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Biosensitive Functionalised Silicon Surfaces: towards Biosensitive Field-Effect-Transistors

A thesis submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy

By
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February 2013

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I hereby certify that this submission is the product of my own work, except where otherwise acknowledge. I also declare that the work embodied in this thesis has not previously submitted for the award of a degree in any institution.

________________________

Guo Chuan

February 15, 2013
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Abstract

In the early diagnosis of certain diseases, the applications of protein analysis can be used to detect the presence of specific proteins in the plasma or serum. The traditional methods require fluorophores, active enzymes or magnetic beads as a label attached to the target proteins. When the target protein in the solution interacts with the complementary proteins immobilized on the surface, it changes the electrical properties of the surface, e.g. dielectric constant, resistance and capacitance. These changes can identify the presence of the target proteins. Ideally, no label is required for the assay to detect proteins.

Electrical impedance spectroscopy (EIS) is a method that characterizes the electrical properties of a system of interest and provides a non destructive method for studying its structure and function. In this study, EIS was used to determine the electrical properties and physical structure of the formation of monolayers of biological materials on silicon surfaces. The electrical properties and physical structures of the interaction of human antibodies with carboxylic acid groups attached to highly and low doped n-type silicon (111) are presented. The specific binding test of anti-human antibodies with human antibody modified silicon surfaces were analysed by EIS and confirmed by X-ray photoelectron spectroscopy (XPS).

Self-assembled monolayers (SAMs) were used to attach biomolecules to the silicon
surface. The results of SAMs on both n- and p-type silicon surfaces indicated that monolayers grafted on n-type silicon surfaces were more effective than those on p-type silicon. In other words, n-type silicon wafers offer advantages over p-type silicon as a substrate for a biosensor based on UV mediated attachment technology.

Plasma polymers were the second major topic in this research. They were used as alternative linkers to attach biomolecules to the silicon substrate. Compared to the self-assembled monolayers technology, plasma polymers provided higher protein coverage and required simpler preparative processes. EIS results revealed the changes in the electric properties after each step of the attachment process and binding of ligands. XPS measurements and wetting tests confirmed the EIS results. The results in this thesis show that EIS is a fast and reliable detection method and sensitive enough to detect small changes on the molecular monolayer level. Measurements can be performed directly without requiring any special treatment. The SAMs or plasma coated polymers immobilized directly on the silicon surfaces provides a platform for antibody immobilization in biosensor development that can be tailored to detect antigen-antibody binding.

The coupling of the biomolecules on low doped silicon surface induced a field effect inside the silicon. This field effect was dependent on the charge of the biomolecules. This would therefore allow the construction of an actual experimental BioFET that could detect the antigen-antibody interactions.
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Chapter 1

Introduction

1.1 Antigen-antibody interactions

The applications of diagnostic biosensors based on the interaction between an immobilized antibody (Ab) and an antigen (Ag), or the inverse, in a solution has expanded in recent years.\(^1\) An antigen is a substance from the extended environment or formed within the body, and usually has a molecular weight (MW) higher than 1.5 KDa. The immune system will try to respond to antigens by synthesizing antibodies that destroy or neutralize any antigen bearing biological entity recognized as a foreign and potentially harmful invader.

In placental mammals, immunoglobulin is another name for antibodies and can be divided into five different classes: IgA, IgD, IgE and IgM. All these antibodies produced by B-cells have a similar Y-shaped structure. The paratope in the tip of the “Y” of an antibody is specific to one particular epitope in an antigen, allowing these two proteins to bind together with precision.

The structure of an antibody is shown in Figure 1.1. It consists of four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds.\(^2\) All chains have a constant region and a variable region. One constant and one variable region of each of the heavy and light chains form the antigen-binding site, called antigen-binding fragment (F\(_{ab}\) fragment), and two
identical protein fragments from the constant region of the antibody’s two heavy chains form the fragment crystallizable region (F\textsubscript{c} region) where an antibody can interact with the cell surface receptor (F\textsubscript{c} receptor).

![Diagram of antibody structure](image)

**Figure 1.1:** Basic structure of an antibody.\textsuperscript{3}

Non-covalent interactions between an antibody and antigen are supported by non-polar hydrophobic interactions, Coulomb interaction, van der Waals interactions, London dispersion attractive forces and steric repulsion forces. The interaction is controlled by association and dissociation reaction rate constants, K\textsubscript{a} and K\textsubscript{b} respectively.

\[
\text{Ab} + \text{Ag} \overset{K_a}{\underset{K_d}{\rightleftharpoons}} \text{Ab:Ag}
\]

The association constant K\textsubscript{a}, which ranges from $10^{-5}$–$10^{11}$ M\textsuperscript{-1}, can be described by:

\[
K_a = \frac{K_a}{K_d} = \frac{[\text{Ab:Ag}]}{[\text{Ab}][\text{Ag}]}
\]

Where [Ab], [Ag] and [Ab: Ag] are the concentrations of antibody, antigen and complex in the solution, respectively.\textsuperscript{3}
A short overview of the applications of the biosensor methods in antibody-antigen interaction measurements is given in the next section with a focus on direct electrochemical detection of proteins without any labels.

1.2 Background of biosensors

A biosensor is a self-contained analytical device that is capable of quantitatively detecting chemical species at very low concentrations in a complex mixture. In the general model, a biosensor has three components: a sample recognition element (or selector), a signal transducer and a detector. The selector supports selectivity in the biosensor and is able to recognize the analyte (appointed biological species). The recognition element on binding the analyte generates a chemical signal. The function of the transducer is to convert these chemical changes into a physically measurable response that can be further processed by the detector, which creates an electrical output. A schematic diagram of a biosensor is shown in Figure 1.2.

![Figure 1.2: The scheme of a biosensor.](image)

In 1962, Clark et al.\(^5\) invented one of the earliest practical biosensors. It used glucose
oxidase as the recognition element and an amperometric oxygen electrode as the transducer to deduce the concentration of glucose. A glucose oxidase coated electrode can oxidize glucose into gluconic acid when the electrode contacts a solution containing glucose and oxygen; the reduction in oxygen partial pressure provides a measure of the glucose concentration. Since then, many approaches based on physics, chemistry and material sciences have been used in developing more sensitive, reliable and robust biosensors for applications in the fields of biotechnology, agriculture and medical diagnosis. The most commercially successful biosensor so far is for blood glucose sensing in diabetes patients. However, most potential applications in other fields are limited by technological difficulties due to substrate selection, biosensor contamination and storage, selectivity and sensitivity.

1.3 Field-effect transistors (FETs)

Electrical biosensors depend on a change in current and/or voltage to detect binding between an immobilized capture probe and a target or analyte in solution. Based on the methods of electrical measurements; electrical biosensors can be defined by voltammetric, amperometric/coulometric and impedance sensors. Compared to voltammetry and amperometry which apply DC or pseudo-DC at an electrode as a function of an applied electrode-solution voltage, impedance biosensors use an AC steady state, with a possible constant DC bias to measure the electrical impedance of an interface. The measurements are made by injecting a small sinusoidal voltage at a particular frequency and measuring the resulting current; the process can be repeated
at different frequencies. This approach is known as electrochemical impedance spectroscopy (EIS) and has been used to study a variety of electrochemical phenomena over a wide range of materials. Compared to other methods, impedance biosensors have the significant advantage of being able to measure impedance or capacitance change of the interface without the need for any special reagents. A general schematic drawing of a FET device is shown in Figure 1.3.

![General schematic drawing of a FET device](image)

**Figure 1.3**: General schematic drawing of a FET device. In the FET, current flows along a semiconductor path called channel. Two ohmic contacts at both end of the channel are the source and drain. The gate terminal controls the channel current.

Field-effect transistors (FETs) used as a switch controlling electrical power to a load. They are a closely related but separate class of biosensor that are controlled by the field-effect modulation of carriers in a semiconductor due to nearby charged particles, e.g., metal-oxide-semiconductor field-effect transistors (MOSFETs), ion-sensitive field-effect transistors (ISFETs), and biologically active field-effect transistors (BioFETs). These field-effect transistors depend on the interaction of external charges with carriers in a nearby semiconductor, leading to enhanced
sensitivity at low ionic strengths where counter ion shielding is reduced. The principle of operation of FETs is similar in semiconducting nanowires, electrolyte-insulator-semiconductor structures, and suspended gate thin film transistors. FETs can directly translate the analyte-surface interaction into a readable signal, without the requirements for sophisticated instrumentations. These devices utilize the electronic properties of the sensing element, such as its capacitance or conductance, to produce the signal output. However, it should be noted that measurements of the biological layer-insulator-semiconductor interface where conductance or capacitance changes might occur within the semiconductor (due to field-effects) and also within the biological layer.

In general, an FET sensor’s three electrodes are: Source (S) is the electrode which the majority carriers enter the channel. Conventional current entering the channel at S is designated by $I_s$. Drain (D) is the electrode which the majority carriers leave the channel. Conventional current entering the channel at D is designated by $I_D$. Drain to Source voltage is $V_{DS}$. Gate (G) is the electrode that modulates the channel conductivity. Applying the voltage to gate could be used to control $I_D$. The semiconductor channel is used to sense component of the device. In order to provide selectivity toward a unique analyte, a specific ligand is anchored to the surface of the semiconductor channel. This ligand is typically chosen to recognize the analyte with a high degree of both specificity and affinity. It has been noted that in the case of MOSFET, the gate is electrically isolated from the source and drain electrodes;
however, in ISFET and BioFET, the gate represents for an ion-selective membrane, electrolyte and a reference electrode. The voltage between the source and drain of an ISFET sensor regulates the current flow in the gate voltage. In the case of ISFET, the amount of current flow is controlled by changes in the pH of the electrolyte, the ion concentration, etc. In this study, we use the mechanism of n channel ISFET sensor in which the current is conducted by electrons (major carriers in n-type semiconductors). When a positive voltage is applied to the gate, the free holes are repelled from the near-surface region of the substrate, and pushed downward into the bulk, leaving behind a carrier-depletion region (space-charge region). The depletion region is built up by the bound negative charge associated with the acceptor atoms. At the same time, the positive gate voltage pulls the electrons from the bulk of the substrate into the near-surface region. When enough electrons are pulled into the near-surface region, the n channel is in effect created, electrically bridging the source and drain electrodes. If a voltage is applied between the source and the drain with the created channel, a current will flow through this p channel via the mobile electrons. In the case of n-type semiconductors, applying a positive voltage accumulates carriers (electrons) and increases the conductance, whereas applying a negative voltage leads to the depletion of carriers and reduces the conductance. The electric field created by the applied voltage on the gate develops in the vertical direction in the substrate. In most case, the voltage applied between the source-drain and gate electrodes is constant for an ISFET sensor. Any change in the current can be related to a change in the conductance of the ion-selective membrane. When a charged molecule links to the ion-selective
membrane, an electric field is created near the surface and exerts an effect on the semiconductor channel. If the bond molecules carry the same charge as the carriers in the semiconductor, the carriers will deplete from the surface into the bulk, thus causing a decrease in the conductance, i.e. when a negatively charged molecule such as 1-undecenoic acid binds to an n-type silicon surface it causes electrons to be repelled, thus resulting in a decrease in conductance.

1.4 Analysis methods

The impedance measurements are made by injecting an alternating current (AC) across the system at a known angular frequency, $\omega = 2\pi f$ (where $f$ is the frequency in Hertz) and a small amplitude $i_0$. EIS measures the amplitude $v_o$ and phase difference $\Phi$ of the response voltage that develops across the system.

The alternating current (AC), $\tilde{I}$, is defined as:

$$\tilde{I} \equiv I_o \sin(\omega t)$$

The relationship between conductance, $G$ and resistance, $R$ is

$$G \equiv \frac{|I|}{|V|} \equiv \frac{1}{R}$$

Therefore, the voltage response is

$$\vdash \tilde{V} \equiv I_o R \sin(\omega t)$$

Using the identities $e^{j\theta} \equiv \cos\theta + j \sin\theta$ and $j \equiv \sqrt{-1}$, the AC voltage can be expressed in phasor as:

$$\tilde{V} = V_0 e^{j(\omega t + \Phi)} \quad \text{and} \quad \tilde{I} = I_0 e^{j(\omega t)}$$

The impedance is
\[ Z = \frac{\varphi}{I} = \frac{V_o}{I_o} e^{j\theta} = |Z| e^{j\angle Z} \]

Where \( |Z| = \frac{V_o}{I_o} \) and \( \angle Z = \varnothing \)

The impedance of a complex system consists of a real part (resistance, \( R \)) and an imaginary part (reactance, \( X \)) is

\[ Z = R + jX \]

Where \( R = \frac{V}{i} \) and \( X = -\frac{1}{\omega C} \)

Then, the total impedance of elements in series is defined as

\[ Z_N(\omega) = \sum_{n=1}^{N} Z_n(\omega) \]

And the total impedance of elements in parallel is given by

\[ \frac{1}{Z_N(\omega)} = \sum_{n=1}^{N} \frac{1}{Z_n(\omega)} \]

Thus, \( Z_N(\omega) = \frac{1}{\sum_{n=1}^{N} \frac{1}{Z_n(\omega)}} \)

Since the admittance is the reciprocal of the impedance, it follows that

\[ Y_n(\omega) = \frac{1}{Z_n(\omega)} \]

Then, the total impedance of elements in parallel can be defined as

\[ Z_N(\omega) = \frac{1}{\sum_{n=1}^{N} Y_n(\omega)} \]

Admittance can be defined as a complex of conductance and susceptance

\[ Y(\omega) = G + jB \]

Where susceptance can be defined as \( B = \omega C \)

Therefore, the admittance is expressed as

\[ Y(\omega) = G + j\omega C \]

In the case of a homogeneous material, the individual layer of materials shows no frequency dependency. Each layer is represented by a resistor and capacitor in parallel.
and series of such layers forms a Maxwell-Wagner circuit. The Maxwell-Wagner models were used as the equivalent circuits for the evolution of the charge buildup at the two-material interface on the basis of the difference of charge carrier relaxation times in these two materials. The mechanism of charge buildup is due to the different dielectric constant and conductivity which creates an interface where charge could build up and create an electrical time constant. The effect attributed to the charge buildup is called Maxwell-Wagner effect, and this effect occurs in all kinds of two-material interface, such as metal-insulator interface, semiconductor-insulator interface, and others. Finally, plasma polymer was used as an alternative to self-assembled monolayers for the coupling of biological matter with silicon. The impedance can be considered as a resistor and capacitor in parallel. The impedance of the resistor can be represented by a conductance $Y_{\text{resistor}}(\omega) = G$, and the impedance of the capacitor can be represented by a capacitance $Y_{\text{capacitor}}(\omega) = C$

Thus, the impedance of the system is

$$Z_N(\omega) = \frac{1}{\sum_{n=1}^{N} Y_n(\omega)} = \frac{1}{Y_{\text{resistor}}(\omega) + Y_{\text{capacitor}}(\omega)} = \frac{1}{G + j\omega C}$$

Conductance and capacitance describe the ability of a homogeneous material to conduct and store electric charge, respectively. If a homogeneous material has cross-sectional area $A$ and thickness $d$, the conductance and capacitance can be
expressed by

\[ G = \sigma \frac{A}{d} \text{ and } C = \varepsilon \frac{A}{d} \]

Where \( \sigma \) and \( \varepsilon \) are the electrical conductivity and dielectric permittivity of the material, respectively.

For two homogeneous systems,

The combinational impedance of the system is then

\[ Z(\omega) = \frac{1}{G_1 + j\omega C_1} + \frac{1}{G_2 + j\omega C_2} \]

And the total admittance is

\[ Y_n(\omega) = \frac{G_1 G_2 + j\omega C_1 C_2 + j\omega C_1 G_2 - \omega^2 C_1 C_2}{(G_1 + G_2) + j\omega(C_1 + C_2)} \]

Conductance and capacitance are the real and imaginary parts of \( Y_n(\omega) \), respectively.

\[ G(\omega) = \frac{G_1 G_2 (G_1 + G_2) + \omega^2 (G_1 C_2^2 + C_1^2 G_2)}{(G_1 + G_2)^2 + \omega^2 (C_1 + C_2)^2} \]

\[ C(\omega) = \frac{G_1^2 C_2 + C_1 G_2^2 + \omega^2 C_1 C_2 (C_1 + C_2)}{(G_1 + G_2)^2 + \omega^2 (C_1 + C_2)^2} \]

At low frequencies, the capacitor in parallel with the resistor is essentially an open circuit. Consequently the impedance is dominated by the highest resistor if its value is much larger than the other one as shown in Figure 1.4a and b. The overall parallel conductance and capacitance at low frequencies are therefore given by:

\[ \lim_{n \to \infty} G(\omega) = \frac{G_1 G_2}{G_1 + G_2} \text{ and } \lim_{n \to \infty} C(\omega) = \frac{C_1 G_2^2 + C_2 G_1^2}{(G_1 + G_2)^2} \]
For example, in the case of the two layer system, $C_1 = 5 \text{ mF}$; $G_1 = 0.002 \text{ S}$; $C_2 = 55 \text{ mF}$; $G_2 = 6 \text{ S}$ shown in Figure 1.4, when $G_1 \ll G_2$, then $G (\omega) \approx G_1$ and $C (\omega) \approx C_1$. These results show that the overall parallel conductance and capacitance at low frequencies are dominated by the layer with lower conductance.

![Figure 1.4: (a) Equivalent Maxwell-Wagner circuits of single-layer and two-layer systems. The values of the capacitors and resistors used to calculate the dispersion curves in this figure are: $C_1 = 5 \text{ mF}$, $G_1 = 0.002 \text{ S}$ and $C_2 = 55 \text{ mF}$, $G_2 = 6 \text{ S}$.](image)

(b) and (c) Plots of the overall parallel conductance $G (\omega)$ and capacitance $C (\omega)$ of the same two systems represented in the Bode plot above. $G (\omega)$ and $C (\omega)$ are constant in frequency for the single-layer system (solid line) but disperse strongly for the two-layer system (dotted line). (Refer to Boecking’s PhD thesis)

In high frequency regions, the impedance is dominated by the capacitance and the overall conductance and capacitance are given by:

$$G (\omega) = \frac{1}{\omega C_1}$$

$$C (\omega) = \frac{1}{\omega G_1}$$
At high frequencies, the conductance of the system is determined by the conductance of the electrolyte and the capacitance is very small.

1.5 Self-assembled monolayers (SAMs)

Self-assembled monolayers (SAMs) are single layers of highly ordered molecules formed by the adsorption of a reactive organic molecule on a solid surface. From the energetic point of view, a self-assembling molecule can be divided into three parts (as illustrated in Figure 1.5):

i) Functional head groups: these are where SAMs are exposed to the gas or liquid interface. i.e. amine (-NH$_2$), aldehyde (-COH), carboxylic acid (-COOH) which can be used to conjugate biological matters.

ii) Hydrocarbon segments: usually alkyl chains via van der Waals interactions laterally tilt to assist in the formation of ordered molecular structures which rely on the pinning density of the head groups.

iii) Terminal attachment groups: these form chemical bonds with special substrate sites, i.e. silanes, thiols and alkenyl groups, and can be used for bonding to silicon oxide, gold and hydrogen terminated silicon surfaces, respectively.
When the terminal attachment groups of a self-assembling molecule bind to the surface atoms of a substrate, the alkyl chains form a canting angle ($\theta$) from the surface of the substrate, as shown in Figure 1.6.

The alkyl chains take on a densely-packed “ordered” or “solid-like” state. Loosely-packed alkyl chains form a “disordered” or “liquid-like” state. The lower the canting angle, the higher the packing density, and vice versa.

There are many methods for preparing densely-packed SAMs on the surface of various solid substrates. A review of the procedures is given in the following section.
1.6 SAMs on gold

An early suggestion for using alkylthiol on an Au surface was proposed by Nuzzo and Allara in the 1980s.\textsuperscript{23-26} They realized the utility of combining a relatively inert gold surface with a biofunctional organic molecule in a well-ordered regularly oriented array. The structure of alkylthiol is made of an n-alkyl chain with a thiol group at one end and a functional group at the other end (Figure 1.7a). The procedure for the preparation of SAMs on a gold surface is very simple: upon immersion of the gold substrate into an alkylthiol solution at room temperature for 24 hours, alkylthiols coordinate to the Au substrate and reduce to alkylthiolates.

\[
\text{RSH} - \text{Au}(0)\text{n} \rightarrow \text{RS}^-\text{Au}(1)\text{Au}(0)\text{n} + \frac{1}{2}\text{H}_2
\]

In this review, we will only discuss Au (111). Au (111) surface has a lower energy crystalline than Au (110), Au (100) or Au (001) and its atoms are spaced 2.88 Å apart. The sulfur atoms of alkylthiols spontaneously coordinate to the Au (111) surface and are spaced 5 Å apart (Figure 1.7c). The n-alkyl chains are tilted 30° off the normal axis (Figure 1.7b). The energy between the Au – S bond is approximately 40 Kcal mol\textsuperscript{-1} and each methylene unit in the alkyl chains is about 1 kcal mol\textsuperscript{-1}.\textsuperscript{27, 28}
**Figure 1.7:** Generic alkylthiol structure and coordination on an Au (111) surface: (a) the structure of an alkylthiol molecules is composed of an n-alkyl chain with a thiol group (-SH) at one end and a biofunctional group at the other end; (b) when alkylthiols molecules assemble on an Au surface, thiol groups chemically bond with atoms of Au and alkyl chains are tilted 30° off the normal axis; (c) the atoms of Au (111) are spaced 2.88 Å apart and thiols bonded to Au are spaced 5 Å apart. 27, 28

Alkylthiol molecules with short alkyl chains form monolayers faster than long ones, 28-30 but some evidence 24 shows that longer alkyl chains yield more stable and densely-packed monolayers. To meet the basic requirements of a biosensor, long alkylthiols are usually chosen.

