorb unreacted starting material; by reducing adsorbed 4.2 electrochemically, desorption is achieved through coulombic repulsion [of 4.2 anion with a negatively poised CP6 electrode].
Figure 4.2. Cyclic voltammogram of 4.2 (left). DFT calculated LUMO for 4.2 (left insert). Cyclic voltammogram of CP6 (right). Electrochemistry was done in acetonitrile with TBAPF₆ as the supporting electrolyte.

Finally, we also reacted CP2 with 4.3 to afford CP7, hypothesizing that the methyl group on 4.3 could slow the competing unfavorable side reaction (see Scheme 4.2). The ratios of I to N on the surface after each method are listed in Table 4.1. From the table, we see that iodide fouling is significantly diminished for both CP6ₜ₂ as well as CP7.

<table>
<thead>
<tr>
<th></th>
<th>N/N</th>
<th>O/N</th>
<th>I/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>1.0</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>CP6ₜ₁</td>
<td>1.0</td>
<td>2.4</td>
<td>0.8</td>
</tr>
<tr>
<td>CP6ₜ₂</td>
<td>1.0</td>
<td>3.1</td>
<td>0.3</td>
</tr>
<tr>
<td>CP7</td>
<td>1.0</td>
<td>1.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>
4.3.3 Surface Michel Additions to Tether Cysteine Derivatives (Generation of CP8 and CP9)

CP6\textsubscript{T1}, CP6\textsubscript{T2} and CP7 were all subject to a Michael addition reactions in acetonitrile with 4.4 to afford CP8\textsubscript{T1}, CP8\textsubscript{T2} and CP9 (see Scheme 4.4). The voltammogram for each ferrocene appended substrate (CP8\textsubscript{T1}, CP8\textsubscript{T2} and CP9) demonstrated a clear feature associated with the ferrocene/ferrocenium redox couple (see Figure 4.3). Noticeably the capacitive current for CP8\textsubscript{T2} was enhanced with significantly less faradaic area under the voltammetric peaks. While the peak at -1.1 V in Figure 4.2, right) was attributed to the reduction of adsorbed 4.2, this potential could just as well be compromising the integrity of the molecular wire on CP6. Reduction of the immobilized maleimide group (leading to the destruction of the molecular wire units) leaves less moieties for 4.4 to react with resulting in an attenuated signal in the voltammogram.
Scheme 4.4. Michael addition of cysteine appended ferrocene 4.4 with immobilized maleimide.

**CP9** shows a redox feature for the immobilized ferrocene, but with significantly less iron coverage then for **CP8**. Increasing the reaction time to 24 hours increased the iron content to comparable values to **CP8** but without the significant iodide contaminant.
The XPS data for CP8 shows the presence of Fe 2p and S 2p after the conjugation in a 1:1.2 ratio. CP9 also shows the presence of Fe 2p and S 2p after the conjugation but in a ratio of 1:2. The source of this inflation for the atomic percentage of sulfur has yet to be determined. The Fe 2p and S 2p high resolution spectra for CP8_T1, CP8_T2 and CP9 are shown in Figure 4.4.
4.3.4 Surface Michel Additions to Tether Thiol Appended Ru (tris)bipyridine Derivative (Generation of CP10)

While the maleimide capped electrodes offer a platform ready for bioconjugation, these substrates could also be used for immobilizing any thiol derivative. Although immobilizing 4.4 onto the surface was successful, it did exhibit some challenges such as iodide fouling and inflated atomic accountability (the increased sulfur content measured for CP9). These results highlight the complexity of surface chemistry. For these reasons, we sought another avenue for CP7. Instead of
introducing more complex cysteine based biological material to our surfaces, we tried Michael addition chemistry with a ruthenium tris(bipyridine) thiol derivative, 4.5, to better understand the surface chemistry. 4.5 also has the advantage of being an electrochemiluminescent active luminophore. Functionalization of 4.5 not only provides another compound to better understand our surface chemistry but also successful immobilization produces an ECL device that could be used in sensor development (see Scheme 4.5).
Scheme 4.5. Generation of an immobilized ECL device from surface Sonogashira cross coupled maleimide.
4.3.4.1 XPS Analysis of Surfaces

CP7 was reacted with 4.5 to afford CP10 and characterized by XPS. Carbon surface functionalized ruthenium compounds have been analyzed by XPS before but several factors present a challenge by XPS because of the proximity of the Ru 3d$_{5/2}$ (for ruthenium (II)) at 281.4 eV$^{36-37}$ to the C 1s peak at 284.6. While the 3.2 eV shift is ample distance to resolve, the intensity of the C 1s peak form the underlying electrode and the very small atomic percent of ruthenium incorporated makes the analysis of ruthenium only qualitative. The high-resolution spectra of the Ru 3d$_{5/2}$ peaks for a blank electrode, CP1, CP2, CP7, and CP10 (1 hr and 24 hr) are shown in Figure 4.5. No peak is observed for ruthenium until after Michael addition with 4.5. In addition to the ruthenium peak we also see the presence of sulfur after the Michael addition as compared to a blank carbon paper (Figure 4.6). The ruthenium atomic percentages were always less than 0.1% far below the limit of detection for XPS, even after 24 hr reaction times. Future experiments at higher temperatures were not carried out and are considered the ideal future work to facilitate higher surface coverage.
Figure 4.5. Ru 3d$_{5/2}$ XPS high-resolution spectra for (from bottom to top) blank carbon paper, CP1, CP2, CP7, CP10 reacted for 1 hr and CP10 reacted for 24 hr.

Figure 4.6. S 2p XPS high-resolution spectra for blank carbon paper (black) and CP10 reacted for 24 hr (blue).
4.3.4.2 Electrochemistry and Electrochemiluminescence

As mentioned earlier, Ru(tris)bipyridine is a luminophore and common reagent used in electrochemiluminescence. Since we have successfully tethered this compound to a surface we looked to see if CP10 can generate an ECL signal. Figure 4.7 shows the cyclic voltammogram of a blank carbon paper in phosphate buffer in the absence and presence of TPA. Also shown in Figure 4.7 is CP10 in the absence and presence of TPA (see appendix for overview of ECL). Comparing the cyclic voltammograms for blank carbon paper, we see a large oxidative current in the presence of TPA due to the oxidation of TPA to TPA radical cation. When the electrode is replaced with CP10 we see a catalytic enhancement of the current. The increase in current is not the sum of the oxidative current from the immobilized ruthenium as we can see that in the absence of TPA the ruthenium oxidation peak is not observable by CV. This corroborates the data found by XPS, that there is a very minimal amount of ruthenium incorporated on the surface. The additional oxidative current is better explained as catalytic current from the ruthenium with TPA as the sacrificial reductant. It is known that TPA radical cation can deprotonate and reduce Ru(III) to Ru(II) (generating the catalytic current) and is a source for the ECL signals. The reaction between TPA radical cation and Ru(III) is highly exergonic and produces Ru(II) in the excited state which then emits light upon relaxation. To test for this, we measured the emission at 595 nm during the cyclic voltammetry. In the presence of TPA, we measured light emission at the potentials that can oxidize TPA and ruthenium. Figure 4.8 (left) shows the light intensity overlaid with the cyclic voltammogram for CP10 in the presence and absence of TPA. To further validate that CP10 was in fact an immobilized ECL device we also collected the emission spectrum from CP10 in the presence of TPA.
Figure 4.8 (right) shows the emission spectrum of CP10 showing a peak at approximately 580 nm.

Figure 4.7. Cyclic voltammogram of native carbon paper in the absence (solid black line) and presence (dashed black line) of TPA. Cyclic voltammogram of CP10 in the absence (solid blue line) and presence (dashed blue line) of TPA.
Figure 4.8. Cyclic voltammogram (left, top) and light emission (left, bottom) of CP10 in the presence (blue) and absence (black) of TPA. Emission of CP10 in the presence of TPA (right).

4.3.5 Surface Thiol-yne Reaction with Thiol Appended Ru (tris)bipyridine Derivative (Generation of CP11)

Two control reactions were set up to test the validity of the Michael addition with CP7 to produce CP10 shown in Scheme 4.6. The reaction on blank paper afforded no ruthenium or sulfur on the surface, but a substantial amount of ruthenium and sulfur was found for the reaction with 4.5 and CP2 (see Figure 4.9). The incorporation of ruthenium and sulfur on this surface can be explained by a thiol-yne reaction to afford CP11. CP11 was tested for its activity as a luminophore, and did produce light emission during cyclic voltammetry but was not stable at the oxidative potentials of 1.35 V for long periods of time (see Figure 4.10). The emission spectrum for CP11 shows no emission. The reason for the lack of emission is suggested to be from the instability of the molecular wire at such oxidizing potentials which degrades during analysis so that no emission is observed.
Scheme 4.6. Control reaction between blank carbon paper and 4.5 (top). Control reaction between CP2 with 4.5 to afford CP11 (bottom).
Figure 4.9. Ru 3d_{5/2} (left) and S 2p (right) XPS high-resolution spectra of CP11 and blank carbon paper after 24 soaking in SI.5.

Figure 4.10. Cyclic voltammogram (left, top) and light emission (left, bottom) of CP11 in the presence (orange) and absence (black) of TPA. Emission of CP11 in the presence of TPA (right).
4.4 Conclusion

We have shown that we could extend the Sonogashira reaction coupling conditions to include a maleimide type functional group. Maleimide modified electrodes (CP6, CP7) could be further reacted with thiol derivatized compounds to afford electrodes with very specific applications such as reactions with cysteine derivatives for biological applications or a thiol appended luminophore to afford an immobilized ECL device. While the subsequent sulfur chemistry was successful it highlighted the complexity of surface chemistry and characterization. To date things that still need to be fully understood are 1) the iodide contamination (efforts were successful to minimize the contamination, but did not eliminate it completely), 2) the inflated sulfur atomic percentages and 3) the instability of the thiol-ene linker for CP11.
REFERENCES


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(15) *Oncology Assays*; Brochure for Mesoscale: Gaithersburg, MD, 2011.


