Abstract

The controlled modification of gold nanorods has important implications for their successful applications in a wide variety of fields. In this work, electrochemical and electroless methods for the surface-specific modification of gold were optimized with the aim of developing a site-specific strategy for the functionalization of gold nanorods.

Electrochemistry and fluorescence microscopy techniques were used to investigate the surface-specific modification of alkanethiol-coated gold bead electrodes, which served as a macroscopic model system for the nanorods. 11-mercaptoundecanoic acid (MUA) was partially removed from the electrodes by reductive desorption and the uncovered regions were modified with a fluorophore-functionalized, thiolated DNA molecule. Single crystal gold bead electrodes were employed in order to study and optimize the modification methods on all crystallographic surfaces under identical conditions.

Two methodologies for the surface-specific modification of gold bead electrodes were investigated. In the first, a potential was applied to the electrode using a potentiostat, and it was determined that the SAM could be reductively removed selectively from the Au{111} surfaces of the electrodes by a 5 minute electrochemical application of any potential from -0.75 V to -0.8 V vs. Ag|AgCl. In the second method, the electrode potential was set electrolessly by adding a strong reducing agent, sodium borohydride, to the electrolyte. In the absence of oxygen, it was found that the electroless MUA desorption closely resembled the results obtained electrochemically, and that Au{111}-selective modification of the gold bead electrode was achieved at potentials near -0.75 V vs. Ag|AgCl.

The electroless modification strategy was then applied to MUA-stabilized gold nanorods. Our preliminary results indicate that sodium borohydride successfully removes alkanethiol from gold nanorod surface, enabling them to be modified with thiolated, fluorophore-labelled DNA.
Preface

All experimental results presented in this thesis are the original and unpublished work of the author. The experimental design and data analysis was performed in collaboration with the research supervisor, Dr. Dan Bizzotto.

Several key contributions to this work by others must be highlighted:

1. Preliminary work on this project, including the development of the AutoLab data collection protocols, was performed by Kaylyn Leung, Jannu Casanova-Moreno and Tamiko Masuda.

2. The GNR experiments presented here were performed in collaboration with Jonathan Massey-Allard and Dr. Jeff Young of the UBC Department of Physics and Astronomy.

3. The spectroelectrochemical cell used in this work was made by Brian Ditchburn, the UBC Department of Chemistry glassblower.

4. TEM imaging was performed by Bradford Ross of the UBC BioImaging Facility.
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## Nomenclature

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<td>$\Gamma$</td>
<td>Surface excess</td>
</tr>
<tr>
<td>$\Gamma_{\text{max}}$</td>
<td>Maximum surface excess</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency</td>
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<tr>
<td>$\theta$</td>
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<td>Intrinsic lifetime</td>
</tr>
<tr>
<td>$\Phi_2$</td>
<td>Potential at OHP</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Angle</td>
</tr>
<tr>
<td>$A_{\text{ox}}$</td>
<td>Oxidation area</td>
</tr>
<tr>
<td>$A_{\text{red}}$</td>
<td>Reduction area</td>
</tr>
<tr>
<td>$C$</td>
<td>Capacitance</td>
</tr>
<tr>
<td>$C_{\theta=0}$</td>
<td>Capacitance of fully uncovered surface</td>
</tr>
<tr>
<td>$C_{\theta=1}$</td>
<td>Capacitance of fully covered surface</td>
</tr>
<tr>
<td>Nomenclature</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>$C_{\text{bare, }E_{\text{treat}}}$</td>
<td>Capacitance at treatment potential of bare electrode</td>
</tr>
<tr>
<td>$C_{\text{bulk}}$</td>
<td>Concentration of ions in the bulk solution</td>
</tr>
<tr>
<td>$C_{\text{dl}}$</td>
<td>Double layer capacitance</td>
</tr>
<tr>
<td>$C_{D}$</td>
<td>Capacitance of the diffuse layer</td>
</tr>
<tr>
<td>$C_{MUA, E=0}$</td>
<td>Initial capacitance of MUA-coated electrode</td>
</tr>
<tr>
<td>$C_{MUA, E_{\text{treat}}}$</td>
<td>Capacitance at treatment potential of MUA-coated electrode</td>
</tr>
<tr>
<td>$C_{\text{OHP}}$</td>
<td>Capacitance of the OHP</td>
</tr>
<tr>
<td>$d_0$</td>
<td>Limit of resolution</td>
</tr>
<tr>
<td>$E_{\text{eq}}$</td>
<td>Potential at equilibrium</td>
</tr>
<tr>
<td>$E_{\text{mix}}$</td>
<td>Mixed potential</td>
</tr>
<tr>
<td>$E_{\text{OCP}}$</td>
<td>Open circuit potential</td>
</tr>
<tr>
<td>$E_{\text{treat}}$</td>
<td>Treatment potential</td>
</tr>
<tr>
<td>$Fl.\text{Int.}$</td>
<td>Fluorescence intensity</td>
</tr>
<tr>
<td>$Fl.\text{Int.}_{\text{meas}}$</td>
<td>Measured fluorescence intensity</td>
</tr>
<tr>
<td>$i$</td>
<td>Current density</td>
</tr>
<tr>
<td>$i_{\text{ox}}$</td>
<td>Oxidation current</td>
</tr>
<tr>
<td>$i_0$</td>
<td>Exchange current density</td>
</tr>
<tr>
<td>$i_{\text{red}}$</td>
<td>Reduction current</td>
</tr>
<tr>
<td>$i_r$</td>
<td>Imaginary current</td>
</tr>
<tr>
<td>$i_r$</td>
<td>Real current</td>
</tr>
<tr>
<td>$k_{nr}$</td>
<td>Rate of non-radiative decay</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>$k_r$</td>
<td>Rate of radiative decay</td>
</tr>
<tr>
<td>$n$</td>
<td>Refractive index</td>
</tr>
<tr>
<td>$R_{ax}$</td>
<td>Axial resolving power</td>
</tr>
<tr>
<td>$R_s$</td>
<td>Solution resistance</td>
</tr>
<tr>
<td>$t_{exp}$</td>
<td>Exposure time</td>
</tr>
<tr>
<td>$V_{ac}$</td>
<td>AC voltage</td>
</tr>
<tr>
<td>$x_2$</td>
<td>Outer Helmholtz Plane</td>
</tr>
<tr>
<td>AC</td>
<td>Alternating current</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>CE</td>
<td>Counter electrode</td>
</tr>
<tr>
<td>CTAB</td>
<td>hexadecyl-trimethyl-ammonium bromide</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammogram</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double-stranded DNA</td>
</tr>
<tr>
<td>$E$</td>
<td>Potential</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
</tr>
<tr>
<td>fcc</td>
<td>Face-centred cubic</td>
</tr>
<tr>
<td>GCS</td>
<td>Gouy-Chapman-Stern model of the electrical double layer</td>
</tr>
<tr>
<td>GNR</td>
<td>Gold nanorod</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>LIA</td>
<td>Lock-in amplifier</td>
</tr>
<tr>
<td>LSPR</td>
<td>Localised surface plasmon resonance</td>
</tr>
<tr>
<td>MCH</td>
<td>Mercaptohexanol</td>
</tr>
<tr>
<td>MUA</td>
<td>11-mercaptoundecanoic acid</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>OHP</td>
<td>Outer Helmholtz Plane</td>
</tr>
<tr>
<td>Ox</td>
<td>Oxidized species</td>
</tr>
<tr>
<td>QY</td>
<td>Quantum yield</td>
</tr>
<tr>
<td>R</td>
<td>Gas constant</td>
</tr>
<tr>
<td>RCF</td>
<td>Relative centrifugal force</td>
</tr>
<tr>
<td>RE</td>
<td>Reference electrode</td>
</tr>
<tr>
<td>Red</td>
<td>Reduced species</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SCE</td>
<td>Saturated calomel electrode</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Single-stranded DNA</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>UHV</td>
<td>Ultrahigh vacuum</td>
</tr>
<tr>
<td>WE</td>
<td>Working electrode</td>
</tr>
<tr>
<td>z</td>
<td>Signed charge of an ion</td>
</tr>
</tbody>
</table>
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Chapter 1

Introduction

1.1 Identifying the Problem

Gold nanorods (GNRs) have generated a significant amount of interest in recent years as a result of their unusual optical properties.\(^1\) GNRs have been demonstrated to have a wide variety of uses, such as in biomolecular sensing\(^2\), drug delivery\(^3\), and as components in light-emitting diodes\(^4\). Due to their geometric anisotropy, GNRs have two localised surface plasmon resonances (LSPR), one which corresponds to the short axis of the rod and one which corresponds to the long axis. The longitudinal LSPR is of particular interest as it exhibits extremely high sensitivity to the rod aspect ratio and can be tuned to a wide range of frequencies from the visible to near IR.\(^5\) In addition, due to their shape, electromagnetic fields near the GNR ends experience significant enhancement as compared to the incident radiation. This can be used to enhance the emission of proximally located fluorophores, though the enhancement effect is strongly dependent on the GNR-fluorophore distance, generally 10s of nanometers.\(^6\)

For applications that take advantage of the field enhancement effects near the GNR ends, methods for the precise, end-selective, modification of GNRs are needed. The simplest of such methods exploits the binding affinity of hexadecyl-trimethyl-ammonium bromide (CTAB) for the GNR end-caps. CTAB, which is a commonly used stabilizing GNR ligand, has been observed to bind more strongly to the long axes of GNRs than to their ends.\(^7\) As such, upon the introduction of another molecule which can bind to the GNR it will do so preferentially on the ends. This method, however, is not highly controllable and requires the use of CTAB-coated GNRs, which is undesirable for biological applications as CTAB is known to be cytotoxic.\(^8\)

As stated by Burrows \textit{et al.}\(^9\), a general method for the site-specific modification of GNRs, ideally one that is customizable to a variety of stabilizing ligands, is needed. Additionally, the
1.2 Scope of the Thesis

ability to precisely tune the length of the end-selective ligands is desired in order to take full advantage of field enhancement effects. The intention of this work is to design an electrochemistry-based method to accomplish this by optimizing electrochemical and electroless methods for the surface-specific modification of single-crystalline gold bead electrodes. These electrodes exhibit the complete gold stereographic triangle and so the modification methodologies can be studied on all crystallographic surfaces under identical conditions. It is postulated that an electroless method could be adapted for use on GNRs by exploiting of their intrinsic crystallography.

1.2 Scope of the Thesis

This thesis describes the use of electrochemical and electroless methods for the surface-specific modification of single crystal gold electrodes. The methods are evaluated using in-situ fluorescence microscopy techniques in order to correlate the electrochemical measurements to the underlying crystallography of the gold electrode. They can then be optimized toward the surface-specific modification of alkanethiol-stabilized GNRs.

The specific objectives of this work are:

1. To determine the optimal electrochemical treatment potential for the surface-specific modification of the MUA-coated gold bead electrodes.

2. To characterize the electroless removal of a MUA SAM from gold using sodium borohydride and to determine the conditions required for the surface-specific removal of MUA.

3. To evaluate the electroless desorption of MUA on GNRs toward end-selective modification.
Chapter 2

Theoretical Background

2.1 Electrochemistry

This research is concerned with examining reactions which occur at electrode/solution interfaces and as such a detailed description of such interfaces is given. This section will outline the basic theory underlying the understanding of these interfaces, as well as the methods used to probe them. An excellent description of electrochemical interfaces has been written by Bard and Faulkner.10

2.1.1 The Electrode Interface

Faradaic electrochemistry is largely concerned with the movement of charge across an interface: in particular across the interface formed between a metal (the electrode) and a solution (the electrolyte). In metals, charge is accumulated on the surface of the metal, whereas on the solution side, this charge is balanced by dissolved ions which exist as a gradient of concentration into the electrolyte some distance from the electrode surface, called the electrical double layer. As a result of this separation of charge the metal|solution interface resembles a parallel plate capacitor.

The Gouy-Chapman-Stern (GCS) model of the electrical double layer models systems where the charge on the electrode is balanced by solvated ions that interact with the metal surface through electrostatic interactions. The closest distance that these ions can exist from the electrode surface is termed $\chi_2$, or the outer Helmholtz plane (OHP). As the distance from the electrode surface increases, the concentration of solvated ions decreases until all of the electrode’s charge is compensated and the ion concentration becomes equal to that of the bulk solution. The distance between $\chi_2$ and the point at which all electrode charge is compensated...
2.1. Electrochemistry

is referred to as the diffuse layer. The change in potential as the distance from the electrode surface increases can be described electrically using an equivalent circuit. For the GCS model of the electrical double layer, the potential can be adequately modelled as two capacitors and a resistor in series (see Figure 2.1); the first capacitance being from the electrode surface to \( x_2 \), \( (C_{OHP}) \) and the second represented by the capacitance of the diffuse layer \( (C_D) \). The capacitance of the electrical double layer \( (C_{dl}) \) can be represented as follows:

\[
\frac{1}{C_{dl}} = \frac{1}{C_{OHP}} + \frac{1}{C_D}
\]  

(2.1)

Mathematically, \( C_{dl} \) as described by the GCS model in a symmetric \( (i.e. \ z:z) \) electrolyte can be calculated according to Equation 2.2, where \( \varepsilon \) and \( \varepsilon_0 \) are the solution and vacuum permittivities, \( z \) is the charge of an ion in solution, \( F \) is the Faraday constant, \( c_{bulk} \) is the concentration of ions in solution, \( R \) is the ideal gas constant, \( T \) is the temperature in Kelvin and \( \Phi_2 \) is the potential at the OHP with respect to that of the bulk solution.

\[
\frac{1}{C_{dl}} = \frac{x_2}{\varepsilon \varepsilon_0} + \frac{1}{(2\varepsilon \varepsilon_0 z^2 F^2 c_{bulk}/RT)^{1/2} \cosh(z F \Phi_2/2RT)}
\]  

(2.2)

2.1.2 Gold Crystallography

This work will investigate electrochemical processes on electrodes made from gold, and so a description of gold crystallography is required. In its solid form, gold adopts a face-centred cubic (fcc) crystalline structure, in which the gold atoms form a periodic assembly of cubes having an atom at each of the corners as well as at the centres of each of the cube’s six faces. In bulk gold electrode surfaces, different arrangements of atoms are exposed depending on the orientation of the surface with respect to the fcc crystal orientation. The exposed surfaces can be thought of as the fcc crystal having been ‘cut’ to expose a plane of a particular atomic arrangement. These surfaces can be identified according to their Miller index, some examples of which are given in Figure 2.2. While Miller indices distinguish between different planes within the cubic lattice that are related by point symmetry, this work will treat these geometrically identical planes as an indistinguishable and will denote them by curly brackets. For example, the \( (\mp100) \), \( (0\mp10) \),
Figure 2.1: Schematic of the Gouy-Chapman-Stern model of the electrical double layer, where $C_{\text{OHP}}$ and $C_{\text{D}}$ refer to the capacitances of the outer Helmholtz plane and the diffuse layer, respectively. $R_{\text{sol}}$ indicates the solution resistance.
and (00±1) planes will be referred to simply as the Au{100} plane.\footnote{11}

Crystallographic planes exhibit differences in their reactivities, largely as a result of differences in their surface free energies. Crystal surfaces have varying degrees of 'roughness', which can be characterized in terms of the density of broken bonds for a given surface. Considering, for example, the Au\{111\} surface shown in Figure 2.2, there are three 'broken' nearest-neighbour bonds per atom that would exist if there were another layer of atoms on top of those shown.\footnote{12} The more broken bonds that exist for a particular surface, the higher its surface energy. For the surfaces shown in Figure 2.2, both the orders of increasing density of broken bonds and increasing surface energy is \{111\}<\{100\}<\{110\}.

### 2.1.3 Electrochemical Techniques

A variety of electrochemical techniques that were used in this work to study electrode interfaces of interest are described in the following sections.

#### 2.1.3.1 The Electrochemical Cell

Electrochemistry is performed in an electrochemical cell that consists of three electrodes: a working electrode (WE) at which the electrochemical reaction of interest occurs, a reference electrode (RE) which maintains a known potential, and a counter electrode (CE) through which current is passed. Potential is applied to the working electrode via a high input impedance potentiostat, which alters the amount of current at the CE until the desired potential difference between the WE and the RE is obtained. It is particularly important that the current passes through the CE rather than the RE as the current could alter the composition of the RE, thereby
2.1. Electrochemistry

Figure 2.3: Schematic of a three electrode electrochemical cell. The blue circle represents the electrolyte solution. Electronic and ionic conduction is represented by black and grey lines respectively. $R_S$ represents the solution resistance. $V$ and $i$ indicate the measurement of potential and current, both of which are performed by a potentiostat.

Changing its potential.$^{10}$

Current through the electrochemical cell is transported by charge carriers (ions) in solution which are inherently less mobile than the electrons within the metal electrodes. Consequently, the solution acts as a resistor ($R_S$), and as a result a potential drop occurs across the solution which is encompassed within the measurement of the applied potential. The potential drop across solution can be minimized by decreasing the resistance of the solution, as demonstrated by Ohm’s law (Equation 2.3). Practically, this can be achieved by increasing the solution conductivity by increasing the concentration of charge carriers in solution.

$$E = iR$$  \hspace{1cm} (2.3)

2.1.3.2 Cyclic Voltammetry

A cyclic voltammogram (CV) is plot of current as a function of applied potential measured from a working electrode. A CV is obtained by applying a potential to the WE and sweeping it in a linear manner between two limiting potentials while measuring the current flowing through the WE. In the absence of any electroactive species in solution, the only current measured is due to the charging or discharging of the double layer capacitor. When electroactive species are present, however, the amount of current measured increases as a result of electron-transfer reactions.
2.1. Electrochemistry

2.1.3.3 Differential Capacitance

Capacitance can be a sensitive measure of changes at the electrode interface. In the absence of any faradaic reactions occurring at the electrode, the double layer can be modelled as a simple RC circuit: that is a resistor \( R_{\text{sol}} \) and a capacitor \( C_{\text{dl}} \) in series. The differential capacitance, which is defined as the change in surface charge over the change in voltage across the capacitor, is equivalent to the double layer capacitance. Differential capacitance can be measured experimentally using impedance methods. The addition of a sinusoidal AC voltage perturbation to the applied potential results in a sinusoidal current response. The in-phase and out-of-phase components of the resulting current can be measured by a phase sensitive detector (lock-in amplifier) and the differential capacitance calculated according to Equation 2.4, where \( i_{\text{re}} \) and \( i_{\text{im}} \) are the real (in-phase) and imaginary (out-of-phase) components of the current, \( V_{\text{ac}} \) is the amplitude of the voltage perturbation in V rms, and \( \omega \) is the angular frequency in Hz of the applied voltage.\(^{13}\)

\[
C_{\text{dl}} = \frac{i_{\text{m}}}{V_{\text{ac}} \omega} \left[ 1 + \left( \frac{i_{\text{re}}}{i_{\text{im}}} \right)^2 \right] \tag{2.4}
\]

2.1.4 Mixed Potential

At times, multiple redox active species can exist in the same electrolyte. For a one-step, one-electron reaction of the form \( Ox + e^- \rightarrow Red \), the dependence of the measured current density, \( i \), on the applied potential can be expressed by the Butler-Volmer equation\(^{10}\), shown in Equation 2.5. Here, \( i_{\text{o}} \) is the exchange current density, \( \alpha \) is the transfer coefficient, \( F \) is the Faraday constant, \( E \) is the applied potential, \( E_{\text{eq}} \) is the equilibrium potential, \( R \) is the gas constant and \( T \) is the temperature. This equation is applicable only for well-stirred solutions, when the surface concentrations are approximately equal to the bulk concentrations. The exchange current density is defined as the amount of either the reductive or oxidative current per unit area flowing at equilibrium when the net current flow is zero.