The process of SAMs formation on an Au surface occurs in distinct stages. First, alkylthiols adsorb on the Au surface by laying down (Figure 1.8a) and this continues until 80 ~ 90% coverage is achieved 30 (Figure 1.8b). Next, partial monolayers are formed through nucleation and the growth of islands (Figure 1.8c). 31-34 These islands are surrounded by other alkylthiols laying flat. Finally, densely-packed islands
continue to nucleate and expand until a fully covered monolayer is formed (Figure 1.8d)

(a)

(b)

(c)

(d)

Figure 1.8: Stages of alkylthiolate SAMs formation on Au (111): (a) alkylthiol molecules initially adsorb onto the Au surface by laying down; (b) this continues until 80 ~90% of the surface is coated; (c) islands of densely-packed monolayers begin to build up; and (d) continue to grow until a complete monolayer forms.  

31,32
Thiolated DNA can be attached to a gold surface through an Au-S bond. This DNS layer can be readily detected using EIS.\textsuperscript{35} The advantages of a gold substrate over other materials are that the chemical preparations for a gold surface are simpler and the electrical measurements are easy to make. Chilcott et al. (2007)\textsuperscript{35} reported a conductance in the order of 1-100 Sm\textsuperscript{-2} for an Au-DNA system compared to 0.001-0.01 Sm\textsuperscript{-2} for a Si-alkyl monolayer-DNA system. The higher conductance of the gold substrate results in a higher signal to noise ratio for detection which makes it easier for EIS to detect small changes on the substrate surface. However, gold also has disadvantages such as the desorption of thiolated molecules in an organic solvent or at elevated temperatures.\textsuperscript{36} In addition, the atomic roughness of the gold surface makes it more difficult to prepare a large sample. Once the gold contacts with an aqueous solution, an ionic double layer forms between the gold and solution. The electrical impedance of the ionic double layer is very high and serves as an electrical barrier to the coupling of inorganic devices to biological systems. This layer induces an electrical drift which reduces the sensitivity to detect the target analyte. Moreover, a metal surface is prone to electrochemical oxidation corrosion and contamination with physiological or biological fluids that alters the electrochemistry of the surface. Published EIS studies by Chilcott et al.\textsuperscript{35} characterized interfaces that gold formed with potassium chloride electrolytes of concentrations ranging from 1 – 300 mM as 2 – 3 nm thick layer of conductivity many orders of magnitude less than that of the bulk electrolyte. Unfortunately, most SAMs are in the range of 1-2 nm thick. Therefore, the ionic double layer on the rough gold surface adds an electrical impedance dispersion
that can strongly mask that due to the SAMs attached to the gold. Some studies\textsuperscript{36-38} have demonstrated that enhancement of the working surface area of gold contributed to errors in signal calculation due to the roughness of gold itself. Guiducci et al. (2006)\textsuperscript{39} reported that an accurate effective area of a rough surface was too hard to model and calculate, leading to greater difficulty in measuring actual changes in the electric properties of the system than for an atomically flat surface. Although Losic et al. (2001)\textsuperscript{37} produced a flatter gold surface with roughness reduced from 30 nm to 1.2 nm; it was still too rough to be used for a DNA biosensor. Moreover, this flatter gold surface required complex methodologies and many reagents. In contrast, silicon wafers can achieve an atomically flat hydrogen-termination surface by treatment with ammonium fluoride or hydrogen fluoride solution for only a few minutes.\textsuperscript{40}

1.7 SAMs on silicon

In the early stages of biosensor investigation, metals were used as substrates for supporting the biomolecules or other organic moieties. However, the use of metals was not a long-term solution for making favorable biosensors because of a variety of problems, including possible corrosion of exposed metal and for implantable electrodes unfavorable interaction with tissues. However, one of the major problems with metal substrates is that the surface will invariably be rough at the atomic scale and this leads to exacerbated problems associated with ionic double layers at the metal-solution interface. Compared to metal-based biosensors, silicon-based architectures offer advantages that include: (1) it is based on a well-established
technology for the bulk manufacture semiconductor materials; (2) its widespread use in the semiconductor industry leading to relatively inexpensive high-purity silicon wafers; (3) the ability to tailor the bulk properties of the silicon substrate by altering the dopant type,\textsuperscript{41} (4) the ability to tune the chemical properties of the silicon surface via modification with very stable self-assembled monolayers (SAMs),\textsuperscript{42} and the atomically flat surface of the silicon wafers eliminates many of problems with the frequency-dependent electrical properties of ionic double layers at the surface. Indeed, the low electric constant (12, that is much less than that of the aqueous solution) of the silicon substrate compared with that of a metal greatly reduces the effects of ionic double layers. Therefore, semiconductors, especially porous silicon, have taken the place of metals to play an increasingly important role in biosensor research. Two alternative strategies have been employed for the formation of SAMs on silicon substrates:

(a) SAMs deposited on native silicon oxide by silanization or vapour deposition.

(b) Direct attachment of SAMs on a silicon surface via Si-C bond formation.

Silicon forms an ultra-thin (10 ~ 15 Å) native oxide layer when it is exposure to the air. Silanes (RSiX\textsubscript{3} with X = Cl, OMe or OEt) organometallics (RLi or RMgX) and alcohols (ROH) are widely used to build up the self-assembled monolayers (SAMs) on a silicon oxide surface. The high density of silanol groups (Si-OH) with high surface energy is formed by cleaning the substrate. These silanol groups, which are later used as coupling sites for silanization reactions or converted into more reactive functions (i.e. Si-Cl or Si-NEt\textsubscript{2}) suitable for alkylation or alkoxylation reactions allow
molecules to diffuse on the physisorbed ultra-thin water layer due to their highly hydrophilic properties.

The traditional method to yield clean and hydroxylated thin oxide layers on silicon surfaces is the Root Cause Analysis (RCA) cleaning process, as described in Figure 1.9a. It is still widely employed in the industry.\textsuperscript{43, 44} First, the silicon wafer is immersed into a Piranha solution (a mixture of concentrated sulfuric acid and hydrogen peroxide) and then into an alkaline mixture of dionised (DI) water, ammonium hydroxide and hydrogen peroxide. Any contaminants such as dust, silica, oil or metal are removed via these two treatments. Secondly, the SiO\textsubscript{2} layer is removed using dilute hydrofluoric acid. Finally, the hydroxylated surface is achieved by treating the silicon wafer in a bath of hydrochloric acid and hydrogen peroxide.

An alternative method including the five steps shown in Figure 1.9b can also yield a hydroxylated surface.\textsuperscript{45-47} First, organic contaminants are removed by sonication in chloroform. Second, the wafer is photochemically cleaned by UV radiation in an oxygen atmosphere for 15 minutes. Third, cleaning in Piranha solution for about 10 minutes. Next, the silicon wafer is rinsed with DI water. Finally, about 45 minutes of photochemical oxidation by UV radiation is used to remove the last traces of contamination. Both of these methods can yield a density of about $10^{15}$ hydroxylated groups/cm\textsuperscript{2}
Silanization is a process in which silane (RSiX₃; where X = Cl, OCH₃ or OC₂H₅) in an alkane solvent, such as dicyclohexyl or n-hexane, forms self-assembled monolayers (SAMs) on a hydrated silicon surface. The reactions are usually completed at temperatures between -30°C and room temperature, depending on the alkyl length. Short chain lengths of silane having high vapour pressure require a lower temperature for the formation of a densely-packed monolayer by evaporating pure molecules either under argon flow at room temperature⁴⁸, ⁴⁹ or by heating in a confined medium or under vacum.⁵⁰ The process of SAMs formation during silanization occurs in four steps⁴⁶, ⁴⁷ as shown in Figure 1.10:
i. Physisorption: the silane molecules are physisorbed at the hydrated silicon surface

ii. Hydrolysis: the head groups of saline hydrolyze into highly polar trihydroxysilane –Si (OH)₃ groups in the presence of absorbed water layers on the silicon surface.

iii. Covalent grafting to the substrate: the –Si (OH)₃ groups covalently bond to the hydroxyl groups on the SiO₂ surface. During the initial period, monolayers form on the substrate in a disordered or liquid state.

iv. In plane reticulation: the silanol functions of neighboring silane molecules are driven by lipophilic interaction. Over longer periods of time, ordered or solid state monolayers are obtained on the surface.

Figure 1.10: Schematic showing the mechanism of SAMs formation on a hydrated silicon surface.
The process in which a hydroxylated silicon dioxide surface is coupled with alcohols, lithium compounds or Grignard reagents is called halogenations or amination (Figure 1.10). Chloro-terminated silicon dioxide is prepared either by reaction with thionyl chloride in tetrahydrofuran or tetrachlorosilane in toluene or in the presence of pyridine under vacuum. Amino-terminated surfaces can be obtained by the treatment of a hydroxylated surface with aminosilane Si(NEt₂)_4 in dry toluene. Because –OH, -Cl and –NEt₂ surfaces are not stable in air, all the activated surfaces must be used immediately for the preparations of self-assembled monolayers (SAMs).

The organic lithium (RLi) or organ magnesium compounds (RMgX) can easily react with halogenated or aminated surfaces to form densely-packed monolayers via the formation of Si-R bonds. Activated silicon dioxide coupled with alcohol yields alkoxylated surfaces through the formation of Si-OR bonds.

**Figure 1.11:** Halogenation or amination process of SAMs formation on the hydroxylated silicon dioxide surface.
1.8 SAMs directly on Si-H

Without any method of protection, silicon will form a native oxide that can prevent the use of silicon electrodes for electrochemical functionalization. The natural formation of silicon dioxide on the surface of silicon has a low isoelectric point, meaning that under physiological conditions (pH= 7.4), silicon dioxide is negatively charged\(^5\) and adds to the electrical resistance.\(^6\) These surface charges could become an electrical barrier and potentially limit the sensitivity of certain nanoelectronic biomolecular sensor devices. Thus, the ideal biofunctionalization strategy for electrochemically activating Si surfaces should begin with silicon surfaces free of oxide. Two etching methods can be employed to obtain H-terminated silicon surfaces, as shown in Figure 1.12. Si (100) wafers etched with 1-2% HF aqueous solution yield atomically flat dihydride-terminated Si surfaces (=Si-H). Treatment of Si (111) wafers with 40% NH\(_4\)F produces monohydride-terminated Si surfaces (=Si-H). The H-terminated Si surfaces are only stable in air for a few minutes and easily reoxidize if immersed in an aqueous solution.

Figure 1.12. Chemical etching processes to obtain H-terminated Si surfaces.
Silicon dioxide removal in HF solution was one of the most widely used techniques to clean silicon surfaces in the semiconductor industry.\textsuperscript{57} There were many arguments about the mechanism of silicon surface cleaning such as F passivation,\textsuperscript{58} bond strength (6.0 eV of Si-F and 3.5 eV of Si-H, respectively) and hydrogen passivation.\textsuperscript{59} Trucks \textit{et. al} (1990)\textsuperscript{60} reported a theoretical understanding of hydrogen termination of Si surfaces etched by HF acid. Their conclusions were that HF insertion into Si-Si bonds was the dominant reaction pathway. This theoretical mechanism is applicable to all forms of H termination (monohydride, dihydride, and trihydride) depending only on the initial structure of the Si/SiO\textsubscript{2} interface. However, the disadvantage of this HF etching method is that HF leaves \(\sim10\%\) of the surface covered with Si-F species (Figure 1.13a).\textsuperscript{61} The surface produced thus had about 10% coverage of hydroxyl group when it is rinsed with water. Houston, Maboudian and Howe (1995)\textsuperscript{62} investigated the stability of Si-H surfaces produced by ammonium fluoride and concluded that a saturated (40%) ammonium fluoride solution with no HF had a slower oxide etch rate, which led to a cleaner, more hydrophobic surface. On the NH\textsubscript{4}F treated silicon surface (Figure 1.13b), there were no fluorine groups after etching and, therefore, there was no fast mechanism for water attacks. Consequently, silicon etched in 40% ammonium fluoride led to a more hydrophobic surface compared to that obtained with hydrogen fluoride.

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![Image of Silicon etching in HF and NH\textsubscript{4}F](image.png)

\textsuperscript{10}\% Si-F coverage

- a. Silicon etched in HF
- b. Silicon etched in NH\textsubscript{4}F
**Figure 1.13:** Termination and morphology of a silicon surface. The surface Si-F groups in (a) will be replaced by –OH groups upon rinsing, leading to a less hydrophobic surface compared to that obtained in (b) with ammonium fluoride.

Infrared spectroscopy (IR) studies demonstrated that the stability of hydrogen-terminated silicon surfaces depends on the humidity.\textsuperscript{63} Therefore, hydrogen-terminated silicon surfaces are only stable for a few minutes. Later on, water will attack the surface by donating an electron to form Si-OH groups. Then OH moves into Si-Si bonds to regenerate a Si-O surface. This reaction does not stop until all of the back bonds are oxidized. The observation of IR reported by Mitura et al.\textsuperscript{63} identified that the (SiO)\textsubscript{3}Si-H species are the predominant species on the surface after a few hours in humid air.

The high bond strength and low polarity of a Si-C bond allow it to form a stable self-assembled monolayer on a silicon surface once the reactive Si surface are obtained. Organic molecules with an unsaturated bond such as 1-alkene or 1-alkyne can be grafted on hydride-terminated silicon surfaces form self-assembled monolayers. This strategy is known as hydrosilylation and was first reported by Linford and Chidsey in 1993.\textsuperscript{41} The reaction is most commonly initiated by heating or irradiating with UV/white light to produce a surface radical where the unsaturated molecules can attack and form a Si-C bond. The proposed mechanism of monolayer formation is depicted in Figure 1.14.
This proposed mechanism includes three steps:

1. The initiator breaks homolytically to form two acyloxy radicals which eventually break apart to form an alkyl radical and carbon dioxide.

2. The alkyl radical absorbs the hydrogen atom from the hydrogen-terminated Si surface to produce a silicon radical.

3. The silicon radical reacts with 1-alkene to form a Si-C bond.

Sieval et. al (1998)\textsuperscript{67} used the thermal induction method to prepare densely-packed monolayers on both Si (100) and Si (111) surfaces by heating the hydrogen-terminated silicon surfaces to 200°C in the presence of 1-alkenes. Infrared spectra and x-ray reflectivity measurements showed that the monolayers on silicon (100) prepared from long-chain alkenes were similar to those prepared on the silicon (111) surface. Moreover, they found that the advancing contact angles for water were similar, but the receding angles were somewhat lower due to the roughness of the silicon (100) surface. The silicon (100) surface was not flat and consisted of SiH, SiH\textsubscript{2} and SiH\textsubscript{3} groups.\textsuperscript{68} In addition; they examined ester functionalities at various
distances from the reactive alkene moiety. The results showed that functionalities that reacted with the hydrogen-terminated surface, like carboxyl acids and alcohols, gave rise to disordered monolayers. In contrast, well-ordered monolayers were obtained with the ester-protected analogous of compounds such as CH$_2$=CH-C$_8$H$_{16}$-C(O)O-CH$_3$, CH$_2$=CH-C$_8$H$_{16}$-C(O)OC$_3$H$_7$ and CH$_2$=CH-C$_8$H$_{16}$-CH$_2$OC(O)CH$_3$. The contact angles revealed that the ester groups were indeed at the outside of the monolayer. As there was enough space for the ester groups outside of the monolayer, it allowed for dense packing. Some compounds with demanding ester group close to the reactive site, such as CH$_2$=CH-CH$_2$-(O)C-C$_{11}$H$_{23}$ and CH$_2$=CH-CH$_2$-O(O)C-C$_{17}$H$_{35}$, resulted in the disordered monolayers because not enough space was available for the ester groups near the silicon surface. Bateman et al., (1998)$^{69}$ reported that the alkyne C≡C bond could react twice with a surface Si-H group, depending on the reaction conditions, compared to the alkene C=C bond which only reacted once with a Si-H group.$^{70}$ Sieval et al. (2000)$^{71}$ showed a mechanism for the reaction of a 1-alkyne with a H-terminated Si surface to form two Si-C bonds as shown in Figure 1.15.
Figure 1.15: The mechanism of the reaction of a 1-alkyne with an H-terminated Si surface.\textsuperscript{71}

Figure 1.15 depicts how 1-alkynes could form two Si-C bonds per molecule on silicon (100) instead of only one as in the case of alkenes and how this could protect the neighboring unreacted surface sites from further reaction, leading to improved passivation properties. Observations using infrared spectroscopy identified that 1-alkynes form two Si-C bonds per molecule to the Silicon (100) surface because of the absence of alkene moieties in the monolayers of 1-alkynes on the H-terminated silicon (100) surface. X-ray reflectivity (XRR) measurements\textsuperscript{71} showed that an intermediate layer is present between the Si surface and the alkyl chains, which might be the result of the formation of Si-C bridges in the monolayers. Therefore, combined with the results from IR spectroscopy and XRR measurements on the monolayers, Sieval et al.\textsuperscript{70} concluded that 1-alkynes form two Si-C bonds per reacting molecule on
the H-terminated silicon (100) surface. Covalently attached, well-ordered alkyl monolayers on H-terminated silicon surfaces play an essential role in the modification of silicon surface. Therefore, it is very important to investigate the substitution percentages of the Si-H for Si-alkyl groups in the monolayer structures. The nearest-neighbor distance between Si atoms on an unreconstructed 1x1 H-Si (111) surface is 3.84 Å, and the surface area of a Si-H group on a Si (111) surface is 12.77 Å². Therefore, only some chemical groups such as methyl groups or halogen groups are small enough to fit atop every Si (111) surface site, leading to a complete coverage of the Si (111) surface. But such methylated Si (111) surfaces are not available for further functionalization because of their strong surface protection from other chemical attachments. In the case of other molecules, such as long alkyl chain attachments (surface area of 24 Å²) reported by Alexander et al.(2001), approximately 50-55% of the Si-H groups on the surface had reacted with 1-alkene due to steric constraints (Figure 1.16), whereas the other Si-H groups remained intact.

![Figure 1.16: Structure of substitution pattern with alkyl chains. Note: a projected surface area of 24 Å² per alkyl chain in the monolayer and a tilt angle of 28-29° were derived from IR measurements and x-ray reflectivity experiments.](image)

31
Further candidates for complete coverage on a silicon (111) surface include unsaturated organic species such as ethynyl or propynyl moieties (-C≡C-R, with R=H, CH₃), because of the linearity of these molecules. Moreover, the functionalization of Si surfaces by C≡C groups opens new reaction pathways for further organic modification of these surfaces to introduce functional groups by click chemistry. Albert et al (2009) compared the reactivity of alkynes and alkenes on silicon (100) surfaces. They used two different approaches to investigate both alkynes and alkenes on hydrogen-terminated silicon (100) surfaces via hydrosilylation. One approach was to form monolayers from a series of solutions containing different mole fractions of an alkyne with a trifluorothioacetate distal moiety and an alkene with a terminal carboxylic acid functional group. XPS measurements showed that the mole fraction of alkyne on the surface was larger than that in the respective alkyne/alkene mixture (ratio of alkyne to alkene: 1.7-2.0) when monolayers were formed at 120°C. The other approach used a molecule with an alkyne at one end and an alkene at the other (non-1-yne-8-ene), so that, both ends could attach to the hydrogen-terminated silicon surface. Azidoferrocene which can only attach to free alkyne moieties was used as an additional reagent to determine the orientation of this molecule. The quantity of the ferrocene moieties attached to the self-assembled monolayers via electrochemical analysis revealed that the alkyne end reacted preferentially.