5.1 Introduction

The chemical structure of deoxyribonucleic acid (DNA), solved by Watson and Crick in 1953\(^1\) consist of a repeating deoxyribose sugars backbone units connected by phosphoester linkages. The C1’ position of the ribose sugar is covalently bound to one of four base pairs: adenine, guanine, cytosine or thymine. These bases are the units in which DNA forms a double helix through hydrogen bonding with a complimentary strand. Because the bases preferentially pair to one another (adenine to guanine and cytosine to thymine), a chain of single stranded DNA (ssDNA) can be thought of as a marker with a unique and specific complimentary sequence (cDNA) that it can bind. This unique binding event is called hybridization. Hybridization is the foundation for DNA biosensors and microarrays, in which single strands of DNA are immobilized onto a substrate and upon hybridization with its compliment can transduce a signal, either electrochemically,\(^2,3\) optically\(^4\) or piezoelectrically.\(^5\) This technology has contributed towards major discoveries in gene delivery and mutation detection,\(^6\) and remains at the forefront of emerging research by identifying single polymorphisms.\(^4\) In addition to the scientific efforts regarding immobilized ss-DNA and hybridization with its compliment DNA, this technology can be carried a step farther by probing the interaction between sequence specific dsDNA and a transcription factor. An example of such an event is the binding of an NF-κB transcription factor with the dsDNA
sequence GGG ACT TTCC\textsuperscript{7,8} or GGGACTTTCC.\textsuperscript{9} This binding interaction signals an over-regulation of pro-inflammatory cytokines which is the underlying cause for Crohn's disease or ulcerative colitis.\textsuperscript{10,11} Immobilizing the dsDNA sequence onto a support, in which the dsDNA is the probe, can produce a signal upon its interaction with the transcription factor, NF-κB. This scheme, incorporated into a microelectrode array provides a platform to survey a library of small molecules that could potentially inhibit this interaction.

5.1.1 Types of Biomolecular Immobilization

Immobilization of DNA, or more broadly, biomolecules, can be classified by the strength of the biomolecular-substrate association. Methods to immobilize DNA include, chemisorption of thiol modified DNA to gold,\textsuperscript{12,13} physisorption,\textsuperscript{6,14} entrapment\textsuperscript{15,16} and affinity.\textsuperscript{12} Each of these immobilization procedures comes with the assurance (at some level) that if the species does not evaporate then it remains on the substrate; basic chemical knowledge of solute/solvent interactions replaces rigorous characterization of the substrate.\textsuperscript{17} Another method to immobilize DNA is through covalent attachment. For the purposes of this introduction, covalent attachment is defined as a chemical bond made between the DNA and substrate that is greater than 50 kcal/mol, distinguishing this type of immobilization from chemisorption of thiol modified DNA on Au, which has a Au-S bond dissociation energy of about 40-50 kcal/mol.\textsuperscript{18,19} Covalent attachment is the most robust attachment, but is the most tedious to characterize. This is because evidence must now come directly from the covalent interaction itself and relies on more sophisticated techniques. Typical characterization techniques of immobilized DNA include impedance spectroscopy,\textsuperscript{6,14,20} cyclic voltammetry of redox active intercalators or
indicators,\textsuperscript{14} X-ray photoelectron spectroscopy,\textsuperscript{13-21} quartz crystal microbalance,\textsuperscript{6-12} atomic force microscopy,\textsuperscript{22-25} fluorescence labeled experiments,\textsuperscript{13-26-27} and ir and raman spectroscopy.\textsuperscript{28-29} While these techniques (and the combination of these) are worthy evidence of immobilization, it is important to distinguish that evidence of conjugation must come from the bond formed (for example, in an IR spectrum, the removal of an azide stretch after copper catalyzed azide alkyne cycloaddition would constitute as evidence of covalent formation of the triazole ring). It is not uncommon to find in the literature instances where there is no characterization of the linkage at all, and evidence of the immobilization event come indirectly from subsequent chemistries with a tagged compound.\textsuperscript{30-33} It is also important to note the contribution non-specific binding plays in biomolecular surface chemistry,\textsuperscript{34-39} adding to the complexity of determining analytical results. Because biomolecules are particularly large (on the order of 12 kDa for a 20 mer ssDNA sequence, (a typical DNA size for immobilization), they often adopt secondary and tertiary structures. What causes these secondary and tertiary structures are usually at the heart of what causes them to be very good non-specific adsorbers (phosphate anionic backbone in DNA, hydrogen bonding, disulfide bonds in proteins, etc...). Non-specific binding of biomolecules on surfaces can lead to false positives when trying to characterize a conjugated immobilized biomolecule.\textsuperscript{40}

The favorable interaction between the NF-κB transcription factor and the ds-DNA sequence GGG ACT TTCC (13.29 kcal per mole of NF-κB and DNA)\textsuperscript{41} can act as a driving force for DNA desorption if the association energy exceeds the adsorption energy corresponding to the physisorbed DNA. Because of this, we chose conjugation of DNA onto our substrate (as opposed to chemisorption, physisorption, entrapment or
affinity), despite the challenges associated with characterizing conjugated immobilized DNA.

5.1.2 Supports for Immobilization

The support in which DNA is immobilized on is as important as the nature of the immobilization discussed in 5.1.1. The support is often a function of its application, and can range from nanoparticles,\textsuperscript{2,27-42} conducting substrates,\textsuperscript{43} non-conducting interfaces,\textsuperscript{44} polymers,\textsuperscript{45} and others. Our application is to screen small molecules that actively inhibit the NF-κB protein/DNA binding interaction. The way this project will screen the presence or absence of the interactions is through electrochemiluminescence. The NF-κB protein will be tagged with a luminophore, such as tris(bipyridine)ruthenium(II) chloride, so that upon binding in the presence of a sacrificial reductant, such as tripropylamine (TPA), the transcription factor will emit light (scheme 5.1). The support must be a conductive electrode whose voltage can be regulated so that oxidation of both the luminophore and sacrificial reductant can be controlled. Also, because we want to screen many small molecules, a material that can be developed into microarrays is ideal to achieve a high throughput system. Electrode substrates were narrowed down to substrates that are easily masked. Finally, transparency allows optimization of light output from ECL experiment as well as removes a one-sided detection constraint imposed by an opaque electrode. Indium tin oxide (ITO) electrodes provide the conductivity, patterning ability and transparency we sought.
Scheme 5.1. Electrochemiluminescence from the biomolecular interaction of DNA and luminophore tagged transcription factor in the presence of a sacrificial reductant.

5.2 Materials and Methods

5.2.1 General Considerations

ITO plates were purchased from nanocs, in 1” x 3” sheets with resistances of 100 ohms. Sodium hydroxide (NaOH), 4-aminobenzoic acid (≥99%), sodium nitrite (NaNO₂), hydrochloric acid (10 N), N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride 98% (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino)ethanesulfuric acid (MES), sodium citrate tribasic dehydrate, sodium chloride (NaCl), tris(hydroxymethyl)aminomethane HCl (Tris-HCl), magnesium chloride (MgCl), dithiothreitol (DTT), bovine serum albumin (BSA) and glycerol.
were purchased from one of the following major chemical suppliers: Fisher, Sigma Aldrich, Acros, or Alfa Aesar. The DNA sequence GAGTAGGATCAGTACAGCTCTGAGGGACTTTCTGATC (5’ → 3’) modified with hexyl amine at the 5’ end (ssDNA-NH$_2$) and its compliment were purchased from Eurofins MWG Operon LLC. MES buffer stock solution was made by dissolving MES (195 mg) in Millipore water (10 mL) and adjusting the pH to 6.0 with HCl or NaOH. NHS stock solution was made by dissolving NHS (10 mg) in MES stock solution (1 mL). 2x SSC buffer stock solution was made by dissolving sodium citrate tribasic dehydrate (441 mg) and NaOH (879 mg) in Millipore water (50 mL) and adjusting the pH to 7.0 with HCl or NaOH. Binder buffer stock was made by dissolving Tris-HCl (316 mg), NaCl (292 mg), MgCl$_2$•H$_2$O (20 mg), DTT (16 mg), BSA (5 mg) in glycerol (5 mL) and Millipore water (95 mL).

5.2.2 Instrumentation

5.2.2.1 Electrochemistry

All electrochemistry was performed using either a CHI-620D or a CHI-760D bipotentiostat. All electrochemistry was performed using the standard 3 electrode cell, with ITO as the working electrode, a platinum gauze as the auxiliary and a Ag/AgCl as the reference. Faradic impedance measurements were performed in the presence of 1 mM K$_3$Fe(CN)$_6$ redox probe and 0.1 M NaCl electrolyte. The impedance spectra were recorded at the foot of the wave of the bias potential of the redox probe (0.15 V vs. Ag/AgCl), by applying 5 mV alternative voltage in the frequency range of 1 to 100,000 Hz. Impedance data were fit using the ZIVE ZMAN 2.3 software.
5.2.2.2 X-Ray Photoelectron Spectroscopy

All XPS spectra were acquired using a Thermo Fisher K-alpha+ spectrometer. The X-rays used were monochromatic Al Kα X-Rays (1486.7 eV) with a power of 72 W (12 kV, 6 mA). The operating pressure in the main chamber was less than $1 \times 10^{-8}$ mbarr. The X-Ray spot size was elliptical in shape with a semi-major axis of 400 µm. Angle resolved XPS was performed with an angle resolved stage from 0° to 60° in 7.5° increments. Film thickness was determined by taking the ratio of intensities of oxygen in the film from the carboxylic acid group, and oxygen from the ITO bulk material in accordance with the following equation:

$$\frac{I_{\text{COOH}}(530.5 \text{ eV})}{I_{\text{ITO}}(530.5 \text{ eV})} = R = R^\infty \left[ \frac{d}{\lambda_0 \cos \theta} \right] - 1$$

Where $I_{\text{COOH}}(530.5 \text{ eV})$ is the intensity of the oxygen peak from the carboxylic acid layer, $I_{\text{ITO}}(530.5 \text{ eV})$ is the intensity of the oxygen peak from the ITO, $\lambda_0$ is the attenuation length of oxygen through the film (which is assumed to be the same when analyzing the same element in very thin films) and $\theta$ is the deviation angle of the surface normal relative to the detector.

5.2.2.3 Contact Angle Goniometry

Contact angle measurements were acquired using a First Ten Angstroms Series 100 goniometer. An approximately 20 µL sized Millipore water droplet was suspended from a pipette. The pipette was gradually lowered so that the water droplet could contact the surface and then the pipette was quickly retracted. FTA 32 2.0 software was used to measure the angles of the water droplet on the surface.
5.2.2.4 Time of Flight Secondary Ion Mass Spectrometry

A ToF-SIMS IV with the upgraded capabilities of a ToF-SIMS V (ION-TOF, GmbH) was used. With a bismuth/manganese primary ion source, all spectra and images were collected using the high-current ‘bunched’ mode: 25 keV Bi$_3^+$ clusters of pre-bunched pulse width of 640 ps and target current of ~0.3 pA. Image pixel density was 128 x 128 pixels with the ion dose density being set at the static SIMS limit of 1 x 10$^{12}$ ions/cm$^2$. The extraction cone used to attract ejected ions to the time of flight-mass spectrometer was pulsed ± 2 kV depending on the secondary ion charge of interest, with a 10 kV post-acceleration for the detector. To prevent charge build up on the surface, a low-energy (75 eV) electron flood gun was utilized. The mass spectra were calibrated using ubiquitous ions for positive ion modes. The positive ion mass calibrations were made using: H$^+$, H$_2^+$, H$_3^+$, C$^+$, CH$^+$, CH$_2^+$, CH$_3^+$, C$_2$H$_3^+$, C$_3$H$_5^+$, C$_4$H$_7^+$, C$_5$H$_5^+$, C$_6$H$_5^+$, and C$_7$H$_7^+$. ION-TOF Measurement Explorer (version 6.2) was used for all data analysis. The ion spectra and images were normalized to its CH$_3^+$ intensity.