\[
i = i_{\text{o}} \left[ \exp \left( \frac{(-\alpha)F(E-E_{\text{eq}})}{RT} \right) - \exp \left( \frac{(1-\alpha)F(E-E_{\text{eq}})}{RT} \right) \right] \tag{2.5}
\]
2.1. Electrochemistry

When the overpotential, \( E - E_{eq} \), applied to the system is large, then the Butler-Volmer expressions for the oxidation and reduction reactions can be simplified to the following expressions in the absence of mass transport:

\[
i \approx i_0 \exp \left[ -\alpha \frac{F(E - E_{eq})}{RT} \right] \quad \text{and} \quad i \approx i_0 \exp \left[ \frac{(1 - \alpha)F(E - E_{eq})}{RT} \right]
\] (2.6)

More than one redox couple reacting on the same surface gives rise to a more complex system. In particular, at the open circuit potential (OCP), when no external potential control is being applied, a so-called mixed potential can occur. If no external potential is applied to the system and no net current is flowing, then the magnitudes of the oxidation and reduction area-normalized currents must be equivalent: \( i_{red}A_{red} = i_{ox}A_{ox} \), where \( A \) is the area on which each reaction is taking place. The potential can be expressed by the current densities of both reactions in terms of the Butler-Volmer equation. For simplicity, a few assumptions are made. First, it is assumed that \( \alpha \) is equal to 0.5, and second, it is assumed that the overpotential for each reaction is sufficiently large that no back-reaction is occurring. Therefore, the simplified versions of the Butler-Volmer equation (from Equation 2.6) can be used. The oxidation and reduction currents are shown in Equations 2.7 and 2.8.

\[
i_{red} = i_{0,red} \exp \left[ \frac{(E_{mix} - E_{eq,red})F}{2RT} \right]
\] (2.7)

\[
i_{ox} = i_{0,ox} \exp \left[ -\frac{(E_{mix} - E_{eq,ox})F}{2RT} \right]
\] (2.8)

The current density equations can be combined into an expression to find the mixed potential using \( i_{red}A_{red} = i_{ox}A_{ox} \), as shown in Equation 2.9.

\[
\exp \left[ \frac{E_{mix}F}{RT} \right] = \left( \frac{A_{ox}i_{ox}}{A_{red}i_{red}} \right) \exp \left[ \frac{F}{RT} \cdot \frac{(E_{eq,red} + E_{eq,ox})}{2} \right]
\] (2.9)

This can be further rearranged to Equation 2.10,

\[
E_{mix} = \frac{\ln \left( \frac{A_{ox}i_{ox}}{A_{red}i_{red}} \right)}{F/RT} + \frac{(E_{eq,red} + E_{eq,ox})}{2}
\] (2.10)
2.2 Self-Assembled Monolayers

Self-assembled monolayers (SAMs) are stable, monolayer-thick assemblies of chemisorbed molecules supported on some solid substrate. SAMs are so named as they form spontaneously from either the solution or the vapour phase as a result of interactions between the surface and a molecular head-group which possesses a high affinity for that substrate. The structure of the assembly may then undergo re-organization on the substrate surface and can achieve a high degree of order due to intermolecular interactions between the adsorbed molecules.

In general, as shown in Figure 2.5, molecules which form SAMs can be considered to have...
2.2. Self-Assembled Monolayers

Three components:

- A headgroup, which interacts with the substrate surface and regulates self-assembly;

- A backbone, generally composed of a hydrocarbon chain of variable length, which contributes stability to the SAM through intermolecular van der Waals interactions;

- A terminal group, which can be modified in order to create SAMs with a wide variety of functionalities.

While many types of adsorbates and substrate combinations have been studied: such as organosilane molecules on various substrates like silicon dioxide\(^{15}\), glass\(^{16}\) and mica\(^{17}\); and fatty acids on metal oxides\(^{18-20}\), one of the most ubiquitously researched systems is that of alkanethiolate SAMs on gold.

2.2.1 Alkanethiolate SAMs on Gold

Since the first reports of metal-supported SAMs\(^{22}\), gold has been consistently used as an alkanethiolate SAM substrate\(^{21,23,24}\). While other substrates can and have been studied, gold remains an attractive option for several reasons. First, gold is widely available in high purity in a variety of forms, such as thin films and polished crystals of a single crystallographic surface. Second, it is quite inert and does not react with most chemicals, including atmospheric oxygen, meaning that ultrahigh vacuum (UHV) conditions are not generally required. Lastly, gold-thiolate assemblies are particularly attractive due to the strong nature of the Au-S bond,
which is found to be around 40-50 kcal/mol (~160-210 kJ/mol). By virtue of gold's comparative unreactivity toward most other functional groups, the SAM functionality can be modified by changing the alkanethiolate terminal group without risk of disrupting the thiol-mediated self-assembly process.

Alkanethiols of the form R-SH adsorb onto gold surfaces from the gas according to Equation 2.11, where Au(0) indicates an open adsorption site on the gold surface. From the solution phase, however, this production of hydrogen has not been categorically confirmed. It has been speculated that the proton may react with solution oxygen and convert to water.

\[
\text{RSH} + \text{Au}(0) \rightleftharpoons \text{AuSR} + \frac{1}{2}\text{H}_2
\]  

(2.11)

Alkanethiolate SAMs are most commonly prepared by the immersion of a cleaned gold surface into an ethanolic (typically 1-10 mM) solution of the thiol. The self-assembly of alkanethiols from the solution phase has been found to undergo a two-step adsorption process. The first step involves a rapid adsorption of alkanethiols onto the surface, on the order of just a few minutes, after which the SAM has reached 80-90% of its maximum film thickness. The second step is a significantly slower process in which the full film thickness is obtained over the course of several hours. The initial adsorption of the SAM is strongly related to the concentration of alkanethiol in solution, with higher concentrations resulting in a faster film formation, whereas the second step is found to be correlated with the backbone chain length, with longer chains resulting in a faster film completion. This is likely due to longer chains resulting in more intermolecular van der Waals interactions, leading to faster film re-organization and crystallization.

2.2.1.1 Alkanethiolate SAMs and the GCS Model of the Electrode Interface

The addition of an adlayer of alkanethiolate molecules at the electrode interface necessitates some modifications of the GCS model of the interface discussed previously. Most notably, the double layer capacitance of SAM-covered electrodes is markedly different from electrodes only in contact with the electrolyte. As seen in Figure 2.6, the addition of a SAM extends the distance defined as \(x_2\). Furthermore, the dielectric constant for alkanethiolate SAMs is considerably
2.2. Self-Assembled Monolayers

smaller than that of water (2 to 3 for long alkyl chains and 80 for water). As can be seen in Equation 2.2, an increase in $\chi_2$ and a decrease in $\varepsilon$ result in a decrease in the value of the double layer capacitance.

Measurement of the double layer capacitance can provide insight into the extent of the electrode’s alkanethiolate coverage. The surface coverage ($\theta$) is defined as the fractional coverage of the surface, as shown in Equation 2.12, where $\Gamma$ is the surface excess and $\Gamma_{\text{max}}$ is the maximum possible amount of an adsorbate on the surface.

$$\theta = \frac{\Gamma}{\Gamma_{\text{max}}}$$

(2.12)

The coverage can be calculated by first modelling the inner Helmholtz plane as a two capacitors in parallel. The first capacitor represents the part of the electrode that is not covered by the absorbate (with capacitance $C_{\theta=0}$), and the second represents the part that is fully covered surface (with capacitance $C_{\theta=1}$). The total capacitance of a partly covered electrode ($C$) can then be represented as the weighted average of these two capacitances as shown in Equation 2.13. This can be rearranged to solve for the surface coverage, as in Equation 2.14.26,27

$$C = C_{\theta=0}(1-\theta) + C_{\theta=1}\theta$$

(2.13)

$$\theta = \left( \frac{C - C_{\theta=0}}{C_{\theta=1} - C_{\theta=0}} \right)$$

(2.14)

2.2.1.2 Desorption of Alkanethiolate SAMs

Alkanethiolate SAMs are capable of spontaneous desorption. Schlenoff et al.28 found that, while they showed no evidence of desorption in air at room temperature, octadecanethiol SAMs did desorb when immersed in various solvents. In water, the surface coverage drops to approximately 80% within 12 to 24 hours, but that level of coverage remains steady for several days. In ethanol, however, the surface coverage is reduced to approximately 75% in 6 hours and then continues to decrease for about three days until a steady coverage of 50-55% is reached. The mechanism for the spontaneous desorption of alkanethiol is unclear, but the authors note that
Figure 2.6: Schematic of the electrical double layer, potential profile and equivalent circuit modified to include an adsorbed organic monolayer, where $C_{OHP}$ and $C_D$ refer to the capacitances of the inner Helmholtz plane and the diffuse layer, respectively. $R_{sol}$ indicates the solution resistance.
2.2. Self-Assembled Monolayers

more desorption was not necessarily observed for better octadecanethiol solvents.

Alkanethiolate SAMs can also be removed from the gold surface electrochemically by a reductive desorption via the application of a sufficiently negative potential, as described by Equation 2.15. This results in the solvation of both the thiolate and the gold adsorption site.

\[
\text{AuSR} + x\text{H}_2\text{O} + e^- \rightleftharpoons \text{RS}^-(\text{aq}) + \text{Au(H}_2\text{O})_x
\]  

(2.15)

Studies have suggested that the mechanism for the reductive desorption process is that the alkanethiolate SAM first begins to desorb from defect sites, grain boundaries and vacancies within the monolayer.\(^{29,30}\) Subsequent monolayer desorption spreads inwards from the defects and grain boundaries, shrinking the islands of well-ordered monolayer, likely due to the edge molecules experiencing a higher electric field than those within more tightly packed domains.\(^{31}\) Next, small, pin-hole defects appear within the well-ordered regions of the monolayer that grow in size via the same process that causes the domain shrinking. This process is referred to as nucleation and growth.\(^{31,32}\)

Investigations into the influence of the underlying surface crystallography on the reductive desorption of alkanethiolate SAMs have found a strong correlation between the crystallography and the desorption potential. It has been demonstrated that the potential at which SAMs are removed from the low index planes is related to the relative surface energies of those surfaces. For the three low index surfaces discussed in Section 2.1.2, Au\{111\} is desorbed at the least negative potential, followed by Au\{100\} and Au\{110\} (see Figure 2.7).\(^{33,34}\)

Imabayashi and co-workers determined that the electrochemical desorption of an alkanethiolate is dependent on the alkane chain length. By preparing mixed SAMs via the coabsorption of two different alkanethiols, they found that the two species tended to segregate into domains. The domains of the alkanethiolate possessing the shorter chain length could be selectively desorbed by precisely controlling the substrate potential, leaving uncovered gold adsorption sites that could be backfilled with another thiolated molecule, resulting in highly modifiable surfaces.\(^{35}\)

Multi-component alkanethiolate SAMs can also be prepared via place exchange reactions between a single species alkanethiolate SAM exposed to a solution containing another alka-
Figure 2.7: Cyclic voltammograms of decanethiolate SAMs on polished single crystal gold bead electrodes recorded in 0.1 M KOH at a scan rate of 50 mV s$^{-1}$. Adapted from Doneux et al.$^{33}$ with permission from Elsevier.
Similar to the reductive desorption of alkanethiolate, the replacement process occurs most quickly at defect sites and grain boundaries (on the order of hours) while replacement within a well-ordered region of a SAM may take days to occur. The rate of replacement is also dependent on the alkanethiolate structure, with shorter chain thiolates generally experiencing a faster displacement.\textsuperscript{24,36}

Other methods for the removal of alkanethiol from gold surfaces have been proposed. For example, the use of iodine for the decomposition of alkanethiol-stabilized gold nanoparticles via the release of the thiolate SAM in the form of disulphide is widely known.\textsuperscript{37} Recently, it was demonstrated that sodium borohydride could be used to remove alkanethiolate from both gold and gold nanoparticles.\textsuperscript{38,39} The mechanism proposed is that the thiolate is displaced from the surface by hydride adsorption.

2.2.2 Applications of Alkanethiolate SAMs

Due to their ease of preparation and modification via functional terminal groups, alkanethiolate SAMs have a wide range of possible applications across diverse disciplines. For example, they have been used as platforms for biosensing\textsuperscript{40}, as coatings on metals to prevent corrosion\textsuperscript{41,42}, in microcontact printing\textsuperscript{43}, and as stabilizing ligands for nanoparticles\textsuperscript{44}.

2.2.2.1 Alkanethiol-stabilized Gold Nanorods

Gold nanorods (GNRs) are rod-shaped gold nanoparticles, with dimensions of both the long and short axes on the order of 10s or 100s of nanometers. Due to their anisotropy, GNRs exhibit shape-dependent properties.\textsuperscript{9} For example, whereas spherical gold nanoparticles have a single plasmon absorbance band, GNRs have two: one for the short axis, called the transverse plasmon, and one for the long axis, called the longitudinal plasmon, which has been found to be strongly dependent on the GNR aspect ratio (length/diameter).\textsuperscript{45}

Initial GNRs syntheses utilized soft templating methodologies with hexadecyltrimethylammonium bromide (also called cetyltrimethylammonium bromide, or CTAB) as the particle stabilizing surfactant. CTAB was chosen for this application as it is known to form cylindrical micelles in solution in concentrations above its second critical micelle concentration and it was specu-
2.2. Self-Assembled Monolayers

lated that this might aid in the growth of rod-like particles. In 1997, Yu et al. developed an electrochemical method for the preparation of aqueous solutions of GNRs. A gold plate anode and a platinum cathode were immersed in a solution of CTAB and various co-surfactants. Oxidation of the gold anode, along with sonication, resulted in reduction of gold into CTAB-stabilized GNRs at the cathode, though several other morphologies, including spheres, plates and prisms were concurrently formed.

In 2001, Murphy et al. introduced a seeded growth method of preparing aqueous GNRs. Here, small (<5 nm) single crystal seed particles were added to solutions of hydrogen tetrachloroaurate(III) (HAuCl₄), CTAB and a small amount of silver ions. Ascorbic acid, a mild reducing agent, was then added in order to reduce the gold salts and initiate growth of the seed particles into rods due to the cylindrical nature of the CTAB micelles. Because the ascorbic acid is too weak a reducing agent to result in much nucleation of new seed particles, this synthesis was found to produce a more monodisperse product than the previous electrochemical method. This, as well as being more simple and scalable, resulted in the seeded growth method being quickly developed and improved into a robust synthetic procedure with high yield and monodispersity as well as a wide range of possible GNR sizes and aspect ratios.

For certain applications CTAB is an undesirable GNR coating. In particular, for biological applications, CTAB-stabilized GNRs have been found to exhibit cytotoxicity even when care is taken to remove unbound ligand. Furthermore, CTAB itself is largely unmodifiable, resulting in difficulties in preparing functional GNRs. As such, GNRs stabilized by other, biocompatible and modifiable surfactants is of significant interest. Alkanethiolates SAMs, which have historically been used as spherical gold nanoparticle surfactants, are an attractive option due to their greater stability as compared to the CTAB bilayer and their ease of functionalization through different terminal groups. In particular, 11-mercaptoundecanoic acid (MUA) capped nanoparticles are often used for biological applications as they are soluble in aqueous media at biological pH due to the negative charge of the carboxyl group, and they are easily further modified by biological molecules of interest.

Several methods for exchanging the CTAB bilayer for a MUA SAM exist, including organic solvent aided exchange, place exchange resins, through a thiolated-polyethylene glycol intermediate and using a double phase transfer ligand exchange. It has been observed, however, that in some cases the CTAB is not completely
removed from the GNR surface and that methods for better CTAB removal are needed.\textsuperscript{9}

### 2.2.2.2 GNR Crystallography

The synthesis of GNRs using CTAB has become the most commonly employed synthesis method. In this method, the growth of the rods is dependent on the surfactant’s interactions with the metal surface of the seed particles. In particular, the crystallographic direction of growth will be dictated by how strongly the CTAB interacts with each of the crystallographic surfaces,\textsuperscript{56} with stronger binding resulting in a restriction of growth. Numerous investigations\textsuperscript{51,57–59} have found that generally the long axes of GNRs are composed primarily of Au\{100\} and Au\{110\} while in general Au\{111\} is only found near the GNR end-caps.

### 2.2.2.3 End-Functionalization of GNRs

The longitudinal plasmon band of GNRs has been found to be extremely sensitive to not only the aspect ratio\textsuperscript{45} but also to the immediate dielectric properties surrounding the GNR\textsuperscript{2}. For this reason, GNRs have generated significant attention for their optical properties possible biosensing applications, among others. In particular, due to the enhancement of the local electric field near GNR end-caps\textsuperscript{60}, the proximity of a molecule, such as a fluorophore, to the GNR ends can result in significant fluorescence emission enhancement.\textsuperscript{61–63} This has created a need for methods for the selective attachment of molecules of interest to GNR ends as well as for tuning the GNR-molecule distance.

As discussed previously, the attachment of alkanethiol molecules to GNRs is attractive due to the strong Au-S interaction as well as the highly tunable functionality of alkanethiol for biocompatibility. In addition to the complete replacement of the CTAB bilayer with MUA which was

---

Figure 2.8: Cartoons of general GNR crystallographies. Adapted from Keul \textit{et al.}\textsuperscript{59} with permission of The Royal Society of Chemistry.
discussed in Section 2.2.2.1, the selective attachment of thiol to CTAB-GNR ends has been reported. In all of these cases the thiol selectively attached to the GNR ends following a simple incubation period due to the comparatively weaker binding of CTAB to Au\{111\}. While reasonably good specificity is achieved using this method, the long axes of the GNRs remain coated in CTAB, which brings with it all the concerns regarding biocompatibility. A method which allowed for the Au\{111\}-specific attachment of an alkanethiol with a particular functionality to an already alkanethiol-coated GNR would be ideal. To our knowledge, no such method exists. Recently, Novo et al. demonstrated that electrochemical charging of GNRs immobilized on indium-tin-oxide (ITO) electrodes is possible and resulted in changes in the measured surface plasmon frequency. If GNR charging is possible, this suggests that the electrochemical reductive desorption of alkanethiolate ligands from GNRs may also be possible.

2.2.3 DNA

Deoxyribonucleic acid (DNA) is a biopolymer composed of repeating units called nucleotides. In turn, each nucleotide is composed of three important structures: a DNA base, a deoxyribose sugar and a phosphate group. The four DNA bases are attached at the 1’ carbon of the deoxyribose sugar through a glycosidic bond, and can be divided into two groups: the purines, which comprise guanine and adenine, and the pyrimidines, cytosine and thymine. The phosphate groups act as the linkages between the sugars, attaching the hydroxyl group on the 3’ carbon of one sugar ring to the methyl group on the 5’ carbon of the next. This sugar and phosphate polymer is referred to as the phosphate backbone, which is deprotonated and negatively charged at neutral pH. As a result of the 3’ to 5’ phosphate group linkages, this chain of DNA, called a single strand of DNA, or ssDNA, is left with unbonded 3’ and 5’ carbons on either end of the strand. The ends of the ssDNA are often differentiated by referring to them as the 3’ or the 5’ end. ssDNA can be described by listing the sequence of the nucleotides, designated by a single-letter code: A, T, G and C. Conventionally, the oligonucleotide sequence is listed starting from the 5’ end.

The structure of DNA, which was elucidated by the combined work of Chargaff, Watson and Crick, and Wain-Hobson, is a duplex of two strands of DNA. This double stranded
DNA (dsDNA) generally takes the form of a rigid double helix (though other duplex structures exist) and is held together by specific, hydrogen-bonding interactions between the bases of the two strands. Guanine and cytosine interact through three hydrogen bonds and adenine and thymine interact through two. The two strands of DNA within the double helix are called complimentary DNA strands as their ability to form the duplex relies heavily on the nucleotide sequence.