### 1.9 Photochemical functionalization
#### 1.9.1 UV irradiation
Fleming et al. reported that UV irradiation can promote the hydrosilylation of
unsaturated compounds due to the homolytic cleavage of Si-H bonds. Comparing UV irradiation to thermal functionalization, the latter approach requires a thermal input which could cause slight damage to a silicon wafer, while the former takes place at room temperature and thus avoids any thermal damage. Cicero et al. (2000) modified a hydride-terminated Si (111) surface with UV light (254 nm) in the presence of a range of alkenes at room temperature for two hours. Observations of the XPS spectrum of the hydride-terminated Si(111) surface in the presence of 1-octene showed approximately one alkyl group per two silicon atoms, an ideal number based on molecular modeling. The thickness of this monolayer (9Å) measured by ellipsometry coincided with the calculations. They used the thickness formula \[ d=0.126x(n-1)xcos0 + 0.186 \] where, \( n \) is the carbon atoms and it is assumed that the Si-C and C-C bond lengths projected along the molecular axis are 0.184 and 0.126 nm, respectively. Effenberger et al. examined Si(111)-H with wavelengths up to 350 nm. They found that longer wavelengths of UV light promoted hydrosilylation without oxidation, but at the cost of prolonging irradiation for 20-24 hours and an accompanying increase in temperature to 50°C.

The mechanism for UV-induced hydrosilylation of a Si-H bond surface is shown in Figure 1.16.

![Proposed mechanism for the hydrosilylation of Si(111)-H under UV condition.](image)
Based on the Si-H bond dissociation energy, a silicon surface requires a minimum of 3.5 eV, a wavelength of shorter than 350 nm UV light is necessary to obtain surface silicon radicals on H-terminated flat Si (111). Silicon radicals react rapidly with unsaturated carbon-carbon bonds to form Si-C bonds, accompanied with absorption of neighboring hydrogen to complete hydrosilylation. The use of UV irradiation methods to induce hydrosilylation could yield stable and densely packed monolayers, but there have been some reports about loss of hydrides and concomitant oxide formation under UV irradiation. Related to Cicero et al.’s observation, clearly UV mediated methods plays a critical role in activating the surface for reaction with both alkenes/alkynes and dioxygen. Therefore, hydrosilylation of Si-H surfaces via UV irradiation, in the presence of deoxygenated alkenes/alkynes, must be done in unexposed areas (such as under inert gas atmosphere), otherwise it may result in an oxide/alkyl pattern formation. Consequently, a new method was developed for the attachment of high-quality organic monolayers on crystalline silicon surfaces by using visible light source from 447 to 658 nm.

### 1.9.2 White light:

White light (22-44mW/cm²) can induce the hydrosilylation of alkenes and alkynes on hydride-terminated silicon surfaces at room temperature. Since Effenberger et al. showed that the water contact angle was only 95°C after modification of a flat hydrogen-terminated silicon surface by UV light (380 nm) in the presence of 1-octadecene, to improve the quality of the monolayer, Hamers et al. used visible
light (514 nm) to modify partially iodinated Si(111) and Si(100) surfaces. Although this worked well, it required an extra step and partially iodinated silicon surfaces provided poorly defined substrates. Recently Boukherroub et al.\textsuperscript{84} obtained well-ordered and stable self-assembled monolayers on silicon surfaces by irradiating a Si-H silicon wafer with visible light (447 nm) at room temperature in the presence of 1-alkene or 1-alkyne. They compared monolayers prepared by a thermal method with UV light and visible light and found that the maximum contact angle of an n-hexadecyl monolayer was obtained by using visible light. They also pointed out that the use of visible light of 371-658 nm wavelength did not result in any side effects unlike irradiation with UV light (at 254 nm) which made 1-alkene solutions turn yellow. The authors investigated the influence of the doping type, doping concentration of the silicon, and variation of the irradiation wavelength. They concluded that the contact angles of monolayers on n-type Si (100) were higher compared to those of monolayers on p-type Si (100). In addition, the efficiency of photochemical monolayer formation on n-type Si (100) was higher than on p-type Si (100). By observing the water contact angles of the monolayers as a function of irradiation time, they identify the rate order of covalent attachment: highly doped n > lowly doped n > lowly doped p > highly doped p.

Stewart et al. proposed an alternative mechanism (Figure 1.18) to explain how visible light promotes the hydrosilylation of photoluminescent nanocrystalline silicon\textsuperscript{86, 87}. Excitation of visible light promotes the formation of delocalized radical cations at the
silicon surface and the electrons are temporarily moved to the bulk, leading to a surface that is susceptible to nucleophilic attacks. Si-Si bonds are not very strong when they contain radical cations, resulting in the Si-centered cations at the surface being highly stabilized by the neighboring Si atoms. The structure of alkenes/alkynes is covalently attached on the silicon surface with a β-CH radical site. Later on, this radical can absorb an H atom and leave a Si radical at the surface that is available for the attachment of a second alkene/alkyne.

**Figure 1.18**: Proposed mechanism for the hydrosilylation of Si-H with visible light.86, 87

### 1.10 Antibody immobilization on SAMs modified silicon

Antibodies immobilized on various supports have been widely used for different purposes due to their high specificity. The applications of immunosensors in which antibodies are immobilized on electrodes or semiconductor chips for the detection of antigens88-90 in diagnostic immunosensors have expanded considerably in recent years91. The most common of the numerous coupling strategies for immobilizing antibodies on different supports is through the formation of crosslinkers in which carbodiimides (EDC) and succinimide esters (NHS ester) are widely used. Water-soluble EDC is called a zero length crosslinker, because it promotes the formation of amide linkages without leaving a spacer molecule. The optimal reaction medium of EDC is at a pH between 4.7 and 6.0. EDC not only reacts with
carboxylates, it can alone form a stable complex with sulfhydryl groups, and tyrosine residues can react with EDC via the phenolate-ionized form of its side chain. Therefore, NHS and sulfo-NHS are used to reduce the side reactions and enhance the yield. The ratio of the concentration of EDC to that of NHS strongly affects the yield too. Jang et al. reported that the 3:1 EDC/NHS ratio and reaction time of 4 hours in alcohol provided the best offset compared to other combinations.

Once antibodies have been covalently grafted on the surfaces of solid supports, the specific binding capacity of antibodies is usually less than that of antibodies in solution. This is attributed to the random, but fixed, orientation of antibodies on the surfaces. In Section 1 we mentioned that the immunoglobulin G (IgG) is composed of two different fragments: the Fc region which contains an antibody effector without antigen binding affinity and the F(ab')2 regions which contains an antigen binding site at the amino end of each fragments. Without any special treatment, the coupling of antibodies with supports could occur at any possible attachment point, resulting in a random orientation of antibodies on the surface of supports which might prohibit the formation of an antibody-antigen complex (Figure 1.19).

![Figure 1.19: Antibodies immobilized by random coupling procedure.](image)

To eliminate these drawbacks, several approaches have been developed for achieving
oriented antibody immobilization. In 1978, Gersten and Marchalonis\textsuperscript{92} immobilized an antibody to an F\textsubscript{c} receptor (protein A) on a solid surface, thereby improving its antibody-antigen capacity. Although protein A has been successfully utilized for binding the F\textsubscript{c} portion of antibodies from many mammalian species, it has failed to react with antibodies from goats, sheep, cows and horses.\textsuperscript{93} Protein G, which is found in the cell wall of Streptococcus human pathogenic strains of the Lancefield group G, is used as an alternative to react with antibodies that protein A cannot bind to; however, protein G does not bind strongly to several antibodies that protein A reacts well to. Another strategy to orient antibody immobilization on solid supports relies on the oxidation of the antibody’s carbohydrate moieties.\textsuperscript{94, 95} It was found that the activity was enhanced by a factor of three compared to that of the same antibody bound to the same support through its ω-amino groups.\textsuperscript{96} However this reaction is highly controlled by the pH of the binding condition and coupling time. Matson and Little\textsuperscript{96} reported that affinity purification and excessive oxidation caused damage to both the antigen binding sites and carbohydrate moieties of a monoclonal antibody.

In conclusion, several studies have revealed that oriented antibodies on solid supports have 2-8 times higher ligand binding efficiencies than those of randomly immobilized ones. However, this leads to more complicated preparation processes and depends highly on reactive conditions such as the pH, coupling time and temperature. Another important issue is that the more the compounds are immobilized on the solid surface, the more complicated equivalent circuits are required to describe the whole system.
using electrical impedance spectroscopy. Many papers have shown that, based on impedance analysis, it is possible to detect antibody-antigen binding without any antibody orientation treatment. 98-100

1.11 Polymer films

It is advantageous that proteins retain their function over a long period of time after immobilization on solid surfaces, especially in biochemical fields and in applications such as in vivo implants and biosensors. 101-104 The traditional approach to prevent proteins from immobilizing on solid surfaces with non-specific and uncontrolled weak interactions is to use chemical linkers such as self-assembled monolayers (SAMs), Langmuir-Blodgett layers or organic polyelectrolyte layers. 103, 105, 106 The drawback of these approaches is that they involve complex wet chemical reaction steps prior to and during the attachment of the bioactive proteins. Another issue is the duration of the strength of the traditional linkers at retaining proteins immobilized on solid surfaces.

Two techniques can simplify the steps of the process with strongly covalent binding layers to substrates for coupling with proteins: plasma modification of polymers and plasma deposition of polymer-like materials on any solid surface by adding polymer precursors into plasma. Strong binding of proteins to plasma deposited surface was reported by Bohnert et al. 107 in 1990. Later Kiaei et al. 108 tested the resistance of protein layers to detergent cleaning. The results showed that plasma polymers were capable of immobilizing proteins, possibly by covalent binding, but there was no
direct proof.

Yin et al.\textsuperscript{109} reported that adding a pulsed voltage to a conventional plasma enhanced chemical vapor deposition system for biasing substrates could result in the additional freedom of tailoring the density of activated sites for covalent immobilization of proteins, thus enhancing covalent coverage and increasing the shelf life of the surfaces. Mixing of nitrogen with the plasma during deposition led to a high protein immobilization capacity and stable activity over a long period of time.\textsuperscript{110} In 2009, Yin et al.\textsuperscript{111, 112} coated solid materials of various shapes using an acetylene plasma polymerization technique adapted from plasma enhanced chemical vapor deposition by adding a pulsed voltage electrode to a substrate holder immersed in plasma. The result showed that approximately one monolayer of proteins was strongly bound to the solid surface. Analysis with a quartz crystal microbalance with dissipation (QCM-D) detected that further layers of protein beyond the monolayer were physically absorbed during prolonged incubation in the protein-containing solution. These subsequent physisorbed protein layers were removed easily by SDS, but the first monolayer resisted removal by 5\% SDS detergent even at 90°C.

\subsection*{1.12 Aims of thesis}

The objective of this thesis is to investigate the biosensitive functionalised silicon surfaces towards development of biosensitive Field-Effect-Transistors (BioFETs). The characteristics of a FET are dependent on the nature of the coupling of external
signals to the gate and the doping levels of the silicon. When a charged molecule is immobilized on the gate, an electric field effect is created near the low-doped silicon surface. This electric field exerts an effect both inside and outside the silicon. In order to understand how the electric field affects the electric properties of the FETs. It is preferred to study the electric properties of the coupling of external signals first. Therefore, highly doped silicon wafers were chosen. The charged molecules, such as the self-assembled molecules, linked on highly doped silicon surfaces do not induce the formation of a depletion layer in the silicon. Therefore, any change in the electric properties can be related to a change in the coupling of external signals. Although this research is focused on the self-assembled monolayers covalently attached directly to the silicon replacing the silicon dioxide layer, the silicon dioxide layer has to be studied before the self-assembled monolayers. This is due to two factors: one is that the silicon surface is always covered by the silicon dioxide without any special treatments, and the electric properties of the silicon dioxide is similar to those of the self-assembled monolayers; the other one is that the self-assembled monolayers do not provide long-term protection of the surfaces from re-oxidation. During the process of an analyte immobilized on the self-assembled monolayers, the silicon surfaces may partially re-oxidize. Therefore, the studies of the silicon dioxide help us figure out the difference between the electric properties attributed to the immobilization of the analyte and that attributed to the surface re-oxidation.

To study how the coupling of external signals exerts a field effect inside the silicon,
low-doped silicon wafers were chosen. If the coupling of external signals carries a charge the same as that of the main carriers in the low-doped silicon (e.g. electrons in n-type silicon), then the charge carriers will be repelled under the bound analyte and form a depletion layer, causing a decrease in conductivity. In contrast, analytes with molecular charges opposite to the main carriers in the low-doped silicon lead to accumulation of main carriers beneath the bound analyte, thus causing an increase in conductivity. This is also the principle of how a FET device could potentially detect a target analyte.
1.13 References


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Chapter 2
Self-assembled monolayers (SAMs) directly immobilized on the Si (111) surface

2.1 Introduction

Functionalized self-assembled monolayers (SAMs) on gold via gold-thiol bonds or organosilanes on silica surfaces have been widely used for the immobilization of biomolecules. However, the limited stability of Au-S bonds restricts the applications of thiol SAMs on gold. Although SAMs deposited on silicon dioxide by silanization or vapour deposition have higher stability, the negatively charged silicon dioxide under physical conditions (pH: 7.4) becomes an electrical barrier and potentially limits the sensitivity of certain nanoelectronic biomolecular sensor devices. Thus, the ideal biofunctionalization strategy for electrochemically activating Si surfaces should begin with a free silicon dioxide surface.

The covalent attachment of self-assembled monolayers (SAMs) directly to silicon surfaces that can be used for preparing dense and biologically compatible monolayers have interesting physical and electrical properties.\textsuperscript{1-4} The electric potential of the surface changes when the charged molecules outside of the semiconductor interact with the semiconductor, altering the conductivity. Most biological matter, such as DNA and proteins, are charged at commonly accessible pH values. Therefore, these charged molecules grafted on the surface induce an electric field that can be directly detected by a label-free method. The covalent attachment of SAMs terminated with
biorecognitive reactive groups directly to silicon without a SiO$_2$ layer could serve as an insulating FET gate and prevent the re-oxidative processes of the silicon substrate. The Si-C is superior to the metal-biochemistry interface as inert metals form an ionic double layer interface with an aqueous biological environment. The high electrical impedance of the ionic double layer is a substantial electrical barrier to the coupling of inorganic devices to biological systems and the sensing based on impedance measurements would be affected by the strong frequency dependence of the impedance of the electric double layer. Moreover, electrochemical oxidation corrosion and contamination by metal could alter the electrochemistry of the surface. In contrast, the Si-C not only supports more stable and desirable applications for sensing and actuating functions, but it can also be produced by the thermally-, photochemically- or catalytically-activated reaction of hydride terminated silicon surfaces with 1-alkenes.$^{5,6}$

While photochemical hydrosilylation can induce alkenes directly grafting on Si surfaces via Si-C bonds, many mechanistic aspects of the functionalization reactions remain poorly understood. The proposed mechanism for the formation of Si-C bonds under UV is a radical reaction suggested by Linford. et. al., in 1995.$^7$ First, the UV light cleaves the Si-H bond to form a silyl radical. Then the silyl radical reacts with the alkene to form a Si-C bond and the carbon radical may absorb a hydrogen from a neighbouring Si-H site to create a new reactive radical. Based on the bond strength of Si-H on a silicon surface (~3.5 eV), this requires a wavelength smaller than 350 nm.
UV light to homolyse the Si-H bond. In fact, direct single photo-induced homolysis requires deep UV (157 nm, 7.9 eV). However, Stewart et al. obtained alkenes directly immobilized on Si-H surface with longer wavelength (>400 nm) UV. Later, Alexander et al. (2005) reported that UV irradiation could only offer Si-C formation on an atomically flat Si-H surface, not on a Si-SiO$_2$-H surface. These studies rejected the idea of simply cutting off Si-H bonds to form a radical. The exciton mechanism suggested instead of the direct Si-H bond breaking.

Since the characteristics of a FET device are dependent on the type and doping levels of the silicon, one of the principal issues in realizing the potential of sophisticated Si-C FET devices for biological sensing and simulation is the efficiency of the biochemistry-device link that couples biochemical processes with silicon based devices. Cai et al. (2002) reported that much greater photo-attachment efficiency is obtained on heavily doped n type silicon than on p type silicon. Sun et al (2005) concluded that the efficiency of photochemical monolayers grafted on silicon (100) surfaces follows the following rate order: highly doped n > lowly doped n > lowly doped p > highly doped p. It is proposed that photo-attachment processes occurring on n-type samples drift carriers (holes) toward the surface where they induce oxidation reactions with nucleophilic molecules. Conversely, in p type samples the photoexcited holes drift away from the surface, making the surface more negatively charged and repelling nucleophilic attacks by alkenes. All of these results are based on XPS and IRRA measurements. However, limited information has been reported
about the comparison of electrical properties between n and p type silicon modified by alkenes via photochemical hydrosilylation reactions.

In this chapter, the preparation of Si-C linked monolayers with various functional end groups on a Si (111) surface by photochemical hydrosilylation of UV mediated method is described. The quality, chemical composition and structure of the monolayers were characterized by X-ray photoelectron spectroscopy (XPS), water contact angle measurements and high resolution electrical impedance spectroscopy (EIS). We also investigated the differences in the electrical properties that occur between n- and p-type silicon substrates when silicon surfaces are modified directly with alkenes. A comparison between n- and p-type silicon substrates via high resolution electrical impedance (EIS) showed the field-induced effect. Our results reveal that the changes in electrical properties were associated with chemical reactions on the surface.

2.2 Experimental methods

2.2.1 Materials

All materials were reagent grade and used as received. MilliQ water (18 M) was used for the rinsing of samples and preparation of solutions. 1-tetradecene (98%, Sigma) and undecenoic acid (98%, Aldrich) were stored at 4°C. Early samples were cleaned using 98% H₂SO₄, ARSelect grade (Malinckrodt), and 30% H₂O₂, SEMI grade (Olin), and were subsequently etched with 40% NH₄F solution, CMOS grade (Baker). Single
side polished and backside etched p-Si (111) wafers of 100 mm in diameter, 630 ± 25 µm in thickness, and resistivity of <0.002ohm-cm (Boron-doped) and n-Si (111) of 100 mm in diameter, 550 ± 25 µm in thickness, and the resistivity of <0.01ohm-cm (phosphorus-doped) were purchased from Virginia Semiconductor Inc. For UV light, 254 nm light was supplied from an ozone-free Hg pen lamp (Spectroline, model 11SC-1, 4.5mW/cm² intensity at 2.54 cm).

2.2.2 Preparation of hydrogen-terminated silicon substrates

Si (111) wafers were cleaved into pieces (approximately 15 mm x 15 mm) and cleaned ultrasonically with acetone, ethanol and MilliQ water several times in an ultrasonic bath for five minutes each time. The silicon wafers were treated in concentrated H₂SO₄ : 30% H₂O₂ (3:1, v/v) at 90°C for 30 minutes followed by copious rinsing with MilliQ water. (Caution: concentrated H₂SO₄/H₂O₂ solution reacts violently with organic materials and should be handled with extreme care.) Hydrogen-terminated Si (111) surfaces were obtained by etching in a deoxygenated 40% NH₄F for 20-30 minutes (Caution: NH₄F solution cannot be handled in glass test tubes or apparatus during laboratory work). The 40% NH₄F solution was deoxygenated by bubbling with nitrogen gas for at least 30 minutes. Hydrogen bubbles, a reaction product, could cause significant roughening on many length scales with several characteristic morphological features. The simplest, although most arduous method was utilized to prevent bubble-induced surface roughness; the sample was mechanically removed and reinserted into the etchant every 15 seconds. A
smooth surface was obtained in this manner after up to 40 minutes.

2.2.3 Photochemical hydrosilylation of alkenes

The reactions of 1-tetradecene and undecenoic acid with both n and p type H-Si (111) were performed under UV illumination. The photochemical reaction was performed in a nitrogen-purged sealed vessel as shown in Figure 2.1.

Figure 2.1: Set up for the photochemical hydrosilylation.
Figure 2.2: The fresh Si-H sample immersed into the alkenes solution.

Figure 2.3: Set up used in photochemical hydrosilylation.
The freshly prepared H-Si (111) wafers were then functionalized by photochemical hydrosilylation reactions in neat 1-tetradecene or undecenoic acid (Figure 2.2). 1-tetradecene and undecenoic acid were deoxygenated with nitrogen gas for at least one hour. Because the hydride-terminated silicon surfaces were only stable for a few minutes in the air, the chamber had to be firmly sealed by a G-clamp as soon as possible. A vacuum pump extracted the air from the chamber, and then let dry nitrogen gas fill up the chamber before the UV light was turned on. The nitrogen gas was kept flowing through the chamber during hydrosilylation to prevent ingress of oxygen from the air which could otherwise lead to oxidization of the sample and also to reduce the temperature in the chamber (Figure 2.3).

After irradiation with 254 nm light from a low-pressure mercury lamp (~4.5mW/cm² intensity at 2.54 cm) for 2-3 hours, the samples were rinsed with dichloromethane, ethanol, MilliQ water and blown dry under a stream of nitrogen.

**2.2.4 Physical measurements**

The self-assembled monolayers grafted on the silicon surface were examined by water contact angle, x-ray photoelectron spectroscopy (XPS) and electrical impedance spectroscopy (EIS).

**Water contact angle measurement:** The samples were placed horizontally on a microscope stage and 2µl of water was dropped on top of the samples. At least three
measurements of the contact angle were made using tangential images for each sample. The uncertainty of the measurements was about ±1°.