5.2.3 ITO Preparation

ITO plates were cut into 2 x 1 cm$^2$ pieces and prepared by five different methods. Method 1: Solvent Treatment. ITO plates were scrubbed with glass cleaner and sonicated in glass cleaner for 5 min followed by sonication in Millipore water, acetone, ethanol and finally Millipore water for 5 min each. Method 2: Plasma Treatment. Following the solvent treatment, the ITO plates were placed in a plasma cleaner (Harrick PDC-32G) and allowed to react under air plasma on high RF power for 20 minutes. Method 3: Ammonium-Peroxide Treatment. Following solvent treatment, ITO plates were added to a 100 mL round bottom flask containing a hot
solution of NH₄OH (27%) and H₂O₂ (30%) in Millipore water (45mL) in a ratio of 1:1:5. *Method 4: UV Treatment.* Following the solvent treatment ITO plates were left under a UV lamp (254 nm) in a fume hood for 30 min. *Method 5: NaOH Treatment.* ITO plates were sonicated in a 10 wt % solution of NaOH for 10 min twice and finally sonicated in Millipore water to remove any excess NaOH rinsing with water in between. NaOH base treated ITO plates were used for all subsequent chemistries.

### 5.2.4 Electrochemical Deposition of 4-Diazobenzoic Acid

4-aminobenzoic acid (2.3 mg) and a small stir bar was added to a 20 mL vial. To that was added Millipore water (19.1 mL) and HCl (0.9 mL). NaNO₂ (200 µL) was added from a stock solution (1 M) and allowed to stir for 5 min. A cleaned 2 x 1 cm² ITO plate was used as the working electrode. The stirring was stopped after 5 min. A cyclic potential was swept from 0.2 V to -0.4 V and back at a scan rate of 0.4 V/s and then immediately used for the immobilization of ssDNA.

### 5.2.5 Surface Immobilization and Hybridization of DNA

EDC (11 mg) was added to a 1.5 mL Eppendorf tube with NHS stock (170 µL) and MES buffer stock (330 µL). This solution was combined with the H₂N-ssDNA (40 µM stock) in a ratio of 1:1. 50 µL of the final EDC/NHS/ H₂N-ssDNA was delivered to the COOH terminated ITO surface and allowed to react for 3 days in a chamber with 75% relative humidity.

Following the immobilization of H₂N-ssDNA, the ITO plates were soaked in 2x SSC buffer 5 consecutive times for 5 minutes each time, followed by a soak in Millipore water 2 consecutive times for 5 minutes each time, rinsing with water in between to remove any excess H₂N-ssDNA. The ITO were dried under a stream of N₂.
dsDNA stock (100 µM) was diluted in 2x SSC buffer in a ratio of 1:49. 50 µL of the final solution was delivered to the ssDNA modified ITO surface and allowed to react for 4 hrs in a chamber with 100% relative humidity.

5.2.6 Binding of NF-κB Protein

Conditions for the binding of NF-κB protein to the immobilized ITO-dsDNA electrode were determined based on the electrophoretic mobility shift assay (EMSA).

5.2.6.1 Electrophoretic Mobility Shift Assay Protocol

To 20 µL binding buffer, 2 nM hybridized or single stranded DNA was added with 1:1, 1:10 or 1:100 molar ratio of purified NF-κB. dH2O was added to bring the total volume to 30 µL. Samples were incubated at 20ºC for 30 minutes. 6 µL of 6X EMSA gel-loading solution was added to each sample and 30 µL was loaded on to a 6% DNA retardation gel. The gel was run at 120 V for 1 hour in 1X TBE buffer at 4º C. The gel was then stained for 20 minutes in 50 mL 1X TBE with 5 µL SYBR Green EMSA nucleic Acid gel stain, protected from light. The gel was washed with 50 mL dH2O for 10 seconds three times. The gel was visualized using a Gel Doc EZ imager (Bio-rad).

5.2.6.2 Surface Protein Binding

Hybridized ITO electrodes were subject to 50 µL of protein (2 µM) in binding buffer for 30 minutes in a chamber with 100% relative humidity and rinsed copiously with Millipore water before further analysis.
5.3 Results and Discussion

5.3.1 Pretreatment of ITO Electrodes

Several methods have been adopted in the literature for preparing ITO electrodes including: plasma treatment,\textsuperscript{46-51} UV treatment,\textsuperscript{46,50-51} base treatments,\textsuperscript{52-55,56} and sonicating successively with various organic solvents.\textsuperscript{48-49,51,55,57} ITO must be treated due to a layer of organic contaminants from handling in the atmosphere. ITO was treated with 5 of the suggested techniques: sonication in successive solvents, plasma treatment, ultraviolet radiation treatment, NH$_4$OH base treatment and NaOH base treatment. It was found by contact angle goniometry and XPS that treatment with base (10 w% NaOH) was most effective at removing the organic contaminants. Table 5.1 shows the atomic percent of C, O, In and Sn. The percentage of carbon gives an indication of the amount of contaminants on the surface. Another indicator of contamination is in the oxygen region. The peak at 530.5 eV represent the oxygen in In$_2$O$_3$ and the oxygen at higher binding energies can either represent a hydroxyl group attached to the indium or other carbonyl type contaminants.\textsuperscript{58} Figure 5.1 shows the components fit for the O 1$s$ region for 4 of the 5 different treatment methods. Table 5.1 shows the atomic percentages of carbon and the ratio for oxygen at 530.5 eV to the other two oxygen components. Both the plasma treatment and the NaOH base treatment remove comparable amounts of carbonaceous contamination, but inspection of the O 1$s$ region for the plasma treated ITO shows a large oxygen component at 532.0 eV, while the NaOH base treated ITO plates preserves more of the metal oxide. Sonication treated ITO plates show a comparable preservation of the In$_2$O$_3$ component with NaOH base treated ITO, but with more carbonaceous contamination. Figure 5.2 shows the contact angle of a water droplet on the treated ITO plates as a function of
time the ITO plates rest in atmosphere. Figure 5.2 shows that NaOH base treated ITO plates are more hydrophilic (due to the removal of organic contaminants) and more stable over time than ITO plates treated with any other method.

Table 5.1. Atomic percentages of C, In, Sn and O and the ratio of In$_2$O$_3$ oxygen peak (530.5 eV) to the remaining oxygen components.

<table>
<thead>
<tr>
<th></th>
<th>C 1$s$</th>
<th>In 3$d$</th>
<th>Sn 3$d$</th>
<th>O 1$s$</th>
<th>O 1$s$ (530.5 eV)/ O 1$s$ remaining components</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sonication in Solvents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>20.9 ± 9.6</td>
<td>32.2 ± 4.5</td>
<td>3.6 ± 0.6</td>
<td>43.3 ± 4.6</td>
<td>1.37 ± 0.19</td>
</tr>
<tr>
<td><strong>Base treatment (NH$_4$OH)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base treatment</td>
<td>26.1 ± 9.6</td>
<td>28.4 ± 5.9</td>
<td>2.8 ± 0.5</td>
<td>42.7 ± 3.2</td>
<td>1.16 ± 0.34</td>
</tr>
<tr>
<td>(NaOH)</td>
<td>17.4 ± 2.5</td>
<td>34.1 ± 0.6</td>
<td>3.8 ± 0.3</td>
<td>44.7 ± 2.0</td>
<td>1.40 ± 0.07</td>
</tr>
</tbody>
</table>

Figure 5.1. High resolution O 1$s$ XPS spectra of ITO plates cleaned using A.) plasma treatment, B.) base treatment with NH$_4$OH, C.) base treatment with NaOH, D.) sonication in successive solvents.
Figure 5.2. Contact angle measurements of Millipore water droplet onto treated ITO plates as a function of time the ITO plates rested in atmosphere. Droplets were 20 µL.

5.3.2 Functionalization of ITO electrode with diazonium salt

Electrochemical deposition of 4-aminobenzoic acid on ITO was done by preparing the corresponding 4-benzoic acid phenyl diazonium salt in situ in a dilute acidic solution. The concentration of the acid solution must be enough to dissolve the 4-aminobenzoic acid and facilitate the diazonium reaction, but not strong enough to etch into the ITO. Molarities below 3 M satisfy the latter condition\(^5\), and the in-situ deposition procedure utilized concentrations of 0.5 M. Figure 5.3 shows the cyclic voltammogram of 4-aminobenzoic acid in 0.5 M HCl solution before and after
addition of NaNO$_2$. The peak at -0.28 V in Figure 5.3 is only present when the electrochemical active diazonium is available. This corresponding COOH appended aryl film is prone to multilayer formation through attack to the meta position of the aryl moiety with subsequent electrochemically generated phenyl radicals. The carboxylic acid unit at the 4’ position is not large enough to have any blocking effect, and the meta positions are exposed for attack from aryl radicals. The total charge passed was calculated by integrating the cyclic voltammogram from $t = 0$ secs to $t = 3$ secs in accordance with the equation (1)

$$Q = \int_0^t i(t) \, dt$$

(1)

The total charge passed represents the total number of diazonium molecules that are reduced to form the phenyl radical. Taking all reductions as discrete sites of film construction (as opposed to some phenyl radicals reacting with other species in solution) we obtain an upper limit of theoretical film density. The upper limit was determined to be $8.4 \times 10^{14} \pm 3.1 \times 10^{14}$ sites per cm$^2$. Although the upper limit ($8.4 \times 10^{14} \pm 3.1 \times 10^{14}$ sites per cm$^2$) is less than monolayer coverage for silicon atoms ($1 \times 10^{15}$ atoms/cm$^2$), it is important to realize two things. First, the cross-sectional area for benzene (the attached molecular units on the electrode surface) are significantly larger than silicon atoms; this area needs to be accounted to estimated monolayer coverage (see below). Second, the total charge passed cannot distinguish between a packed monolayer or a loosely packed multilayer. For this, we used angle resolved XPS (ARXPS).
5.3.3 Angle Resolved X-ray Photoelectron Spectroscopy (ARXPS)

The electrochemical deposition of 4-aminobenzoic acid by generation of the diazonium \textit{in-situ} is unlike the diazonium salts that are utilized in chapters 2, 3 and 4 as there are no triisopropylsilyl groups that prevent multilayer formation through steric congestion. Electrochemical deposition of 4-benzoic acid phenyl diazonium salt is expected to form multilayers. Adjusting the cyclic voltammetric dynamic parameters, we could attain a surface coverage maximum of $8.4 \times 10^{14} \pm 3.1 \times 10^{14}$ (\textit{vide infra})
which is less than three densely packed monolayers. (A densely packed monolayer of benzene rings on a 1 cm$^2$ area is 2.8 x 10$^{14}$ molecules based on a 0.29 nm$^2$ cross sectional area of a benzene ring). To gather evidence for the thickness of the film we used angle resolved XPS.