### 2.2.3.1 DNA SAMs

This sequence-specific nature of the hybridization has lead to significant interest in DNA as a biosensor, either by detecting the presence of complementary strands of ssDNA, or by detecting other molecules on the basis of how they bind with DNA. ssDNA can be immobilised on gold surfaces in order to develop sensors through the attachment of a thiolated hydrocarbon 'linker' to one end of a strand of ssDNA.\(^{71–73}\) The strong Au-S interaction results in the formation of a SAM, as described in Section 2.2.1. A schematic of a general DNA biosensor platform is shown in Figure 2.9.

In order effectively utilize the DNA hybridization phenomenon for detection purposes, the
2.2. Self-Assembled Monolayers

Surface density of the ssDNA must be considered. If the DNA layer is too densely packed, then incoming target molecules are unable to effectively hybridize due to steric effects between neighbouring molecules.\textsuperscript{71} It was found by Peterson \textit{et al.}\textsuperscript{74} that probe densities as low as $2 \times 10^{12}$ probes/cm$^2$ were required in order to achieve even a 73\% hybridization efficiency. The theoretical maximum probe density for thiolated ssDNA is approximately $8 \times 10^{13}$ probes/cm$^2$.\textsuperscript{75} Though this varies slightly with the length of the oligonucleotide. Methods for control or limiting the density of ssDNA on the surface are needed.\textsuperscript{75}

Herne and Tarlov\textsuperscript{76–78} found that the surface density of thiolated DNA could be reasonably well-controlled using a short-chain alkylthiol as a ‘spacer’ molecule. Following the immobilization of the DNA on the surface, the substrate is exposed to a solution of mercaptohexanol (MCH) which fills in the gaps between the DNA probes. The authors found that this two-step surface assembly method had the added advantage of effectively removing non-specifically adsorbed DNA. This method has remained widely used to prepare DNA-based biosensor surfaces.

Later studies\textsuperscript{79} on fluorescently-labelled thiolated DNA, however, found that MCH backfilling methods resulted in heterogeneous surfaces, with some parts covered in large aggregates of DNA. This undesirable surface characteristic could be mediated by altering the surface preparation method. It was demonstrated that by first preparing an MCH SAM and then exposing the substrate to a solution containing the thiolated DNA resulted in mixed DNA/MCH monolayers with very low DNA densities (~1\% of the theoretical maximum) due to place exchange reactions between the DNA and the MCH. This method has proven to be more successful for preparing more homogeneous DNA/MCH SAMs, especially for electrochemical studies.\textsuperscript{79–82} It has been speculated that, due to the larger footprint of an oligonucleotide as compared to an alkane chain, a thiolated DNA molecule on a surface will occupy the same space as two MCH molecules.\textsuperscript{83}

Other methods for the preparation of mixed alkanethiol/thiolated DNA SAMs exist. For example, Satjapipat \textit{et al.}\textsuperscript{84} demonstrated that by first preparing a mixed monolayer of two alkanethiols with different chain lengths, the shorter thiolate can be reductively desorbed and the uncovered gold can be subsequently modified with thiolated DNA. Other groups have shown that probe density can be controlled simply by co-adsorbing the thiolated DNA and an alkanethiol in various relative concentrations.\textsuperscript{85,86} Both of these methods, however, result in the
2.3 Fluorescence Microscopy

Figure 2.10: Schematic of the re-orientation of dsDNA (A) and ssDNA (B) as a result of changing the charge on the electrode. Adapted with permission from Rant et al. Copyright 2007 National Academy of Sciences.

formation of phase-segregated domains within the SAM (as determined by atomic force microscopy) rather than a surface of more uniform probe density.  

DNA-modified surface exhibit interesting effects by virtue of DNA's negatively charged backbone. Within a limited potential range, when the metal is negatively charged, the DNA will be repelled from the surface by electrostatic interactions. When a positive charge is applied, the DNA will become attracted to the surface. This is represented in Figure 2.10. For dsDNA, which acts as a rigid rod, this effect results in a significant re-orientation of the DNA and so a large change in the measured fluorescence is measured. For ssDNA, which is far less rigid, the fluorescence change is less significant.

2.3 Fluorescence Microscopy

This work is primarily concerned with SAMs on gold surfaces, and so an electrochemical approach to their investigation is logical. However, electrochemical methods, such as differential capacitance, only provide an average measure of the total electrode surface. In order to correlate the electrochemical data to the underlying electrode surface structure a method for visualizing the electrode surface is needed. Combining electrochemical methods with fluorescence microscopy allows for this. A brief review of the main principles of fluorescence microscopy is provided in the following sections.
2.3. Fluorescence Microscopy

2.3.1 Fluorescence

Luminescence occurs when a molecule in an excited state releases energy via the emission of a photon. The absorbance of a photon of appropriate energy excites an electron in some molecule from the lowest vibrational energy level of the ground electronic state ($S_0$) into one of the vibrational energy levels of an excited singlet electronic state ($S_1$). The molecule relaxes to lowest vibrational energy level of $S_1$ due to, generally, the loss of thermal energy as a result of intermolecular collisions. From there, the molecule spontaneously emits the excess energy in the form of a photon as it relaxes back to the ground state. This process is referred to as fluorescence. In solution, the energy of the emitted photon is lower than that of the absorbed photon because of the energy lost due to collisions which results in vibrational relaxation. This shift in the emitted photon’s energy is called the Stokes shift.

A molecule in an excited electronic state can also release that energy through other means, such as phosphorescence. In this case, the excited electron is transferred from $S_1$ to a triplet state, $T_1$, via a process called intersystem crossing. From there the electron can relax back to the ground state via the emission of a photon. Phosphorescence is a much slower process than fluorescence because the relaxation of the photon from the triplet state involves “forbidden” energy state transitions. The fluorescent and phosphorescent pathways of relaxation can be depicted in a Jablonski diagram, as shown in Figure 2.11.

The ratio of the number of emitted photons to the number of absorbed photons is defined as the quantum yield (QY) and can be expressed according to Equation 2.16,

$$ QY = \frac{k_r}{k_r + k_{nr}} $$

where $k_r$ is the rate of radiative emission and $k_{nr}$ represents the rates of all possible non-radiative deactivation pathways. The intrinsic lifetime of a fluorophore, $\tau_0$, is represented by $\frac{1}{\tau_0} = \sum k_i$, where $k_i$ is the rates of all radiative and non-radiative deactivation pathways. The quantum yield of a fluorophore can then be represented in terms of the fluorescence lifetime under experimental conditions ($\tau$) as shown in Equation 2.17.
Figure 2.11: A Jablonski diagram showing the processes of electronic excitation and emission. An electron in the ground state, $S_0$, is excited by the absorption of an incident photon. This excited electron can then relax back to the ground state either by fluorescent emission, or by intersystem crossing to a triplet state, $T_1$, followed by phosphorescent emission.
2.3. Fluorescence Microscopy

\[ QY = \frac{\tau}{\tau_0} \]  

(2.17)

2.3.2 Non-radiative Deactivation Pathways

There are several processes which can result in a decrease in a fluorophore’s quantum yield, or its fluorescence intensity. Perhaps most importantly for this work is the process by which a fluorophore can be quenched as it approaches a bulk metallic surface. Near a metal surface, the fluorophore, which can be considered an oscillating dipole, induces electron oscillations in the bulk and at the surface of the metal. When the fluorophore-metal distance is sufficiently small, coupling between the fluorophore dipole oscillation and the surface oscillation can occur, and consequently the energy from the excited electronic state is transferred to the metal rather than emitted as a photon. Once transferred, most often the energy is lost to heat and so no fluorescence is observed. It has been determined that for fluorophore very close to bulk metals, the decay rate is a function of the inverse cube of the fluorophore-metal distance (d^{-3}).

Another phenomenon that decreases the quantum yield is that of photobleaching. This is a process by which exposure to light leads to the irreversible destruction of the fluorophore though some photochemical reaction. It was found by Zheng et al. that this destructive process can be worsened by the presence of molecular oxygen in the fluorophore solution.

2.3.3 Instrumentation

Fluorescence experiments in this work were performed using an inverted epi-fluorescence microscope. In fluorescence microscopy, a set of filters is used to select the desired wavelength for sample excitation as well as for detection. A schematic of these filters is presented in Figure 2.12 a. First, radiation from a light source is passed through the excitation filter, which blocks all wavelengths other than the required band of excitation wavelengths. A wavelength-selective dichroic mirror directs the excitation light (and reflects away other wavelengths) through the objective where it is focused onto the fluorescent sample. Following excitation, the sample emits photons in all directions. Some of the emitted light passes back through the objective. This emitted light is directed once again toward the dichroic mirror where it passes through toward
another filter: the emission filter. The purpose of this is to remove all light which does not correspond to the fluorescence wavelengths. This ensures that any reflected excitation light, or any stray light from other sources, is not collected. Finally, the emission light is measured by a detector, such as a charge-coupled device (CCD) array.

2.3.4 Microscope Resolution

At high magnifications, the resolution of an optical microscope is limited by the diffraction of light. As mentioned above, because a fluorophore emits light in all directions, only a part of that light can be collected using an epi-fluorescence microscope. The amount of light collected is dependent on the numerical aperture (NA) of the objective, which is defined as:

\[ NA = n \sin(\theta) \]  

(2.18)

where \( n \) is the refractive index of the material between the sample and the objective and \( \theta \) is the angle formed between the objective lens normal and the light collected by the objective at the most oblique angle.\(^94\)

As light passes through the objective it diffracts, with smaller NA objectives resulting in more diffraction. Light that passes through different points of the objective will diffract and interfere with one another, resulting in a circular diffraction pattern called an Airy disk. Airy disks are somewhat blurred spots surrounded by concentric rings of minimum and maximum light intensities. The Rayleigh criterion states that two objects can be said to be resolved as long as the central maxima of one Airy disk is sufficiently far from the next Airy disk to overlap with its first minima, as shown in Figure 2.12 b. The limit of resolution, \( d_o \), can be represented mathematically in terms of the wavelength of the light (\( \lambda \)) and the NA of the objective according to Equation 2.19.\(^94,95\)

\[ d_o = \frac{1.22\lambda}{2NA} \]  

(2.19)

Another important parameter to consider is the axial resolving power (\( R_{ax} \)), or depth of focus, of the objective. This parameter, which defines the vertical thickness of the region in
focus, normal to the objective, can be calculated according to Equation 2.20, where $n$ is the refractive index of the medium between the objective and the sample.\textsuperscript{95}

\[
R_{ax} = \frac{n\lambda}{(NA)^2}
\]  

(2.20)
2.3. Fluorescence Microscopy

Figure 2.12: a) Schematic representation of the filter set for an inverted epi-fluorescence microscope. The green line represents the excitation light and the blue the emitted light. b) A representation of two overlapping Airy disks. The limit of resolution, $d_0$, is shown.
Chapter 3

Materials and Methods

3.1 Reagents

3.1.1 Water

All water used was ultra high purity water with a resistivity of 18.2 MΩ cm and a total organic carbon content of less than 3 ppm was prepared using a Milli-Q Integra 5 system.

3.1.2 General Reagents

A list of all reagents used in this work can be found in Table 3.1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Purity</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-mercaptoundecanoic acid (MUA)</td>
<td>C_{11}H_{22}O_{2}S</td>
<td>95%</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Argon gas</td>
<td>Ar</td>
<td>&gt;99.998%</td>
<td>Praxair</td>
</tr>
<tr>
<td>Gold wire (d = 0.5, 1.0 mm)</td>
<td>Au</td>
<td>99.999%</td>
<td>Alfa Aesar</td>
</tr>
<tr>
<td>Gold wire (d = 0.5mm)</td>
<td>Au</td>
<td>99.9985%</td>
<td>Alfa Aesar</td>
</tr>
<tr>
<td>Hexadecyl-trimethylammonium bromide (CTAB)</td>
<td>C_{19}H_{42}BrN</td>
<td>≥99.0 (BioUltra)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Hydrochloric acid (37%)</td>
<td>HCl</td>
<td>ACS</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate</td>
<td>MgCl_{2}·6H_{2}O</td>
<td>≥99.0 (BioXtra)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Monobasic potassium phosphate</td>
<td>KH_{2}PO_{4}</td>
<td>99.0%</td>
<td>Sigma</td>
</tr>
<tr>
<td>Nitric acid (68-70%)</td>
<td>HNO_{3}</td>
<td>ACS</td>
<td>VWR Analytical</td>
</tr>
</tbody>
</table>
3.1. Reagents

<table>
<thead>
<tr>
<th>Potassium chloride</th>
<th>KCl</th>
<th>≥99.0 (ACS)</th>
<th>Fluka</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium hydroxide</td>
<td>KOH</td>
<td>99.99% (Semiconductor grade)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>KNO₃</td>
<td>≥99.0 (BioXtra)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>≥99.5 (BioXtra)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Sodium phosphate dibasic dihydrate</td>
<td>Na₂HPO₄·2H₂O</td>
<td>99.5%</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Sodium phosphate monobasic</td>
<td>NaH₂PO₄</td>
<td>99% (Enzyme grade)</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Sulfuric acid (95.0-98.0%)</td>
<td>H₂SO₄</td>
<td>ACS</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Tris(2-carboxyethyl) phosphine (TCEP)</td>
<td>C₉H₁₅O₆P</td>
<td>≥98%</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Tris(hydroxymethyl) aminomethane (Tris base)</td>
<td>C₄H₁₁NO₃</td>
<td>≥99.9 (BioPerformance)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl)</td>
<td>C₄H₁₁NO₃·HCl</td>
<td>≥99.0 (BioPerformance)</td>
<td>Sigma-Aldrich</td>
</tr>
</tbody>
</table>

### 3.1.3 Buffer Solutions

Several general-purpose aqueous buffer solutions, which were used repeatedly throughout this work, are described in Table 3.2. All buffer solutions were used within 4 weeks of preparation.

### 3.1.4 DNA

A custom, 30 base pair oligonucleotide was purchased from Integrated DNA Technologies (IDT) with the following sequence: 5’-CTG-TAT-TGA-GTT-GTA-TCG-TGT-GGT-GTA-TTT-3’. The 3’ end of the oligonucleotide was modified by an AlexaFluor 488 fluorophore, and the 5’ end by a C6-thiol functionalization, which was shipped protected as a disulfide (see Figure 3.1). High performance liquid chromatography (HPLC) purification was performed by the manufacturer. The oligonucleotide, which was shipped as a dry pellet, was dissolved into Tris/NaCl buffer and stored at -18 °C. Prior to use, the disulfide-protected DNA was reduced in order to obtain the free thiol functionality at the 5’ end as described in Section 3.4.1. This doubly-modified
3.2. Materials

Table 3.2: Compositions and primary uses of all aqueous buffer solutions used in this work.

<table>
<thead>
<tr>
<th>Buffer name</th>
<th>Composition</th>
<th>pH</th>
<th>Primary use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immobilization Buffer (IB)</td>
<td>0.806 mM Tris-HCl 0.195 mM Tris-base 100 mM NaCl 50 mM MgCl₂</td>
<td>7.5</td>
<td>Preparing DNA solutions for immediate use</td>
</tr>
<tr>
<td>Phosphate Buffer (PB)</td>
<td>9.3 mM Na₂HPO₄·2H₂O 0.7 mM NaH₂PO₄</td>
<td>8.0</td>
<td>Electrolyte for gold bead potential treatments</td>
</tr>
<tr>
<td>Tris/KNO₃</td>
<td>0.806 mM Tris-HCl 0.195 mM Tris-base 10 mM KNO₃</td>
<td>7.5</td>
<td>Electrolyte for all spectroelectrochemical measurements</td>
</tr>
<tr>
<td>Tris/NaCl</td>
<td>10 mM Tris 100 mM NaCl</td>
<td>7.5</td>
<td>Preparing DNA solutions not for immediate use</td>
</tr>
<tr>
<td>10% Phosphate Buffered Saline (PBS)</td>
<td>1 mM Na₂HPO₄·2H₂O 0.18 mM KH₂PO₄ 13.7 mM NaCl 0.27 mM KCl</td>
<td>7.4</td>
<td>Electrolyte for all gold nanorod experiments</td>
</tr>
</tbody>
</table>

An oligonucleotide will be referred to as HSC6-DNA-AF488.

3.1.5 Gold Nanorods

Gold nanorods (GNRs) (40 x 112 nm) capped with 6-mercaptophexanoic acid (C₆H₁₂O₂S) were purchased from Nanopartz. The GNRs were shipped in a 3 mg/mL solution of approximately 10% PBS and 100 mM CTAB. The GNRs were used as received from the manufacturer. Information regarding the size polydispersity and the possible inclusion of Ag in the nanorods as a result of the synthesis was unavailable.

3.2 Materials

3.2.1 Gold Bead Working Electrodes

Multi-crystalline gold bead working electrodes were prepared using either 0.5 or 1.0 mm gold wire (99.999% pure, Alfa Aesar). The end of a length of gold wire was immersed in freshly prepared aqua regia (~1:3 by volume concentrated nitric and hydrochloric acids) to etch away
3.2. Materials

Figure 3.1: (Top) Structure of AlexaFluor 488- and disulfide-modified oligonucleotide (HSC6-DNA-AF488). (Bottom) Excitation and emission spectra of AF488, data obtained from Chroma website.\textsuperscript{96}
any surface oxides or contaminants. The end of the wire was then melted using a butane torch until a ~2-5 mm diameter bead was formed. The presence of facets of particular crystalline orientations as well as surface defects is largely determined by the rate of cooling as the bead solidifies, with slower cooling generally yielding larger facets and fewer defects. Repeated cycling of aqua regia treatment of the gold and subsequent melting and cooling was performed until a bead displaying several large, well defined Au{111} and Au{100} facets was obtained, as shown in Figure 3.3.
3.2.2 Cleaning of Glass and Teflon Equipment

All glassware as well as any Teflon pieces that came into contact with solution were acid cleaned prior to use in order to remove potential contaminants, such as organic material. These materials were submerged in a mixture, approximately 1:1 by volume, of concentrated sulphuric and nitric acids. The mixture was then heated gently below the boiling point for approximately 3 hours. After cooling, the pieces were thoroughly rinsed with water and stored overnight filled with or submerged in water.

3.2.3 Electrochemical Cells

Three electrochemical cells, described below, were used throughout the course of this work. All reference electrodes described below were tested periodically (approximately once per month) to ensure stability of the measured potential.

**General Electrochemical Cell**  For all electrochemical measurements where the exclusion of oxygen was not required a three-electrode electrochemical cell was set up in a beaker containing ~30 mL of electrolyte, as shown in Figure 3.4 (left). A BASi RE-6 Ag|AgCl reference electrode and a coil of gold wire serving as the counter electrode were used.

![Figure 3.4: Schematic of the cells employed for electrochemical experiments. General electrochemical cell (left), heart-shaped electrochemical cell (centre) and spectroelectrochemical cell (right). Figures are not drawn to scale.](image)
Heart-shaped Electrochemical Cell For electrochemical measurements where oxygen was purged from the electrolyte, a heart-shaped electrochemical cell containing 2 or 3 mL of electrolyte was used along with the same reference and counter electrodes as for the general electrochemical cell. In this case, the reference electrode was separated from the rest of the cell using an electrolyte-filled glass tube terminated by a glass frit (see Figure 3.4 (centre)). Prior to beginning experiments the electrolyte was bubbled with argon for a minimum of 15 minutes and the headspace above the electrolyte was continuously purged with argon to keep the electrolyte free of oxygen.