**XPS analysis:** A photoelectron spectroscopy (XPS) system, (model: SPECS-XPS, from SPECS, Germany) was used to analyse the element concentration and binding energies of the SAM modified silicon surfaces. The XPS system was equipped with a PHOIBOS 150-9 MCD energy analyser capable of detecting medium sensitivity elements to a trace level of 100ppm. Al K-alpha x-ray source (1486.74 eV) was used in the analysis. The atomic concentration was calculated using a commercial code (CasaXPS, Version 2.3). In the atomic concentration calculation, each peak area was integrated and scaled with a sensitivity factor of the peak followed by normalization with the total area of all peaks.

**EIS measurement:** High resolution impedance spectroscopy (INPHAZE. Sydney, Australia) was used for the electrical impedance measurements over frequencies from 10 mHz to 1 MHz. The silicon wafers were mounted in an INPHAZE three-electrode chamber with the functionalized surface facing up. The back side of the silicon was covered with a gallium-indium eutectic in order to make a low ohmic contact with the copper connecting electrode. The three-electrode chamber was filled up with a 100 mM potassium chloride solution (KCl) and an Ag/AgCl/KCl electrode was used as a reference electrode. A schematic of the measurement chamber is shown in Figure 2.4. The impedance spectrometer was capable of measuring both $i$ and $\nu$ to a resolution of
0.0025% as well as the phase difference $\theta$ between the sinusoidal voltage and current to a resolution of 0.001 degrees. The dielectric substructure of the monolayers was determined using Dielectric Structure Refinement (DSR) software (INPHAZE, Sydney, Australia) which decomposed the data for each monolayer into a series of sub-structural layers comprised of a capacitance shunted by a conductance element. In all of the measurements, the amplitude of the AC signal was kept smaller than 30 mV in order to ensure that the sample was not perturbed to a significant degree.

![Schematic photograph of a three terminal chamber.](image)

**Figure 2.4:** Schematic photograph of a three terminal chamber.

### 2.3. Results and discussion

#### 2.3.1 Characterization of hydrogen-terminated silicon surfaces

Ideally flat hydride-terminated silicon (111) surfaces can be prepared by etching in
40% ammonium fluoride solution (NH₄F). Although Si surfaces are always
terminated by a monolayer of hydrogen atoms, the atomic structure of the
hydride-terminated n-Si (111) surfaces highly depends on the pH. Ideal flattening is
obtained at approximately a natural pH (pH~8) of NH₄F solution. A lower or higher
pH will produce different rough morphologies and the roughness increases with time
and it has been found that it presents no preferential directions. Dissolved oxygen
can form etch pits, hence the NH₄F solution must be deoxygenated by bubbling with
an inert gas such as nitrogen or argon prior to the immersion of the silicon wafers.

Previous studies have shown that ideally flat surfaces are observed with n type silicon
(111) wafers but p⁺ type is rough. It has also been shown that atomically flat
surfaces are only obtained by using single side polished wafers, not double-side
polished wafers. This is attributed to cathodic protection by the rough side which acts
as a sacrificial anode.

X-ray photoelectron spectra of silicon wafers before and after etching with 40%
ammonium fluoride solution were used to assess the quality of the Si-H surfaces. XP
survey scans of n-Si (111) wafers after cleaning with hot Piranha solution (a mixture
of concentrated H₂SO₄ and 30% H₂O₂: 3:1 in volume) and rinsing with MilliQ water
displayed the well-known binding energies for bulk silicon 2p and 2s signals at ~100
eV and ~150 eV respectively, as well as a large 1s signal for oxygen at ~533 eV due
to the presence of the native silicon dioxide layer (Figure 2.5). A small peak at ~285
eV attributed to carbon 1s was also detected and was assumed to be the result of
organic contaminants deposited on the surface. The silicon 2p narrow scan (Figure 2.6a) shows a broad peak between 102 and 104 eV, indicating the presence of silicon dioxide. This broad peak disappeared after etching in 40% NH₄F solution for 20 minutes followed by rinsing with MilliQ water (Figure 2.6b).

![Figure 2.5: XPS survey scan of the nSi (111) wafer after cleaning in Piranha solution, then rinsing with MilliQ water](image)

![Figure 2.6: Narrow scan spectra of the silicon 2p region of the silicon wafer (a) before and (b) after etching in 40% NH₄F solution. The removal of the broad peak between 102 – 104 eV indicates the elimination of the native silicon dioxide layer.](image)

Previous studies²³,²⁴ have reported that rinsing with water after etching in ammonium fluoride solution causes the re-oxidation of the Si-H terminated surface. The freshly
prepared Si-H surface is hydrophobic, so the sample emerges dry from the 40% ammonium fluoride solution. Therefore, it is suggested that in order to obtain completely oxide-free Si-H terminated surfaces, the sample must not be rinsed with water after it is withdrawn from the etching solutions. However, our XPS scans detected the residues of carbon, nitrogen, oxygen and fluorine on the silicon surface after the etching step without rinsing (Figure 2.7a), even cleaning with tissues did not show any improvement (Figure 2.7b). The surface rinsed with water after etching did not show any residues of nitrogen or fluorine ions; however the surface still contained residual carbon and oxygen peaks. It was supposed that the adventitious carbon and oxygen were the contaminants during the water rinsing step. The XP narrow scan of the silicon 2p region (Figure 2.7d, e and f) did not show any detectable silicon dioxide signals, indicating that quickly rinsing the samples with MilliQ water did not lead to the re-oxidation of Si-H terminated surfaces.
Figure 2.7: XP survey spectra and Si 2p narrow scans of a Si (111) wafer after NH$_4$F solution etching (a and d), without any cleaning (b and e) and with tissue cleaning (c and f) with deionized water. It was found that fluorine and nitrogen ions from nitrogen gas bubbled through the ammonium fluoride solution could remain on the freshly etched silicon surfaces unless the fresh surface was quickly rinsed with MilliQ water. This rinsing step did not lead to the formation of
detectable traces of silicon dioxide, but was associated with low levels of adventitious carbon and oxygen contaminants.

2.3.2 Characterisation of alkyl monolayers: photochemical hydrosilylation of 1-tetradecene and undecenoic acid

Alkyl monolayers directly grafted on silicon surfaces were produced by the reaction of unsaturated terminal alkenes with hydride terminated silicon surfaces under UV illumination and characterised by XPS, water contact angle and EIS measurements to assess the quality of the variously prepared layers in comparison with other literature data.

2.3.2.1 Influence of reaction condition and reagent purity on monolayer quality

The quality of the alkyl monolayers was found to be highly dependent on the reagent purity and the handing process. Dissolved oxygen and water have a higher reactivity with Si-H surfaces than alkenes. It has been reported\textsuperscript{25} that employing strict Schlenk line conditions or bubbling the alkenes for at least one hour with an inert gas can minimize the effects of oxygen on alkyl monolayers. In this research oxygen was excluded by bubbling the alkenes with nitrogen gas for at least 90 minutes. Secondly it is necessary to use semiconductor chemicals of the highest grade available for the etching and cleaning steps during the preparation of the Si-H surfaces. Low grade 40\% ammonium fluoride solution showed moderate to high levels of silicon dioxide residue. The freshly etched surface using a high grade NH\textsubscript{4}F solution was found to be oxide-free (see above), but contaminated by fluoride and nitrogen ions, as has been
reported elsewhere. Although quickly rinsing the samples after etching can remove the fluoride and nitrogen ions, it should be noted that the hydride terminated silicon surface is stable for a few minutes in the air, but re-oxidated in water in only a matter of seconds.

In addition, when Si-H surface samples react with alkene molecules during the photochemical hydrosilylation, the reaction must be carried out under an inert atmosphere. UV light can convert oxygen into ozone, the latter having a higher reactivity than alkenes with Si-H surfaces which can lead to silicon dioxide formation during the preparation of alkyl monolayers. Thus while high quality alkyl monolayers on Si (111) were obtained (see example in Figure 2.8a), the XP narrow scan of the silicon 2s and 2p regions of other samples exhibited a broad peak at 102 - 104 eV, demonstrating that an amount of silicon dioxide was formed (Figure 2.8b).

![Figure 2.8: XPS narrow scan of the silicon 2p region of (a) high quality and (b) low quality tetradecyl monolayers on Si (111) surfaces, respectively. The broad peak between 102-104 eV shown in (b) indicates the trace of silicon dioxide which was attributed to re-oxidization during the preparation of alkyl monolayers.](image)

XP narrow scans of the carbon 1s of high and low quality alkyl monolayers are shown.
in Figure 2.9a and 2.9b respectively. The carbon 1s peak of the high quality monolayer was fitted with a single peak at 285.0 eV, which corresponded to the C-C binding energies of the carbons in the alkyl chains. The XP narrow scan of the carbon 1s of low quality layer exhibited a shoulder peak at C-O (~286.5 eV), which indicated the degradation of the alkyl monolayers.

**Figure 2.9:** XPS narrow scan of the carbon 1s region of the high quality and low quality tetradecyl monolayers on Si (111) surface. One intensive peak at 285.0 eV corresponded to the C-C binding energies of the carbons in the alkyl chains. An additional peak found at 286.5 eV (C-O) in (b) indicated that the combination of UV and oxygen could degrade the alkyl monolayers.

The oxygen 1s signal of the high quality alkyl monolayers (Figure 2.10a) displayed a high binding energy at 533 eV; however, the low quality alkyl monolayers (Figure 2.10b) showed the oxygen 1s signal at 532 eV. These peaks are in agreement with the ones usually reported for C=O groups in the binding range from 531 eV to 532 eV and for C-O bond typically observed for binding energies of 533 eV – 534 eV. However, some caution must be taken in the relative intensity of the peaks, since the non-bridging oxygen (Si-O: 533 eV) and bridging oxygen (Si-O-Si: 532 eV) have
similar binding energies and we therefore cannot neglect the possibility of a small amount of such linkages. We suppose that non-bridging oxygen formation contributed to the contamination after cleaning the NH₄F solution etching silicon wafers, but before the preparation of the self-assembled monolayers. The non-bridging oxygen signal observed on high quality alkyl monolayers was ascribed to adventitious sources and not oxidized silicon species. In contrast, bridging oxygen was attributed to the degradation of alkyl monolayers when the monolayers were prepared under both UV and oxygen conditions. The oxygen 1s region coincided with the Si 2p region (Figure 2.10b) where an obvious peak at 102-104 eV identified the formation of silicon dioxide layers.

![Figure 2.10: XPS narrow scan of the oxygen 1s region of (a) high quality and (b) low quality alkyl monolayers on Si (111) surfaces.](image)

Our results coincide with other reports to the possible microscopic mechanisms of alkyl monolayer degradation. The combination of UV and oxygen does not only induce re-oxidation on Si-H surfaces, but it also degrades the alkyl monolayer. XPS results showed a broad peak at 102 ~ 104 eV, which is associated with silicon dioxide. An additional peak found at 286.5 eV of the narrow scan of the
carbon 1s region indicated a loss of C-C chains. It is possible that atomic oxygen may penetrate a few carbons into the alkyl monolayer and react with the H on the alkyl chains. In addition, the radicals formed during the hydrogen abstraction process may recombine, leading to cross-linking (Figure 2.11).

![Figure 2.11: Possible complex components formed on silicon surface due to the degradation of alkyl monolayer under the combination of UV and oxygen.](image)

2.3.2.2 Carboxylic acid-terminated monolayers: photochemical hydrosilylation of undecenoic acid

Alkyl monolayers with methyl terminal groups produce a stable surface, but they are not suitable for further reaction. The choice of functional end group is restricted by its chemical reactivity toward the silicon surface. Previous studies have reported that some alkyl monolayers terminated with –OH, -CHO- or NH₂ cannot be obtained directly by the thermal reaction because the function of Si-O-C or Si-N-C linkages is to compete with the addition to the C=C double bond. Carboxyl end groups also react with the silicon surface to form siloxane esters SiO(CO)R by thermal reaction. Therefore, the surface must be reacted with a precursor bearing a protected end group such as an ester (-COOCH₃), which then produces ester-terminated alkyl monolayers.
that can subsequently be hydrolysed to obtain the acid-terminated alkyl layer. The drawback of this technique involves the difficulty in achieving complete hydrolysis of the ester-terminated groups and the final surface produced in this manner is likely to have both ester and acid end groups. However, it was reported that the photochemical hydrosilylation of undecenoic acid on a Si-H surface does not lead to an appreciable reaction between the carboxyl groups and the silicon surface.\(^{34}\) It should also be noted that a thorough rinse with hot CH\(_3\)COOH solution followed by water can obtain a 100% acid-terminated silicon surface.\(^{35}\)

![Graph](image)

**Figure 2.12:** XP survey spectrum and silicon 2p narrow scan (insert) of a carboxyl acid terminated alkyl monolayer directly on a Si (111) surface formed by photochemical hydrosilylation of undecenoic acid.

Figure 2.12 shows the XP survey spectrum of a hydrogen-terminated Si (111) surface coated with a monolayer of undecenoic acid in a UV mediated hydrosilylation reaction. Silicon 2p and 2s (99 and 151 eV), carbon 1s (285 eV) and oxygen 1s (532 eV) were detectable as expected. No other residual contaminations such as fluoride or
nitrogen ions were seen. The high resolution XP spectrum of the silicon 2p region (insert) did not show a significant peak between 102 and 105 eV characteristic of oxidized silicon species, confirming the high quality of the samples.

The high resolution spectra of the carbon 1s and oxygen 1s regions shown in Figure 2.13 were consistent with the presence of carboxyl acid terminated monolayers. The XP narrow scan of the carbon 1s region (Figure 2.13a) consisted of a large peak centred at 285.0 eV due to the C-C bond of the alkyl chain and a smaller peak at a higher binding energy (~290.0 eV) assigned to the –COOH group. The oxygen 1s region exhibited the characteristic binding energies of –C-OH and –C=O oxygen at 534.4 eV and 533.0 eV attributed to the carboxyl acid from the monolayer.

![Figure 2.13: XP narrow scan of the carboxyl acid terminated monolayer on the Si (111) surface for (a) the carbon 1s region and (b) the oxygen 1s region. In the XPS survey spectrum, the carbon 1s signal and oxygen 1s signal were detectable at 285 eV and 532 eV respectively. Very little silicon dioxide was found, which would otherwise be revealed in the silicon 2p narrow scan. The XP narrow scan of the carbon 1s region was composed of two peaks: one large peak at 285 eV due to the alkyl chain (C-C) and one at a higher binding energy about 290 eV assigned to the carboxylic acid group (–COOH). The oxygen 1s region showed a broad peak at about 533 eV which corresponded to the C=O oxygen of the carboxylic acid group of the alkyl monolayer.](image)
2.3.3 Water contact angle measurements

Water contact angle measurements were performed to provide further assessment of the quality of the surfaces. Examples are shown in Figure 2.14. The contact angle for native silicon dioxide was 23° and attributed to its hydrophilic nature. When the Si (111) surface was terminated by hydrogen after being etched in 40% ammonium fluoride solution, the water contact angle for the surface changed to 86°. For the high quality tetradecyl monolayer, the contact angle was found to be 101°, which is in good agreement with the formation of dense-packed monolayers with a uniform surface of pure methyl character. The low quality tetradecyl monolayer showed a
smaller contact angle of 81°, suggesting less order of packed alkyl chains as shown in Figure 2.11. The Si (111) surface coated with undecenoic acid exhibited a much smaller contact angle in the order of 55°. Two factors could influence the properties of the contact angle for this type of organic molecules: one is the hydrophilic nature of the carboxyl acid (-COOH) group due to the charge separation and the other is the loss of close-packed chains due to the spatial structure of the acid group.

2.3.4 Electronic Impedance Spectroscopy (EIS) studies

We used the equivalent circuit shown in Figure 2.15 to model our EIS results. It consists of a solution resistance (R_s) in series with a parallel network of a capacitor (C) and a conductor (G).

![Equivalent circuit](image)

**Figure 2.15:** Equivalent circuit used for the evaluation of the impedance data. R_s denotes the solution resistance, C stands for the layer capacitance, and G is used to evaluate the conductance of the layer.

EIS measurements are made by injecting a known alternating current, \( i = i_0 \sin(\omega t) \), of small amplitude \( i_0 \) (15 millivolts root-mean-square) and angular frequency \( \omega \) into the sample via the working and counter electrodes. The voltage response \( i = i_0 \sin(\omega t + \theta) \) is measured using the reference and working electrodes whence the impedance magnitude \( (v_0/i_0) \) and phase \( \theta \) is calculated. The current and
voltage are frequency-dependent and have different phases and therefore the impedance $Z$ is defined as $Z = \frac{V}{I} = Z' + jZ''$. EIS results are often presented as plots of the magnitude ($|Z|$) and phase angle ($\theta$) of the impedance as a function of frequency $f$. In the present study, the measurements were made over wide ranges of frequency from 10 mHz to 1 MHz. Alternatively, it is also presented as capacitance (C) and conductance (G) as a function of frequency.

2.3.4.1 EIS analysis of silicon dioxide

Figure 2.16a shows the impedance spectra of native silicon dioxide on a Si (111) surface in a 100 mM KCl solution, measured at a constant DC potential. The highest impedance, in excess of 500 $\Omega \text{m}^2$, was observed at the lowest frequency. The impedance decreased linearly on the log-log plots of impedance vs. frequency with increasing frequencies, while the phase angle ($\theta$) shown in Figure 2.16b is close to -90° (~89° at $f = 1$ Hz) up to a frequency of 100 Hz. Since an ideal capacitor will manifest a phase angle of -90°, in the frequency range from 0.1 Hz to 100 Hz, the native silicon dioxide behaves like a pure capacitor. At the lowest frequencies (0.01 Hz < $f$ < 0.1 Hz), the phase angle deviates slightly from -90°. This is attributed to the presence of a conductive component due to the structure of silicon dioxide, leading to a resistance in parallel with capacitance. Above ~1 kHz, the impedance reaches a limiting value and the phase angle approaches 0° (~3.1° at 100 kHz); in this region the impedance is dominated by the solution resistance. Because the solution resistance is determined in part by the physical separation between the sample and reference.
electrode, the apparent transition from capacitive to resistive behaviour (~ 10 kHz) is dependent on the cell geometry of silicon dioxide. Moving the reference electrode (Ag/AgCl) closer to the sample would induce a decrease in the solution resistance and extends the capacitive region out to higher frequencies. The capacitance measurements as a function of frequency shown in Figure 2.16c lead to an estimate of the thickness of the layers as follows:

$$d = \frac{\varepsilon_0 \varepsilon_r}{C/A}$$  \hspace{1cm} (1)

where $\varepsilon_0$ is the dielectric permittivity of free space ($= 8.85 \times 10^{-12} \, \text{F} \, \text{m}^{-1}$), the $\varepsilon_r$ of silicon dioxide is 4.4 and the area of the sample is $1.81 \times 10^{-5} \, \text{m}^2$. A capacitance of 0.028 F/m$^2$ is obtained by fitting the insert circuit shown in Figure 2.16c for silicon dioxide, which yields a layer thickness of about 1.4 nm. This is in agreement with the thickness of the structure.$^{36-39}$
Figure 2.16: Electrical measurements of silicon dioxide on a Si (111) surface in contact with a 100 mM KCl solution. (a) Impedance as a function of frequency, biased at the constant DC potential, (b) Phase angle as a function of frequency, (c) Capacitance as a function of frequency. The points are the average of 5 spectra and the error bars (generally smaller than the size of the symbols used in the plots) show the standard deviations of the errors for measurements.

2.3.4.2 Characterization of a methyl-terminated alkyl monolayer on silicon (111) surfaces

The impedance spectra of tetradecyl monolayers attached directly on Si (111) surfaces as a function of frequency are presented in Figure 2.17a. These show a similar trend,
as the results obtained with silicon dioxide, except that the impedance at the lowest frequency was highest. Significantly, at higher frequencies where the impedance is dominated by the solution resistance, the impedance data was constant while the phase angle was near zero. In the low frequency region for the monolayer derivatized Si (111) surface, the capacitance of the monolayers represents significant impedance. Therefore, log |Z| decreases linearly with the increasing of log f; with a slope of -1 as the phase angle is approximately -90° (Figure 2.17b). This is consistent with the tetradecyl monolayer being an insulator. At extra low frequencies the phase angle shifts away from -90° toward 0 as the frequency is decreased; we attribute this behavior to the presence of a few conductive defect sites in the monolayers. This view is consistent with the XPS measurements presented in Section 3.2.1 which demonstrated the presence of contaminants and a slight re-oxidation of the Si (111)-H surface in the preparative process of photochemical hydrosilylation.