The surface sensitivity of XPS comes from the inelastic mean free path (IMFP), or more appropriately for XPS, the effective attention length (EAL) of electrons within a solid.$^{60}$ While the X-ray source penetrates deep into the surface, the electrons emitted cannot travel far within a solid without colliding with other particles and losing their energy. Because of this, the electrons constrained to depths that are less than the EAL have the highest probability of escaping the solid and traveling through vacuum (where the IMFP is meters, a distance that is less than that between the solid analyte and the detector). The intensity of the electrons detected follows the Beer Lambert relationship (equation 2)

$$I = I_0 e^{-\frac{d}{\lambda \cos \theta}}$$

where $I$ is the intensity of the electrons emitted from the sample, $I_0$ is the intensity of the electrons emitted from the sample of an infinitely thick film, $d$ is the distance from the surface of the sample, $\lambda$ is the EAL and $\theta$ is the angle between the surface normal and the emitted electrons that are detected. Plotting this equation as a function of $d$ in units of $\lambda$ shows 95% of the XPS signal comes from distances that are less than 3$\lambda$ when the surface normal is align with the detector ($\theta = 0$, light gray area, Figure 5.4). Typically, EAL values can be up to several nanometers, meaning the XPS signal is primarily generated from electrons that reside in the top 10 nm of a surface, with an exponential dependence on this distance. By changing the angle of the substrate with respect to the detector we can enhance the surface sensitivity by decreasing the depth.
of analysis. **Figure 5.4** shows how the sample depth decreases with increasing $\theta$. The depth analysis is reduced to $1.5\lambda$ when $\theta$ increases to 60° (dark gray area, **Figure 5.4**).

**Figure 5.4.** XPS signal intensity as a function of sample depth in units of $\lambda$ for incident angle 0° and 60°.
Figure 5.5. Image depicting how the sample depth changes as the incident angle changes. The dark gray region represents the analysis depth.

The common method to determine film thickness is to integrate equation 2 from $z = 0$ to $z = d$ for an electron from the thin film, and from $z = d$ to $z = \infty$ for the underlying substrate. The EAL used should be appropriate for the material through which one is integrating. Our system comprises of a Ph-COOH film on an ITO substrate. The EAL for the C 1s core electrons from the Ph-COOH film and the O 1s core electron from the ITO substrate was calculated using the NIST standard reference database (parameters for the C 1s and In 3d used are tabulated in Table 5.2).\textsuperscript{61}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C 1s</th>
<th>O 1s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic Energy</td>
<td>1197</td>
<td>955.5</td>
</tr>
<tr>
<td>Beta asymmetry parameter</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Valence electrons</td>
<td>45</td>
<td>22</td>
</tr>
<tr>
<td>Band Gap Energy</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>X-ray incident angle</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>EAL (calculated)</td>
<td>3.15</td>
<td>1.55</td>
</tr>
</tbody>
</table>

Integrating equation 2 from $z = 0$ to $z = d$ for the Ph-COOH thin film gives us
\[ I_{P_{\text{H-COOH}}} = I_{P_{\text{H-COOH}}}^{0} \left[ 1 - e^{\left(-\frac{d}{\lambda_{P_{\text{H-COOH}}}}\cos\theta\right)} \right] \]  \hspace{1cm} (3)

and integrating equation 2 from \( z = d \) to \( z = \infty \) for the ITO substrate gives us

\[ I_{\text{ITO}} = I_{\text{ITO}}^{0} e^{\left(-\frac{d}{\lambda_{\text{ITO}}\cos\theta}\right)} \]  \hspace{1cm} (4)

Taking the ratio of these two equations gives us

\[ \frac{I_{P_{\text{H-COOH}}}}{I_{\text{ITO}}} = \frac{I_{P_{\text{H-COOH}}}^{0}}{I_{\text{ITO}}^{0}} \left[ e^{\frac{d}{\lambda_{\text{ITO}}\cos\theta}} - e^{\frac{d}{\cos\theta} \left(\frac{1}{\lambda_{P_{\text{H-COOH}}} - \frac{1}{\lambda_{\text{ITO}}}}\right)} \right] \]  \hspace{1cm} (5)

A thick film of 4-aminobenzoic acid shows a significant peak at 289.0 eV corresponding to the carboxylic acid moiety. The O 1s region of the thick film of 4-aminobenzoic acid is fit to 3 peaks, labelled V, VI and VII. Peaks V and VI at 532.2 eV and 533.3 eV correspond to the alcohol oxygen and the ketone oxygen of the carboxylic acid functional group respectively. A third peak, labeled peak VII was also fit to the data to increase the goodness of fit and can be assigned to adventitious oxygen or water adsorption. The peak heights of the C 1s peak at 289.0 eV were chosen for \( I_{P_{\text{H-COOH}}} \) and \( I_{P_{\text{H-COOH}}}^{0} \) instead of the O 1s peak because of the minimal overlap C 1s has with native ITO. The O 1s peaks (V and VI) have significant overlap with O 1s (peaks II and III) in native ITO. **Figure 5.6** shows the C 1s and O 1s high resolution spectra for native ITO, ITO-Ph-COOH, and a thick film of 4-aminobenzoic acid standard.
Native ITO is fit to 4 peaks labeled I, II, III and IV at 530.5 eV, 531.7 eV, 532.7 eV and 534.0 eV. Peak I corresponds to the oxygen in In$_2$O$_3$. Peak II has been assigned in the literature as oxygens bound to oxygen deficient indium (indium with less than 6 coordinated oxygens). Peak III is assigned to hydroxyl groups on the surface and peak IV is added for goodness of fit and can be ascribed to adsorbed water/adventitious oxygen. Peak I was chosen for I$_{ITO}$ and I'$_{ITO}$ for four reasons: a) it corresponds to the O in In$_2$O$_3$, b) it is readily resolved from its neighboring peaks, c) it’s signal intensity is the most intense, and d) it has minimum interference from the oxygen peaks from the Ph-COOH film (the oxygen peaks grow in at 532.2 eV and 533.3 eV). Table 5.3 shows the O 1s component ratios at varying incident angles. For native ITO, an increase in peak II with increasing incident angle is expected since the hydroxyl groups cap the surface and would contribute more to the total signal as the
measurement becomes more surface sensitive. An increase in peak III with increasing incident angle suggests that oxygen vacancies are denser near the surface.

Once the Ph-COOH film is electrodeposited there is a recognizable increase in the amount of the higher binding energy oxygen relative to the 530.5 eV peak even at incident angle = 0° due to oxygen peaks V and VI from Ph-COOH. Figure 5.7 compares native ITO with ITO-COOH at 0° and 60°.

Table 5.3. Area ratios for O 1s components for native ITO and ITO-Ph-COOH at varying incident angles

<table>
<thead>
<tr>
<th>incident angle</th>
<th>530.5 (I)</th>
<th>531.7 (II)</th>
<th>532.7 (III)</th>
<th>534 (IV)</th>
<th>530.5 (I)</th>
<th>531.7 (II)</th>
<th>532.7 (III)</th>
<th>534 (IV)</th>
<th>532.2 (V)</th>
<th>533.3 (VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.00</td>
<td>0.95</td>
<td>0.46</td>
<td>0.13</td>
<td>1.00</td>
<td>0.73</td>
<td>0.51</td>
<td>0.30</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>7.50</td>
<td>1.00</td>
<td>0.90</td>
<td>0.42</td>
<td>0.10</td>
<td>1.00</td>
<td>0.80</td>
<td>0.53</td>
<td>0.31</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>15.00</td>
<td>1.00</td>
<td>0.90</td>
<td>0.38</td>
<td>0.06</td>
<td>1.00</td>
<td>0.78</td>
<td>0.50</td>
<td>0.26</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>22.50</td>
<td>1.00</td>
<td>0.93</td>
<td>0.49</td>
<td>0.09</td>
<td>1.00</td>
<td>0.77</td>
<td>0.39</td>
<td>0.30</td>
<td>0.27</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Figure 5.7. (Left) O 1s high resolution spectra of native ITO at 0° and 60° incident angle. (Right) O 1s high resolution spectra of ITO-COOH at 0° and 60° incident angle.
Equation 5 was solved for $d$ at each incident angle. $I_{\text{Ph-COOH}}^\circ$ and $I_{\text{Ph-COOH}}$ refer to the C 1s peak heights at 289.0 eV for a thick film of 4-aminobenzoic acid and ITO-Ph-COOH respectively. $I_{\text{ITO}}^\circ$ and $I_{\text{ITO}}$ refer to the heights of peak I for native ITO and ITO-Ph-COOH respectively. $\lambda_{\text{ITO}}$ and $\lambda_{\text{Ph-COOH}}$ were 1.55 and 3.15 respectively (from Table 5.2). A thickness of 1.4 ± 0.2 nm was determined by taking the average of the film thickness at each angle. Typically, aryl diazonium multilayer films are grown through subsequent attack of the aryl radical to the immobilized phenyl group at the meta position. 1.4 nm is the length of a Ph-COOH film that is 3 layers thick. This is in excellent agreement of the upper limit of $8.4 \times 10^{14} \pm 3.1 \times 10^{14}$ of functionalized molecules which corresponds to 3 times a densely packed monolayer.

### 5.3.4 Immobilization of ITO-Ph-COOH with DNA

Following electrochemical deposition to form ITO-Ph-COOH, a 50 µL droplet of amine terminated ss-DNA, EDC, NHS in MES buffer was delivered to the surface and left to react for 3 days (Scheme 5.2). The long reaction times were used to ensure reaction completion. The pKa of COOH and hexylamine is 4.8 and 10.56 respectively (the pendant group on ss-DNA). Operating the reaction at pH 6.0 induces a pre-concentration of the hexylammonium near the deprotonated carboxylate anion. This process is slow, since the negatively charged phosphate backbone of the DNA molecule will be repelled by the immobilized COO⁻.