Electrochemical Cell for Spectroelectrochemical Measurements Spectroelectrochemical measurements were performed in a custom-built cell fitted with a bottom made of a 250 μm thick glass optical window (see Figure 3.4 (right)). The reference electrode was held in a separate reservoir connected to the cell with an electrolyte volume of ~1.5 mL. Separation between the reference electrode reservoir and the cell solutions was maintained by a PTFE stopcock in order to prevent cross contamination. A thin, coiled platinum wire served as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. An Ag|AgCl electrode could not be used in this cell because Tris/KNO₃ was the electrolyte used, Tris and silver form an insoluble precipitate which can block the frit at the end of the reference electrode and impede its function. As described above, the electrolyte was bubbled with argon for a minimum of 15 minutes prior to beginning experiments and the headspace was continuously purged with argon.

3.3 Instrumentation

3.3.1 Electrochemistry

Electrochemical measurements were performed using a Metrohm / Eco Chemie Autolab PG-STAT12 potentiostat. Differential capacitance measurements were performed using an EG&G 5208 lock-in amplifier (LIA) as described in Section 2.1.3.3. Briefly, a sinusoidal perturbation (200 Hz, ~5 mV rms) was produced by the LIA, fed into the potentiostat and added to the applied potential. The measured current was then fed back into the LIA and separated into
3.3. Instrumentation

its in-phase and 90° out-of-phase components were measured by the PGSTAT12 and the capacitance of the layer, assuming a series RC circuit, was calculated according to Equation 2.4, which has been reproduced below.

\[ C_{dl} = \frac{i_m}{V_{ac}\omega} \left[ 1 + \left( \frac{i_{re}}{i_{im}} \right)^2 \right] \]

3.3.2 Spectroelectrochemistry

In situ fluorescence imaging of the gold bead electrodes was done with an Olympus IX70 inverted fluorescence microscope. The microscope was equipped with a Photometrics Evolve 512 charge-coupled device (CCD) camera (512x512 pixels), controlled via in-house developed National Instruments LabVIEW routines. The microscope used an X-Cite eXacte mercury lamp for illumination. All fluorescently-labelled gold bead electrodes were imaged with an Olympus LMPanFl 5x objective (numerical aperture 0.13), through Chroma filter sets (450-490 nm excitation filter, 495 nm dichroic mirror, 500-550 nm emission filter) as shown in Figure 3.5, unless otherwise stated. An analogue FHI ELAB potentiostat in conjunction with an EG&G Princeton 5208 Two Phase Lock-in Analyzer was used during all imaging experiments in order to simultaneously measure differential capacitance as described in Section 2.1.3.3.

Figure 3.5: Excitation and emission spectra of AlexaFluor 488 as well as the spectra of the Chroma excitation and emission filter set employed for spectroelectrochemical measurements. Data obtained from the manufacturer website.\textsuperscript{96}

For the above objective the limit of resolution was found to be ~1.3 \( \mu \)m for a wavelength of 500 nm. This was calculated according to Equation 2.19 which was presented in Section 2.3.4.
3.4. Methods

The axial resolving power was calculated according to Equation 2.20, using a refractive index of 1.333, and was found to be ~39 μm. Using this objective, for a bead that is centred within the field of view, a circular area with a diameter of approximately 0.7 mm will be in focus.

3.3.3 Transmission Electron Microscopy

Transmission electron microscopy (TEM) was performed on a Hitachi H7600 TEM operating at an accelerating voltage of 80 kV. Images were collected using a 5 megapixel AMT XR50 CCD camera.

3.4 Methods

3.4.1 Reduction of Disulfide-Protected DNA

In order to generate a free thiol from the disulfide-protected thiolated-DNA received from the manufacturer, a tris(2-carboxyethyl)phosphine (TCEP) reduction procedure was followed. 40 μL of a solution of 20 μM DNA, 10 mM TCEP in Tris/NaCl was prepared and allowed to react at room temperature for approximately 3 hours with periodic gentle vortexing. This solution was then purified using an illustra Microspin G-50 Column (GE Healthcare) to remove the excess TCEP and free protecting group.

The column was prepared by draining the storage buffer by centrifuging for 30 seconds with a relative centrifugal force (RCF) of 735 and then by washing three times with 300 μL of Tris/HCl. Each wash was drained by centrifuging for 1 minute at 375 RCF. The 40 μL DNA/TCEP solution was loaded onto the column dropwise as then centrifuged for 2 minutes at 375 RCF. The reduced HSC6-DNA-AF488 was stored at -18 °C for no more than 2 weeks before using.

The concentration of the reduced HSC6-DNA-AF488 solution was determined by measuring the absorbance at 260 nm (ε = 3.277 x 10^{-5} M^{-1}cm^{-1}) using an Ocean Optics USB2000 Spectrometer equipped with a DH2000 Deuterium Lamp. It was necessary to use a UV-Vis with a monochromator located between the sample and the detector as the fluorescence resulting from the AF488 interfered with the measurement of the DNA absorbance.
3.4.2 Cleaning Gold Bead Working Electrodes

Crystalline gold bead electrodes were cleaned of any surface contaminants by flame annealing. The bead was held within the blue flame of a butane torch until the metal glowed red-hot. This colour was maintained for 30 seconds while ensuring that the gold did not melt. The bead was then removed from the flame, allowed to cool slightly and then rinsed with MilliQ water. This flaming and rinsing process was repeated a minimum of three times.

3.4.3 Preparation of MUA SAMs

Freshly cleaned gold bead electrodes were immersed in 100 µL of a 1 mM ethanolic solution of MUA for 90 minutes, after which they were rinsed with 100 µL of ethanol and then stored in 100 µL of ethanol for no more than eight hours.

3.4.4 Potential Treatment of MUA SAMs

MUA SAMs were partially reductively desorbed from gold bead electrode surfaces via the administration of various potentials sufficiently negative to initiate the desorption process. The potential of the electrode was set in one of two ways: electrochemically or electrolessly. In both cases, which are described in detail below, the potential treatment was performed in magnetically stirred 3 mL of deoxygenated 10 mM PB, pH 8, in the heart cell described in Section 3.2.3.

**Electrochemical Potential Treatment** In the electrochemical treatment, the desired potential, called the treatment potential (E\text{treat}), was applied for 5 minutes. In addition, a 5 mV perturbation from the lock-in amplifier was added to the potential in order to measure the capacitance as described in Section 2.1.3.3.

**Electroless Potential Treatment** For the electroless potential treatment, rather than applying the treatment potential using a potentiostat, the electrode potential was set via the addition of small volumes (~1-10 µL) of aqueous sodium borohydride solutions (0.03-0.3 M). In this case, the potentiostat was used to alternatingly monitor the open circuit potential (E\text{OCP}) of the
3.4. Methods

system for 10 seconds, and then apply the measured $E_{OCP}$ for five seconds along with a 5 mV AC pertubation in order to measure the differential capacitance. Upon completion of the experiment, the bead was removed from the electrolyte and immediately rinsed with water in order to remove all traces of reducing agent.

3.4.5 Control Samples

Control experiments were performed by exposing MUA-coated gold bead electrodes to the same conditions as the electrochemically and electrolessly treated beads, but without the application of a treatment potential or the addition of sodium borohydride. These SAM-covered beads were immersed in 10 mM PB in a heart-shaped electrochemical cell as described above, and the potentiostat was used to alternatingly monitor and apply OCP and measure capacitance in the same manner as was done for the electroless potential treatment procedure.

3.4.6 Immobilization of Fluorophore-labelled DNA

Following the electrochemical or electroless potential treatment, the MUA-coated gold bead electrodes, including the control beads, were rinsed with water and immersed in 40 $\mu$L of a 1 $\mu$M solution of HSC6-DNA-AF488 dissolved in IB for 10 minutes to allow for the thiolated DNA to adsorb to any open adsorption sites. Next, the MUA/HSC6-DNA-AF488-coated gold bead electrodes were removed from the DNA solutions, rinsed with 100 $\mu$L of fresh tris/NaCl and then immersed in IB overnight in order to remove any physisorbed DNA molecules.

3.4.7 Spectroelectrochemical Measurements

In situ fluorescence imaging of the MUA/DNA-AF488-coated gold bead electrodes was performed with simultaneous capacitance measurements. The procedures for the following spectroelectrochemical investigations of these samples were described in Sections 3.2.3 and 3.3.2. Prior to performing any spectroelectrochemical measurements, a brightfield image of the bead electrode was taken in order to correlate the locations of the Au{111} and Au{100} facets with the fluorescence images.
3.4.7.1 Electrochemical Removal of Physisorbed DNA

First, the MUA/HSC6-DNA-AF488 SAMs were cycled within a limited potential range (0 to -0.4 V vs. SCE) in order to dislodge any physisorbed HSC6-DNA-AF488 from the surface. Due to the negatively charged backbone of DNA, proximity to a negatively charged electrode results in the repulsion of the DNA from the electrode surface. Physisorbed DNA, which is not as strongly bound as chemisorbed HS-DNA, can be irreversibly dislodged from the electrode by the application of potentials that are insufficiently negative to cleave the S-Au bond of chemisorbed thiolated DNA but that are sufficiently negative to repel the DNA away from the surface.\textsuperscript{87}

The potential was cycled between 0 V and -0.4 V vs. SCE three times with sequential 50 mV steps, with each potential being applied for 4 seconds, as shown in Figure 3.6. At each potential step, the differential capacitance was measured and a fluorescence image of the electrode was taken. This cycling of the electrode potential, which will be referred to as a \textit{DNA flipping procedure}, was performed for a minimum of six cycles for each SAM-covered bead electrode.

![Figure 3.6: Potential profile of the DNA flipping procedure.](image)

3.4.7.2 Electrochemical Reductive Desorption of Chemisorbed SAM

Next, the MUA/HSC6-DNA-AF488 SAM was reductively desorbed from the electrode surface by stepping the potential from 0 V to -1.4 V vs. SCE in 20 mV potential steps, each lasting 8 seconds. $E = -1.4$ V was then held for a total of 2 minutes in order to fully desorb the SAM before the potential was stepped back to 0 V. A measurement of the capacitance of the electrode as
3.4. Methods

well as a fluorescence image was performed every 4 seconds. The potential profile for this stepping potential scan is shown in Figure 3.7.

![Potential profile of the stepping potential scan with 20 mV, 8 second steps used for all spectroelectrochemical measurements. The inset shows a zoomed-in section of the potential steps.](image)

Following desorption of the SAM via the stepping potential scan, a second, identical stepping scan was performed on the desorbed gold bead electrode in order to obtain a measurement of the bare electrode capacitance as well as the background fluorescence of the bead electrode. This background fluorescence image, which includes any light which was reflected from the gold surface, was used for the background correction of all fluorescence images.

3.4.7.3 Fluorescence Image Analysis

All fluorescence images were processed using ImageJ. The fluorescence images were normalized by the exposure time according to Equation 3.1, where $Fl.\ Int_{\text{meas.}}$ is the fluorescence intensity and $t_{\text{exp}}$ is the exposure time. The threshold value of the camera employed (500 counts), must be subtracted from all images before normalizing. All fluorescence images presented in this work are shown on a logarithmic intensity scale and have been falsely coloured using ImageJ’s 'Fire' look-up table.

$$Fl.\ Int. = \frac{(Fl.\ Int_{\text{meas.}} - 500)}{t_{\text{exp}}}$$ (3.1)

The different crystallographic surfaces of interest could be identified using methods previ-
ously reported by Yu et al.\textsuperscript{82}, as shown in Figure 3.8. The fluorophore-labelled thiol species used by Yu et al. differs from that used in this work and has different desorption potentials, but the order in potential in which the different crystallographic planes desorb remains the same. Once identified, the Au\{111\} and Au\{100\} facets were selected as regions of interest (ROIs) using ImageJ. The average fluorescence intensities within these ROIs could then be calculated for all fluorescence images obtained at all potentials. In the interest of clarity, further analysis of the fluorescence images will be discussed in later chapters as the data is presented.

Figure 3.8: (a) Brightfield optical image at $E_{\text{OCP}}$ with Au\{111\} facets outlined in red and fluorescence images of a gold bead electrode at potentials approximately corresponding to the reductive desorption of the (b) Au\{111\}, (c) Au\{100\} and (d) Au\{111\} surfaces. Overlayed on the fluorescence images are the surface crystallographic planes. The fluorescence intensity is represented by a colour as indicated by the calibration bar. Adapted with permission from Yu et al.\textsuperscript{82}. Copyright 2015 American Chemical Society.
Chapter 4

Selective Electrochemical Modification of Single Crystal Gold Bead Electrodes

MUA-coated single crystal gold bead electrodes were modified by the application of potential sufficiently negative to selectively reductively desorb MUA only from the Au\{111\} facets while leaving the SAM on the remaining surface largely intact. Desorbed gold was then backfilled with HSC6-DNA-AF488 in order to fluorescently label the modified parts of the bead. In order to quantify the selectivity of the MUA desorption process, the amount of DNA on the Au\{111\} facet was compared to the changes observed on the Au\{100\} facet of the electrodes. DNA was specifically chosen for this purpose as it has a highly customizable length. The eventual goal of this work is to develop a method for the end-selective modification of GNRs, and so a ligand of variable length is desired in order to exploit the filed-enhancement effects of GNR ends.

4.1 Characterization of the Selective Electrochemical Desorption of a MUA SAM Using Capacitance

4.1.1 Electrochemical Reductive Desorption of MUA SAMs

In order to determine how the MUA SAM is reductively removed from the electrode surface, the change in the capacitance of the electrodes with respect to the applied potential was measured. Figure 4.1 shows the capacitance of a MUA-coated gold bead electrode (black) and a bare gold bead electrode (red) as the potential was linearly scanned (5 mV/s) from 0 V to -1.4 V vs

Figure 4.1: Capacitance of a MUA-coated gold bead electrode (black) and a clean gold bead electrode (red) as the potential was scanned at a rate of 5 mV/second from 0 to -1.4 V vs. Ag|AgCl. The arrow indicates the scan direction. The data obtained was smoothed using a 10 point moving average.

Comparing these two scans, it is apparent that the capacitance of the electrode near 0 V vs Ag|AgCl is significantly lower for the MUA SAM (~1.8 μF cm⁻²) than for the uncovered Au (>20 μF cm⁻²). As described in Section 2.2.1.1, a decrease of the capacitance is expected for a SAM-covered electrode as compared to an electrolyte-covered electrode. For the MUA-coated electrode, starting at 0 V, the capacitance remains relatively constant as the potential becomes more negative, which is characteristic of a reasonably well-ordered SAM. Near -0.825 V, the capacitance begins to sharply increase, which suggests desorption of the MUA SAM, until -1.2 V where the potential levels off at approximately 20 μF cm⁻². At -1.2 V, the capacitance of the bare Au electrode is equal to that of the MUA-coated electrode, which suggests at this potential all alkanethiolate has been reductively desorbed from the electrode surface.

It has been previously demonstrated that alkanethiolates reductively desorb from the Au{111} of a single crystalline gold bead electrode at the least negative potential, and as such it is likely that the initial rise in capacitance near -0.825 V is a result of MUA desorption from these regions. It follows that by applying a potential near -0.825 V vs Ag|AgCl for a given amount of time that an alkanethiolate SAM could be selectively reductively desorbed from Au{111}.
4.1.2 Partial Electrochemical Reductive Desorption of MUA SAMs

Various potentials were applied to MUA-coated gold bead electrodes in order to determine the required potential for the optimal selective desorption of Au(111). These potentials (electrochemical treatment potentials, or $E_{\text{treat}}$) were each applied for a total of 5 minutes, along with the measurement of the capacitance, as described in Section 3.4.4. The capacitance of each electrode is presented in Figure 4.2.

Changes in the capacitance of the MUA-covered electrodes can be indicative of changes in the SAMs. The fractional surface coverage, $\theta = \frac{\Gamma}{\Gamma_{\text{max}}}$, which was introduced in Section 2.2.1.1, is related to the capacitance according to Equation 4.1. By differentiating with respect to time (Equation 4.2), it is found that the rate of change of the capacitance is proportional to the rate of change of the surface excess. Therefore, by monitoring the capacitance of the MUA-coated electrodes, we can correlate the capacitance changes to changes in the rate of MUA desorption.

$$C = C_\theta \cdot \frac{\Gamma}{\Gamma_{\text{max}}} + C_{\theta=0} \left(1 - \frac{\Gamma}{\Gamma_{\text{max}}}ight)$$

(4.1)

$$\frac{dC}{dt} = \frac{d\Gamma}{dt} \left(\frac{C_\theta - C_{\theta=0}}{\Gamma_{\text{max}}}\right)$$

(4.2)

The capacitance for several $E_{\text{treat}}$ more positive than -0.725 V (Figure 4.2 a) remain more or less constant over the 5 minute potential application. Considering the linear scan results shown in Figure 4.1, no change in capacitance between 0 V and -0.8 V is expected, indicating that the MUA SAMs are stable at these potentials. The inset of Figure 4.1 a, however, reveals that a small increase in the capacitance was observed for some of the electrodes immediately following the application of $E_{\text{treat}}$. This was also observed for several of the control experiments performed, and as such the increase is likely not a result of the applied treatment potential. It is possible that this increase is related to the wetting of the electrode. Small changes in the wetting of the gold surface will result in changes to the electrode area, which in turn causes slight shifts in the measured capacitance.

For $E_{\text{treat}} = -0.75$ V, shown in Figure 4.2 b, a small but noticeable change in capacitance

Figure 4.2: Capacitance measured during the electrochemical potential treatment of MUA-coated gold bead electrodes. The $E_{\text{treat}}$ shown, which were applied for 5 minutes, are: a) -0.725 V, -0.7 V, -0.65 V, -0.6 V and -0.4 V, b) -0.75 V, c) -0.775 V, d) -0.8 V, e) -0.825 V and f) -0.85 V vs Ag|AgCl. Individual traces represent different electrodes.
occurs over the 5 minute potential application, indicating the removal of some MUA from the electrode surface. This is not expected when compared to the linear scan shown in Figure 4.1, which shows no change in capacitance at this potential. This change is therefore likely a result of the difference in the time taken for the two measurements, and implies that alkanethiolate desorption kinetics at -0.75 V are too slow to be noticeable at the scan rate studied, 5 mV/sec, but becomes significant when the potential is applied for several minutes.

As seen in Figure 4.2 c, at $E_{\text{treat}} = -0.775$ V, a larger increase in capacitance is observed which suggests that the desorption kinetics are faster at this potential and that more of the MUA SAM is being removed over the course of the 5 minute potential application. At this $E_{\text{treat}}$ it also becomes evident that the rates at which the capacitive increase occurs varies between electrodes, with the partial desorption of some SAMs seemingly initiating more quickly than others. This is likely due to either differences in the initial quality of the SAM or differences in the electrodes themselves. As described in Section 2.2.1.2, the reductive desorption of alkanethiol occurs first at defective regions in the SAM. Any increase in the number of these defective regions will result in an increase of the desorption kinetics. Furthermore, because capacitance is an average measure of the total electrode surface, any differences in the relative amounts of different atomic arrangements, such as Au{111}, could result in different rates of desorption.

This sample to sample variation of the SAM desorption results becomes even more apparent at $E_{\text{treat}} = -0.8$ V, shown in Figure 4.2 d. Here, the maximum capacitance that was observed for $E_{\text{treat}} = -0.775$ V ($\sim 4 \mu$F cm$^{-2}$) is observed much earlier during the application of $E_{\text{treat}} = -0.8$ V. This implies that the MUA SAM is being desorbed much more quickly at this more negative treatment potential. Furthermore, the different SAMs studied at this potential demonstrate significantly different rates of desorption, but all show capacitance - time curves with a fast initial increase before a plateau is reached. This sigmoidal shape has been previously observed by Doneux et al.$^{32}$ and is indicative of alkanethiolate desorption which propagates first from defective regions of the SAM followed by shrinking of well-ordered SAM domains, as is discussed in Section 2.2.1.2. It is likely that the variance in the shape of the capacitance - time curves at this $E_{\text{treat}}$ are a result of differences in the initial MUA SAMs, with more defective SAM regions resulting in a faster SAM desorption.