Figure 2.17c shows the measured capacitance of a tetradecyl monolayer on the Si (111) surface in contact with a 100 mM KCl electrolyte. The curve was fitted using a parallel capacitor C and conductor G combination. It clearly shows that C is given by the measurements below 1 kHz that are characterized by the frequency-independence of the capacitance over this frequency range. A capacitance of 0.011 Fm⁻² was obtained for the tetradecyl monolayer. Equation (1) then yields an estimates thickness of d = 1.7 nm (assuming ε_r is 2.1), which is in agreement with the study by Wong et.al. in 2007.39
Figure 2.17: Electrical measurements of alkyl monolayers on a Si (111) surface in 100 mM KCl solution. (a) Impedance as a function of frequency, biased at the constant DC potential, (b) Phase angle as a function of frequency, (c) Capacitance as a function of frequency. The points are the average of 5 spectra and the error bars (generally smaller than the size of the symbols used in the plots) show the standard deviations of the errors for measurements.

When alkene molecules assemble on a Si (111) surface, C=C groups chemically bond with atoms of Si and alkyl chains are titled off the normal axis as shown in Figure 2.18. The thickness, d, is the distance of the reference electrode from the monolayer, which is normal to the silicon surface, not the length of the alkyl chains. The chain length of 1-tetradecene is calculated to be 2.14 nm\(^4\) and the experimental result for estimation of thickness of 1-tetradecene attached on the silicon surface is 1.65 nm.

Since both the thickness of the layer and the molecule length are known, the canting angle, \(\theta\), could be obtained using simple trigonometry below:

\[
\cos \theta = \frac{\text{thickness}}{\text{chain length}} = \frac{d}{l}
\]

The canting angle for 1-tetradecene on the silicon surface is 39°.
2.3.4.3 Characterization of an acid-terminated monolayer on silicon (111) surfaces

The Si (111)-H surface with an attached acid-terminated monolayer had impedance spectra that were very different from those of the methyl-terminated monolayer described in Section 3.4.2. The absolute value of the impedance on a logarithmic scale (Figure 2.19a) and the phase angle (Figure 2.19b) are plotted as a function of frequency (on a logarithmic scale). The impedance spectrum reveals that region where the impedance is dominated by the series resistance of the solution is well defined and the impedance becomes largely independent of the frequency. The phase angle in this region is essentially zero. In the frequency region where the capacitance dominates the impedance, the logarithm of the impedance magnitude no longer varies with log-frequency and dispersion with frequency of the impedance is observed at ~1 Hz which is also reflected in the phase angle as a function of frequency. We attribute this behaviour to the presence of the carboxyl acid functional groups (-COO\(^{-}\)) at the
terminus of the monolayer. The dispersion at frequencies smaller than 1 Hz represents the acid groups and the frequencies between 1 Hz and 100 Hz is associated with the hydrocarbon groups of the monolayer.

The Si (111)-H surface with an attached acid-terminated monolayer can be described by an equivalent circuit consisting of two GC elements, as shown in Figure 2.19c. The capacitance $C_1$ is in parallel with a conductance $G_1$ and represents the region containing the acid groups. $C_2$ and $G_2$ represent the layer containing the hydrocarbon, alkyl groups of the monolayer.

Fitting the data to this model yielded a value of $0.072 \text{ Fm}^{-2}$ for the capacitance $C_1$ of the layer containing the acid groups, corresponding to a thickness of 0.26 nm for COO$^-$ layer. The capacitance $C_2$ of the hydrocarbon groups in the monolayer was similarly found to be $0.033 \text{ Fm}^{-2}$, which yields a thickness of about 0.97 nm for the layer. Therefore, the total thickness of the acid-terminated monolayers on the silicon surfaces ~1.3 nm and is in good agreement with other observations.\(^{40}\)
Figure 2.19: Electrical measurements of an acid-terminated monolayer on the Si (111) surface in 100 mM KCl solution. (a) Impedance as a function of frequency, biased at the constant DC
potential, (b) Phase angle as a function of frequency, (c) Capacitances as a function of frequency. The points are the average of 5 spectra and the error bars (generally smaller than the size of the symbols used in the plots) show the standard deviations of the errors for measurements.

2.3.5 Silicon doping effects

2.3.5.1 Doping of silicon

Silicon is intrinsically a semiconductor. This essentially means that the electrons fill the available energy levels below the Fermi level and electrical conduction involves the elevation of electrons across an energy band-gap above the Fermi level from the conduction band above the Fermi level. When silicon is “doped” with a low concentration of either a tri-valent or penta-valent element the material becomes a much better conductor. When doped with a penta-valent element the extra electron available provides electrons as charge carriers and the material is said to be an “n type” or if doped with a trivalent element electronic vacancies or “holes” are created and the material is known as “p type” in which the charge carriers are positively charged “holes”.

2.3.5.2 Tetradecyl monolayers on highly doped p-Si (111) surfaces

To assess the effects of doping of the silicon, self-assembled monolayers directly grafted on p type Si (111) surfaces were studied using EIS and compared with layers on n type silicon surfaces.

Figure 2.20a shows that the capacitance of the model fitted to the data yields a value of 0.028 Fm\(^{-2}\) for the SAMs of 1-tetradecene molecules grafted on p type Si (111)-H
surfaces, which yield a monolayer thickness of about 0.66 nm. This is not in agreement with the geometric length. It should be recalled (Section 3.4.2) here that for the case of the 1-tetradecane SAMs on n type silicon, the monolayer thickness deduced from the EIS data did agree with the molecular dimensions of these molecules. If we assume that in the case of the p type silicon, the capacitance value deduced was actually that of silicon dioxide, then the thickness of the layer would have to be 1.4 nm (Figure 2.20b), which indeed is in the range of known thickness of the silicon dioxide layer present in bare silicon surface. Comparing the electronic properties of the layers on n type Si (111) surfaces to those on highly doped p type Si (111) surfaces, the capacitances of the latter were two times higher than the former and were similar to the capacitances of native silicon dioxide; however, the conductance of the layers on p type Si (111) were about twenty times higher than the conductance of native silicon dioxide. Therefore, it seems plausible that the layers on highly doped p type Si(111) surfaces are composed of both silicon dioxide and tetradecyl monolayers. In the low to medium frequency range, a tetradecyl monolayer grafted on an n Si(111)-H surface behaves like a pure capacitor and its phase angle is close to -90° (Figure 2.17b), while a tetradecyl monolayer attached on p type Si (111)-H surface shifts away from -90° and toward 0 (Figure 2.20c), indicating that the monolayer is not densely-packed, consists of many defect sites and has a much greater conductance.
Figure 2.20: Electrical measurements of a tetradecyl monolayer on a p type Si (111) surface in 100 mM KCl solution. (a) Assuming the capacitance was that of the tetradecyl monolayer only, (b) Assuming the capacitance was that of a layer of silicon dioxide (c) Phase angle as a function of frequency. The points are the average of 5 spectra and the error bars (generally smaller than the
size of the symbols used in the plots) show the standard deviations of the errors for measurements.

The canting angle of the alkyl monolayer on both n- and p-type Si-H surfaces was calculated by the thickness formula \( d = 0.126 \times (n-1) \times \cos \theta + 0.186 \), \( n = 14 \) for carbon atoms, assuming that the C-C bond and Si-C bond lengths projected along the molecular axis are 0.126 nm and 0.186 nm respectively.\(^{41}\) The canting angle of the alkyl monolayer on an n type Si-H surface is 39º which is in agreement with other experimental data,\(^{42-44}\) with canting angles in the range of 30º - 45º for \( n > 10 \). However, the canting angle for the monolayers on p type silicon calculated from the dimensions deduced from the EIS measurements were 52º. This is not reasonable for a densely-packed monolayer. The quality of the monolayer was found to be strongly dependent on the doping type, indicating that the silicon surface potential plays an important role in depositing the alkyl monolayer. The band-bending near the n type silicon surface exhibits an upward movement\(^{11}\) that drives the electrons into the bulk of the silicon and accumulates holes near the surface, leaving a more positively charged surface that is more susceptible to nucleophilic attack by alkenes. Conversely, the downward band-bending of the p type silicon surface leads to an accumulation of electrons near the surface which can repel nucleophilic attack. This view is confirmed by X-ray photoelectron spectroscopy data presented below in Figure 2.21.
Figure 2.21: XPS narrow scan of the silicon 2p region of tetradecyl monolayer formation (a) on an n type Si (111), (b) p type Si (111) surface, and (c) the narrow scan of the carbon region of the tetradecyl monolayers on both n and p types of silicon.

The silicon 2p signals of the tetradecyl monolayer grafted on n type Si(111)-H surface (Figure 2.21a) indicate that no oxygen reformed during the photochemical hydrosilylation reactions (absence of peaks in the range from 102 ~ 106 eV). However, the broad peak between 102 eV – 104 eV detected on the p type Si (111)-H surface reveals that the surface was re-oxidized in the process of monolayer preparation (Figure 2.21b). The carbon 1s signals of the alkyl monolayer surface on the n type silicon surface (Figure 2.21c) produced a main peak centered at 285 ev which was in response to the alkyl chain (C-C), but the monolayer on the p type silicon surface
showed an additional peak at a higher binding energy around 290 eV assigned to the carboxylic acid group (-COOH). Moreover, the intensity of C 1s indicates that the tetradecyl monolayer formed on the n type silicon surface was approximately two times higher than the p type which was in broad agreement with our EIS results.

2.4 Conclusion:

The results presented in this chapter revealed that UV mediated hydrosilylation reactions of both methyl-terminated and acid-terminated alkenes can form dense-packed and homogenous monolayers on n-Si (111) surfaces. The quality of the monolayers was highly dependent on the methods of preparation. In general, we found that rinsing silicon samples with water after 40% ammonium fluoride etching could eliminate the contamination of nitrogen and fluorine ions and that rapid rinsing of the etched silicon samples did not induce the re-oxidation of Si-H surfaces. The photochemical hydrosilylation reactions of alkenes must perform under inert gases, such as nitrogen or argon, because the combination of UV light and oxygen not only re-oxidizes the hydrogen-terminated silicon surface, but also degrades the alkyl monolayer. The combined analysis of XPS, water contact angle and impedance spectroscopy measurements revealed the insulating nature of the monolayers. The estimated thickness of the methyl-terminated monolayer (1-tetradecene) was 1.7 nm and the acid-terminated monolayer (undecenoic acid) was 1.3 nm. For the undecenoic acid monolayers, the layer containing the acid moiety could be clearly distinguished as it manifested a clear additional dispersion in the capacitance as a function of
frequency.

The effect of doping of the silicon on the properties of tetradecane monolayers grafted onto the silicon surface was investigated using both XPS and EIS. It was shown that well-ordered monolayers could be easily obtained by irradiating an n type Si (111)-H surface with UV (254 nm) light in the presence of alkenes, but not on a p type Si (111)-H surface. Based on our results the proposed mechanism concerning the splitting of Si-H under UV irradiation to form a radical should be rejected, because if this proposed mechanism were real, the alkene molecules could form dense-packed monolayers on both n and p type silicon (111) surfaces. We believe that our results can be explained by the excitation mechanism. Under UV irradiation the band-bending near the n type silicon surface exhibits an upward movement that drives the electrons into the bulk of the silicon and accumulates holes near the surface, leaving a more positively charged surface that is more susceptible to nucleophilic attack by alkenes. Conversely, the downward band-bending of the p type silicon surface leads to an accumulation of electrons near the surface that can repel nucleophilic attack.

In conclusion, high quality monolayers with functional groups formed on n type Si (111) surface could protect the silicon from oxidation serve as cross-linkers for further chemical reactions with biomolecules with potential applications for biosensors.
2.5 References


37. Bard, A. J., Faulkner, L. R., 2001 *Electrochemical Methods, Fundamentals*


Chapter 3

Antigen-antibody interactions on Si-C surfaces

3.1 Introduction

It is well known that an antigen has a high affinity to its corresponding antibody and that this has great potential in clinical and research studies. Therefore, the use of antigen or antibody labeled conjugates to quantify analytes of interested has been extensively investigated. A review is given by Mirsky et al.\(^1\) A commonly used method of analysis based on antigen-antibody binding known as enzyme-linked immunosorbent assay (ELISA) involves the following processes. First, an antibody is chosen which has the property of binding to a particular antigen. Next, when the antigen is immobilized, then an antibody (secondary antibody) is added in the reaction. This antibody has the ability to detect the antigen. Once the antibody enters the reaction, it finds the antigen, attaches to it and forms a complex. Then, either an enzyme or another antibody is used to link to the secondary antibody. Finally, an enzymatic substrate is added in the process, to that the quantity of the antigen in the sample can be indicated. This enzyme releases a signal through which detection of quantity of antigen is made possible.\(^2,3\) There are two types of ELISA:

**Competitive ELISA:** in this method, unlabeled antibody and its antigen are incubated together. These antigen-antibody complexes are then brought near the antigen-coated surface. The secondary antibody is added which detects this complex. This secondary antibody is coupled to the enzyme. A substrate is added and remaining enzymes release a specific signal.\(^3,4\)
**Sandwich ELISA**: ELISA can also be described as a sandwich model with the antigen located between two specific complementary antibodies. Because the second antibody is commonly tagged by an enzyme, which could produce molecules that are coloured or electroactive, the analyte is detected by a change in colour or current. Because the ELISA can detect either the presence of antigen or the presence of antibody in a sample, it can be used to determine serum antibody concentrations in the diagnose chronic infection. It is also useful for detecting potential food allergens in the food industry. However, ELISA is time-consuming and requiring several steps and labels.

Over the past few decades with studies on biosensors, efforts have moved from labeled immunoassays to non-labeled techniques which could lead to simplification, increased speed and reliability as well as lower costs. Newman et al developed a label-free capacitive biosensor based on changes in the dielectric constant due to antigen-antibody interaction. The majority of capacitive signals should derive from the antibody layer, so the choice of substrate used to immobilize the antibodies is very important. Although many different types of surfaces such as metals or semiconductors have been used as substrates for antibody attachment, the requirement for reproducible and stable surfaces has placed increased emphasis on the preparation of antibody-modified surfaces that are extremely homogeneous. The rough surface of gold induces the presence of apertures and grooves where the ions in the solution penetrate the film and discharge directly onto the gold surface. This would form an
ionic double layer with very high impedance. The ionic double layer serves as an electrical barrier to the coupling of inorganic devices to biological systems. The electrical drift caused by the ionic double layer reduces the sensitivity to detect the analyte in the solution.\textsuperscript{10} Immobilization of the antibody directly on a silica substrate yields a change in capacitance with the antigen-antibody interaction, but with low sensitivity.\textsuperscript{11} This phenomenon can be explained by the difficulty in controlling the density of Si-OH groups generated on the surface that are necessary for chemical linkages. In addition, the change in the dielectric constant that occurs on binding of antigen and antibody is relatively small. Charged silica under physiological condition (pH=7)\textsuperscript{12} adds to the electrical resistance\textsuperscript{13} and therefore limit the sensitivity of the sensor devices. Ideally, the signal change should only arise from the biological recognition event, not from the charged silicon dioxide or defect sites in the film.

It has been demonstrated that hydrogen-terminated silicon surfaces\textsuperscript{14,15} allow for the construction of a densely-packed organic film via direct Si-C covalent bond formation.\textsuperscript{16-19} This Si-C bond formation serves as a starting point for antibody attachment and results in high selectivity, good stability\textsuperscript{20,21} and a low density of electrically active defects.\textsuperscript{22-25} In the past few years, there have been significant developments for chemical functionalization and passivation of hydride-terminated silicon surfaces using various silylation reactions in the presence of alkenes to form organic monolayers directly on Si (111) surfaces via Si-C bonds. Previous experiments\textsuperscript{26,27} have shown that the attachment of proteins was achieved by first
attaching a long-chain alkene bearing a protected end group at the surface. Next, the protected end group was hydrolysed to a carboxylic group which remained chemically accessible and could be replaced with a variety of standard chemical reagents, and, finally, it was linked to the antibody. Recent studies have demonstrated that carboxyl groups can be introduced onto silicon surfaces via a single step reaction between hydride terminated silicon (Si-H) and undecenoic acid rather than undecenoic methyl ester. 28-30

In this chapter, we describe further research bases on Boecking’s work 31 to integrate the binding of antigen-antibody on Si-C linked carboxylic acid-terminated monolayers on silicon (111). we report investigations of the changes in electrical impedance and capacitance that occur when silicon (111) surfaces were modified with human IgG using direct Si-C bond formation chemistry and then exposed to complementary and non-complementary human IgG molecules in solution. We chose monolayers based on 12-carbon chains with a carboxylic acid terminal group. The carboxylic acid group was then activated by carbodiimide (EDC) and succinimide ester (NHS ester) to form an active ester which preferred to react with the primary amine group from the antibody.
3.2 Experimental methods

3.2.1 Materials

Single sided polished and backside etched n-Si (111) wafers of 100 mm in diameter, 550 ± 25 µm in thickness, and resistivity of < 0.01 ohm-cm (phosphorus-doped) were purchased from Virginia Semiconductor Inc. All cleaning and etching were clean-room grade. Sulfuric acid, 98% (H₂SO₄), hydrogen peroxide, (30% H₂O₂) and ammonium fluoride, (40% NH₄F) were supplied by Sigma.

All other chemicals were reagent grade or higher and unless otherwise specified, were used as received. MilliQ water (18 MΩ) was used for all experiments. 1-undecenoic acid (98%), N-ethyl-N-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Aldrich.

Investigations were performed using human immunoglobulin G (Human IgG, Sigma I4506) and human immunoglobulin M (Human IgM, Sigma I8206) grafted onto silicon (111) surfaces which was functionalized with an organic SAM terminating in a NHS-ester. The complementary antibodies anti-human IgG (F_ab specific, Sigma F9512) and anti-human IgM (µ-chain specific, Sigma F5384) were used to investigate antigen-antibody binding. Phosphate buffered saline (PBS, pH 7.4, 10mM) was used in all experiments and stored at 4°C. It was prepared by dissolving 1 PBS tablet in 200 mL of DI water. Glycine (0.1 M, Sigma G7126) was used to block excess carboxylic acid groups.
3.2.2 Surface characterization

Water contact angle measurement, X-ray photoelectron spectroscopy (XPS) analysis and electrical impedance spectroscopy (EIS) measurement were performed as described in chapter 2.2.4.

3.2.3 Functionalization of Si (111)-H surface

**Acid-terminated surface:** Functionalization of the Si (111) wafer to produce a surface with an undecanoic acid terminated SAM was performed as described in chapter 2.2.3.

**NHS-ester surface:** After the acid-terminated surfaces were obtained, we immediately continued with the conversion of the acid function to succinimidyl ester as follows: the acid-terminated surfaces were covered with 500 μL of an aqueous solution of NHS (25 mM) and 500 μL of an aqueous solution of EDC (75 mM) was added drop by drop and allowed to react at room temperature in the dark for 1 hour. It should be noted that NHS is not stable in an aqueous solution, therefore the solution containing NHS and EDC was prepared immediately prior to use. Similar to the previous step, rinsing is critical following NHS/EDC coupling. The sample was then rinsed with DI water for 30 seconds, soaked in DI water for 3 minutes and rinsed again with DI water for 20 seconds.

**Antibody immobilization:** After the conversion of acid-terminated surfaces to NHS
ester surfaces, the proteins were attached on the substrate. Here, we used human IgG and human IgM as a typical immunological model to show the procedures of antibody immobilization. The antibodies were preserved in a freezer (-20°C) to maintain their bioactivity. Before making the antibody solution, the antibodies were slowly thawed in a refrigerator at 4°C. PBS solution is widely used in biological reactions due to its constant pH value. The pH value and the concentration of the PBS buffer are two major issues: a higher or lower pH could change the bioactivity of the proteins and unsuitable concentrations of PBS could destroy the structure of the proteins. In this work, the PBS buffer solution had a pH value of 7.0 at room temperature and a concentration of 10 mM. The concentration of human IgG and human IgM was 10 μg/mL. A high concentration of antibody may induce a large aggregation on the surface, leading to large background noise. In contrast, a low concentration of antibody may not form a uniform layer on the silicon surface. The NHS-ester active surfaces were immersed in 1 ml of human IgG or human IgM and left to incubate for 45 minutes. NHS esters are highly reactive compounds suitable for the modification of amino groups. Since amino groups are always contained in proteins and peptides, the reaction of NHS esters with amines is especially common. In order to achieve a high degree of surface covering, the excess unbound antibodies can be removed by rinsing several times with PBS and the DI water. In this work, we rinsed the surface after antibody immobilization for 30 seconds with cold PBS solution, and then soaked the sample in DI water for 3 minutes. Next, the samples were rinsed with flowing water for 3 minutes and rinsed with DI water for another 30 seconds. This cleaning
procedure was repeated twice. Finally, a solution of 50 mM glycine in 10 mM PBS was incubated for 1 hour to block the non-reacted carboxyl acid sites. After rinsing with cold PBS and DI water, the samples were dried under a stream of nitrogen and stored in a nitrogen desiccator at room temperature.

**Anti-antibody attachment:** The human IgG-modified samples were immersed in a PBS buffer solution containing anti-human IgG or anti-human IgM (1mg/mL) and left to incubate for 1 hour at 37°C. Afterwards, the excess unbound anti-antibodies were removed with two cold PBS washes followed by rinsing with DI water. The sequence of the steps of anti-antibody attachment on antibody-modified surfaces is shown in Figure 3.1.