Following immobilization of the $H_2N$-ss-DNA, a 50 µL droplet of the compliment strand (cDNA) in 2x SSC buffer was delivered to the surface and left to
react for 4 hours. A parallel experiment was performed in which ss-DNA and its compliment were left to react in 2x SSC buffer for 4 hours. Figure 5.8 shows the gel that demonstrates the success of hybridization of solution ssDNA under these conditions. DNA modified ITO surfaces were characterized by XPS, for the presence of phosphorous, nitrogen, oxygen and carbon and by TOF-SIMS for the presence of the guanine, adenine, cytosine nucleotides as well as the conjugated functional group.

Scheme 5.2. EDC/NHS coupling of ssDNA and subsequent hybridization.

![Scheme 5.2](image)

Figure 5.8. From left to right. Ladder, 40 nM ssDNA, 40 nM dsDNA, 400 nM ssDNA and 400 nM dsDNA. dsDNAs were hybridized for 4 hrs in 2x SSC buffer.
5.3.4.1 XPS Analysis

Figure 5.9 shows the XPS spectra for the oxygen, nitrogen, phosphorous and carbon region of native ITO, ITO-Ph-COOH, ITO-ss-DNA and ITO-ds-DNA. Due to the complexity of the carbon and oxygen species present from adventitious and native ITO material, and the multiple carbon and oxygen species within the DNA structure, peak fitting was not utilized to analyze the data. Despite the lack of peak fitting for the C 1s and O 1s region, important observations can still be made about the spectra that signify DNA conjugation.
Figure 5.9. High resolution spectra for C 1s (top left), N 1s (top right), O 1s (bottom left), and P 2p (bottom left) for native ITO (bottom, pink), ITO-Ph-COOH (second from bottom, black), ITO-ss-DNA (second from top, red) and ITO-ds-DNA (top, blue).

5.3.4.1.1 C 1s region

The C 1s region shows a shift for of the major peak to higher binding energy as well as additional peaks growing in at 287 eV and 289.5 eV for ITO-ss-DNA and
ITO-ds-DNA compared to ITO-Ph-COOH. Adventitious carbon peaks (such as the peak present on native ITO) is primarily hybridized carbon bound to one or two carbons and two or one hydrogens respectively (carbons such as aliphatic, aromatic, vinyl, etc..). After the electrochemical deposition of 4-aminobenzoic acid, the peak does not shift because we have added more of the same type of carbon (6 sp² type carbons, each bound to 2 carbons and 1 hydrogen within the immobilized phenyl ring). Once DNA is conjugated and then subsequently hybridized, the shift to higher binding energies becomes noticeable. Looking at the structure of DNA, we can see that only 3 of 19 carbons (red carbons, Scheme 5.3) that make up the nitrogenous bases share the binding energy of adventitious carbon. The carbons responsible for the shift in binding energy, and growth of additional higher binding energy peaks are from the carbons bound to nitrogen, oxygen and carbonyl type carbons in the nitrogenous bases in DNA. Likewise, the sugar phosphate backbone contains only 1 in 5 carbons (blue carbons, Scheme 5.3) that share the same binding energy of adventitious carbon.

Scheme 5.3. DNA Structure.
5.3.4.1.2 O 1s Region

The O 1s region shows an enhancement at 532.6 eV due to the oxygens within DNA after conjugation. Peaks could not be fit responsibility to the O 1s spectrum for ITO-ss-DNA and ITO-ds-DNA due to the severe overlap with peaks I-VII (vide infra) for native ITO and ITO-Ph-COOH. For example: it is expected that peaks I-III, from the native ITO would decrease in intensity, since they would be buried beneath the oxygen from the DNA signal; but the extent in which the signal would decrease in intensity is strongly dependent on the orientation of the DNA on the surface. In addition, peak V (the alcohol) reacts with the H$_2$N-ss-DNA to form an amide group, manifesting in a shift in binding energy. Furthermore, the intensity of peak VI would decrease immensely, as the oxygen density within the ss-DNA overpowers the signal from the oxygen within ITO-Ph-COOH.

The O 1s spectrum of DNA can be fit to two peaks; a larger peak at 533.4 and a smaller one at 531.7. The increase of the O 1s spectrum for ITO-ss-DNA and ITO-ds-DNA at 532.6 eV can be attributed to the presence of DNA overlapping with the native ITO signals (peaks II and III).

5.3.4.1.3 N 1s and P 2p region

Minimal nitrogen is found on native ITO substrates, but in some cases, can be up to 1% (as shown in Figure 5.9), while in no case is phosphorous found on native ITO nor ITO-Ph-COOH. Electrochemical deposition of the diazonium salt results in a significant increase in the nitrogen component by XPS, but still only a noise ridden signal form P 2p region. The nitrogen is due to some film growth through diazo linkages upon electrochemical deposition. After conjugation with ss-DNA and further hybridization with ds-DNA the nitrogen increases, and phosphorous is detected. The
P:N ratio for ITO-ss-DNA and ITO-ds-DNA is 0.12 ± 0.02 and 0.14 ± 0.02 respectively. This is much lower than the theoretical P:N ratio of 0.27 for DNA. The low number is due to the inflated atomic percent of nitrogen from the diazo linkages. When taking the nitrogen from the diazo linkages into account, the P:N ratio for ITO-ss-DNA and ITO-ds-DNA is 0.24 ± 0.07 and 0.23 ± 0.06 respectively, in close agreement to the theoretical value of 0.27.\textsuperscript{65}

5.3.4.1.4 Non-Specific Adsorption

The XPS analysis shows the presence of DNA on the ITO, but cannot distinguish whether the DNA is conjugated onto the ITO or non-specifically binding. In response to trying determine the extent DNA physisorbs onto ITO-Ph-COOH, the same reaction was carried out on ITO-Ph-COOH with H\textsubscript{2}N-ss-DNA in the absence of EDC and NHS. \textbf{Figure 5.10} shows the ITO-Ph-COOH after being soaked with H\textsubscript{2}N-ss-DNA, and further soaked with the c-DNA. For ITO-Ph-COOH, there is no phosphorus, and after soaking in H\textsubscript{2}N-ss-DNA there is phosphorous present, indicating that the DNA adsorbs onto the ITO-Ph-COOH. Following a soak with the c-DNA the phosphorous peak is removed suggesting hybridization induces desorption of the ss-DNA. This observation was demonstrated by Liu and Liu on ITO nanoparticles.\textsuperscript{66} They noted hybridization acts as a driving force for desorption of ss-DNA. ss-DNA’s flexibility allows favorable adsorption by adopting different conformations to facilitate adsorption, while ds-DNA’s negatively charged more rigid structure prohibits the plasticity needed to adsorb.
It is worth noting the indium 4s plasmon peak located at 139 eV that is present for adsorbed ss-DNA and desorbed ss-DNA (red and blue, Figure 5.10). The indium 4s plasmon peak is noticeable on native ITO, but is attenuated in ITO-Ph-COOH due to the Ph-COOH layer (see Figure 5.9, pink and black). After ITO-Ph-COOH is soaked with 20 µM solution of ss-DNA in the absence of EDC/NHS, the indium 4s plasmon reappears, suggesting displacement of the Ph-COOH film with ss-DNA. In
the presence of EDC/NHS, the indium 4s plasmon is not visible, suggesting the Ph-COOH is preserved during conjugation with ss-DNA and hybridization with its cDNA (see Figure 5.9, red and blue).

5.3.5 TOF-SIMS Analysis

TOF-SIMS is considered a complimentary technique to XPS providing long range information in the form of mass fragments. Native ITO, ITO-Ph-COOH, and ITO-ds-DNA were all subject to TOF-SIMS analysis. Negative and positive mode was collected. Positive mode found cytosine (C$_4$H$_6$N$_3$O$^+$, m/z 112), adenine (C$_5$H$_3$N$_5$ $^+$, m/z 136), and guanine (C$_5$H$_5$N$_5$O$^+$, m/z 151) mass fragments for conjugated ITO-ds-DNA. These mass fragments were also found for the control, but in much smaller quantities suggesting the hybridization step does not effectively remove 100% of the non-specifically adsorbed ss-DNA. This is corroborated by the fact that TOF-SIMS is a much more sensitive technique than XPS and would be able to detect small quantities of residual hybridized ss-DNA. The mass fragments of the nucleic acid bases are further evidence of DNA, but still doesn’t specify between conjugation or physisorption (or both). The mass fragment for the conjugated component of the immobilized DNA was found by TOF-SIMS offering solid evidence for the DNA covalent linkage. The amide product mass fragment (C$_7$H$_{13}$NO$_2^+$, m/z 129) from the reaction with H$_2$N-C$_6$-ss-DNA and ITO-Ph-COOH was detected by TOF-SIMS. This peak was not present in a standard DNA sample, nor in the control reaction. Figure 5.11 shows the spectra for the cytosine, adenine, guanine and amide product for ITO-ds-DNA and the control (ITO-Ph-COOH soaked with H$_2$N-ss-DNA, and further soaked with c-DNA).
Figure 5.11. TOF SIMS spectra for ITO-ds-DNA conjugated in the presence (black) and absence (red) of EDC/NHS. The y axis is normalized to the CH$_3^+$ peak for each spectrum.

5.3.6 Electrochemical Impedance Spectroscopy (EIS)

EIS is often employed as a complementary technique to characterize DNA immobilization and hybridization on electrode substrates. Typically, either the capacitance is obtained (called non-faradaic EIS), or the resistance to charge transfer ($R_{CT}$) of a redox probe in solution is obtained (called faradaic EIS) through analyzing a sinusoidal current response to a frequency varying sinusoidal voltage input. This method is especially useful in characterizing DNA modified electrodes because it does not require the use of labels and relies solely on changes at the interface.
Scheme 5.4. ss-DNA conjugation, cDNA hybridization and protein reversible interaction with dsDNA.

Figure 5.12. EIS circuit used to fit the impedance data. Q denotes a constant phase element. R denotes a resistance. W denotes Warburg impedance. Each grouping of a constant phase element and resistance indicate a time constant.
Figure 5.13. Impedance data of modified ITO electrodes in a solution of 1 mM K$_3$Fe(CN)$_6$ and 0.1 M NaCl fit with the circuit shown above. Insert shows the high frequency region in more detail. The $r^2$ values for the fit are 0.992, 0.997, 0.995, 0.999 and 0.996 for ITO, ITO-ssDNA, ITO-dsDNA and ITO-protein before and after association respectively.