The trend of a more negative $E_{\text{treat}}$ leading to faster SAM desorption continues at $E_{\text{treat}} =

-0.825 V and -0.85 V, shown in Figure 4.2 e and f, respectively. In both cases the sigmoidal shape of the capacitance - time curves is observed with the increase in capacitance occurring earlier and more quickly. Furthermore, the final capacitance measured at 5 minutes increases as \( E_{\text{treat}} \) becomes more negative, in general indicating that more MUA becomes reductively desorbed from the electrode surface.

In order to more easily see the differences between the capacitance of the MUA SAM linear scan presented in Figure 4.1 and the capacitances resulting from the potential step to the various \( E_{\text{treat}} \) after 5 minutes, those results have been summarized and presented in Figure 4.3 for comparison. Also shown are the capacitances of the electrodes after one minute at \( E_{\text{treat}} \). It is evident that the increase in capacitance, and therefore the desorption of the MUA SAM, is shifted to more positive potentials for the \( E_{\text{treat}} \) steps than for the linear scan, though the 5 minute steps seem to follow the same trend as the linear scan with a plateau near a capacitance of 6 \( \mu \)F cm\(^{-2}\). This indicates that for \( E_{\text{treat}} \leq -0.75 \) V vs Ag|AgCl, as the duration of the potential step increases, the rate of the MUA reductive desorption also increases. For \( E_{\text{treat}} > -0.75 \) V, no change in the capacitance was observed during the 5 minute potential treatment. It is important to note that for each of the values of \( E_{\text{treat}} \) studied multiple replicates were performed. For all beads where little or no capacitance change was observed, the sample to sample error was very small, as represented by the range of values presented in Figure 4.3. As the changes in capacitance became more significant, the error also increased.

Figure 4.4 shows the capacitance measured for a MUA-coated gold bead electrode during three stepping potential scans, each having 50 mV steps of different step durations. Also shown is the linear scan of a MUA SAM which was presented at the beginning of this section as well as the final measured capacitances after the 5 minute application of various \( E_{\text{treat}} \). As the step duration increases, the initial MUA SAM reductive desorption shifts to more positive potentials. Note that between 0 and -0.2 V the capacitance measured for the 5 minute stepping scan decreased steadily before suddenly increasing sharply back to a capacitance of approximately 2 \( \mu \)F cm\(^{-2}\). This decrease was due to the evaporation of the electrolyte and the increase a result of the subsequent re-positioning of the bead electrode in solution. It was found that the capacitances measured for the step and 5 minute hold to \( E_{\text{treat}} < -0.75 \) V are more or less identical to those measured for the 5 minute stepping scan and that the 15 second stepping

Figure 4.3: Average capacitances of MUA-coated gold bead electrodes at 1 minute (blue) and 5 minutes (red) into the application of \( E_{\text{treat}} \). The y-error bars show the highest and lowest measured values at each potential, and the x-error bars show the highest and lowest OCP measured for the control experiments. For the controls, as well as all \( E_{\text{treat}} \) from -0.725 V to -0.85 V, a minimum of three experiments were performed. (Black) Measured capacitance during the desorption of a MUA SAM from a gold bead electrode as the potential was scanned at a rate of 5 mV/second from 0 to -1.4 V. The arrow indicates the scan direction.

scan is virtually indistinguishable from the linear scan between 0 V and -0.9 V.

4.1.3 Quantifying the MUA SAM Partial Desorption

The changes in the capacitance of the MUA-coated gold bead electrodes as a result of the application of various \( E_{\text{treat}} \) can be used to estimate the amount of MUA that was reductively desorbed from the electrode surface, as discussed in Section 2.2.1.1. The surface coverage of defects, \( \theta \), is calculated according to Equation 4.3\textsuperscript{26,27},

\[
C_{\text{MUA, } E_{\text{treat}}} = (\theta)C_{\text{MUA, } E=0} + (1-\theta)C_{\text{bare, } E_{\text{treat}}}
\]  

(4.3)

where \( C_{\text{MUA, } E_{\text{treat}}} \) and \( C_{\text{bare, } E_{\text{treat}}} \) is the measured capacitance at the various \( E_{\text{treat}} \) for the MUA-coated electrode and a bare electrode, respectively and \( C_{\text{MUA, } E=0} \) is the initial capacitance of the MUA-coated electrode, which is assumed to be the same over all potentials. This equation can be re-arranged to solve for \( \theta \) as shown in Equation 4.4.

Figure 4.4: Potential-time profiles for potential staircase scans with 50 mV steps from 0 to -1.05 V vs Ag|AgCl with step durations of 15 seconds, 1 minute and 5 minutes (top). The resulting normalized capacitance of the staircase scans for MUA-coated gold bead electrodes, a 5 mV/sec linear scan of a MUA-coated gold bead electrode, and the capacitances at 5 minutes for various E_treat applications (bottom). The arrow indicates the scan direction.
4.2 Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

Table 4.1: The percentage of the MUA SAM remaining on the electrode surface following the 5 minute application of various \( E_{\text{treat}} \).

<table>
<thead>
<tr>
<th>( E_{\text{treat}}(V) )</th>
<th>( \theta \times 100% )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to -0.700</td>
<td>99.7</td>
</tr>
<tr>
<td>-0.725</td>
<td>98.5</td>
</tr>
<tr>
<td>-0.750</td>
<td>89.8</td>
</tr>
<tr>
<td>-0.775</td>
<td>88.8</td>
</tr>
<tr>
<td>-0.800</td>
<td>76.1</td>
</tr>
<tr>
<td>-0.825</td>
<td>74.0</td>
</tr>
<tr>
<td>-0.850</td>
<td>71.9</td>
</tr>
</tbody>
</table>

\[
\theta = \frac{C_{\text{MUA}, E_{\text{treat}}} - C_{\text{bare}, E_{\text{treat}}}}{C_{\text{MUA}, E=0} - C_{\text{bare}, E_{\text{treat}}}} \tag{4.4}
\]

The results of this calculation are presented in Table 4.1. According to this estimation, for \( E_{\text{treat}} = -0.75 \text{ V} \) to \(-0.85 \text{ V}\), the MUA SAM is approximately 10 to 30 percent reductively desorbed.

4.2 Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

The capacitance measurements discussed in the previous section provide an excellent means of estimating the amount of MUA which was desorbed from the electrode surface. However, because capacitance is an average measurement of the entire electrode surface, it is therefore impossible to ascertain from capacitance measurements alone which parts of the MUA SAM on a gold bead electrode have been reductively desorbed. In order to identify which surfaces of the gold bead electrode experienced a disruption of the MUA SAM, and to what degree, a method for labelling any uncovered adsorption sites following potential treatment is needed. To that end, the partially desorbed MUA-coated gold bead electrodes were immersed in solutions of HSC6-DNA-AF488 in order to immobilize the fluorophore-labelled DNA onto any defective regions of the MUA SAM which had been created by the electrochemical potential treatment process, as described in detail in Section 3.4.6. This backfilling process, along with fluorescence microscopy of the bead electrode surface, allows for the identification of the parts of the MUA SAM which were more greatly disrupted by the potential treatment.
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

All the experiments presented in this section were performed in the spectroelectrochemical cell, as described in Section 3.4.7. An Ag|AgCl reference electrode could not be used for these measurements as they are incompatible with the Tris\KNO\textsubscript{3} electrolyte used, and so an SCE reference electrode was used instead. The results presented in this section have therefore been converted to E vs. Ag|AgCl in order for them to be more directly comparable with the previously presented results.

4.2.1 Examining the Effect of the Electrochemical Potential Treatment on the SAM Stability

Following the application of the treatment potential, it is expected that for some of the more negative values of \(E_{\text{treat}}\) large regions of thiol will have been desorbed from the SAM given that as much as 30\% of the monolayer has been desorbed. Although some of this uncovered gold will have been backfilled with HSC6-DNA-AF488, it is likely that the 10 minutes the gold bead electrodes spend immersed in the DNA solution is insufficient to completely replace all the MUA lost during the potential treatment. Figure 4.5 shows the average measurement of the capacitance at 0 V vs Ag|AgCl (\(C_{0V}\)) of these mixed MUA/HSC6-DNA-AF488 SAMs, which provides some insight into the status of the monolayer. For \(E_{\text{treat}}\) = -0.6 V to -0.75 V, the \(C_{0V}\) remains very similar to the values measured for the control experiments, which are represented as the grey region of the figure. Although not shown, similar values were obtained for \(E_{\text{treat}}\) = -0.4 V. For \(E_{\text{treat}}\) ≤ -0.775 V the measured \(C_{0V}\) is noticeably higher than the controls, indicating that despite the HSC6-DNA-AF488 backfilling, uncovered absorption sites (defective regions) remain on the electrode surface. This is likely due in part to the larger surface area that the DNA occupies on the surface, as described in Section 2.2.3.1.

The effects of these defective regions on the SAM can be observed by reductively desorbing the mixed MUA/HSC6-DNA-AF488 SAMs from the electrode surface and measuring their capacitances. By comparing them to the capacitance measured during the desorption of a well-ordered MUA SAM, some estimate of how defective are the post-potential treatment MUA/HSC6-DNA-AF488 SAMs can be obtained. Figure 4.6 shows the measured capacitance of selected MUA-coated gold bead electrodes during a spectroelectrochemical stepping poten-
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

Figure 4.5: The average measured capacitance at 0 V vs Ag|AgCl of the mixed MUA/HSC6-DNA-AF488 SAMs on gold bead electrodes following the potential treatment and DNA backfilling. The y-errorbars show the highest and lowest measured values. The grey region between the dashed lines represents the range of $C_{0V}$ values measured for the control sample MUA SAMs after exposure to HSC6-DNA-AF488.

tial scan (described in Section 3.4.7) after various $E_{treat}$: -750, -800 and -850 mV, as well as a control sample. Also shown is the linear scan of a MUA SAM that was first presented in Figure 4.1.

It was shown in Figure 4.4 that the capacitance for a MUA SAM during a stepping scan with 50 mV, 15 second steps is more or less indistinguishable from a linearly scanned MUA SAM. The stepping potential scan used for all spectrotelectrochemical measurements (20 mV, 8 second steps) is quite similar in step size and duration, so the capacitance measured during this stepping potential scan should be similar to that of the linear scan.

The capacitance of the MUA SAM control sample shown in Figure 4.6 a, closely resembles the linear scan capacitance profile, indicating that the application of the measured $E_{OCP}$ and the exposure to the HSC6-DNA-AF488 did not alter the integrity of the MUA SAM in any notable way. For samples treated with $E_{treat} = -0.75$ V, shown in Figure 4.6 b, the capacitance begins to increase at a slightly more positive potential than -0.8 V during the stepping scan than for the linear scan. The mixed MUA/HSC6-DNA-AF488 SAM began to reductively desorb from the surface at a more positive potential than for a well-ordered alkanethiolate SAM. This suggests that a treatment potential of -0.75 V is sufficiently negative to disrupt the MUA layer enough such that the 10 minute exposure to HSC6-DNA-AF488 does not adequately repair the defects. Both
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

Figure 4.6: Capacitance of various MUA-coated gold bead electrodes that have been backfilled with HSC6-DNA-AF488 for a) a control sample as well as following the 5 minute electrochemical application of b) -750 mV, c) -800 mV and d) -850 mV. The measurements were performed during a stepping potential scan from approximately 0 to -1.4 V vs. Ag|AgCl with 20 mV, 8 second steps. The capacitance of a MUA-coated gold bead electrode during a 5 mV/second linear potential scan from 0 to -1.4 V vs. Ag|AgCl is shown in black. The arrows indicate the scan direction.
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

$E_{\text{treat}} = -0.8\text{ V}$ and $E_{\text{treat}} = -0.85\text{ V}$ (Figure 4.6c and d) show increases in the capacitance at much less negative potentials: -0.55 V and -0.4 V, respectively. This indicates that the MUA SAMs at these treatment potentials were much more significantly disrupted.

### 4.2.2 Assessing the Selectivity of the Electrochemical Modification of MUA SAMs

In this section, the fluorescence images obtained during the stepping potential scan of the mixed MUA\HSC6-DNA-AF488 SAMs will be discussed. In order to quantify the selectivity of the partial desorption process for, the fluorescence intensities measured on the Au\{111\} facet will be compared with those measured on a different portion of the surface. Au\{100\} has been selected for comparison because, according to previous studies\(^82\), it is the surface which desorbs at potentials slightly more negative than for the desorption of Au\{111\}. Therefore, any modification observed on Au\{100\} indicates that the modification process is not selective for Au\{111\}.

The fluorescence intensities of the mixed SAMs increase dramatically as it is desorbed from the electrode surface. This is due to the increasing distance between the fluorophore and the gold surface, which decreases the metal-mediated quenching effect. By comparing the maximum intensities obtained on Au\{111\} and Au\{100\} surfaces as the SAM is desorbed, an estimation of the fluorophore’s selectivity, which corresponds to the selectivity of the MUA partial desorption via the application of $E_{\text{treat}}$, for the Au\{111\} surface can be determined. This is achieved by calculating the difference between the intensities measured for the desorbing SAM and any background reflection that exists for the same, bare gold bead electrode. Figures 4.7 and 4.8 show the fluorescence results for the SAM desorption of two representative gold bead electrodes: a control sample, and $E_{\text{treat}} = -0.75\text{ V}$, respectively.

First, consider the control experiment shown in Figure 4.7. The fluorescence image I shows relatively constant, low fluorescence across the electrode surface, though there is a greater fluorescence in an approximately hexagonal region surrounding the Au\{111\} facets. Because the control samples did not experience any significant increase in their capacitances during the potential treatment process, it can be assumed that all the HSC6-DNA-AF488 on the surface was
adsorbed through place exchange reactions with the MUA SAM as demonstrated previously using mercaptohexanol (MCH) SAMs on the same surface.\textsuperscript{82}

Figure 4.7, bottom, shows that during the stepping potential scan, the maximum fluorescence from the Au\{111\} facet occurs at a potential of approximately -0.85 V, which is slightly more negative than -0.8 V, the potential at which an alkanethiolate SAM begins to reductively desorb from the surface. This is due to the fluorescence intensity increasing as the distance between the fluorophore and the gold surface increases. It is therefore expected that the fluorescence would reach a minimum some time after the desorption occurred, as the fluorophore diffuses away from the surface. While minimal HSC6-DNA-AF488 is present on the Au\{111\} facet itself, it is apparent from Figure 4.7 II that the fluorescence of the hexagonal region around the facet is desorbing near -0.8 V as well, which suggests that this hexagonal region is a surface similar to Au\{111\}. It is likely that this region is composed of large Au\{111\} terraces interspersed with step edges. The presence of these steps gives this region of the electrode a higher density of defects, which enables the HSC6-DNA-AF488 and the MUA to place exchange more easily than other regions of the electrode. At a slightly more negative potential, ~-0.9 V, fluorescence from the Au\{100\} facet reaches its maximum, and by E = -1.4V (image III) the SAM has been entirely removed from the electrode surface.

The fluorescence image shown in Figure 4.7 IV is of a stepping potential scan of the completely desorbed gold bead electrode. This scan was performed immediately following the one described above. The average intensity from both the Au\{111\} and Au\{100\} facets are shown by the dashed lines, and remain constant with respect to the potential. The low intensities measured here are a result of reflected excitation light reaching the detector. The maximum fluorescence intensity for each facet was calculated by determining the difference in the intensity between the maximum obtained during the SAM desorption and the average intensity measured from the bare gold bead electrode, as illustrated in Figure 4.7, bottom. The ratio of the fluorescence intensities of Au\{111\}/Au\{100\} can then be calculated. This ratio provides an estimate of the selectivity of HSC6-DNA-AF488 for Au\{111\} over other regions. A higher ratio corresponds to a greater selectivity. For this control sample the ratio was found to be approximately 0.68, indicating a slightly higher fluorescence on Au\{100\} than on Au\{111\} due to preferred, selective thiol exchange.
Figure 4.7: (Bottom) Average fluorescence of Au\{111\} (shown in red) and Au\{100\} (blue) facets of a gold bead electrode control experiment during two stepping potential scans. Solid lines indicate the first stepping potential scan and the dashed lines represent the second scan of the now bare electrode. The arrow indicates the direction of the scan. (Above) Select fluorescence images of the first stepping scan at (I) -0.4 V, (II) -0.8 V and (III) -1.4 V. Image (IV) is of the second stepping scan at 0 V. The false colour represents the fluorescence intensities, which have been shown on a log scale. The vertical lines demonstrate the calculation of peak height.
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

A gold bead electrode that was subjected to $E_{\text{treat}} = -0.75$ V is shown in Figure 4.8. Here, the maximum of the fluorescence peak of Au{100} appears at -1.2 V, far more negative than the same peak appears in the control sample shown in Figure A.1. This is inconsistent with the previous results. Examining the fluorescence image in Figure 4.8 II, it appears that the fluorescent HSC6-DNA-AF488 molecules desorbed from the adjacent Au{111} surface are drifting over the Au{100} facet as they dissipate into solution. This drift remains visible even in image III at a potential of -1.4 V. This distortion of the Au{100} fluorescence peak results in an inaccurate calculation of the Au{111}/Au{100} fluorescence intensity ratio. This problem of fluorophore drift, which proved impossible to control or predict, occurred for many of the samples examined, more of which are shown in the Appendix.

The results of the Au{111}/Au{100} fluorescence intensity ratios as calculated by the intensities from the analysis of the SAM desorption are presented in Figure 4.10, b, with the highest value of the ratio occurring for $E_{\text{treat}} = -0.8$ V. The error for all potentials more negative than -0.725 V, and in fact particularly for -0.8 V, is quite large. This error can most likely be attributed to the problems associated with the slow dissipation of desorbed HSC6-DNA-AF488, which has been described above. In order to validate these presented results, a second method of calculating the selectivity of the potential treatment for the desorption of MUA from the Au{111} facets that is not subject to drift-related problems is required.

4.2.3 An Alternative Method for Assessing the Selectivity of the Electrochemical Modification MUA SAMs

The method of calculating the Au{111}/Au{100} fluorescence intensity ratios presented above is subject to error associated with the drifting of desorbed HSC6-DNA-AF488 molecules across the electrode surface. It would therefore be useful if that same ratio could be calculated accurately without the need for desorbing the SAM. To that end, the fluorescence images obtained during the DNA flipping procedure, which was described in Section 3.4.7.1, were examined. Here, the potential was cycled within a limited potential range (0 to -0.4 V vs. SCE, see Figure 3.6), and so no chemisorbed DNA was removed from the surface. For our purposes, only the fluorescence intensities measured at an applied potential of -0.4 V were considered. At this
Figure 4.8: (Bottom) Average fluorescence of Au\{111\} (shown in red) and Au\{100\} (blue) facets of a gold bead electrode, $E_{\text{treat}}$ = -0.75 V, during two stepping potential scans. Solid lines indicate the first stepping potential scan and the dashed lines represent the second scan of the now bare electrode. The arrow indicates the direction of the scan. (Above) Select fluorescence images of the first the first stepping scan at (I) -0.4 V, (II) -0.9 V and (III) -1.4V. Image (IV) is of the second stepping scan at 0 V. The false colour represents the fluorescence intensities, which have been shown on a log scale.
potential, the Au-S bonds remain intact, but the fluorophore-metal distance is increased (and thus the fluorescence increased) due to the negative charge of the DNA backbone being repelled by the negative electrode surface. The fluorophore bound to the Au\{111\} and Au\{100\} facets should be equally effected by the potential application. For each electrolessly modified bead electrode, the \textit{DNA flipping procedure} resulted in three applications of -0.4 V, and so three measurements of the intensities and three calculations of the Au\{111\}/Au\{100\} ratio were performed. The fluorescence images were background corrected using the measured intensity for the bare gold electrode as described in the previous section.