In summary, acid-terminated organic monolayers were first grafted on Si (111)-H surfaces through Si-C bonds. Second, acid groups were converted to NHS ester at room temperature for 1 hour. Third, human IgG attachments were obtained by immersing NHS ester surfaces in a human IgG solution. Next, glycines were used to block the remaining non-reacted sites. Finally the samples were ready for the antigen-antibody binding test.
Figure 3.1: Detection of anti-IgG molecules using IgG-modified substrate. (a) acid-terminated surface, (b) NHS-ester, (c) IgG attachment, (d) glycine blocking and (e) anti-IgG coupling with IgG (the schematic is not drawn to scale).
3.3 Results and discussion

Stepwise functionalizations of acid-terminated n type silicon (111) surfaces were studied using high resolution impedance spectroscopy (EIS) and confirmed by XPS measurements. EIS involves measuring an AC current that flows in response to a small, sinusoidal AC voltage applied across the interface. The current is generally not in phase with the applied voltage. The impedance $Z = \frac{V}{I}$ is described as a complex number $Z = Z' + iZ''$, describe the electrical properties of the interface. In a very simple model, $Z'$ arises from the conductance and $Z''$ is associated with the capacitance. Because biomolecular interfaces can have complicated structures, the impedance represents combined electrical response of the underlying silicon, the molecular interface and the buffer solution. Each of these physical structures has its own unique electrical properties and could be affected by the others. By measuring the conductance and capacitance as a function of frequency, it is possible to separate these contributions.

3.3.1 NHS-ester formation

Chemical activation of the acid functionalized surface with N-hydroxysuccinimide molecules obtains the NHS ester activated surfaces.

$$\text{Si(111)-C}_{10}\text{H}_{20}\text{COOH} + \text{NHS/EDC} \xrightarrow{1\text{hr at RT}} \text{Si(111)-C}_{10}\text{H}_{20}\text{COONHS}$$

We measured the impedance changes after the acid groups were converted to NHS
esters and exposure to the same electrolyte. Figure 3.2-a and b show the changes in impedance and phase angles. The same data is presented in the alternative form of conductance and capacitance in Figure 3.2-c and d. The conductance and capacitance plots clearly showed dramatic differences between the behaviors of the two systems at low frequencies. This indicates that this would be the frequency region where the best sensitivity was obtained for detecting the conversion of acid groups to NHS esters.
Figure 3.2: The (a) impedance, (b) phase angle, (c) conductance and (d) capacitance as a function of frequency before and after the acid groups converted to NHS esters (blue-acid group; red-NHS ester). The points are the average of 5 spectra and the error bars (generally smaller than the size of the symbols used in the plots) show the standard deviations of the errors for measurements.

At frequencies lower than 1 Hz (Figure 3.2a), the increasing impedance was due to NHS ester formation. The carboxyl acid group (COOH) in the aqueous solution at pH 7 is usually negatively charged (COO⁻) and can be neutralized by
N-hydroxysuccinimide molecules to form a neutral NHS ester. Figure 3.2-b shows that the phase angle of the acid functionalized silicon (111) at 1 Hz shifted away from -90° due to the negatively charged carboxyl acid groups. After neutralization with N-hydroxysuccinimide molecules, the phase angle was closer to -90° which is that expected for a pure capacitor. We assume that the increasing phase angle at extra low frequencies is due to the more hydrophilic nature of NHS esters compared carboxyl acid groups which we confirmed also by water contact angle measurements. NHS-ester activated surface showed much smaller contact angles in the order of 55°, since partial carboxyl groups were converted to NHS esters which were more hydrophilic nature.

At frequencies lower than 100 Hz, the EIS spectra measured after NHS ester formation were distinct with both the conductance and capacitance decreasing after the acid groups converted to NHS esters. At intermediate frequencies between 10^2 and 10^4 Hz, the EIS spectra were nearly overlapping. At high frequencies greater than 10^4 Hz, the EIS spectra did not depend on the chemical reactions that occurred on the organic monolayer surface. This demonstrates that, at high frequencies, the impedance is largely only sensitive to the composition of the electrolyte and the high frequency limit corresponds to the ohmic resistance of the solution.

To verify the stability of the organic monolayer in the aqueous solution and the changes in conductance and capacitance arising solely from the chemical reactions
between carboxyl acid groups and N-hydroxysuccinimide molecules, control experiments (not shown) were conducted in which an acid functionalized silicon (111) surface was exposed to the same electrolyte. Capacitance measurements showed that the acid-terminated silicon surface produced less than a 2% change at 1Hz, while a 36% decrease was observed when the acid groups were converted to NHS esters. This result confirms that the electrical changes observed in Figures 3.2a-d arise from the formation of NHS esters and not due to other factors such as physisorption or electronic drift.

An electrical circuit model can be used to understand the physical structure of a system in which the electrical response of the physical interface is fitted using various circuit elements. Because the samples were based on highly doped silicon wafers, we assume that the interface can be divided into two physical regions (neglecting the space-charged layer in the silicon because it is highly doped and has a high conductivity): the organic monolayers and the electrolyte. Previous studies have found that simple series and parallel combinations of resistors and capacitors can be used to model homogenous layers (Figure 3.3). Because the alkyl chain region of the undecenoic acid molecules and N-hydroxysuccinimide molecules have the same dielectric properties ($\varepsilon_r=2.1$), we used the same circuit to model the structures before and after converting the acid groups to NHS esters. The thickness of the organic monolayers was defined by $d = \frac{\varepsilon_0 \varepsilon_r}{C/A}$ where $\varepsilon_0$ is the dielectric permittivity of free space ($= 8.85 \times 10^{-12} \text{ F m}^{-1}$), $\varepsilon_r$ of the organic monolayer is 2.1 and the area of the
sample is $1.81 \times 10^{-5}$ m$^2$. A capacitance of 0.012 F/m$^2$ was obtained for the NHS esters layers which yielded a thickness of about 1.6 nm.

![Figure 3.3: Equivalent circuit used for the evaluation of the impedance data. $R_s$ denotes the solution resistance, $C$ stands for the layer capacitance, and $G$ is used to evaluate the conductance of the layer.](image)

Figure 3.3: Equivalent circuit used for the evaluation of the impedance data. $R_s$ denotes the solution resistance, $C$ stands for the layer capacitance, and $G$ is used to evaluate the conductance of the layer.

Figure 3.4a displays the XPS survey of Si-H surfaces modified with undecylenic acid showing signals due to carbon and oxygen at 285 and 535 eV. After the activation of carboxyl acid group with NHS/EDC (Figure 3.4b) at room temperature for 1 hour, new signals due to N 1s were observed. This is consistent with the chemical components of the organic layers on the surface.

![Figure 3.4: XPS survey of (a) undecenoic acid functionalized Si (111) surface and (b) after conversion of the acid to NHS ester.](image)

Figure 3.4: XPS survey of (a) undecenoic acid functionalized Si (111) surface and (b) after conversion of the acid to NHS ester.

Figure 3.5a shows that the narrow scan of the Si 2p region of the undecanoic acid
functionalized Si (111) surface displayed a slight silicon oxidation peak around 104 eV. A similar curve was obtained after conversion of the acid functional groups to NHS esters. The absence of broad peaks around 104 eV in the high resolution spectrum of the silicon surface modified with undecanoic acid is consistent with a process occurring without any apparent oxidation of the surface. Furthermore, the activation of the NHS/EDC ester process did not induce any measurable oxidation of the surface, indicating the good quality of the surface of the acid group. Figure 3.5b shows the high-resolution XP spectrum of the N 1s region before and after activation of NHS-activated surface. The two peaks at 400 and 402.6 eV correspond to C-N and N-O, indicating the formation of NHS esters. The carbon 1s signals of the undecanoic acid surface (Figure: 3.5c) were resolved into two characteristic peaks. The main peak centered at 285 eV was in response to the alkyl chain (C-C), while the peak at 290 eV indicated the carboxylic acid functional groups (O-C=O). After the conversion of the acid monolayer to an NHS-activated surface, the peaks at 285, 287 and 290 eV were attributed to the chemically different carbons of C-C, O=C-N and O-C=O, respectively. The high resolution spectrum for the O1s peak at 535.6 eV (Figure: 3.5d) shifted about 1.2 eV with respect to the corresponding peak (534.4 eV) in the acid spectrum due to the formation of a C-O-N bond in the NHS ester.
Figure 3.5: Narrow scan of (a) Si 2p, (b) N 1s, (c) C 1s and (d) O 1s of the (blue) undecanoic acid functionalized Si (111) surface and (brown) after conversion of the acid to NHS ester.
3.3.2. Antibody immobilization

Previous EIS studies\textsuperscript{35,36} focused on high frequencies (> 10 kHz) where the sensitivity of detecting proteins is highest. In their logarithmic plots of real and imaginary components of the complex admittance, the changes in electrical properties were hard to be observed at low frequencies, but became apparent at higher frequencies. Because the total impedance was small at high frequencies, the biological reaction-induced changes in electrical properties were more clearly by plotting the impedance as a function of frequency. However, the silicon and double-layer impedances are comparable in size at high frequencies, where proteins are most easily detected; separating the individual contributions is difficult. In our study, the plots of capacitance and conductance were used as alternatives to real and imaginary plots. It was found that it was easier to detect biomolecular layers at higher frequencies, but SAMs at lower frequencies. Figure 3.6a shows that at high frequencies (>10\textsuperscript{5} Hz) where the sensitivity to human IgG attachment is highest, the overall impedance is primarily controlled by the silicon space-charge region and the resistance of the biomolecular layer (human IgG). In this work, we used highly-doped n type silicon (111) wafers in which the impedance of the silicon substrate is very small, thus the total impedance at high frequencies becomes more sensitive to the biomolecular layers. At low frequencies (<1 kHz), the overall impedance is controlled by SAMs and the solution double layer. Therefore, the lower frequencies provide less sensitivity to biomolecular layer.
Figure 3.6b reveals that after human IgG attached to the NHS ester functionalized surface, the conductivity increased at high frequencies due to the charge groups in the antibodies. At low frequencies (from $10^{-2}$ to $10^{2}$ Hz), the impedance became dominated by the initially densely-packed self-assembled monolayers and the ionic environment. Since the addition of the additional new antibody leads to an increase in the thickness of the capacitive layer, the effective capacitance was, at first, expected to decrease. The EIS spectrum of the capacitance as a function of frequency was found to move downwards with the addition of N-hydroxysuccinimide molecules as shown in Figure 3.4d. However, with the introduction of human IgG, the capacitance curve (Figure 3.6c) at low frequencies moved slightly upwards. Since the theoretical change in capacitance is due to the addition of new layers over the silicon substrate, the capacitance model used to fit the system is a capacitor in parallel with a resistor as shown in Figure 3.3. However, this model is only suitable for homogenous and uniform electric fields. When antibodies are randomly grafted on a silicon surface, the surface becomes rough, leading to an unknown local modification of the electric field. In 2006, Albina et al.\textsuperscript{33} reported that capacitance values increased with surface roughness and, moreover, that the smaller the dielectric thickness, the larger the increase in capacitance. Therefore, the classical analytic formulation of capacitance, $d = \varepsilon_0\varepsilon_r C/A'$, does not apply and cannot help identify whether the changes in capacitance arise from chemical or biological reactions. We assume that the increase in capacitance not only arises from a roughness effect; but also from charged groups on the protein that changes the double layer. The underlying organic SAMs may indeed
develop some defects. The attachment of very large molecules such as antibodies is likely to lead to distortions of the otherwise well aligned alkyl chains of the SAMs. Therefore, the SAMs may become much more conducting. When a conducting element is paralleled with the capacitance of the SAMs, the overall capacitance with the antibody attached will increase. This is not because the underlying SAMs have become thinner; but because they have become more conductive due to the defects introduced by attachment of the very large antibodies. In addition, human IgG made the surface more hydrophilic; this leads to the possibility of a higher concentration of ions trapped in the organic monolayers and, hence, an increase in the monolayer capacitance and conductance (Figure 3.6d). A similar increase in capacitance with the addition of antibodies to a silica substrate was observed by Sibai et al.\textsuperscript{34} This hypothesis is confirmed by XPS measurements.
Figure 3.6: Capacitance and conductance of acid monolayer functionalized surfaces before and after human IgG immobilization: (a) and (b) in log scales; and (c) and (d) in linear scales.

To confirm that the changes in capacitance at low frequencies are due to human IgG attachment, the stability of the acid monolayer functionalized surface was tested in 10 mM PBS solution with and without human IgG, as shown in Figure 3.7.
Figure 3.7: Stability of acid-terminated monolayer surfaces in 10 mM PBS solution with and without 1mg/ml human IgG for 1 hour.

Figure 3.7 shows that at low frequencies (0.01, 0.1, 1 and 10 Hz) the acid-terminated monolayer samples immerse in 10 mM PBS solution without human IgG for 1 hour did not experience a change in capacitance (e.g. 0.013 Fm$^{-2}$ at 0.01 Hz), while exposure to human IgG caused the capacitance to increase significantly to 0.016 Fm$^{-2}$ at 0.01 Hz. These results demonstrate that the changes in capacitance are derived from human IgG attachment.

We used X-ray photoelectron spectroscopy (XPS) to analyse the chemical composition of the surfaces before and after human IgG attachment to evaluate the nature of the chemical bonding associated with the transformations that occurred on the surface. Figure 3.8a shows the XPS survey of the NHS-activated ester monolayer surfaces showing silicon 2p and 2s signals at 99 and 151 eV, carbon 1s signal at 285 eV, nitrogen 1s signal at 402 eV and oxygen 1s signal at 532 eV.
human IgG to the monolayers via EDC/NHS activation is verified by the large increase in the nitrogen, carbon and oxygen signals in the XPS survey spectrum shown in Figure 3.8b.

**Figure 3.8:** XP survey spectra of (a) undecanoic acid monolayers after activation with EDC/NHS and (b) after coupling with human IgG.

Figure 3.9a shows the high-resolution spectrum of the silicon 2p region before and after human IgG attachment. There are apparent peaks at a higher binding energy around 104 eV after coupling with human IgG, which is indicative of adventitious oxidation of the surface. Since the NHS-terminated monolayer is less compact and more hydrophilic than the undecanoic acid-terminated monolayer on the silicon (111) surface, it can be expected that reactive oxyanions\(^ {35} \) will penetrate this monolayer more easily during the process of human IgG attachment. As a result, oxidation of the underlying silicon is more easily initiated for the NHS-modified silicon. Local re-oxidation makes the organic monolayer separate from the silicon surface and results in the formation of pores in the monolayer where the ions from the electrolyte...
could penetrate. This would increase the dielectric difference of the organic monolayer and, hence, increase the monolayer capacitance associated as revealed by the results obtained by EIS.

Figure 3.9b shows the narrow scans of the nitrogen 1s region before and after human IgG coupling. The peak at 400 eV is relatively broad, presumably due to the peptide backbone of the antibody. The absence of a nitrogen 1s binding energy at 402 eV suggests that the NHS moiety was replaced by the primary amine group in the antibody as a result of aminolysis and competing hydrolysis reactions. The absence of an oxygen 1s peak at 535 eV in the oxygen 1s narrow scan (Figure 3.9c) characteristic of the C-O-N oxygen of the NHS ester modified surface also confirms the removal of the NHS moiety, which is in agreement with the study by Boecking et al in 2004.27

Figure 3.9d shows the carbon 1s narrow scan before and after the surface was modified with antibodies. The peak at 258 eV is attributed to most of the C-C chains of the alkyl groups in the monolayer. The peaks at 286.2 eV and 288.3 eV are mainly due to the carbon bonded to the amide carbon C-(C=O)N and C-N groups present in the antibody. The observed carbon 1s binding energies are in agreement with other reports of the corresponding carbons in polymers.36,37 The area of the peak at 290 eV assigned to carboxyl acid groups in the monolayers is reduced because most of the acid terminal groups have been activated and reacted with the antibody to form amides.
Figure 3.9: XP narrow scans of the (a) Si 2p region, (b) N 1s region, (c) C 1s region and (d) O 1s region before and after human IgG attachment.
3.3.3 Antigen detection

The human IgG-coated silicon wafers were immersed in a solution containing complementary antibody (anti-human IgG). This led to an increase in the capacitance at low frequency region (Figure 3.10a) which is normally associated with the organic monolayers (see section 3.3.2). The reason for an increase in the capacitance on binding the complimentary antibody may be twofold. The first reason is the continuing introduction of defects in the otherwise very well aligned alkyl chains of the SAMs; that leads to the SAMs becoming more electrically conducting. The second possible reason is the large increase in the hydrophilicity of the surface. This effect was observed in the measurements of contact angle which was reduced to 0°; an increase in the hydrophilic nature of the surface resulted in an increase in the electrical capacitance of the thin organic layers.

At high frequencies (>10^5 Hz) (Figure 3.10b) where sensitivity to antigen-antibody reactions was observed, the capacitance was found to decrease to a larger extent (see Figure 3.10b) compared to the decrease due to the human IgG immobilized on the acid-terminated silicon surface (Figure 3.6a).

3.3.4 Non-specific binding test

To study the specificity of the antigen-antibody interaction, a solution containing 50 µg/mL of anti-human IgM was added to the human IgG-modified surface and incubated at 37°C for 30 minutes. The sample was then flushed with 10 mM cold
PBS (pH 7.4) solution for 5 minutes to eliminate any anti-IgM physically absorbed on the surface. EIS measurements were then made with the system in contact with PBS solution. The results obtained for the capacitance as a function of frequency is shown in Figure 3.10a and b (green line), which also shows for comparison the capacitance dispersion for the IgG modified surface alone (blue line). The conductance spectrum was also measured, as indicated by the green line in Figure 3.10c and d. Both the capacitance and conductance spectra indicate that the interaction of anti-human IgM with human IgG-modified silicon produces only minimal changes in the electrical response. In contrast, the spectra before and after exposure to anti-human IgG shows that the interaction of anti-human IgG with human IgG-modified silicon produces significant changes in the capacitance and conductance in both high and low frequency regions (Figure 3.10a-d). These spectra show pronounced differences between specific binding (human IgG + anti-human IgG) and non-specific binding (human IgG + anti-human IgM).
Figure 3.10: Capacitance and conductance of the human IgG-modified surface (blue) before and after reacting with anti-human IgG (brown) and anti-human IgM (green): (a) and (b) in linear scales, and (c) and (d) in log scales

XPS surveys were also obtained before and after addition of the anti-IgG. These are shown in Figure 3.11a. These results show that after the addition of anti-human IgG, relative increases in carbon 1s, oxygen 1s and nitrogen 1s were obtained, whereas the amount of silicon significantly decreased. Because anti-human IgG has similar
chemical components as human IgG, no new or shifted peaks were observed, only a relative increase in the intensity (Figure 3.11b and c) was detected. This is the major drawback of XPS. Therefore, it is better for analytes to have different chemical components or be labeled with special tags.

Figure 3.11: (a) XP survey of human IgG-modified silicon before (blue) and after (brown) exposure to anti-human IgG, (b) narrow scan of nitrogen 1s region and (c) narrow scan of carbon 1s region.

The atomic concentrations (%) and relative atomic concentrations of acid-, NHS ester-, human IgG- and anti-human IgG modified-surfaces are summarized in Table 1. Analysis of the atomic concentrations of carbon, nitrogen and oxygen in the
acid-terminated monolayers resulted in values in good agreement with those expected. Sensitivity factors of 0.368 (Si 2p), 0.314 (C 1s), 0.499 (N 1s) and 0.733 (O 1s) were used in the calculations to convert peak areas to concentrations. The significant increase in oxygen 1s after human IgG was immobilized on the surface indicates the starting point of re-oxidation of the silicon surface.

**Table 1:** Relative atomic concentrations (%) from XPS data.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Si 2p</th>
<th>C 1s</th>
<th>N 1s</th>
<th>O 1s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-terminated</td>
<td>77</td>
<td>16</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>NHS ester -activated</td>
<td>66</td>
<td>17</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Human IgG</td>
<td>51</td>
<td>29</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Anti-human IgG</td>
<td>16</td>
<td>57</td>
<td>11</td>
<td>20</td>
</tr>
</tbody>
</table>

3.3.5 Equivalent circuit modeling

Capacitance measurements at low frequencies and high frequencies of a fresh sample after every step of the immobilization on silicon are summarized in Table 2.