5.3.6.1 EIS Measurement for Hybridization

We employed faradic EIS on native ITO, ITO-ss-DNA and ITO-ds-DNA in a solution containing 1 mM K$_3$Fe(CN)$_6$ and 0.1 M NaCl as shown in Scheme 5.4. The $R_{CT}$ reflects the ease in transferring an electron to and from the Fe(CN)$_6^{3-}$. For an unmodified electrode, the $R_{CT}$ reflects the ability of an electron to be transferred from the electrode to the redox probe. It is important to note the sensitivity of this technique and the $R_{CT}$ value is different for the same redox probe on different electrode substrates. When comparing the $R_{CT}$'s of a redox probe as evidence of electrode modification it should be done in comparison to the $R_{CT}$ of the redox probe on the native substrate. The impedance spectroscopy and $R_{CT}$ values for the modified electrodes are shown in Figure 5.13. A large increase for the $R_{CT}$ is seen after the modification with ss-DNA attributed to the covering of the electrode with the DNA molecule. After hybridization, a small decrease in the $R_{CT}$ is observed. In the most
straightforward case, the R\(_{CT}\) should increase after hybridization due to an increase in negatively charged phosphate groups that repel the negatively charged redox probe, making the probe harder to access the electrode for a charge transfer event.\(^{45}\) In other examples though, a decrease in the R\(_{CT}\) is observed, similar to our results.\(^{6-71}\) The decrease is attributed to a conformational change from ss-DNA that more efficiently blocks the electrode due to its flexibility to an upright rigid ds-DNA exposing pinholes available for the probe to migrate close to the surface. This type of adsorption behavior has been observed before on ITO nanoparticles.\(^{66}\)

5.3.6.2 EIS for Protein Binding

Following hybridization of the ds-DNA, the electrode was exposed to 50 µL of 2 µM NF-κB protein in binding buffer and allowed to bind for 30 mins at 100% relative humidity (Scheme 5.4). After the protein binding event, there is an increase in the R\(_{CT}\) indicating the presence of the protein.

Following the association of NF-κB protein to ITO-dsDNA substrates, these substrates were soaked in a 2 µM solution of dsDNA to determine the extent of dissociation of the NF-κB protein (reversed arrow in Scheme 5.4). After 30 minutes of soaking, electrochemical impedance measurements were taken of the ITO-dsDNA. Figure 5.13 shows the R\(_{CT}\) decreased significantly after these substrates were soaked in the dsDNA solution demonstrating the reversibility of the NF-κB protein association.

5.4 Conclusion

In an effort to improve sensing techniques by incorporating electrochemiluminescence, a method to conjugate ITO substrates with a specific
sequence of DNA was developed. XPS was used in conjunction with TOF-SIMS to demonstrate the validity of the conjugation. EIS was used to further show the ability of the DNA film to bind to the NF-κB protein. Multiple surface techniques were used to thoroughly demonstrate step by step the molecular environment of the surface. The base thin film was electrodeposited through a well-known method of electrochemical deposition of 4-aminobenzoic acid. AR-XPS calculated the depth of the film to be 1.4 nm, suggesting the thin film that has three layers of benzoic acid. XPS was used to detect DNA on the surface after coupling with EDC/NHS by detecting the presence of phosphorous and nitrogen. TOF-SIMS was employed to verify the molecule was covalently attached by detecting the fragment produced by the EDC/NHS coupling. EIS was further used to study the resistance to charge transfer of the FeCN₆ redox probe on the native ITO substrates, ITO-ssDNA, and ITO-dsDNA. For ITO-dsDNA, the association and dissociation of NF-κB proteins was also measured by observing an increase in $R_{CT}$ for the association and a decrease in the $R_{CT}$ for the dissociation event.
REFERENCES


Imagine a drop of DNA solution on a glass slide in atmospheric pressure at room temperature. After evaporation of the solvent, there is no need for any analytical technique to be used to determine if the DNA is actually on the glass slide. Analytical techniques would be used to characterize other things about this interaction, such as stability, surface wettability, film thickness, and others, but no technique would be exclusively employed to evidence the presence of the solute after solvent evaporation.


(41) \( \Delta G^\circ = RT \ln K \), \( R = 1.987 \times 10^{-3} \text{ kcal K}^{-1} \text{ mol}^{-1} \), \( T = 298 \text{°K} \), \( K = 0.17 \text{ nM} \)


IMFP is by definition the average distance particles travel in-between collisions given in nm. The EAL gives the distance, also in nm, of the exponential portion of the emission depth distribution function. The values are often close, but the EAL takes into account the phase transfer between a solid and vacuum by addressing the exponential dependence on distance. For definition of terms and derivations see: (a) Standard E 673, Annual Book of ASTM Standards, Vol. 3.06, American Society for Testing and Materials, West Conshohocken, Pennsylvania, 1998 (b) Jablonski, A., Powell, C.J. J. Electron Spectrosc. Relat. Phenom. 1999, 100, 137-160.


The N:In for ITO-COOH is 0.21 ± 0.07. The N 1s atomic percent from the diazo contribution for ITO-ss-DNA and ITO-ds-DNA is calculated from multiplying indium’s atomic percent by 0.21 and subtracting from the total N 1s atomic percentage.


Appendix A

ELECTROCHEMILUMINESCENCE

Electrochemiluminescence (ECL) is a type of luminescence generated through an electrochemical reaction. One essential component for ECL is a luminophore, a compound that after excitation can return to the ground state via radiative decay (fluorescence or phosphorescence as an example). What sets ECL apart from other types of luminescence is the source of the excitation. The source for ECL excitation is voltage and when this voltage is applied to the luminophore, it can trigger subsequent events that invoke photonic emission. The advantage of ECL being controlled through voltage has certainly demonstrated its viability as it is an analytical technique for hundreds of immunoassays.\(^1\)-\(^2\) Below are outlined the two major pathways that generate ECL, an annihilation pathway and a co-reactant pathway. In the below discussion, only ruthenium (II) tri(bipyridine) (Ru(bpy)\(_3\)\(^{2+}\)) will be discussed as a luminophore and in the case for the co-reactant mechanism, only tripropylamine (TPA) will be discussed as the co-reactant. For an exhaustive list of luminophores and co-reactants see table 1 in *Electrochemiluminescence*.\(^3\)

A.1 Ru(bpy)\(_3\)\(^{2+}\) emission process

Before discussing the ECL pathways, it is important to highlight some important features of the Ru(bpy)\(_3\)\(^{2+}\) luminophore. Ru(bpy)\(_3\)\(^{2+}\) has an absorbance feature around 455 nm (corresponding to 2.7 eV).\(^4\) The absorbance at 455 nm is a metal-to-ligand-charge-transfer (MLCT) as the bipyridine ligands are somewhat easy to reduce and the metal center is relatively easy to oxidize. This excitation promotes
an electron residing in the d orbital of the metal in the singlet ground state to the bipyridine ligand in singlet excited state. After this excitation, the complex swiftly undergoes intersystem crossing to the triplet excited state from which it emits light though phosphorescence at about 608 nm (see Figure A.1). This is the same mechanism that Ru(bpy)$_3^{2+}$ emits light in ECL whether the pathway is annihilation or with a co-reactant. The pathway just identifies the means to get Ru(bpy)$_3^{2+}$ in the excited state (from now on denoted as *$^1$Ru(bpy)$_3^{2+}$*).

![MLCT excitation diagram](image)

Figure A.1. MLCT excitation of Ru(bpy)$_3^{2+}$ and phosphorescence emission (left). Ru(bpy)$_3^{2+}$ compound (right).

### A.2 Annihilation Pathway
As mentioned above, ECL is the result of an excitation of a luminophore through a redox event. The annihilation pathway generates the excited luminophore through the recombination of a reduced and oxidized species at the electrode surface. The steps regarding the annihilation pathway for \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} is shown in equations 1-4 and illustrated in Figure A.2.

\begin{align*}
\text{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} + e^- & \rightarrow \text{Ru(bpy)}\textsubscript{3}\textsuperscript{3+} & (1) \\
\text{Ru(bpy)}\textsubscript{3}\textsuperscript{3+} + \text{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} & \rightarrow \text{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} + \text{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} & (3) \\
\text{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} & \rightarrow \text{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} + h\nu \ 608 \text{ (nm)} & (4)
\end{align*}

Here, the electrode is cycled between the oxidation and reduction potential of \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} at a specific frequency. The original report holds the oxidation and reduction potential for 12 secs in-between switching the potentials. Holding the positive potential for such time creates sufficient \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{3+} quantities. Switching to the negative potentials then creates sufficient \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} quantities. When the two species collide, they annihilate the separation of charge and each return to the 2+ oxidation state, one in the excited state. For the excited to state to be generated, and produce ECL, the enthalpy of energy transfer of reaction (equation 3) must be larger than the enthalpy to generate the \textit{\textsuperscript{6a}} Ru(bpy)\textsubscript{3}\textsuperscript{2+. Once the \textit{\textsuperscript{6a}} Ru(bpy)\textsubscript{3}\textsuperscript{2+} is generated, the emission of light can occur as discussed in A.1.
Figure A.2. From left to right: Oxidation of Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{3+}$ at a fixed potential of +1.2 V vs. SCE. Reduction of Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{3+}$ at a fixed potential of —1.2 V vs. SCE and the presence of Ru(bpy)$_3^{3+}$ in the diffusion layer. Oxidation of Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{3+}$ at a fixed potential of +1.2 V vs. SCE and the presence of both Ru(bpy)$_3^{3+}$ and Ru(bpy)$_3^{+}$ in the diffuse layer which collide to form Ru(bpy)$_2^{2+}$ and *Ru(bpy)$_2^{2+}$. Finally the repetition of potential cycling creates a strong ECL signal.

A.3 Co-Reactant Pathway

ECL can also occur without polarizing the luminophore against itself. Another common method is by oxidizing a co-reactant that can be used as a sacrificial reductant to generate the reduce species necessary for ECL. This routes involves applying one potential and relies on the chemistry of the co-reactant to form the excited ruthenium (in contrast to cycling the potential to form two ruthenium species, which then annihilate). A common co-react used is TPA. While the necessity and importance of the oxidized TPA is well known, the subsequent mechanisms are not as straightforward. There are three suggested pathways that follow the oxidation of TPA, one of which invokes the annihilation route as a second step (through in-situ generation of Ru(bpy)$_3^{3+}$. Also, the subsequent pathways following the oxidation of
TPA are dependent on the co-reactant and luminophore concentrations. The proposed mechanisms for ECL through a co-reactant are shown Figure A.3.