Fluorescence images at an applied potential of -0.4 V of several electrolessly treated gold beads are presented in Figure 4.9. The fluorescence intensities of the Au\{111\} and Au\{100\} facets for the two control beads shown in panels a and b are approximately equal, and thus the calculated ratio is near unity. At $E_{\text{treat}} = -0.75\ \text{V}$, shown in Figure 4.9 c, the Au\{111\} surface has far more fluorophore present than the Au\{100\}, resulting in a much higher calculated fluorescence intensity ratio. The same is true for $E_{\text{treat}} = -0.8\ \text{V}$, shown in Figure 4.9 d. Finally, the bead shown in panel e, $E_{\text{treat}} = -0.85\ \text{V}$, shows that at very negative values of $E_{\text{treat}}$ more HSC6-DNA-AF488 begins to appear on Au\{100\}. This results in a calculated ratio that begins to trend back toward unity.

The fluorescence intensity ratios as calculated for the bound SAMs are presented in Figure 4.10, a. Comparing these results with the desorption ratio analysis shown in Figure 4.10, b, it is immediately clear that they follow the same trend, with the highest ratio corresponding to an $E_{\text{treat}}$ of -0.8 V. Because three values of the ratio of the bound SAM were obtained for every sample, a standard deviation could be calculated, which provides a more useful representation of the error associated with this analysis method. These results are presented in Figure 4.11.

Figure 4.11, a, shows the capacitance measured at the end of the 5 minute application of $E_{\text{treat}}$ which was first presented in Section 4.1.2. A comparison between the capacitance after 5 minutes at $E_{\text{treat}}$ and the Au\{111\} Au\{100\} fluorescence intensity ratio will show the relationship between the amount of total desorption (which is related to the capacitance) and the ratio. For all $E_{\text{treat}} \geq -0.7\ \text{V vs. Ag|AgCl}$ the Au\{111\}/Au\{100\} fluorescence intensity ratio remains near 1, which indicates that the fluorescence of the two facets are approximately equal. This indicates that these values of $E_{\text{treat}}$ were insufficiently negative to result in much desorption of
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

Figure 4.9: Select fluorescence images of various electrochemically treated MUA-coated gold bead electrodes following fluorophore-labelled DNA backfilling: a) a control sample, b) another control, c) -0.75 V, d) -0.8 V and e) -0.85 V. The \( E_{\text{treat}} \) are indicated and were measured vs. Ag|AgCl. The Au\{111\} and Au\{100\} facets have been identified. Each image was taken under an applied potential of -0.355 V vs. Ag|AgCl. The false colour represents the fluorescence intensities, which have been shown on a log scale in order to maximize contrast.
4.2. Fluorescence Characterization of The Selective Electrochemical Modification of a MUA SAM

Figure 4.10: Au{111}/Au{100} fluorescence intensity ratios as calculated from (a) the bound HSC6-DNA-AF488/MUA SAM and (b) the desorption of the SAM, which is shown on a logarithmic scale. The y-error bars show the highest and lowest ratios calculated at each $E_{treat}$, and the x-error bars show the highest and lowest OCP measured for the control experiments. For the controls, as well as all $E_{treat}$ from -0.725 V to -0.85 V, a minimum of three experiments were performed. The red curves show the trend of the data.
4.3 General Observations and Conclusions

MUA-coated crystalline gold bead electrodes were treated with a series of potentials sufficiently negative to reductively desorb some of the alkanethiolate SAM. This was done in order to identify the optimal $E_{\text{treat}}$ for the selective desorption of MUA from the Au{111} surfaces. The selectivity of this process was characterized by backfilling the desorbed regions with HSC6-DNA-AF488 so as to fluorescently label the modified parts of the SAM and then by comparing the fluorescence intensities of the Au{111} and Au{100} surfaces.

Two methods for calculating the Au{111}/Au{100} fluorescence intensity ratio of mixed MUA/HSC6-DNA-AF488 SAMs were presented. The first involved analysing the maximum fluorescence intensities observed on each facet as the mixed SAM was reductively desorbed. While this method resulted in large signals and thus high sensitivity, the slow dissipation of the desorbed fluorophores as well as their tendency to drift across the electrode surface as they dissolved into the electrolyte proved problematic and interfered with the accurate calculation the Au{111}/Au{100} ratio.

The second method presented examined the fluorescence intensities of the two facets of interest while the SAM remained bound to the surface, effectively eliminating the problem of fluorophore drift. While this method did yield far lower signal than the desorption approach, nevertheless the results corroborated those obtained using the first method, with a better accuracy for the calculated ratios.

As shown in Figure 4.10, the highest values of the Au{111}/Au{100} fluorescence intensity ratio were obtained for $E_{\text{treat}}$ = -0.8 V. This is the ideal treatment potential in order to obtain the maximum possible selectivity for the desorption of MUA from Au{111} on a crystalline gold
4.3. General Observations and Conclusions

Figure 4.11: a) Average final capacitances of MUA-coated gold bead electrodes following the 5 minute application of $E_{\text{treat}}$. The y-error bars show the highest and lowest measured values at each potential, and the x-error bars show the highest and lowest OCP measured for the control experiments. For the controls, as well as all $E_{\text{treat}}$ from -0.725 V to -0.85 V, a minimum of three experiments were performed. b) $\{111\}/\{100\}$ fluorescence intensity ratios as calculated for the bound SAMs. The y-error bars indicate the standard deviation at each $E_{\text{treat}}$, and the x-error bars show the highest and lowest OCP measured for the control experiments.
bead electrode. However, while this treatment potential results in the highest possible ratio, very good selectivity was also observed for slightly less negative potentials, between -0.75 V and -0.8 V. Any treatment potentials within that range will result in the selective modification of Au\{111\} with minimal disruption of the SAM on all other gold surfaces. This is important to consider when optimizing the electrochemical modification method for possible applications. If the goal is to modify Au\{111\} but having the highest possible density of the backfilled molecule is not required, then potentials nearer to -0.75 V will be sufficient.
Chapter 5

Selective Electroless Modification of Single Crystal Gold Bead Electrodes

5.1 Motivation

The development of methods for controlling the surface coverage as well as the surface distribution of mixed alkanethiolate SAMs is highly desired, as discussed in Section 2.2.2. In Chapter 4, it was demonstrated that the selective desorption of an alkanethiolate SAM from various atomic arrangements of a single-crystalline gold electrode is possible through the application of a controlled reductive potential. This results in the gold surface becoming uncovered on specific crystallographic surfaces, making it free for further modification. Conceptually, the implementation of this electrochemical reductive desorption process to alkanethiol-stabilized colloidal gold systems should be possible by controlling the reductive environment of the colloids. While electrochemical methods for the manipulation of gold AuNPs has been demonstrated, complete and reproducible modification of the AuNPs is not trivial. A solution chemistry approach utilizing an electroless reduction method would likely yield a far more homogeneous reducing environment and therefore more uniform AuNP modification.

The potential adopted by a metal electrode in a solution containing a redox couple will be defined by the Nernst equation (vs. some reference electrode) and the relative concentrations of the reduced and oxidized species. For our purposes, the reducing agent must be capable of obtaining reducing potentials less than -0.8 V vs. Ag|AgCl in order to reductively desorb the MUA SAM, based on the results in Chapter 4. Therefore a strong reducing agent is needed, but it must also be soluble in water without reacting violently, and must decompose completely into products that will not react on, nor adsorb strongly to, the uncovered gold surface. To our
5.1. Motivation

knowledge the only reducing agent which meets all of these criteria is borohydride (BH$_4^-$).

Borohydride has been successfully used to remove alkanethiolate SAMs from gold surfaces$^{39}$ as well as from gold nanoparticles$^{38}$. But despite its widespread use across many areas of research, the chemistry of BH$_4^-$ in aqueous solution is not well understood$^{101,102}$. In highly alkaline solutions (pH = 14) borohydride undergoes an 8 electron oxidation shown in Equation 5.1, but at somewhat lower pHs, the lifetime of borohydride is significantly shorter and the system is complicated by the competing, pH-dependent hydrolysis of the BH$_4^-$. This hydrolysis generates hydroxyborohydride (BH$_3$OH$^-$), which oxidizes at an even more negative potential than BH$_4^-$, as shown in Equations 5.2 and 5.3. A further complication arises from the generation of hydrogen gas, which can also be oxidized according to Equation 5.4.

\[ BH_4^- + 8OH^- \rightarrow B(OH)_4^- + 4H_2O + 8e^- \quad (E^0 = -1.24 \text{ V vs. SHE}) \]  

\[ BH_4^- + H_2O \rightarrow BH_3OH^- + H_2 \]  

\[ BH_3OH^- + 3OH^- \rightarrow B(OH)_4^- + \frac{3}{2}H_2 + 3e^- \quad (E^0 = \sim -1.7 \text{ V vs. SHE}) \]  

\[ \frac{1}{2}H_2 + OH^- \rightarrow H_2O + e^- \quad (E^0 = 0.83 \text{ V vs. SHE}) \quad pH = 14 \]  

As the pH of the solution approaches 7, the concentration of OH$^-$ is small and no longer controls the oxidation of BH$_4^-$, BH$_3$OH$^-$. These species will instead be oxidized according to Equations 5.5, 5.6 and 5.7, respectively, with no change to the expected $E^0$ values.

\[ BH_4^- + 4H_2O \rightarrow B(OH)_4^- + 8H^+ + 8e^- \]  

\[ BH_3OH^- + 3H_2O \rightarrow B(OH)_4^- + 3H^+ + \frac{3}{2}H_2 + 3e^- \]  

A further complication to this system is the possible presence of oxygen in solution, which can be reduced at a gold electrode according to Equation 5.8.
5.2. Borohydride Electrochemistry on Gold

\[ H_2 \rightarrow 2H^+ + 2e^- \quad (E^0 = 0\, V\, vs.\, SHE) \quad pH = 0 \quad (5.7) \]

\[ O_2 + 2H_2O + 4e^- \rightarrow 4OH^- (-0.401\, V\, vs.\, SHE) \quad (5.8) \]

Presumably, the relative solution concentrations of \( BH_4^- \), \( BH_3OH^- \), and their decomposition products should dictate the potential experienced by any electrode (or AuNP) immersed in the same solution. The presence of competing reactions, such as hydrogen oxidation or oxygen reduction, however, will result in an “applied” potential being equal to the mixed potential of all reactions occurring on the electrode surface, as described in Section 2.1.4. In order to minimize the effects of these competing reactions, all results presented in this section were performed in the absence of oxygen, unless otherwise stated. Due to the unpredictability of the relative rates of the above reactions, strict control over the concentrations of these species has proven to be extremely difficult. This in turn makes correlating the reduction potential to a particular initial concentration of \( BH_4^- \) virtually impossible. As such, rather than attempting to predict the potential, the potential resulting from various additions of borohydride solutions to the phosphate buffer (PB) electrolyte was simply measured using a MUA-coated crystalline gold bead electrode against a salt bridge separated reference electrode. This method provides the additional advantage of allowing us simultaneously measure the electrode’s capacitance. Furthermore, the MUA-coated gold bead electrode could be backfilled with a fluorophore-labelled molecule as was done for the electrochemical modification presented in Chapter 4. The surface reaction selectivity of the electroless MUA desorption can then be directly measured and then compared to that of the electrochemical MUA desorption.

5.2 Borohydride Electrochemistry on Gold

In order to test the suitability of borohydride as the reducing agent for the removal of alkanethiolate SAMs form gold, the lack of reactivity of its decomposition products must be assessed. To that end, a CV of a cleaned gold bead electrode was performed both with and without the addition of fully decomposed borohydride in solution. Figure 5.1 demonstrates that the pres-
5.2. Borohydride Electrochemistry on Gold

Figure 5.1: CV of a clean Au bead electrode in 2.5 mL of deoxygenated 10 mM PB, pH = 8, with and without 0.1 mM fully decomposed NaBH₄. For both CVs the scan rate is 0.1 V s⁻¹.

ence of borohydride’s decomposition products did not alter the gold CV in any significant way, confirming that B(OH)₄⁻ shows no redox activity on gold.

Figure 5.2 shows a similar CV of a bare gold electrode following the addition of 10 μL of a 0.2 M solution of sodium borohydride in 0.1 M KOH (prepared in a strongly basic solution to slow the hydrolysis reaction). Once added to the pH 8 buffered electrolyte (at E = 0 V vs. Ag│AgCl), the hydrolysis and the oxidation of BH₄⁻ begin quickly, resulting in a significant increase in the measured current. On the positive-going scan, a much larger amount of current is generated near -0.7 V as borohydride is oxidized. A lot of hydrogen gas bubbles are also generated, which results in an increase in the measured noise.

As described previously, due to borohydride’s complex chemistry, accurate predictions of the potentials measured following small additions to the electrolyte are difficult. An estimation of the initial potential measured immediately following the BH₄⁻ addition can be calculated for the reaction shown in Equation 5.5 using the Nernst Equation. For a solution at pH 8 initially containing 0.1 mM BH₄⁻ and one thousand times lessB(OH)₄⁻, the calculated potential is ≈ -0.740 V vs. SHE, or ≈ -0.540 V vs. Ag│AgCl.

\[
\text{BH}_4^- + 4H_2O \rightarrow B(OH)_4^- + 8H^+ + 8e^-
\]

It is important to note, however, that this estimate does not consider the hydrolysis of borohydride, the possible oxidation of hydrogen, nor the possible reduction of oxygen. In light of
5.3. The Electroless Approach for the Reductive Desorption of a MUA SAM

A MUA-coated gold bead electrode immersed in PB was treated with small additions of a basic solution of borohydride to characterize its capability for reductively removing an alkanethiolate SAM from a gold surface. As described in Section 3.4.4, the open circuit potential ($E_{OCP}$) of the electrode was measured to monitor the potential and then subsequently applied along with a 5 mV rms potential sine wave to determine the capacitance of the interface. Figure 5.3 a, shows the measured $E_{OCP}$ of a MUA-coated gold bead electrode during one such electroless potential treatment experiment. Following each addition of $\text{BH}_4^-$, $E_{OCP}$ drops significantly and then be-

Figure 5.2: CV of an Au bead electrode in 2.5 mL of deoxygenated 10 mM PB, pH = 8. The scan rate is 0.1 V s$^{-1}$. The start of the scan is designated by (†) and the arrow indicated the scan direction. At the point designated by (*), 10 μL of a 0.2 M solution of sodium borohydride in 0.1 M KOH was added to the electrolyte. A zoomed in portion of the CV is shown above.

these inaccuracies as well as our inability to predict the relative concentrations of any species at any given time, the estimated reduction potential, though unreliable, it is entirely possible that borohydride will provide sufficiently negative potentials. To ensure accuracy, however, the potential will need to be measured.

5.3 The Electroless Approach for the Reductive Desorption of a MUA SAM
5.3. The Electroless Approach for the Reductive Desorption of a MUA SAM

Figure 5.3: a) Measured E\textsubscript{OCP} of a MUA-coated gold bead electrode immersed in 3 mL of 10 mM PB, pH 8, during several 1 \( \mu \)L additions of (\*) 0.03 M and (†) 0.3 M NaBH\textsubscript{4} in 0.1 M KOH. b) The resulting capacitance of the same gold bead electrode.

gins to level off or even increase. This increase is likely a result of the complete decomposition of the borohydride which generated hydrogen gas that is oxidized at a more positive potential. In general, as more BH\textsubscript{4}\textsuperscript{-} is added into the solution, E\textsubscript{OCP} decreases accordingly. The magnitude of the changes in E\textsubscript{OCP}, however, seem to diminish upon subsequent additions of BH\textsubscript{4}\textsuperscript{-}, likely due to the increase in the concentration of borohydride’s decomposition products in the solution. In order to achieve more negative values E\textsubscript{OCP}, higher concentrations of NaBH\textsubscript{4} were required.

The OCP measured between about -0.6 V and -0.8 V in Figure 5.3 a, exhibits a relatively steady decrease despite no further additions of BH\textsubscript{4}\textsuperscript{-}. This is possibly caused by the increase in the concentration of BH\textsubscript{3}OH\textsuperscript{-} as a result of the hydrolysis of BH\textsubscript{4}\textsuperscript{-}, which is known to be oxidized at an even more negative potential than borohydride\textsuperscript{101}. The capacitance is also increasing approximately three-fold within this same range, however, and so the decrease in OCP could equally be a result of additional uncovered gold causing an increase in the rate of borohydride decomposition at the gold surface.

Figure 5.3 b, shows the measured capacitance of the same MUA-coated bead electrode during the additions of BH\textsubscript{4}\textsuperscript{-}. Up to an E\textsubscript{OCP} of approximately -0.65 V, the capacitance remains
5.4 The Partial Electroless Reductive Desorption of a MUA SAM

Controlling the $E_{OCP}$ is challenging given the difficulty in controlling the behaviour of $BH_4^-$, but there are two tools for achieving control at our disposal. The first is using small additions of borohydride. As seen in Figure 5.3, following the initial decrease, once $BH_4^-$ has decomposed,
E\textsubscript{OCP} begins to become more positive. More negative potentials, when needed, can simply be accessed via the addition of more BH\textsubscript{4}\textsuperscript{-}. In some cases, however, E\textsubscript{OCP} can decrease without the need for additional borohydride, as was observed in Figure 5.3. In order to prevent E\textsubscript{OCP} from becoming more negative than desired, the rate of BH\textsubscript{4}\textsuperscript{-} and BH\textsubscript{3}OH\textsuperscript{-} hydrolysis can be increased by the addition of H\textsuperscript{+} into solution\textsuperscript{38}, resulting in an increase in E\textsubscript{OCP}. In this section, three electroless potential treatment experiments, with desorption potentials of -0.507 V, -0.742 V and -0.866 V vs. Ag|AgCl, will be discussed\textsuperscript{1}.

An experiment with a MUA-coated gold bead electrode which experience an electroless desorption potential of -0.507 V is shown in Figure 5.5. Here, a single addition of borohydride resulted in the decrease in E\textsubscript{OCP} to somewhere near -0.55 V, followed by a small, gradual increase to approximately -0.51 V. This potential was then maintained by several smaller additions of borohydride, which prevented E\textsubscript{OCP} from increasing further as BH\textsubscript{4}\textsuperscript{-} decomposed. The resulting capacitance, shown in 5.5 b, remains constant at about 1.9 \(\mu\)F cm\textsuperscript{-2} throughout the experiment, as is expected (based on observation from the electrochemical measurements in the previous chapter) for all potentials more positive than approximately -0.7 V vs. Ag|AgCl. It is expected that this MUA SAM is largely undisturbed.