**Table 2:** Capacitance changes at different frequencies of stepwise-modified silicon

<table>
<thead>
<tr>
<th>Sample</th>
<th>0.1 Hz</th>
<th>1 Hz</th>
<th>$10^7$ Hz</th>
<th>$10^9$ Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolayer</td>
<td>0.012 F/m$^2$</td>
<td>0.012 F/m$^2$</td>
<td>0.665 µF/m$^2$</td>
<td>0.394 µF/m$^2$</td>
</tr>
<tr>
<td>Human IgG</td>
<td>0.013 F/m$^2$</td>
<td>0.013 F/m$^2$</td>
<td>0.417 µF/m$^2$</td>
<td>0.178 µF/m$^2$</td>
</tr>
<tr>
<td>Anti-human IgG</td>
<td>0.017 F/m$^2$</td>
<td>0.015 F/m$^2$</td>
<td>0.336 µF/m$^2$</td>
<td>0.075 µF/m$^2$</td>
</tr>
</tbody>
</table>
At low frequencies an increase in capacitance was observed at each stage of the modification of the surface, whereas a significant decrease in capacitance occurred at high frequencies. As mentioned in Section 3.3.2, 3.3.3 and 3.3.4, the curves of capacitance as a function of frequency are divided into two regions: a low frequency region assigned to the insulating monolayer and a high frequency region attributed to the additional biomolecular layers.

In order to understand the physical basis of the capacitance and conductance changes associated with the antigen-antibody interaction (Figure 3.12a), we divided an equivalent circuit model into two parts that were able to reproduce the behaviour of capacitance at both low and high frequencies. As shown in Figure 3.12b, $C_{\text{org}}$ and $G_{\text{org}}$ represent the capacitance and conductance of the initial acid-terminated monolayer and its ionic environment, while $C_{\text{bl}}$ and $G_{\text{bl}}$ reflect the properties of the anti-human IgG + human IgG layers and its associated ionic charge distribution. When an electrical current is applied to this system, its flow path will depend on the frequency. At high frequencies as shown in Figure 3.12b, the impedance of the organic film (acid-terminated alkyl monolayer) is relatively low and the impedance becomes more sensitive to that of the antibody layer. Hence the capacitance that is measured, although much lower than the capacitance at very low frequencies, becomes more dominated by the capacitance of the biomolecular layer where the sensitivity to antigen-antibody interactions is observed. Some studies$^{38-40}$ have reported that the overall impedance at high frequencies is also controlled by another factor, namely the
silicon space-charge region, which depends on the doping. In this study, we used highly doped silicon wafers. The larger conductance and capacitance of these wafers decrease the impedance of the silicon substrate, so the total impedance becomes more sensitive to $C_{\text{bi}}$.

At very low frequencies, the biomolecular layer impedance is very low as a result of the current flowing through surrounding fluids with low resistivity. The impedance becomes dominated by the capacitance and conductance of the initial acid-terminated monolayer, as shown in Figure 3.12d. Based on the XPS measurements discussed in Section 3.3.2, re-oxidation of the silicon substrate occurred in the acid-terminated monolayer, making the insulating monolayer no longer a homogeneous system and causing the physical basis of the impedance changes to become more complicated. Vasconcelo et al.\textsuperscript{41} used a complex equivalent circuit to fit the impedance changes upon antigen-antibody interactions to their electrode which is very similar to ours. By combining impedance spectroscopy and measurements with an HP 4284A LCR meter, they found that an increase in the conductance of antigen-antibody interactions also were associated with an increased capacitance of the insulating monolayer at low frequencies. This is similar to what we report here based on high resolution measurements of the capacitance as a function of frequency.
Figure 3.12: (a) Physical, schematic, representation of anti-human IgG coupling with human IgG-modified silicon; molecular moieties not drawn to relative correct scales, the equivalent circuit models at (b) overall frequencies, (c) low frequencies and (d) high frequencies.

3.4 Conclusion

Our results demonstrate that the use of direct Si-C linked carboxylic acid-terminated monolayers on silicon (111) as the basis for antibody functionalization leads to good biomolecular recognition capabilities. The interaction between the antibody and its complementary or non-complementary antibody can be directly detected using the high evolution impedance spectroscopy (EIS). The results presented here show that by using EIS measurements at higher frequencies, it is possible to directly detect antigen-antibody interactions without any added agents. Moreover, AC measurements provide no direct current flowing through the sample, thereby minimizing the potential for damaging the biomolecular layers. Our results show that the electrical detecting of biomolecular layers is obtained at high frequencies where the electrical properties of the system are mainly dominated by the biomolecular layers. At low frequencies, the electrical properties are controlled by SAMs and the solution double
layer.

An antibody can be immobilized on a silicon substrate via the NHS-ester activated monolayer. The attachment of the antibodies to the alkyl SAMs appeared to lead to defects (disorder) in the SAMs which lead to a marked increase in the conductance of the SAMs, presumably due to penetration of ions via the defects. The increased conductance of the underlying SAMs on attachment of the antibody molecules then leads to an increase in the overall capacitance, despite the fact that the total thickness of the layers on the silicon substrate becomes larger on attachment of the antibodies. Basically, the capacitance of the SAMs in series with the layer of antibodies becomes shunted by a more highly conducting element. In the high frequency region, we found that the capacitance decreased as additional antibody layers were attached. Therefore, we conclude that the change in capacitance after additional biomolecular layers immobilization depends on details of the surface structure, the quality of the molecular layers and the frequency where the capacitance is measured.

Our results clearly show that changes in conductance or capacitance are associated with the antigen-antibody interaction. The results were confirmed by XPS measurements. Moreover, it is well known that the conductance or capacitance changes arise in part from a field-effect in the silicon substrate, indicating that it should be possible to fabricate a biosensitive field-effect transistor (BioFET) via chemical procedures described in this chapter. The changes in the field effect for direct electrical detection of antigen-antibody interaction can be study by using EIS.
3.5 References:


Chapter 4
Antigen-antibody interactions on plasma polymer modified silicon surfaces

4.1 Introduction

In biochemical applications such as in vivo implants and biosensors it is preferred that proteins retain their functions for a long period of time after immobilization on solid surfaces.\textsuperscript{1-4} The traditional approach to immobilize proteins on solid surfaces with non-specific and uncontrolled weak interactions is to use chemical linkers such as self-assembled monolayers (SAMs), Langmuir-Blodgett layers or organic polyelectrolyte layers.\textsuperscript{2, 5, 6} The drawback of these approaches is that they involve complex chemical reaction steps prior to and during attachment of the bioactive proteins. Another issue is the longevity and strength of the traditional linkers for retaining proteins immobilized on solid surfaces. An alternative approach utilizes plasma polymers as linkers.

The traditional method of plasma fabrication uses a radio or microwave frequency power source to generate a plasma in a mixed vapour of polymer precursors and inert gases.\textsuperscript{7-9} When a plasma polymer is immobilized on a nearby surface; the properties of the plasma polymer are highly dependent on the precursor type and the plasma conditions. Some studies\textsuperscript{10-12} have reported that surfaces modified using traditional plasma polymers have drawbacks such as the short lifetime of the attached protein and frequent adhesion failure at the interface between the polymer and the substrate,
Thus, when a polymer is immobilized on a metal surface, the contrasting properties of the two materials, such as their different thermal expansion coefficients, the possibility of water and moisture ingress along the interface, and the dominance of weak physical bonds at the interface, result in interface adhesion failure.\textsuperscript{13} Yin et al.\textsuperscript{14} reported that adding a pulsed voltage to a conventional plasma enhanced chemical vapour deposition system can result in the additional freedom of tailoring the density of activated sites for the covalent immobilization of proteins, thus enhancing the covalent coverage and increasing the shelf life of the surfaces. Mixing nitrogen, argon or nitrogen + argon with the plasma during deposition led to a high protein immobilization capacity and stable activity over a long period of time.\textsuperscript{15, 17, 18} In 2009, Yin et al.\textsuperscript{16} coated solid materials of various shapes using an acetylene plasma polymerization technique adapted from a plasma enhanced chemical vapour deposition by adding a pulsed voltage electrode to a substrate holder immersed in plasma. The result showed that approximately one monolayer of protein was strongly bound to the solid surface. Quartz crystal microbalance with dissipation analysis (QCM-D) of surfaces so modified showed that further layers of protein beyond the monolayer were physically absorbed during prolonged incubation in the protein-containing solution. These subsequent physisorbed protein layers were easily removed by SDS, but the first monolayer resisted removal by 5\% SDS detergent even at 90\°C.

In this chapter we describe further research based on Yin’s work to integrate the
covalent binding capacity into a complete biosensor methodology in which the recognition proteins are immobilized covalently on the plasma polymer modified silicon surfaces, then, freeze dried to retain their activity during storage. The resulting materials so produced were analysed by ellipsometry, electrical impedance spectroscopy (EIS) and X-ray photoelectron spectroscopy (XPS).

4.2 Experimental methods

4.2.1 Materials

All proteins tested were purchased from Sigma and used without further purification including horseradish peroxidase (HRP, Sigma Cat No P6782), bovine liver catalase (Sigma Cat No C3155), 3,3’5,5’ tetramethylbenzidine (Sigma Cat No T0440), HRP antibody (Sigma Cat No P7899), human IgG (Sigma Cat No I4506), anti-human IgG (Sigma Cat No I3266), anti-elastin clone BA-4 antibody (anti tropoelastin, Sigma Cat No E4013) and sodium dodecyl sulphate (SDS).

4.2.2 Plasma polymerization

The pulsed plasma polymerization system was described in detail by Yin. 17, 18 Figure 4.1 shows the pulsed plasma polymerization system. Briefly, it used one radio frequency (RF) source at 13.56 MHz applied to an electrode at the top of the chamber and another at the bottom. This was used to generate a background plasma and chemical vapour reactions through the bottom electrode, and a pulsed voltage source to bias the substrates through the top electrode. The RF power was 150 W. The pulse
voltage was 200 V for plasma polymers and 400 V for diamond-like carbon (DLC) with a duty cycle 10% and 10 KHz frequency. Acetylene, nitrogen and argon gases were injected into the plasma chamber. Acetylene (10 stand-state cubic centimeter per minute (sccm)) was injected into the plasma chamber as polymer precursor. The flow of argon was 8 sccm for the deposited low nitrogen content surface, and 4 sccm for producing nitride plasma polymer with an additional 4 sccm of nitrogen into the system. The pressure of the system was maintained at 20 Pa.\textsuperscript{17} By adding approximately 14% atomic concentration of nitrogen (without counting hydrogen) into the plasma polymers, the covalent binding capacity was increased significantly.\textsuperscript{18}

**Figure 4.1:** Schematic diagram of the pulsed plasma system.
4.2.3 Protein immobilization

The procedure for HRP attachment and activity analysis was as follows. The samples were incubated for 20 hours in HRP (50 μg/ml) in 10 mM phosphate buffer solution (PBS) at pH 7.0. Incubation was performed in 75 mm sterile Petri dishes with rocking. After incubation, the samples were washed 6 times each for 20 minutes in 10 mM cold phosphate buffer (pH 7.0). The first wash was performed in the same Petri dish used for the enzyme incubation. The samples were transferred to clean Petri dishes for the next 5 washes. Active HRP on the surface of the samples was measured by clamping each sample (approx 15x15 mm) between two stainless steel plates separated by an O-ring (inner diameter 8.0 mm, outer diameter 11.0 mm) that sealed the surface. The top plate had a 5.0 mm diameter hole enabling the addition of 75 μl of the HRP enzyme substrate, TMB (3,3’,5,5’ tetramethylbenzidine, Sigma Cat No T0440). After 30 seconds (or the duration specified in the text), 25 μl of reacted TMB was taken and added to 50 μl of 2 M hydrochloric acid to stop the reaction. A further 25 μl of unreacted TMB was then added to bring the volume to 100 μl. The absorbance of the solution at 450 nm was measured using a Beckman DU530 Life Science UV/VIS spectrophotometer. All enzyme activity tests including rocking time were performed at 23±1°C.

4.2.4 Freeze-drying method

The freeze-drying method used in this work was similar to that used by Nosworthy et al.,\(^9\) where 2.5% sucrose in buffer was added prior to freezing to stabilize the
immobilized proteins. The procedure includes the following steps. First, the protein modified plasma polymer surfaces were rinsed and transferred to Falcon tubes containing 2.5% sucrose in PBS solution. Each tube contained one NPP biosensor with 2 ml of the sucrose containing buffer. Next, the tubes were placed in liquid nitrogen and the samples were frozen. Once frozen, the samples were transferred to a drying chamber (a Christ Alpha 2-4 LDplus freeze dryer) with 0.3 mbar pressure for 48 hours until the drying process was complete. The samples were then stored in a desiccator with a Sigma silica drying agent. The desiccated samples were stored at 20°C.

4.2.5 Analysis methods

**Ellipsometry:** The plasma polymer biosensors were characterized using a Woollam M-2000 spectroscopic ellipsometer, which allowed us to obtain complete information at a wide range of wavelengths from 400 to 1000 nm. The ellipsometry system was with typical sensitivity and accuracy (±0.01° for silicon) and had been demonstrated its effectiveness in many analysis.20, 21 A flow cell with 70° incidence angle windows was used during the ellipsometry analysis. All ellipsometry analysis was done at 70° incident angle. Soaking for proteins immobilization or interaction was all conducted for 30 minutes.

**X-ray photoelectron spectroscopy (XPS) and electrical impedance spectroscopy (EIS):** Both XPS and EIS analysis methods were performed as described in chapter
4.3 Results and discussion

4.3.1 Sensing optimization

The concept of our biosensor methodology using ellipsometry is illustrated in Figure 4.2. In Figure 4.2a, spectra for the ellipsometric parameter Psi are calculated for 3 model sensors with different thicknesses of nitrogen-containing plasma polymer (NPP) layers on a 150 nm silicon nitride coated silicon wafer support. The sensors are assumed to be in water with the sensing radiation inclined at an incidence angle of 70°. The positions of the valleys in the spectra correspond to wavelengths that give a pseudo-Brewster angle of 70°. The sensitivity, shown by the change in Psi caused by adding a 1.0 nm thick protein layer with a refractive index of 1.46 (standard result for the quartz), is plotted in Figure 4.2b. The sensitivity near the valley is low and varies significantly; therefore, it is necessary to choose the nitrogen-contained plasma polymer (NPP) thickness to avoid locating the valley of the Psi spectrum near the 632 nm sensing wavelength. The sensitivity is high and stable for wavelengths that are far away from the valley. For comparison, the sensitivity of a silicon sensor bases on a self-assembled monolayer linker layer of 1.0 nm thickness is also shown (Figure 4.2b). The results show that with an appropriately designed sensor structure, the sensitivity of the sensor can be made much higher than for a silicon sensor with a conventional self-assembled monolayer as a linker. Figure 4.2c shows the flow chart of the proposed biosensor methodology using the antigen-antibody interaction as an example. The steps are: design and construct a biosensor according to the principles
shown in Figure 4.2a and Figure 4.2b with an NPP top layer; covalently immobilize antigen on the biosensor (as in immunosensors); carry out the freeze-drying treatment; store the freeze-dried sensors in dry air; and finally, when needed, use the sensor for diagnosis.

**Figure 4.2:** (a) the ellipsometry spectra of Psi with different NPP layer thickness, (b) the sensitivity caused by binding of a 1 nm thick protein layer, for the sensors in (a) as a function of wavelength, compared with a silicon sensor with a 1 nm thick self-assembled monolayer linker. (c) the flow chart of the sensing methodology using antigen-antibody interactions as an example: design and formation of a base sensor with activated polymer surface; antigen attachment; freeze-drying; storage in dry air; usage by an end-user for diagnosis.

### 4.3.2 Covalent immobilization of proteins on plasma polymers without the cross-linkers

Figure 4.3 shows some examples of linker-free protein covalent binding on NPP
surfaces and compares them with reference surfaces. The covalent binding of the proteins to the surfaces was verified by their persistence when exposed to rigorous washing in SDS detergent. In Figure 4.3a, we show the XPS analysis data of human tropoelastin on NPP compared with bare silicon and diamond-like carbon (DLC). In the XPS analysis, the S 2p peak was used instead of the N 1s peak as the nitrogen peak has a major contribution from the NPP. The XPS S 2p peak arises from cysteine or methionine amino groups in the proteins. Rinsing in water after SDS treatment removes almost all SDS molecules. Any trace quantity of unwashed SDS remaining on the surfaces can be subtracted from the S 2p peak as the binding energy of S 2p in SDS is approximately 4-5 eV larger than that of the main S 2p peak of proteins. This method was recommended previously for XPS analysis of immobilized proteins on surfaces containing nitrogen and the results correlated very well with ELISA analysis. The covalent binding capacity (vertical axis in Figure 4.3a) is defined as the ratio of the remaining tropoelastin after SDS cleaning to the protein quantity before SDS cleaning. The significantly higher covalent binding capacity of the NPP surface is evident. The DLC surface has been previously proposed for biosensor applications by Hartl et al. The significantly higher covalent protein binding capacity of NPP suggests that the NPP surfaces are more suitable for biosensor applications. Figure 4.3b shows the ellipsometry analysis of catalase on NPP in buffer in the following sequence: without protein (NPP), after incubation in catalase protein solution followed by rinsing with fresh buffer, after 0.2% SDS treatment without a water rinse (SDS), and after rinsing with deionised water. Figure 4.3c shows the
quartz crystal microbalance with dissipation (QCM-D) analysis of horseradish peroxidase (HRP) on a NPP surface in the following sequence: HRP immobilization from solution (HRP), PBS rinse (buffer), 2% SDS cleaning (SDS), and PBS rinse again. The bound mass after SDS treatment and PBS rinse was about 300 ng/cm², which is approximately equal to a monolayer of HRP. After introducing SDS, the mass initially increased to approximately twice that of the initially immobilized proteins, followed by a steady reduction of the mass.

Figure 4.3d shows the XPS analysis of BMP-7 (human bone morphogenic protein-7) remaining after SDS cleaning using the same analysis method as in Figure 4.3a. Three surfaces were compared: bare PDMS, plasma polymer without nitrogen (PP), and NPP. The PDMS had negligible covalent binding capacity. The plasma polymer (PP) that contained no nitrogen had a lower covalent binding capacity for the BMP-7 protein than NPP, which is consistent with previous observations using other proteins. The high coverage of the covalent binding of the proteins on the NPP surfaces provides a straightforward and reliable platform for biosensor applications.
4.3.3 The effect of freeze-dried on protein binding

In the freeze-drying treatment, sucrose was used in the last step as a stabilizer. Sucrose and other sugars have been successfully used to stabilize freeze-dried non-immobilized proteins$^{27, 28}$ and linker layers$^{29}$ and to retain the activity of
freeze-dried HRP immobilized on ion implantation treated polymers. Figure 4.4a shows the activity as a function of the number of days of rocking in buffer for HRP immobilized on NPP surfaces before freeze-drying (FD-before) and after freeze-drying (FD-after) as well as for HRP immobilized on silicon surfaces (Si) without freeze-drying. Immediately after freeze-drying, the HRP immobilized on NPP had comparable activity to that before freeze-drying and much higher activity than on silicon without freeze-drying. After 5 and 10 days of rocking in buffer, the activity of the enzymes with and without freeze-drying on NPP was still high compared to silicon. The enhanced activity and the ability to withstand prolonged buffer rocking is attributed to covalent binding in a native conformation, implying that the freeze-drying process did not significantly change the conformation status of the immobilized proteins on NPP. This may be due to multipoint covalent attachment of HRP proteins on NPP. It has been suggested that multipoint covalent binding can result in a more rigid conformation of immobilized proteins. Figure 4.4b shows the conformational status of anti-HRP immobilized and freeze-dried on NPP surfaces. The plot shows the time required for the anti-HRP to achieve a binding of approximate one monolayer of HRP as a function of HRP concentration. The results indicate that the anti-HRP remained functional and was capable of binding HRP at very low concentrations: less than 1 ng/ml. Figure 4.4c shows the activity of immobilized HRP as a function of storage time after freeze-drying. Storage was performed in dry air in a desiccator with an ambient temperature in the range of 10 to 20°C. The results indicate that by using the freeze-drying method, the enzyme
immobilized NPP biosensors could be stored at room temperature under dry conditions for a long period of time, which was convenient for storage and transportation without refrigeration. If refrigerated storage were used, an even longer lifetime for immobilized dried proteins would be possible.\textsuperscript{29,32} Freeze-drying did not alter the covalent binding nature of the proteins on the NPP surfaces as shown in Figure 4.4d, where four proteins including HRP, anti-IgG, anti-HRP, and human tropoelastin were analysed using XPS. The covalent binding capacities assessed after the freeze-drying treatment were similar to those without freeze-drying. This is expected because once a protein is covalently bound to a surface, it should be stable under the conditions encountered in the freeze-drying treatment unless the surface itself is unstable. In contrast, for silicon surfaces with self-assembled monolayer linkers, the silicon can gradually oxidize and release the underlying bound self-assembled monolayers which are used as linkers as discussed in Chapter 3.
Figure 4.4: The effect of freeze-drying on conformation of the immobilized proteins and protein binding strength: (a) the activity of immobilized HRP as a function of time in PB buffer for surfaces of silicon without freeze-drying (Si), NPP without freeze-drying (FD-before), and NPP with freeze-drying after HRP immobilization (FD-after); (b) the time required for activity to reach 0.5 for immobilized HRP on anti-HRP attached and freeze-dried NPP surfaces as a function of HRP concentration, showing the freeze-dried and immobilized anti-HRP biosensors were highly sensitive to small traces of HRP in buffer; (c) the retained activity of immobilized HRP on NPP for conditions of without freeze-drying (No FD), immediately after freeze-drying (FD 0 day), and three months after freeze-drying (FD 3 months); (d) covalent binding capacity for four examples of proteins immobilized on NPP surfaces after the freeze-drying process and analyzed using XPS.