Figure A.3. Three proposed mechanisms for ECL through a co-reactant pathway.
In the first case for ECL generation by a co-reactant pathway, TPA is oxidized near the potential that Ru(bpy)$_3^{2+}$ is oxidized. **Figure A.3a** shows TPA and Ru(bpy)$_3^{2+}$ being oxidized together at the electrode surface. Oxidized TPA (TPA$^+$) quickly deprotonates forming TPA$^\cdot$, a very reactive intermediate with a half-life of 0.2 ms.$^7$ TPA$^\cdot$ is a strong enough reducing agent to reduce Ru(bpy)$_3^{3+}$ to $^\ast$Ru(bpy)$_3^{2+}$ and more stable products. The reaction between TPA$^\cdot$ and Ru(bpy)$_3^{3+}$ is highly exergonic, providing enough energy to form the excited state.

The second case involves the oxidation of TPA and Ru(bpy)$_3^{2+}$ at the electrode surface as well forming TPA$^\cdot$ and Ru(bpy)$_3^{3+}$. TPA$^\cdot$, being such a strong reducing agent, can also reduce Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{+}$ (the product formed in equation 2). With Ru(bpy)$_3^{3+}$ and Ru(bpy)$_3^{+}$ both in solution they can react through annihilation as outlined in equation 3.

Finally, the third case involves only TPA being oxidized at the electrode surface to form TPA$^\cdot$ and which subsequently deprotonates to form TPA$^\cdot$. TPA$^\cdot$ goes on to reduce Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{+}$. This species can react with protonated TPA$^\cdot$ to form $^\ast$Ru(bpy)$_3^{2+}$ and the co-reactant TPA. This final mechanism at least explains how ECL is achieved at potentials below the oxidation potentials of Ru(bpy)$_3^{2+}$ (1.2 V vs. SCE).$^8$
Appendix B
CALCULATING REDOX POTENTIALS USING GUASSIAN SOFTWARE

Standard reduction potentials are the tabulated source for electrochemists to compare reduction and oxidation abilities of compounds. These values are helpful but offer a complication when calculating the reduction and oxidation potentials as these values are all in reference to the standard hydrogen electrode. To get around this challenge, one can still calculate the standard reduction potential of a compound, but the final value is in reference to the free electron, or the absolute electrode potential. There is error associated with these calculations, as the absolute electrode potential is estimated and values for the SHE is between 4.44 and 4.78 V.\(^9\) Also, the level of theory used in the Gaussian calculation may also introduce some error. Despite these factors, it is still a helpful exercise to calculate the reduction potential of a compound and visualize using calculated molecular orbitals where such a transition takes place. Below is discussed how one would achieve a calculated redox potential for a compound in the Gaussian software.

Calculating redox potentials in Gaussian are done using the Born-Haber cycle as a guide (shown for ferrocene in Figure B.1.) Equations 1-8 outline how to attain the final redox potential, \(E^\circ\). Since the cycle has more than one route to get to the product, likewise there is more than one way to calculate \(E^\circ\). Equations 2a-4a show one route to calculate \(E^\circ\) and equations 2b-4b show another. “\(G\)” values that are underlined in equations 5-8 are actual numerical output values obtained from the 6 necessary Gaussian calculations. By inputting these 6 numerical values into equations 5-8, one
can solve for \( E^\circ \) using either equations 2a-4a or 2b-4a to get \( \Delta G^\circ \). \( \Delta G^\circ \) is plugged into equation 1 to solve for \( E^\circ \) which is given as the absolute reference potential, which can then easily be converted to \( E^\circ \) vs. SHE be subtracting 4.44 V. \( F \) in equation 1 stands for faradays constant of 23.06, with the units of V•kcal/mol.

\[ \Delta G^\circ = nF E^\circ \quad \text{(1)} \]

\[ \Delta G^\circ = \Delta G_{\text{gas}}^{\text{redox}} + \Delta G_{\text{sol}}^{\text{redox}} \quad \text{(2a)} \]

\[ \Delta G_{\text{gas}}^{\text{redox}} = G_{\text{Fe}^{\text{III}}}^{\text{corr}} - G_{\text{Fe}^{\text{II}}}^{\text{corr}} \quad \text{(3a)} \]

\[ \Delta G_{\text{sol}}^{\text{redox}} = \Delta G_{\text{sol}}^{\text{Fe}^{\text{III}}} - \Delta G_{\text{sol}}^{\text{Fe}^{\text{II}}} \quad \text{(4a)} \]

\[ \Delta G_{\text{Fe}^{\text{III}}}^{\text{corr}} = \Delta G_{\text{Fe}^{\text{II}}}^{\text{corr}} - \Delta G_{\text{Fe}^{\text{III}}}^{\text{sol}} \quad \Delta G_{\text{Fe}^{\text{II}}}^{\text{corr}} = \Delta G_{\text{Fe}^{\text{III}}}^{\text{corr}} - \Delta G_{\text{Fe}^{\text{II}}}^{\text{sol}} \quad \text{(3b)} \]

\[ G_{\text{corr}}^{\text{Fe}^{\text{III}}} = G_{\text{gas}}^{\text{Fe}^{\text{III}}} + \text{thermal correction}^{\text{Fe}^{\text{III}}} \quad \text{(5)} \]

\[ G_{\text{corr}}^{\text{Fe}^{\text{II}}} = G_{\text{gas}}^{\text{Fe}^{\text{II}}} + \text{thermal correction}^{\text{Fe}^{\text{II}}} \quad \text{(6)} \]
The following 6 sections will outline the necessary 6 calculations to obtain values B.1 through B.6.

\[ \Delta G^\text{Fe}^\text{III}_{\text{sol}} = G^\text{Fe}^\text{III}_{\text{sol}} - G^\text{Fe}^\text{III}_{\text{gas}} \]  
\[ \Delta G^\text{Fe}^{II}_{\text{sol}} = G^\text{Fe}^{II}_{\text{sol}} - G^\text{Fe}^{II}_{\text{gas}} \]  

The following sections will outline the necessary 6 calculations to obtain values B.1 through B.6.

**B.1 \( G^\text{Fe}^{II}_{\text{gas}} \)**

The input file to calculate the energy of ferrocene (or your reduced compound) is shown below:

```plaintext
%nprocshared=8
%mem=16000MB
%chk=FERROCENE2_GAS_SCF.chk
# scf=tight b3lyp/genecp

FE2 GAS SCF

0 1
Fe(Iso=55)  0.00038683  -0.00096134  -0.00178003
C(Iso=12)  -1.67457339   0.91404964   0.80030425
C(Iso=12)  -1.68477325  -0.47782596   1.11810739
C(Iso=12)  -1.68494545 -1.20959328  -0.10689621
C(Iso=12)  -1.68197865 -0.27049976  -1.18327668
C(Iso=12)  -1.68078833  1.04165792  -0.62294632
C(Iso=12)  -1.67901928  1.03021372   0.64602700
C(Iso=12)  -1.67752955 -0.29478708   1.17903787
C(Iso=12)  -1.68393432 -1.21039853   0.08308738
C(Iso=12)  -1.68060606 -0.45201458  -1.12703857
C(Iso=12)  -1.68406591  0.93295743  -0.77821069
H(Iso=1)  -1.67162990  1.73234755  1.50732747
H(Iso=1)  -1.62893592 -0.90689511   2.10880061
H(Iso=1)  -1.64441285 -2.28587700  -0.20747212
H(Iso=1)  -1.66496132 -0.51870859  -2.23596414
H(Iso=1)  -1.66517970  1.97258699  -1.17631344
H(Iso=1)  -1.64135246  1.94729920   1.21731820
H(Iso=1)  -1.66273581 -0.56097961   2.22837436
H(Iso=1)  -1.65917709 -2.28906296   0.15498036
H(Iso=1)  -1.67453562 -0.85376303  -2.13275333
```
The number of processors and memory allocated toward the calculation are dependent on your resources and the complexity of the molecule. Typically, the memory allocated to a calculation is twice that of the number of processors (in GB). The third line gives the name of the .chk point file. The final line indicates the job type, the method and the basis set. The job type to obtain the free energy of ferrocene in the gas phase is a single point energy calculation (denoted SCF). The “= tight” instruction indicates a tighter convergence criteria for the energy calculation (giving a more precise result). NOTE: you may want to optimize your compound before doing a single point energy calculation as the atoms in a single point energy calculations are fixed. The command “geneep” generates a split basis set, as metals typically need different basis sets to represent them. For more information on split basis sets the reader is encouraged to reference the Gaussian help manual. Following the title card is the charge and spin of the molecule (which will differ from the oxidized ferrocenium species).

After a successful calculation, you want to extract the SCF energy (in hartrees). You can pursue through the log file and find a line indicating “SCF Done” with a value beside it. It is also helpful to use the ‘grep’ command in a unix based terminal to find this from the output file. The command is underlined and typically
looks like: [name@server]$ grep ‘SCF Done’ filename.log. The energy output for this calculation came out to be -510.5108429960 hartrees. This is the value for \( B_1 \) in equation 6 and 8.