Figure 5.6 shows E\textsubscript{OCP} for a MUA-coated gold bead electrode following a large addition of borohydride. Following the initial drop, E\textsubscript{OCP} continued to decrease gradually, likely as BH\textsubscript{3}OH\textsuperscript{-} was generated via the hydrolysis reaction shown in Equation 5.2. At E\textsubscript{OCP} = \(-0.8\) V, the potential began to decrease more sharply, reaching approximately -1 V before additions of acid decomposed enough of the BH\textsubscript{4}\textsuperscript{-} / BH\textsubscript{3}OH\textsuperscript{-} to result in an increase in E\textsubscript{OCP}. The capacitance measured throughout this experiment demonstrates that a very sharp increase to \(~7\) \(\mu\)F cm\textsuperscript{-2} occurred between the potentials of -0.8 V and -1 V, indicating a significant disruption of the MUA SAM. This sudden change in both the E\textsubscript{OCP} and the capacitance is the result of significant desorption of the MUA SAM. As discussed previously, alkanethiolate reductive desorption begins first from defective regions within the SAM and then from pin-hole defects which are introduced into well-ordered portions of the monolayer. It is likely that at \(~-0.8\) V, enough MUA has been desorbed to allow more reducing agent to penetrate the layer, thereby significantly

\textsuperscript{1}Because of the variable nature of the measured E\textsubscript{OCP}, defining the desorption potential of each electroless experiment can be difficult. In general, larger changes in capacitance occur at more negative potential, and so an average value of the most negative measure E\textsubscript{OCP} for each bead experiment was used.
5.4. The Partial Electroless Reductive Desorption of a MUA SAM

Figure 5.5: a) Measured \( E_{\text{OCP}} \) of a MUA-coated gold bead electrode immersed in 3 mL of 10 mM PB, pH 8, during several 1 \( \mu \)L additions of (*) 0.03 M and (†) 0.3 M NaBH\(_4\) in 0.1 M KOH. The average desorption potential (indicated) achieved was -0.507 V vs. Ag|AgCl. b) The resulting capacitance of the same gold bead electrode.

increasing the rate of subsequent desorption.

We predict, based on the electrochemical results presented in the previous chapter, that the maximum disruption of the MUA SAM on the Au\{111\} surface without removal of MUA from other surfaces should occur near \( E_{\text{OCP}} \) values of -0.75 V to -0.8 V vs. Ag|AgCl. Given the sharp, unprompted decrease in \( E_{\text{OCP}} \) near -0.8 V discussed above, great care must be taken in order to prevent it by the addition of acid if the desired desorption potential is to be maintained. Figure 5.7 shows one electroless potential treatment experiment in which \( E_{\text{OCP}} \) was maintained near -0.75 V by alternately adding borohydride and acid. The resulting capacitance of approximately 5.5 \( \mu \)F cm\(^{-2}\) is very similar to the capacitances measured for electrochemically modified bead electrodes which showed good selectivity for Au\{111\}.

The capacitance results for the three experiments discussed above are summarized in Figure 5.8. As expected, in general, the more negative the \( E_{\text{OCP}} \), the more the measured capacitance increase. The most notable result presented here is the final measured capacitance of the -0.742 V bead, which reaches a value of just below 6 \( \mu \)F cm\(^{-2}\). This is approximately equivalent to the capacitance measured for the electrochemical potential of -0.8 V, which was presented in Figure 5.4, which means that this bead is expected to have had MUA removed.
5.4. The Partial Electroless Reductive Desorption of a MUA SAM

Figure 5.6: a) Measured $E_{OCP}$ of a MUA-coated gold bead electrode immersed in 3 mL of 10 mM PB, pH 8, during several 1 μL additions of (†) 3 M NaBH₄ in 0.1 M KOH. The (X) indicate the addition of 1 μL of a 0.1 M nitric acid solution. The average desorption potential (indicated) achieved was -0.866 V vs. Ag|AgCl. b) The resulting capacitance of the same gold bead electrode.

Figure 5.7: a) Measured $E_{OCP}$ of a MUA-coated gold bead electrode immersed in 3 mL of 10 mM PB, pH 8, during several 1 μL additions of (⋆) 0.03 M and (†) 0.3 M NaBH₄ in 0.1 M KOH. The (X) indicate the addition of 1 μL of a 0.1 M nitric acid solution. The average desorption potential (indicated) achieved was -0.742 V vs. Ag|AgCl. b) The resulting capacitance of the same gold bead electrode.
5.4. The Partial Electroless Reductive Desorption of a MUA SAM

selectively from Au\{111\}.

Figure 5.8: Potential-capacitance profiles measured for electroless potential treatment experiments with average desorption potentials of -0.507 V, -0.742 V and -0.866 V vs. Ag|AgCl.

![Graph showing potential-capacitance profiles for electroless potential treatment with desorption potentials of -0.507 V, -0.742 V, and -0.866 V vs. Ag|AgCl.](image)
5.5 Fluorescence Characterization of The Selective Electroless Modification of a MUA SAM

Following the procedure that was used for the electrochemical approach to the partial desorbed MUA SAMs, the uncovered gold was fluorescently labelled by backfilling with HSC6-DNA-AF488, as described in Section 3.4.6. Once labelled, the gold bead electrodes were imaged using fluorescence microscopy in order to identify the surfaces with the highest degree of HSC6-DNA-AF488 substitution, which well correspond to the most electrolessly modified surfaces.

All the experiments presented in this section were performed in the spectroelectrochemical cell, described in Section 3.4.7. The results presented in this section have been converted from E vs. SCE to E vs. Ag|AgCl for the reasons that were discussed in Section 4.2.

5.5.1 Examining the Effect of the Electroless Potential Treatment on the SAM Stability

Figure 5.9 shows the capacitance measured in the spectroelectrochemical cell at an applied potential of 0 V vs. Ag|AgCl ($C_{0V}$) of the mixed MUA/HSC6-DNA-AF488 SAMs following fluorophore-labelled DNA backfilling. Because capacitance is an indicator of the coverage of the electrode, $C_{0V}$ can provide insight to the status of the SAMs following the electroless potential treatment and backfilling. For the gold bead electrodes that experienced $E_{OCP}$ more positive than $\sim$-0.75 V the $C_{0V}$ remains within the range of values obtained for the control experiments (designated by the grey box). $C_{0V}$ for electrodes that experienced $E_{OCP}$ more negative than -0.8 V, $C_{0V}$ increases, which could be caused by several factors. First, this increase in $C_{0V}$ could imply that despite the DNA backfilling, defective regions still exist within the SAM. Second, because of the larger footprint of a thiolated DNA as compared to MUA (see Section 2.2.3.1), a DNA SAM is expected to have a higher capacitance than a MUA SAM.

The mixed MUA/HSC6-DNA-AF488 SAMs were desorbed from the electrode surface via a stepping potential scan from 0 to -1.4 V vs. Ag|AgCl in -20 mV steps, each lasting for 4 seconds. It is expected that the capacitance profile for the desorption of a well-ordered MUA/HSC6-
5.5. Fluorescence Characterization of The Selective Electroless Modification of a MUA SAM

Figure 5.9: The average measured capacitance at 0 V vs. Ag|AgCl of the mixed MUA/HSC6-DNA-AF488 SAMs on gold bead electrodes following the electroless potential treatment and DNA backfilling. The x-errorbars show the range of $E_{OCP}$ once a minimum was reached. The grey region between the dashed lines represents the range of $C_{0V}$ values measured for the electrochemical control sample MUA SAMs after exposure to HSC6-DNA-AF488.

DNA-AF488 monolayer should closely resemble that of the linear scanning MUA desorption, as discussed in Section 4.2.1. Figure 5.10 shows the measured capacitances of four mixed MUA/HSC6-DNA-AF488 SAMs compared to the linear scan MUA SAM capacitance. Figure 5.10 a) shows the measured capacitance during the desorption of a control bead (no borohydride) SAM that appears similar to the desorption profile of the MUA SAM, indicating that very little of the monolayer was desorbed during the potential treatment process. Figure 5.10 b) shows a MUA-coated bead that was exposed to an electroless potential treatment with an average minimum $E_{OCP}$ of -0.619 V vs. Ag|AgCl. Given that no significant capacitance changes were observed during any electroless potential treatment for potentials more positive than -0.7 V (see Figure 5.8), no significant desorption of the original MUA SAM was expected. This prediction was confirmed by the stepping scan of this mixed SAM which closely resembles that of the MUA SAM desorption.

The mixed SAMs shown in Figure 5.10 c) and d) (-0.742 V and -0.866 V), however, display significant differences with respect to the MUA linear scan. Both of these samples have a higher initial capacitance than the capacitance measured for a well-ordered MUA SAM. This suggests
5.5. Fluorescence Characterization of The Selective Electroless Modification of a MUA SAM

Figure 5.10: Capacitance of MUA-coated gold bead electrodes that have been backfilled with HSC6-DNA-AF488 following the electroless potential treatment: a) a control sample, b) -619 mV, c) -742 mV, d) -866 mV and e) -1.075 V. The measurements were performed during a stepping potential scan from approximately 0 to -1.4 V vs. Ag|AgCl with 20 mV, 8 second steps. The capacitance of a MUA-coated gold bead electrode during a 5 mV/second linear potential scan from 0 to -1.4 V vs. Ag|AgCl is shown in black. The arrows indicate the scan direction.
that some of the MUA has been reductively desorbed and replaced with the thiolated DNA. Additionally, the increase in capacitance for the samples shown in panels c) and d), which indicates the desorption of the mixed MUA/HSC6-DNA-AF488 SAM, begins at less negative potentials than for a MUA SAM, nearer to -0.5 V and -0.4 V, respectively. The capacitance then increases gradually until approximately -0.85 V where the capacitance plateaus and matches the MUA linear scan once again. The early desorption demonstrates that at these electroless treatment potentials the original MUA SAMs were much more significantly desorbed and that despite DNA backfilling the SAM still contains defective regions.

Finally, the borohydride desorption of the mixed MUA/HSC6-DNA-AF488 SAM from an electrode which experienced an $E_{\text{OCP}}$ of -1.075 V, shown in Figure 5.10 e), has been significantly altered. The very high initial capacitance and the increase in capacitance at ~-0.4 V both point to a layer that has a larger amount of removed MUA and that likely has a high density of defects. However, the capacitance alone is unable to indicate on which crystallographic surfaces those defects are present.

### 5.5.2 Assessing the Selectivity of the Electroless Modification of MUA SAMs

The selectivity of the electroless partial desorption of the MUA SAM was characterized by back-filling uncovered gold adsorption sites with HSC6-DNA-AF488 and comparing the respective fluorescence intensities of the Au{111} and Au{100} facets. As discussed in the previous chapter, because the Au{111} surface of alkanethiolate SAMs desorbs at the least negative potential, followed closely by the Au{100} surface, this ratio should provide a good measure of the selectivity of the desorption process for Au{111}.

The ideal method for measuring the fluorescence intensities on each facet is to do so while the mixed SAM is being desorbed from the electrode surface. As the fluorophore desorbs, the intensity increases as a result of the increasing fluorophore-metal distance. However, the desorbed molecules tend to drift across the bead surface, obscuring the intensities of adjacent facets. This problem, which was also observed for the electrochemical mixed SAMs (see Section 4.2.2), means that calculating the ratio of the Au{111}/Au{100} intensities using the maximum intensity measured during the desorption of the layer is not accurate. To circumvent
5.5. Fluorescence Characterization of The Selective Electroless Modification of a MUA SAM

this problem, the fluorescence intensity ratio will instead be calculated while the layer is still bound to the surface, as described in Section 4.2.3. Briefly, the fluorescence intensities of the Au{111} and Au{100} facets were measured three times for each bead at an applied potential of -0.4 V vs. Ag|AgCl.

Fluorescence images of various electrolessly modified gold bead electrodes are shown in Figure 5.11. Panel a) shows a control experiment with an $E_{OCP}$ of -0.075 V. This sample was not exposed to borohydride and therefore none of the original MUA SAM was reductively desorbed. As such, the only HSC6-DNA-AF488 present on the surface was due to place-exchange reactions with MUA. The brightest region of the bead is the hexagonal region around the Au{111} facet, which was identified in the previous chapter as probably being an Au{111}-like region, interspersed with a relatively high density of step edges. The Au{111} and Au{100} facets themselves show approximately equal fluorescence intensities. Similarly, the bead electrode with $E_{OCP}$ = -0.619 V shown in Figure 5.11 b) shows relatively constant fluorescence across the entire bead surface. This demonstrates that at this potential the MUA SAM remained unaffected, as expected from the MUA SAM desorption shown in Figure 5.4 and 5.10. For both of these beads, an Au{111}/Au{100} fluorescence intensity ratio of approximately 1 is expected.

The electrode shown in Figure 5.11 c) experienced an $E_{OCP}$ of -0.742 V, and exhibits much higher fluorescence on the Au{111} facet than on the Au{100} facet. Here, the entire Au{111} facet as well as the hexagonal region around the facet are very bright, indicating a large amount of the original MUA SAM in this region was desorbed. The Au{100} facet remains comparatively dim. This results in a calculated Au{111}/Au{100} fluorescence intensity ratio greater than 1.

For beads which experienced an electroless $E_{OCP}$ more negative than -0.8 V, such as the one shown Figure 5.11 d), the MUA on the Au{100} facet was also partially desorbed from the surface, leading to fluorescence intensities similar to those on Au{111}. For this bead, an Au{111}/Au{100} fluorescence intensity ratio near 1 is expected. A similar result was obtained for a bead which was exposed to a very negative $E_{OCP}$, as shown in Figure 5.11 e). This bead electrode, however, seems to have a significantly different appearance than the other electrodes examined, with large, sharply defined patches of bright fluorescence. The overall chaotic appearance of this particular mixed SAM may have been cause by the extremely negative $E_{OCP}$ reached. At ~-1 V, as demonstrated by the MUA SAM desorption profile shown.
5.5. Fluorescence Characterization of The Selective Electroless Modification of a MUA SAM

Figure 5.11: Select fluorescence images of various electrolessly treated MUA-coated gold bead electrodes following fluorophore-labelled DNA backfilling: a) a control sample, b) -619 mV, c) -742 mV, d) -866 mV and e) -1.075 V. The $E_{\text{OCP}}$ of the electroless treatment are indicated and were measured vs. Ag|AgCl. The Au{111} and Au{100} facets have been identified. Each image was taken under an applied potential of -0.355 V vs. Ag|AgCl. The false colour represents the fluorescence intensities, which have been shown on a log scale in order to maximize contrast.
5.5. Fluorescence Characterization of The Selective Electroless Modification of a MUA SAM

Figure 5.12: Au\{111\}/Au\{100\} fluorescence intensity ratios as calculated from the bound mixed MUA/HSC6-DNA-AF488 SAMs for the electrochemically and electrolessly treated gold bead electrodes. The y-error bars indicate the standard deviation of each ratio, and the x-error bars show the range of E\textsubscript{OCP} measured once a minimum was obtained.

In Figure 5.4, the original MUA SAM was almost completely desorbed from the surface. The subsequent exposure to the HSC6-DNA-AF488 lasted for only 10 minutes, which may have not been long enough to form any regions of well-ordered monolayer if very large areas of the Au was uncovered.

The Au\{111\}/Au\{100\} fluorescence intensity ratios for the electrolessly treated MUA SAMs are presented in Figure 5.12 along with the results obtained for the electrochemically treated electrodes. For all electrolessly treated beads whose measured E\textsubscript{OCP} remained more positive than -0.7 V, a ratio of approximately 1 was observed due to the minimal desorption of the MUA SAM. The largest ratio obtained was for the electroless treatment potential of -0.742 V vs. Ag|AgCl, indicating a high degree of selectivity for the Au\{111\} surface. This ratio is similar to that obtained for the electrochemical process at E\textsubscript{treat} = -0.75 V. For electrolessly treated electrodes with E\textsubscript{OCP} values more negative than -0.8 V, Au\{111\}/Au\{100\} ratios of ~1 were again measured. These unity ratios are a result of significant desorption of the original MUA SAM across many surface crystallographies leading to similar densities of HSC6-DNA-AF488 on both facets of interest.

The lack of bead electrodes that experienced potentials near -0.8 V for the electroless po-
tential treatment does not allow for comparison with the ideal electrochemical result, since this potential was practically difficult to attain using electroless control of the electrode potential, as discussed previously. This phenomenon is most easily demonstrated in Figure 5.6, which shows that once \( E_{OCP} = -0.8 \) V is reached, the potential begins to drop quite dramatically even without the introduction of additional reducing agent. In fact, this drop in \( E_{OCP} \) was observed in spite of the addition of acid, which should decompose the remaining reducing agent, resulting in an \( E_{OCP} \) increase. The quickly changing capacitance at this potential is probably a result of the significant desorption that is occurring on the Au\{111\} facet.

### 5.6 Examining the Effect of Oxygen on the Electroless Modification Process

The electroless modification of the gold bead electrodes serves as a model system for the electroless modification of AuNRs. Ideally, when applying this methodology to the AuNRs, a less complex method would be attractive. As such, the electroless partial desorption of MUA SAMs was attempted in the presence of oxygen, which greatly simplifies the required apparatus and experimental rigour.

Figure 5.13 shows the capacitance of two bare gold bead electrodes as \( E_{OCP} \) was controlled via the addition of borohydride to the electrolyte, each in either the presence or the absence of oxygen. It is immediately apparent that the capacitance begins to increase (and MUA is desorbed from the surface) at much more positive values of \( E_{OCP} \) when oxygen is present. The fluorescence images shown in the bottom panels of Figure 5.13 further illustrate this discrepancy between the degree of surface modification observed for systems with and without oxygen at approximately the same \( E_{OCP} \) after BH\(_4^-\) addition. Without oxygen, shown on the right, very little of the original MUA SAM was desorbed and the fluorescence intensities of the Au\{111\} and Au\{100\} facets are approximately equal. However, with oxygen present (left), the Au\{111\} surfaces were significantly modified as compared to the Au\{100\} facets. The degree of modification of the Au bead in the presence of oxygen is characteristic of an electrode that experienced a more negative \( E_{OCP} \) in the absence of oxygen. In fact, the bead shown in the
5.6. Examining the Effect of Oxygen on the Electroless Modification Process

Figure 5.13: (Top) Measured $E_{\text{OCP}}$ of two MUA-coated gold bead electrodes immersed in 3 mL of 10 mM PB, pH 8, during several additions of a NaBH$_4$ solution in 0.1 M KOH, both in the presence and in the absence of oxygen. The arrow indicates the scan direction. (Bottom) Fluorescence images of MUA-coated gold bead electrodes following electroless potential treatment (both with and without oxygen) and HSC6-DNA-AF488 backfilling. The $E_{\text{OCP}}$ measured during the potential treatment are approximately equal to the potential designated on the capacitance profile by the dotted line. The images were taken at an applied potential of -0.4 V vs. Ag|AgCl.

The lower left of Figure 5.13 ($E_{\text{OCP}} = -0.285$ V) appears very similar to that shown in Figure 5.11 with an $E_{\text{OCP}}$ of -0.742 V.

To explore the origin of the $O_2$ effect, a direct comparison of the same borohydride-containing solution both with and without oxygen is needed. Figure 5.14 shows the $E_{\text{OCP}}$ of a MUA-coated gold bead electrode following several additions of borohydride in the absence of oxygen, which closely resemble the results discussed in Section 5.4. Once a quite negative $E_{\text{OCP}}$ was reached (~-1.2 V), oxygen was allowed to enter the cell. This resulted in a rapid increase in $E_{\text{OCP}}$. When the solution was again bubbled with argon to remove oxygen, the measured $E_{\text{OCP}}$ decreased to approximately the same value as before the oxygen was introduced. This indicates that the
5.6. Examining the Effect of Oxygen on the Electroless Modification Process

![Graph showing potential vs. time](image)

Figure 5.14: Measured E\text{OCP} of a MUA-coated gold bead electrode immersed in 3 mL of 10 mM deoxygenated PB, pH 8, during several 1 μL additions of 3 M borohydride in 0.1 M KOH, designated by (*). At the time marked (A), the argon flow was stopped and air was allowed to penetrate the electrochemical cell. At time (B), the data collection was paused for 15 minutes while the solution was bubbled with argon.