### B.2 Thermal Correction to \( G_{Fe}^{gas} \)

The following input file is to calculate the frequency of ferrocene (or your reduced compound).

\[
\begin{align*}
\text{%nprocshared}=8 \\
\text{%mem}=16000\text{MB} \\
\text{%chk}= \text{FERROCENE2}_\text{GAS}_\text{FREQ}.\text{chk} \\
\text{# freq b3lyp/genecp}
\end{align*}
\]

**FE2 GAS FREQ**

0 1

\[
\begin{array}{cccc}
\text{Fe(Iso=55)} & 0.00038683 & -0.00096134 & -0.00178003 \\
\text{C(Iso=12)} & -1.67457339 & 0.91404964 & 0.80030425 \\
\text{C(Iso=12)} & -1.68477325 & -0.47782596 & 1.11810739 \\
\text{C(Iso=12)} & -1.68499454 & -1.20959328 & -0.10689621 \\
\text{C(Iso=12)} & -1.68197865 & -0.27049976 & -1.18327668 \\
\text{C(Iso=12)} & -1.68078833 & 1.04165792 & -0.62294632 \\
\text{C(Iso=12)} & 1.67901928 & 1.03021372 & 0.64602700 \\
\text{C(Iso=12)} & 1.67752955 & -0.29478708 & 1.17903787 \\
\text{C(Iso=12)} & 1.68393432 & -1.21039853 & 0.08308738 \\
\text{C(Iso=12)} & 1.68060606 & -0.45201458 & -1.12703857 \\
\text{C(Iso=12)} & 1.68406591 & 0.93295743 & -0.77821069 \\
\text{H(Iso=1)} & -1.67162990 & 1.73234755 & 1.50732747 \\
\text{H(Iso=1)} & -1.62893592 & -0.90689511 & 2.10880061 \\
\text{H(Iso=1)} & -1.64441285 & -2.28587700 & -0.20747421 \\
\text{H(Iso=1)} & -1.66496132 & -0.51870859 & -2.23596414 \\
\text{H(Iso=1)} & -1.66517970 & 1.97258699 & -1.17631344 \\
\text{H(Iso=1)} & 1.64135246 & 1.94729920 & 1.21731820 \\
\text{H(Iso=1)} & 1.66273581 & -0.56097961 & 2.22837436 \\
\text{H(Iso=1)} & 1.65917709 & -2.28906296 & 0.15498036 \\
\text{H(Iso=1)} & 1.67453562 & -0.85376303 & -2.13275333 \\
\text{H(Iso=1)} & 1.63897939 & 1.76549021 & -1.46718755
\end{array}
\]
The command line for this calculation replaces SCF=tight with freq and is a frequency calculation for ferrocene. The value obtained from this calculation is the thermal correction to Gibbs free energy. This can also be found using the grep command in a unix based terminal. The value obtained for the thermal correction to Gibbs free energy was 0.134281 and is the value for $B_2$ in equation 6.

\[ G_{gas}^{Fe^{III}} \]

The input file to calculate ferrocenium is shown below. Notice the charge and spin state are the only items that differ from the calculation performed in B.1 (aside from the check point file name and title card which have no bearing on the calculation).

```plaintext
%nprocshared=8
%mem=16000MB
%chk=FERROCENIUM3_GAS_SCF.chk
# scf=tight b3lyp/geneCP

FE3 GAS SCF

1 2
Fe  5.26310000  -0.07700000  0.03640000
C   5.53820000  1.91910000  0.31470000
C   6.05150000  1.52130000 -0.94620000
C   7.05110000  0.54890000 -0.71680000
```
Like the calculation performed in B.1, the important term you want from the output file of a successful calculation is the single point energy, found by the heading “SCF Done”. The value obtained for this calculation was -510.1375756590 hartrees and is the value for B3 in equation 5.

### B.4 Thermal Correction to $G_{\text{gas}}^{\text{Fe}^{++}}$

The input file to calculate the thermal correction for ferrocenium in the gas phase is shown below. Like before, the charge and spin state are the only items that
differ from the calculation performed in B.2 (aside from the check point file name and title card).

```
%nprocshared=8
%mem=16000MB
%chk=FERROCENIUM3_GAS_FREQ.chk
# freq b3lyp/genecp

FE3 GAS FREQ

1 2
Fe(Iso=55) 0.00038683 -0.00096134 -0.00178003
C(Iso=12) -1.67457339 0.91404964 0.80030425
C(Iso=12) -1.68477325 -0.47782596 1.11810739
C(Iso=12) -1.68499454 -1.20959328 -0.10689621
C(Iso=12) -1.68197865 -0.27049976 -1.18327668
C(Iso=12) -1.68078833 1.04165792 -0.62294632
C(Iso=12) 1.67901928 1.03021372 0.64602700
C(Iso=12) 1.67752955 -0.29478708 1.17903787
C(Iso=12) 1.68393432 -1.21039853 0.08308738
C(Iso=12) 1.68060606 -0.45201458 -1.12703857
C(Iso=12) 1.68406591 0.93295743 -0.77821069
H(Iso=1) -1.67162990 1.73234755 1.50732747
H(Iso=1) -1.62893592 -0.90689511 2.10880061
H(Iso=1) -1.64441285 -2.28587700 -0.20747421
H(Iso=1) -1.66496132 -0.51870859 -2.23596414
H(Iso=1) -1.66517970 1.97258699 -1.17631344
H(Iso=1) 1.64135246 1.94729920 1.21731820
H(Iso=1) 1.66273581 -0.56097961 2.22837436
H(Iso=1) 1.65917709 -2.28906296 0.15498036
H(Iso=1) 1.67453562 -0.85376303 -2.13275333
H(Iso=1) 1.63897939 1.76549021 -1.46718755

C H O
6-31g(d)
****
Fe 0
lanl2dz
****

Fe 0
```
Again, like the calculation performed in B.2, the important term you want from the output file of a successful calculation is the thermal correction to Gibbs free energy. The value obtained for this calculation was 0.135893 hartrees and is the value to input for $B_4$ in equation 5.

**B.5 $C_{sol}^{Fe^{II}}$**

The input file to calculate the single point energy for ferrocene in acetonitrile is shown below. This calculation is similar to the calculation done in B.1, with the additional command SCRF=(smd, solvent=acetonitrile). The Gaussian manual can be referenced for a full list of available solvents to use for the smd calculation model.

```plaintext
%nprocshared=8
%mem=16000MB
%chk=FERROCENE2_SOLVENT_SCF.chk
#scf=tight b3lyp/genecp
SCRF=(smd,solvent=acetonitrile)

FE2 SOLVENT SCF

0 1
Fe(Iso=55)  0.00038683 -0.00096134 -0.00178003
C(Iso=12)  -1.67457339  0.91404964  0.80030425
C(Iso=12)  -1.68477325 -0.47782596  1.11810739
C(Iso=12)  -1.68499454 -1.20959328 -0.10689621
C(Iso=12)  -1.68197865 -0.27049976 -1.18327668
C(Iso=12)  -1.68078833  1.04165792  0.62294632
C(Iso=12)  1.67901928  1.03021372  0.64602700
C(Iso=12)  1.67752955 -0.29478708  1.17903787
C(Iso=12)  1.68393432 -1.21039853  0.08308738
C(Iso=12)  1.68060606 -0.45201458 -1.12703857
C(Iso=12)  1.68406591  0.93295743 -0.77821069
H(Iso=1)  -1.67162990  1.73234755  1.50732747
H(Iso=1)  -1.62893592 -0.90689511  2.10880061
H(Iso=1)  -1.64441285 -2.28587700 -0.20747421
H(Iso=1)  -1.66496132 -0.51870859 -2.23596414
```
The required term from this calculation is found beside the “SCF Done” heading of a successful output file similar to B.1 and B.3. The value obtained for this calculation was \(-510.524402371\) hartrees and is the value to input for \(B5\) in equation 8.

**B.6 \(G_{sol}^{Fe^{III}}\).**

The input file to calculate the single point energy for ferrocenium in acetonitrile is shown below, identical to the calculation done in B.5 except with a charge and spin value of 1 and 2 respectively.

```plaintext
%nprocshared=8
%mem=16000MB
%chk=FERROCENIUM3_SOLVENT_SCF.chk
# scf=tight b3lyp/genecp SCRF=(smd,solvent=acetonitrile)

FE3 SOLVENT SCF

1 2
Fe(Iso=55)  0.00038683 -0.00096134 -0.00178003
C(Iso=12)  -1.67457339  0.91404964  0.80030425
```
C(Iso=12) -1.68477325 -0.47782596 1.11810739
C(Iso=12) -1.68499454 -1.20959328 -0.10689621
C(Iso=12) -1.68078833 1.04165792 0.27049976 -1.18327668
C(Iso=12) 1.67901928 1.03021372 0.64602700
C(Iso=12) 1.67752955 -0.29478708 1.17903787
C(Iso=12) 1.68393432 -1.21039853 0.08308738
C(Iso=12) 1.68060606 -0.45201458 -1.12703857
C(Iso=12) 1.68406591 0.93295743 -0.77821069
H(Iso=1) -1.67162990 1.73234755 1.50732747
H(Iso=1) -1.62893592 -0.90689511 2.10880061
H(Iso=1) -1.64441285 -2.28587700 -0.20747421
H(Iso=1) -1.66496132 -0.51870859 -2.23596414
H(Iso=1) -1.66517970 1.97258699 -1.17631344
H(Iso=1) 1.64135246 1.94729920 1.21731820
H(Iso=1) 1.66273581 -0.56097961 2.22837436
H(Iso=1) 1.65917709 -2.28906296 0.15498036
H(Iso=1) 1.67453562 -0.85376303 -2.13275333
H(Iso=1) 1.63897939 1.76549021 -1.46718755

C H 0
6-31g(d)
****
Fe 0
lanl2dz
****
Fe 0
lanl2dz

The value obtained for this calculation was -510.3368779570 hartrees and is the value to input for $B_6$ in equation 7.

**B.7 Putting it all together**

Inserting values obtained in B.1 through B.6 in equations 5-8 gives a final $E^\circ$ value of 0.510 V vs. Ag/AgCl, which is only 3 mV more positive than reported in the literature for ferrocene in acetonitrile using 0.1 M tertbutyl ammonium perchlorate.\textsuperscript{10} The values for $\Delta G_{redox}^{gas}$, $\Delta G_{redox}^{sol}$, $\Delta G_{Fe^{II}}$, $\Delta G_{Fe^{III}}$, and $\Delta G^\circ$ from equations 1, 2a, 2b,
3a and 3b are shown in Table B.1 along with the $E^\circ$ vs. the free electron and vs. the standard hydrogen electrode.

Table B.1. Value obtained from redox potential calculations.

<table>
<thead>
<tr>
<th>Term</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta G_{\text{gas}}^{\text{redox}}$</td>
<td>0.3748793370</td>
</tr>
<tr>
<td>$\Delta G_{\text{sol}}^{\text{redox}}$</td>
<td>-0.1857429230</td>
</tr>
<tr>
<td>$\Delta G_{\text{Fe}^{II}}$</td>
<td>-510.3901213710</td>
</tr>
<tr>
<td>$\Delta G_{\text{Fe}^{III}}$</td>
<td>-510.2009849570</td>
</tr>
<tr>
<td>$\Delta G^\circ$</td>
<td>-0.1891364140</td>
</tr>
<tr>
<td>$E^\circ$ vs. free electron</td>
<td>5.147</td>
</tr>
<tr>
<td>$E^\circ$ vs. SHE</td>
<td>0.707</td>
</tr>
<tr>
<td>$E^\circ$ vs. Ag/AgCl</td>
<td>0.510</td>
</tr>
</tbody>
</table>
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Antonella

http://pubs.rsc.org/En/content/articlelanding/2013/sc/c2sc21413j#!divAbstract


(6) Bard, A. J. In Electrogenerated Chemiluminescence, Bard, A. J., Eds.; Marcel Dekker: New York, NY, 2004; (a) pg 19 (b) pg 231.