Borohydride did not significantly decompose in the presence of oxygen, meaning BH\text{−}4 and O\text{2} do not react homogeneously with one another. Therefore the increase in E\text{OCP} is not a result of a decrease in [BH\text{−}4] but instead suggests that E\text{OCP} cannot be accurately measured in the presence of oxygen. This is likely as a result of a mixed potential that exists when both borohydride oxidation and oxygen reduction reactions are taking place on the electrode.

Using the Butler-Volmer relationship between current and potential, expression for the mixed potential, E\text{mix}, was found as described in Section 2.1.4 and is presented in Equation 5.9.\textsuperscript{14}

\[
\exp\left[\frac{E_{\text{mix}} F}{RT}\right] = \left(\frac{A_{O_2}/I_{O_2}}{A_{BH_4}/I_{BH_4}}\right) \exp\left[\frac{F}{RT} \cdot \frac{(E_{eq}^{BH_4} + E_{eq}^{O_2})}{2}\right]
\]

\[
E_{\text{mix}} = \frac{\ln\left[\frac{A_{O_2}/I_{O_2}}{A_{BH_4}/I_{BH_4}}\right]}{F/RT} + \frac{(E_{eq}^{BH_4} + E_{eq}^{O_2})}{2}
\]  

(5.9)

Again, for simplicity, it was assumed that in this system \(\alpha = 0.5\). In this case, E\text{mix} is found to be proportional to the average of the equilibrium potentials of both the borohydride oxidation and the oxygen reduction reactions. This estimate is complicated, however, by the area on which these reactions are occurring. As the MUA SAM desorbs, the areas available for these two reactions are changing, and E\text{mix} may change as well. Furthermore, the calculation of

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5.7 Testing the Electroless Desorption of Alkanethiol from GNRs

To determine whether the electroless desorption process using sodium borohydride is transferable to GNRs, a solution of GNRs was prepared. 23 μL of as-shipped GNRs (see Section 3.1.5) was diluted into 2 mL of a 10% PBS solution containing 1 mM CTAB. It was found that the GNRs were unstable in any solution containing less than 1 mM CTAB, and so a method

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**Figure 5.15:** Graphical representation of the mixed potential measured between borohydride oxidation and oxygen reduction. Note that the mixed potential occurs between the equivalence potentials for the two reactions, at the potential where the current densities are equal and opposite.

\( E_{\text{mix}} \) shown in Equation 5.9 is a simplification which does not take into consideration things like the reduction of \( H_2 \), which could also be occurring on the same electrode. A graphical representation of \( E_{\text{mix}} \) is shown in Figure 5.15, which demonstrates that as the concentration of oxygen in solution increases, the measured mixed potential becomes more positive.

Because \( E_{\text{OCP}} \) cannot be measured correctly in the presence of oxygen, accurately characterizing the electroless modification of MUA-coated gold bead electrodes is impossible, and evaluating the process in the presence of oxygen toward the modification of AuNRs impractical.
for determining whether CTAB interferes with the electroless desorption process, or with the
HSC6-DNA-AF488 backfilling process, was required. For this reason, a MUA-coated gold bead
electrode was immersed in the GNR solution. The electrode was also used in order to monitor
the $E_{OCP}$ of the solution.

To this solution, several aliquots of a 0.3 M solution of borohydride in 0.1 M KOH were added,
as indicated in Figure 5.16. The $E_{OCP}$, as monitored by the electrode, reached approximately
-0.9 V vs. Ag|AgCl. It is expected that an alkanethiol SAM exposed to such a negative potential
would experience significant desorption. Following the electroless potential treatment, the bead
was rinsed and then immersed in a 1 mM solution of HSC6-DNA-AF488 for 10 minutes, rinsed
and then stored in IB as described in Section 3.4.6. Similarly, HSC6-DNA-AF488 was added
to an aliquot of the GNR solution (to a final concentration of ~1 mM) in the hope of modifying
any uncovered gold on the GNRs. The GNR solution was allowed to incubate undisturbed for
~48 hours.

The resulting fluorescence image of the gold bead electrode is shown in the bottom panel
of Figure 5.16. It is immediately apparent that despite experiencing $E_{OCP} = \sim\sim0.9$ V, very little
of the fluorophore-labelled DNA was incorporated into the monolayer, indicated by the overall
dim fluorescence of the bead. This is almost certainly due to the presence of CTAB in the
electrolyte. Both the Br$^-$ anion and the CTA$^+$ cation are known to adsorb onto gold between 0
V and ~-0.6 V vs. SCE$^{56}$, and so it is likely that any reductively desorbed alkanethiol would
be immediately replaced by Br$^-$ or CTA$^+$ once the potential had increased as a result of the
decomposition of the borohydride. It is possible that the 10 minute exposure to the thiolated
DNA was simply insufficient to displace the adsorbed CTAB.

Interestingly, the aliquot of GNRs that were exposed to HSC6-DNA-AF488 did not remain
stable in solution and irreversibly aggregated during the ~48 hour incubation time. In contrast,
the GNR solution that was exposed to borohydride but was not exposed to DNA remained
well-suspended. Furthermore, a control solution of GNRs that did not undergo the electroless
potential treatment but that was exposed to DNA also remained well-suspended. This sug-
gests that the thiolated DNA may have successfully modified the GNRs following the potential
treatment, but that the resulting GNRs were no longer stable in the 10% PBS and 1 mM CTAB
solution. It is possible that this could occur due to the negatively charged backbone of the DNA,
5.7. Testing the Electroless Desorption of Alkanethiol from GNRs

Figure 5.16: (Top) Measured E_{OCP} of a MUA-coated gold bead electrode immersed in 2 mL of 10% PBS, pH 7.4, containing 1 mM CTAB as well as 23 μL of the as-shipped GNRs. The solution was bubbled with Argon for 30 minutes to remove oxygen. The symbol (†) indicates a 2 μL addition of 0.3 M NaBH₄ in 0.1 M KOH, (X) a 5 μL addition and the (⋆) symbol indicates an addition of 15 μL of 0.1 M nitric acid. (Bottom) Fluorescence image of the MUA-coated gold bead electrodes following the above electroless potential treatment and HSC6-DNA-AF488 backfilling. The image was taken at an applied potential of -0.4 V vs. Ag/AgCl.
which form complexes with the CTA$^+$ cations via electrostatic interactions that are known to be insoluble in water$^{103}$. Should enough DNA become attached to the GNRs as well as associate with the CTA$^+$ cations, it is feasible that this might render the GNRs insoluble in water. Unfortunately, the insoluble nature of the GNRs made further investigation, and confirmation of the modification, challenging.

5.8 General Observations and Conclusions

Sodium borohydride can be used to set the $E_{OCP}$ of MUA-coated gold bead electrodes, resulting in the reductive desorption of the SAM from the electrode surface. By manipulating $E_{OCP}$ via alternately introducing BH$_4^-$ and acid into the electrolyte, reasonably good control was obtained which enabled the selective modification of primarily the Au{111} surfaces. For $E_{OCP}$ = -0.742 V vs. Ag|AgCl an Au{111}/Au{100} fluorescence intensity ratio of ~8.5 was observed. For all other values of $E_{OCP}$ examined, both more positive and more negative, ratios near unity were observed.

The potential which, based on the electrochemical experiments, was expected to result in the optimally selective desorption of MUA, -0.8 V vs. Ag|AgCl, was difficult to precisely attain using BH$_4^-$. We hypothesize that this is a result of two phenomena. First, once the $E_{OCP}$ becomes more negative than ~-0.6 V, a gradual, uncontrolled decrease was observed, likely due to either the generation of BH$_3$OH$^-$ which oxidizes at a more negative potential than borohydride or to the exposure of more bare gold, which increases the rate of BH$_4^-$ oxidation. Second, at potentials very near -0.8 V a sharp decrease in $E_{OCP}$ and a sharp increase in the measure capacitance were both observed. It is possible that at this potential, enough of the MUA SAM desorbs from the electrode to allow for more reducing agent to penetrate the layer and approach the Au surface, increasing the rate of alkanethiolate desorption. Despite the challenge in reliably obtaining an $E_{OCP}$ of -0.8 V, ~-0.75 V was measured which showed excellent selectivity for specific Au{111} modification.

The electroless desorption of MUA from gold bead electrodes was also characterized in the presence of oxygen, where approximately the same fluorescence was observed at ~-0.6 V with oxygen than was observed at ~-0.8 V in the absence of oxygen. Furthermore, it was hypothe-
sized that in the presence of oxygen the occurrence of both borohydride oxidation and oxygen reduction reactions result in the measurement of a mixed potential which is not representative of the reducing capability of the solution.

Lastly, the electroless desorption of MUA from GNRs was attempted. It was found that following potential treatment and exposure to HSC6-DNA-AF488, the GNRs became insoluble in water. This was explained as the possible formation of insoluble complexes between adsorbed DNA and the CTA\(^+\). As such, while there is some evidence that the thiolated DNA was successfully introduced into GNR ligand shell, this could not be confirmed.

The selective modification of gold bead electrodes was successfully performed using a solution chemistry-based, electroless method which correlated well with the electrochemical results, demonstrating that the mechanisms are likely similar and that the potential set by BH\(_4^-\) is acting as the potentiostat. This process, which was performed on bead electrodes to serve as a model for alkanethiol-stabilized gold colloids, was attempted for the selective modification of AuNRs. Initial results for the electroless modification of GNRs are promising, though more work needs to be done to confirm that the modification did, in fact, occur.
Chapter 6

Conclusions

6.1 Summary

The electrochemical reductive desorption of a MUA SAM from a single crystal gold bead electrode was characterized using both electrochemistry and fluorescence microscopy techniques. MUA SAMs were partially reductively desorbed from the electrode surface via the application of various negative potentials. The measured increase in capacitance provided an estimate of the degree to which the SAM was desorbed, but was unable to indicate from which crystallographic surfaces the desorption took place. By backfilling exposed gold adsorption sites with a thiolated, fluorophore-labelled molecule, HSC6-DNA-AF488, the crystallographic surfaces of the electrode which experienced the most desorption could be identified using fluorescence microscopy.

The experiments showed that a 5 minute application of potentials between -0.75 V and -0.8 V vs. Ag|AgCl resulted in the selective desorption of MUA from primarily the Au{111} surfaces while leaving the remaining surfaces relatively unchanged. The selectivity of the modification for Au{111} was quantified by comparing the fluorescence intensities observed on that surface with those measured for Au{100}. Two methods for calculating the Au{111}/Au{100} fluorescence intensity ratio were presented wherein the average fluorescence intensities of the two facets were determined in one of two ways:

1. The maximum intensity from each facet was measured during the desorption of the mixed MUA/HSC6-DNA-AF488 SAM. This method resulted in large fluorescence signals, but was complicated by the movement of desorbed fluorophore across neighbouring facets, obscuring their intensities and possibly resulting in inaccurate ratios.

2. The intensities from the two facets were measured while the SAM remained bound to the
6.1. Summary

electrode surface, but with a potential of -0.4 V vs. SCE applied in order to repel the DNA from the surface, thereby increasing the fluorescence signal. While this method yielded much lower intensities, the problem of fluorophore drift across the electrode surface was effectively eliminated.

Both of the methods above identified the same potential treatment, -0.8 V vs. Ag|AgCl, as having the highest Au{111}/Au{100} fluorescence intensity ratio, indicating that it is the optimal electrochemical treatment potential resulting in the highest degree of selectivity for Au{111} over other surfaces.

A method for electrolessly desorbing the MUA SAMs from gold bead electrodes through the use of sodium borohydride to set the electrode potential was also characterized. It was found that adding BH$_4^-$ to the electrolyte would decrease the $E_{OCP}$ of the electrode, resulting in the reductive desorption of the SAM. In the absence of oxygen, the measured capacitance as a result of this desorption was found to closely resemble the capacitance profiles observed for the electrochemical desorption process (see Figure 5.4). The potential could be adequately controlled in order to partially desorb the MUA SAMs by alternately adding BH$_4^-$ and then acid to hydrolyze the borohydride, making the potential increase. Precisely attaining an $E_{OCP}$ of -0.8 V, however, proved to be difficult, likely due to the changing nature of the SAM near this potential. As the SAM begins to desorb, more reducing agent is able to penetrate the layer and reach the surface, increasing the rate of desorption. Nonetheless, an $E_{OCP}$ near -0.75 V was obtained, which, following HSC6-DNA-AF488 backfilling and the calculation of the fluorescence intensity ratio, was found to demonstrate excellent selectivity for Au{111}.

Next, the effectiveness of the electroless desorption process in the presence of oxygen was investigated. This was done because when applying the method to more complex systems, such as colloidal gold, a simple approach requiring less specialized equipment is ideal. It was found, however, that the $E_{OCP}$ was not a reliable variable since, in the presence of oxygen, the borohydride oxidation and the oxygen reduction reactions result in the measurement of a mixed potential which does not accurately represent the reducing power of the BH$_4^-$. Because of this, the surface-specific modification of the electrode as a response to the solution potential could not be characterized.
Finally, the electroless modification of GNRs was attempted. It was found that following potential treatment and a long (~48 hour) exposure to the thiolated DNA, the GNRs ceased to be soluble in aqueous solution. This could be explained by the DNA adsorbing to the GNR surface, which will then form insoluble complexes with the CTAB in solution due to electrostatic interactions. Enough of these complexes on the GNR surface could result in the aggregation of the GNRs. These initial experiments suggest that the electroless modification of alkanethiol-stabilized GNRs is possible.

6.2 Suggestions for Future Work

In order to develop and optimize electroless methods for the surface-specific modification of GNRs, a more complete investigation into the electroless modification process is needed. In particular, obtaining more data for values of $E_{\text{OCP}}$ near -0.8 V vs. Ag|AgCl would be useful for determining if the electroless and electrochemical data do in fact correlate in this potential region.

Further studies of the electroless modification of GNRs must be done in order to confirm the effectiveness of this method. In the work presented in this thesis there were several factors that complicated the GNR electroless modification process that must be characterized.

First, the role of CTAB in the electroless desorption of MUA and the adsorption of the thiolated DNA must be investigated. This could be done by using the single crystal gold bead electrode to systematically study the electroless desorption process in the presence of CTAB at various potentials. Preliminary results presented here indicate that when CTAB is present, far less fluorophore-labelled DNA was immobilized on the electrode surface following the electroless potential treatment and HSC6_DNA-AF488 backfilling. If CTAB impedes the adsorption of a thiol onto the uncovered gold, then its possible that by simply increasing the incubation time would solve this problem. Additionally, because of interactions between the negatively charged DNA backbone and the CTA$^+$ ions, it is possible that any successfully modified GNRs could become insoluble in aqueous media and so an appropriate solvent for these modified GNRs would be needed.

Perhaps a more straightforward method for mitigating the problems potentially caused by
6.2. Suggestions for Future Work

CTAB would be to remove it altogether. The GNRs used in this work were unable to be dissolved in solutions that did not contain CTAB. MUA-stabilized GNRs that are water-soluble without the need for CTAB have been prepared\textsuperscript{53,55}, though these reports use smaller rods than those studied here. It is possible that the electroless modification of GNRs would be more successful for smaller GNRs.

Finally, and perhaps most importantly, a method for determining the successful modification of GNRs with DNA is needed. In order to optimize the method for the surface-selective modification of GNRs, a procedure for determine the location of any adsorbed DNA on the GNR surface would be ideal. This could be accomplished by staining the DNA using uranyl acetate, which has been demonstrated as effective for TEM imaging of single DNA molecules.\textsuperscript{104} Alternatively, if two samples of GNRs were modified with complementary strands of thiolated DNA and then mixed together, the successful modification would result in the GNRs binding together. Similar approaches have been used in order to confirm the successful end-selective modification of GNRs, which results in the formation of GNR “chains” which are easily seen by TEM.\textsuperscript{7,52,64,65}
References


References


References


References


Appendix A

Additional Data

Presented here is an analysis of several electrochemically treated bead electrodes that follows the discussion found in Section 4.2.2.

A second control experiment is shown in Figure A.1. The initial mixed SAM appears very similar to that of the control experiment discussed in Section 4.2.2: overall low intensities with higher fluorescence on the hexagonal region around the Au{111} facet. The desorption of Au{111} near -0.8 V followed shortly by the desorption of Au{100} also appears very similar. At potentials more negative than about -1 V, however, this sample begins to behave differently than the one presented in Figure 4.7.

Figure A.1 IV shows the fluorescence intensity from the bare electrode. The intensity of the Au{111} facet measured during the stepping scan of the bare electrode shown in Figure A.1, bottom, is significantly higher than that of the Au{100} facet. This is likely a result of the desorbed HSC6-DNA-AF488 remaining in solution near the electrode surface. This 'cloud' of the desorbed HSC6-DNA-AF488 interferes with an accurate calculation of the ratio of Au{111} to Au{100} fluorescence intensities. Because of how the maximum intensity of each facet is calculated, this unexpectedly high bare bead intensity results in a value of the Au{111} fluorescence peak height that is comparatively smaller than that for Au{100}. Furthermore, examining the fluorescence intensity peak for Au{111} for the first stepping scan, the asymmetry of the peak suggests that after the HSC6-DNA-AF488 was desorbed from the Au{111} facet, it did not dissipate into solution evenly, but instead drifted slowly away, resulting in a broadening of the negative side of the fluorescence peak.

Figures A.2 and A.3 show the fluorescence results and selected fluorescence images for electrodes that experienced $E_{\text{treat}} = -0.8$ V and -0.85 V, respectively. Obvious signs of HSC6-DNA-AF488 drift from the Au{111} facets are observed. In both cases the fluorescence intensity
Appendix A. Additional Data

peak from the Au\{111\} facet appears broad and asymmetrical. This is due to the cloud of desorbed HSC6-DNA-AF488 which is quite apparent when examining image III of both figures. While it does not appear as though Au\{100\} is significantly effected by the slowly dissipating fluorophore, the accuracy of our measurement of the Au\{100\} fluorescence intensity is probably compromised.
Figure A.1: Average fluorescence of Au\{111\} (red) and Au\{100\} (blue) facets of a gold bead electrode control experiment during stepping potential scans of the SAM (solid lines) and of the same bare electrode (dashed lines). The arrow indicates the direction of the scan. Select fluorescence images (above) of the first the SAM desorption at (I) -0.4 V, (II) -0.8 V and (III) -1.4V. Image (IV) is of the bare electrode stepping scan at 0 V. The false colour represents the fluorescence intensities, which have been shown on a log scale.
Figure A.2: (Bottom) Average fluorescence of Au\{111\} (shown in red) and Au\{100\} (blue) facets of a gold bead electrode, $E_{\text{treat}} = -0.8$ V, during two stepping potential scans. Solid lines indicate the first stepping potential scan and the dashed lines represent the second scan of the now bare electrode. The arrow indicates the direction of the scan. (Above) Select fluorescence images of the first the first stepping scan at (I) -0.4 V, (II) -0.8 V and (III) -1.4V. Image (IV) is of the second stepping scan at 0 V. The false colour represents the fluorescence intensities, which have been shown on a log scale.
Figure A.3: (Bottom) Average fluorescence of Au\{111\} (shown in red) and Au\{100\} (blue) facets of a gold bead electrode, $E_{\text{treat}} = -0.85$ V, during two stepping potential scans. Solid lines indicate the first stepping potential scan and the dashed lines represent the second scan of the now bare electrode. The arrow indicates the direction of the scan. (Above) Select fluorescence images of the first the first stepping scan at (I) -0.4 V, (II) -1 V and (III) -1.4 V. Image (IV) is of the second stepping scan at 0 V. The false colour represents the fluorescence intensities, which have been shown on a log scale.