4.3.4 Nitrogen-containing plasma polymer biosensors without freeze-dried treatment

The performance of the biosensors was first analyzed without introducing the freeze-drying treatment. In the following section, we describe the experiments performed to demonstrate the high sensitivity achievable with ellipsometry that was predicted earlier. Figure 4.5 shows a range of protein interactions detected using both ellipsometry and XPS methods for sensing. Figure 4.5a and Figure 4.5b show the measurements obtained for the following sequence of operations: before protein immobilization, after incubation in 200 μg/ml anti-human IgG solution and subsequent rinsing in cold PBS buffer solution, after blocking in 1 mg/ml BSA and rinsing in cold PBS buffer solution, after soaking in 200 μg/ml human IgG and rinsing in cold...
PBS buffer solution. The biosensor in Figure 4.5a-b was designed to position the spectral valley in p-type silicon (the wavelength corresponding to the pseudo-Brewster angle of 70°) close to 632 nm. As shown in the discussion relating to Figure 4.2a-b, the case where the minimum in Psi lies close to the sensing wavelength of 632 nm cannot be used for the biosensor readout because the sensitivity in this range is highly wavelength dependent. This means that this choice of NPP layer thickness is not appropriate for sensing with single wavelength ellipsometry at 632 nm. Note that the BSA blocking after anti-IgG immobilization did not introduce significant additional shifts in either Psi or Del. This is interpreted as the initial anti-IgG immobilization on NPP surfaces resulting in a dense covalently bound monolayer, which is in good agreement with the results shown in Figure 4.3 and reported previously.33-37

Figure 4.5c shows the Psi spectra for a correctly constructed biosensor for sensing at 632 nm. It also shows the measurements obtained for the following sequence of operations: before protein immobilization (NPP), after incubation in 50 μg/ml HRP buffer and subsequent rinsing in buffer (HRP), after incubation in 4 μg/ml anti-HRP buffer and rinsing in buffer (HRP+antiHRP), after further incubation in 100 μg/ml HRP buffer and rinsing in buffer (HRP+antiHRP+HRP). The resulting changes in Psi are: HRP binding (0.43°), anti-HRP binding (0.80°), and no change for further HRP exposure. Since the typical angle sensitivity of a low cost ellipsometry system is larger than 0.02°, the results suggest that the NPP sensor provides a reliable analysis.
Using a simple linear relationship between the immobilized protein layer thickness and the change in Psi, we can calculate that the molecular ratio of the anti-HRP to the initially bound HRP is 1:1.5. This indicates that the anti-HRP captured by the initially immobilized HRP also had a high density. Further incubation in HRP (to give the layer HRP + anti-HRP + HRP structure) did not alter the reading. The XPS analysis of the anti-HRP biosensor agrees reasonably well with the ellipsometry data in Figure 4.5c which gave a value of 1.86 for the immobilized protein quantity ratio of anti-HRP to HRP as shown in Figure 4.5d. The shoulder in the C1s spectra near 288.5eV is commonly used to make an approximate quantification of the immobilized proteins. The integrated intensity ratio due to additional anti-HRP immobilization to that due to HRP immobilization is 2.0, which is approximately the same as that obtained using the corresponding S 2p peaks in the XPS analysis. Given the fact that in XPS analysis a thicker protein surface layer would be slightly over-counted due to the higher sensitivity of the top layer unless only one monolayer of protein is immobilized, this is considered good agreement. Figure 4.5e shows another NPP biosensor using human tropoelastin and anti-tropoelastin plus the ellipsometry method for the sensor readout. The Psi readings at 632 nm shown in Figure 4.5e are for the following sequence of conditions: before protein immobilization (NPP), after incubation in 20 μg/ml anti-human tropoelastin and rinsing in buffer (Anti-tropo), after incubation in 5μg/ml human tropoelastin and rinsing in buffer (Tropo). The sensitivity for both the anti-tropoelastin and tropoelastin is good and is smaller than the measurement uncertainty. Figure 4.5f shows the XPS C1s spectra of a NPP
biosensor for the following sequence of conditions: before protein immobilization (NPP), after incubation in 20μg/ml protein-A and rinsing in buffer (PA), after incubation in 50μg/ml human IgG and rinsing in buffer (PA+IgG). Protein-A is widely used in biosensors to orient antibodies. The same type of NPP biosensor was also analysed using the ellipsometry method for the same sample sensing conditions. The values of Psi at 632 nm are given in the inset of Figure 4.5f. Protein-A can accommodate the Fc site of an IgG molecule to leave the Fab sites available for antigen binding reactions. The changes in Psi resulting from protein immobilization are 0.21° and 0.35° for protein-A and human IgG respectively. Using XPS C 1s analysis, the mass ratio of immobilized IgG to protein-A was found to be about 1.64, which agrees well with the ratio of 1.66 obtained using the ellipsometry method. The XPS and ellipsometry analyses shown in Figure 4.5f suggest that approximately every two immobilized protein-A proteins can capture one IgG protein, resulting in an approximately 50% capture efficiency. Considering the larger size of IgG compared to protein-A, the capture efficiency is excellent and it is consistent with the random model of protein adsorption. It also shows that almost all IgG molecules are correctly oriented.
Figure 4.5: Applications of the proposed biosensors for different antigen-antibody interactions using the ellipsometry and XPS diagnosis methods. (a) and (b) ellipsometry spectra of Psi and Del respectively in the range near 632nm for the following conditions: before protein immobilization.
on NPP (NPP), after anti human IgG soaking in 200 μg/ml buffer and rinsing (Anti-IgG), soaking in 1mg/ml BSA solution (BSA) and soaking in 200 μg/ml human antibody (IgG); (c) ellipsometry Psi spectra and (d) XPS C 1s spectra for biosensors before protein immobilization (NPP), after soaking in 50 μg/ml HRP buffer (HRP), after soaking in 4 μg/ml anti-HRP buffer (HRP + anti-HRP); then further soaking in 100 μg/ml HRP buffer and rinsing (HRP + anti-HRP + HRP); (e) the Psi readings at 632 nm for a biosensor at the following conditions: no protein immobilization (NPP), after 20 μg/ml anti human tropoelastin immobilization and rinsing (Anti-tropo), and after 50 μg/ml human tropoelastin immobilization and rinsing (Tropo); (f) XPS C 1s spectra of biosensor at the following conditions: before protein immobilization (NPP), after 20μg/ml protein-A immobilization and rinsing (PA), and after further 50 μg/ml human IgG soaking and rinsing (PA+IgG). The values of Psi in the legends’ brackets are the ellipsometry analysis data for the same conditions at a 632 nm wavelength.

4.3.5 Freeze-dried nitrogen-containing plasma polymer biosensors

The sensitivity of the freeze-dried NPP biosensors using ellipsometric readout at 632nm is shown in Figure 4.6. All of the freeze-dried NPP biosensors had storage conditions similar to those for Figure 4.4c with a storage period between one week and two months. Figure 4.6a shows a NPP biosensor for human IgG measured over the following sequence of operations: incubation in 20 μg/ml anti-IgG followed by freeze-drying (Anti-IgG + FD), incubation in 1mg/ml BSA blocking (BSA), and incubation in 10 μg/ml human IgG. After BSA blocking, the reaction with human IgG resulted in a change in Psi approximately 25 times larger than the change from BSA blocking, demonstrating the high sensitivity of the NPP IgG biosensor.

Figure 4.6b shows the operation of a freeze-dried protein-A biosensor. The sequence of conditions was: protein-A immobilized and freeze-dried (PA+FD), 40 μg/ml antitropoelastin immobilization (Anti-tropo), 1 mg/ml BSA blocking (BSA), and 40 μg/ml human tropoelastin immobilization (Tropo). The freeze dried biosensor can be functionalised with anti-tropoelastin and is capable of detecting tropoelastin in solution.
Figure 4.6c shows the results for a similar freeze-dried protein-A immobilized NPP biosensor for the human IgG and anti-IgG reaction. The following sequence of operations was used: protein-A immobilization and freeze-drying (PA+FD), 20 µg/ml human IgG immobilization (IgG), 1mg/ml BSA blocking (BSA), 1 µg/ml anti-IgG immobilization (anti-IgG), and further 20 µg/ml human IgG immobilization (IgG+). The first IgG immobilization resulted in a large increment in Psi, indicating the excellent efficiency of the capturing ability of the immobilized and freeze-dried protein-A on the NPP biosensor. The large anti-IgG immobilization increment indicates that the function of IgG is preserved during the immobilization. The stability of the triple-layer of proteins (Protein A + IgG + antiIgG) was challenged by a further soaking in 20 µg/ml human IgG buffer (IgG+) for 30 minutes. There was only a small decrease in Psi indicating that the triple layer remained stable. The effectiveness of freeze-drying was further analysed using a NPP biosensor processed with protein-A immobilization and then human IgG soaking prior to the freeze-drying treatment. Figure 4.6d shows the response of the biosensor with the following sequence of operations: after protein-A and human IgG immobilization then freeze-drying treatment (PA + IgG + FD), 1 mg/ml BSA blocking (BSA) and 10 µg/ml anti-IgG immobilization (Anti-IgG). The biological responses of this biosensor were similar to those in Figure 4.6a-c. Such freeze-dried protein double layer biosensors provide an opportunity for a wide range of end-use and applications and offer a particular advantage when rare antigen (or antibody) biosensors are required in medical diagnosis. Figure 4.6e shows a HRP immobilized biosensor with the following
sequence of operations: after HRP immobilization and freeze-drying treatment (HRP+FD), 1 mg/ml BSA blocking (BSA) and 4 μg/ml anti-HRP immobilization (Anti-HRP). The biological responses of the anti-HRP biosensor are consistent with the performances of other biosensors described above (Figure 4.6a-d). Figure 4.6f is an example of a time trace of Psi for an anti-HRP biosensor, showing the response of the anti-HRP immobilization. After approximately 30 minutes, the antigen-antibody reaction was sufficient for the Psi signal to stabilize in a conveniently short time even when a low concentration (0.2 μg/ml) of anti-HRP was used.
4.3.6 EIS study of proteins immobilized on plasma polymer modified silicon surfaces

Silicon surfaces were coated under two different polymerisation conditions (nitrogen or nitrogen + argon). The addition of nitrogen to the acetylene modifies the chemical content, while argon alters the energetic ion bombardment occurring during polymerisation. A previous study reported that the plasma polymers deposited on a silicon surface by introducing both nitrogen (NPP) and nitrogen + argon (NArPP)
enhanced the activity of protein binding.

Increasing the pulse voltage and duty cycle of the bias applied to the silicon substrate during deposition increased the density of excited species in the plasma as observed by the increase in the thickness of the polymer. Figure 4.7a shows the measured capacitance of NPP on a silicon surface in contact with a 100 mM KCl electrolyte. It clearly shows that capacitances measured below $10^4 \text{ kHz}$ were frequency-independent. Therefore, we assume that these three plasma polymers are homogeneous and hence, can be fitted using a simple Maxwell model for the equivalent circuit. The results shown in Figure 4.7a for the capacitance as a function of frequency show that the capacitance decreased with increasing dielectric thickness of the plasma polymer, as determined by ellipsometry. The phase angles (Figure 4.7b) at low frequency regions were approximately $-90^\circ$, which reveals that the plasma polymers acted as pure capacitors and material behaved as an insulator. At extra low frequencies the phase angles shifted away from $-90^\circ$ toward 0 as the frequency was decreased; we attribute this behaviour to the presence of a few conductive defect sites in the plasma polymers which induce the current leakage. This conductive defect was enhanced as the thickness of the polymer was increased, which makes the surface more porous.

The capacitance spectra of NArPP on a silicon surface as a function of frequency, as presented in Figure 4.7c, show dispersion at low frequencies, indicating a heterogeneous surface. The change in capacitance as a function of the thickness of
NArPP shows similar behaviour as NPP. The phase angles in Figure 4.7d indicate that NArPP is not an ideal capacitor and contains many conductive defect sites. Based on our EIS results, it was decided to focus on NPP for protein attachments.

Figure 4.8a shows a 14 nm plasma polymer coating before and after incubating in 100μg/ml human IgG solution at room temperature for 1 hour. At low frequencies the impedance became dominated by the electrical properties of the plasma polymer and its ionic environment. Since the addition of new layers led to an increase in both the thickness and roughness of the capacitive layer, the effective capacitance was expected to change. The curve of the capacitance as a function of frequency was found to move slightly upwards with the introduction of human IgG. In the high frequency region, which is sensitive to detecting antibodies (see Chapter 3.3), the change in capacitance after incubating with human IgG was also insignificant. The conductance curve (Figure 4.8b) does not show any difference. It is difficult to determine whether this slight change in capacitance is due to the immobilization of human IgG or due to instrumental error. We assume that free radicals in the 14 nm plasma polymer volume are too low to form a dense-packed layer of human IgG. Therefore, we doubled the thickness of the plasma polymer to ~25 nm. An increased thickness leads to an increase in volume containing the free radicals. Both the capacitance and conductance spectra (Figure 4.9a-d) after exposure to human IgG showed that the interactions of human IgG with free radicals in the plasma polymer produced significant changes in the low frequency region. A similar change in
capacitance and conductance was described in Chapter 3.3 with the addition of antibodies to a self-assembled monolayer surface. When anti-human IgG was added to the device with immobilized human IgG, the capacitance was found to increase greatly compared to the increase due to the human IgG immobilized on the device.

When the thickness of the plasma polymer was increased to 36 nm, as presented in Figure 4.10a-d, we did not see any change in the capacitance or conductance before or after human IgG immobilization. We assume that compared with 2-4 nm size of human IgG, the 36 nm thick plasma polymer layer produces a large background signal. In such cases, EIS would not detect any response on the addition of layers. However, a previous study\textsuperscript{16} reported that a minimum thickness of 75 nm was required to obtain a saturation of the covalent protein immobilization. This is out range for detection with our EIS spectroscopy.
Figure 4.7: The (a) capacitance and (b) phase angle of nitrogen-containing plasma polymer (NPP) as a function of frequency; (c) the capacitance and (d) phase angle of the nitrogen and argon-containing plasma polymer (NArPP) as a function of frequency.
Figure 4.8: The capacitance and conductance of 14 nm NPP before (red) and after (blue) incubating with human IgG. (a) and (d) in linear scales; (b) and (c) in log scales.
Figure 4.9: The capacitance and conductance of 25 nm plasma polymer before (red) and after (blue) incubating with human IgG followed by (green) anti-human IgG. (a) and (d) in linear scales; (b) and (c) in log scales.
Figure 4.10: The capacitance and conductance of 36 nm plasma polymer before (red) and after (blue) incubating with human IgG. (a) and (d) in linear scales; (b) and (c) in log scales.
4.4 Conclusion

In this chapter, we have presented a workable system for biosensing that in principle could be produced for routine application. The methodology is reliable, rapid, and the end-user would find it easy to use. The reliability of the biosensor results from the high covalent binding capacity of the NPP surfaces for protein and the excellent performance of the biosensors after a freeze-drying treatment. The rapidity and ease of use result from optical design, the simplicity of the diagnostic method, and the simple storage procedure, which ensures minimal involvement at the diagnosis step. XPS and QCM-D were used to confirm the covalent binding nature of the NPP surface before and after the freeze-drying treatment. The antigen-antibody interactions on the NPP biosensors were reconfirmed after freeze drying using the XPS technique. Three different pairs of antigen-antibodies were tested, together with protein-A, to demonstrate the new biosensor methodology. It is apparent that this method is suited not only for immunosensors but also for other types of biosensors as long as the biological reactions result in mass change or involve molecular binding/detaching processes on the surface of the biosensor. The high sensitivity of the biosensors using the antigen-antibody interactions was consistently demonstrated for cases with and without the freeze-drying treatment. Protein-A remained functional after covalent immobilization for the following cases: protein-A only with or without freeze-drying treatment, and antibodies on protein-A with the freeze-drying treatment. BSA blocking showed very little change in the sensor readings, compared to the antigen-antibody interactions, indicating a high density of covalently immobilized
functional protein for all cases with or without the freeze-drying treatment. The ellipsometry Psi signal ratios of antibody to antigen in the biosensor measurements were consistent with dense layers of antibody or antigen that had been captured or immobilized, as shown in Figure 4.5a, 4.5c, 4.5e-f, and 4.6b-c. The uniform response over a wide range of wavelengths, in the vicinity of an aimed single wavelength at 632 nm, showed that the biosensor sensitivity can be controlled with minimal influence from variation in the NPP thickness, providing a convenient approach for the formation of standardized biosensors. The typical data collection time for ellipsometry analysis (single wavelength and single biological reaction step) for each biosensor is expected to be a few minutes. Most of the processing time in the biosensor diagnosis is the time required for the biological reaction to stabilise or be completed. That time is approximately one hour. This means that the bottle neck limiting the biosensor diagnosis throughput would not be the data collection. By standardizing the NPP biosensors, a rapid readout of biosensor diagnosis could be constructed in which the biosensors could be rotated through a simple ellipsometry analysis unit while waiting for the biological reactions to be completed. This would offer a convenient and low cost solution that matches the speed advantage of an diagnosis techniques such as imaging ellipsometry.\textsuperscript{38, 39} However, it should be pointed out that the ellipsometry technology has the disadvantage of high cost and complicated data analysis.

It was found that the NPP biosensor process and properties could be achieved using
silicon substrates and this, in principle, makes it compatible with standard CMOS (complimentary metal oxide silicon) semiconductor integrated device technology.\textsuperscript{31} Thus, the NPP biosensor methodology could be used in miniature biosensors and integrated with CMOS and opo-electronic elements circuit logic elements to produce a “smart” biosensor.
4.5 References


Chapter 5

Electrical properties of low doped silicon surfaces functionalized with
biomolecular layers

5.1 Introduction

Since Bergveld first investigated the use of ion-sensitivite field-effect transistors (ISFETs) three decades ago, the electrical properties of biomolecular integration with semiconductors such as silicon and diamonds have attracted growing interest due to the possibility of using the semiconductor field effect for direct electrical detection of biomolecules. Silicon-based electrochemical biosensors that can detect biomolecular interactions occurring at the gates of metal-oxide-semiconductor field-effect transistors (MOSFETs) are called biologically active field-effect transistors (BioFETs). Compared to the traditional optical and mass spectrometry detection technologies, BioFETs potentially provide label-free, fast, high sensitivity and direct electronic signal readouts. The field-effect-transistor (FET) is a transistor that uses an electric field to control the conductivity of a channel of one type of charge carrier in a semiconductor material. The specific interactions occurred between charged analytes and receptors on the surface of FETs induce changes of the shape and the conductivity in the electric field. These changes could penetrate into the near-surface region and alter the electronic properties of the sensing elements, such as capacitance and conductance, to produce the signal output. It is well known that DNA has a negative charge due to its phosphate backbone and that many proteins are charged at commonly accessible pH values. Consequently, biosensors based on FETs could be utilized to detect a variety of target molecules. As mentioned in Chapter 3, UV mediated functionalization is an excellent way to modify silicon surfaces with
organic monolayers terminated with carboxyl acid groups, which can then convert to NHS-esters followed by covalently linking biomolecules, such as antibodies to silicon surfaces. In this chapter, we describe an investigation into whether or not field effect changes can directly sense the functionalization of silicon surfaces.

5.1.1 Operating principle of BioFETs

The typical structure of a BioFET sensor is illustrated in Figure 5.1. The BioFET performs a similar structure as the ISFET except that in case of the BioFET the oxide gate insulator of the original ISFET has been replaced with an organic monolayer to which functional receptor groups can be attached as desired. In general, a BioFET sensor consists of three electrodes, namely the source and drain electrodes which bridge the semiconductor channel and the gate electrode which modulates the channel conductance. It has been noted that in the case of a MOSFET and an ISFET, the gate is electrically isolated from the source and drain electrodes. However, in a BioFET, the gate is a reference electrode. The voltage between the source and drain of a BioFET sensor produce a current flow which is regulated by the gate voltage. In the case of an ISFET, the amount of current flow is controlled by changes in the pH of the electrolyte, the ion concentration, etc. By contrast, the current flow in a BioFET is controlled by changes in the charged biomolecules linked on the substrate. In this study, we use the mechanism of an n channel BioFET sensor in which the current is conducted by electrons (major carriers in n-type semiconductors). When a positive voltage is applied to the gate, the free holes are repelled from the near-surface region.
of the substrate, and pushed downward into the bulk, leaving behind a carrier-depletion region (space-charge region). The depletion region is built up by the bound negative charge associated with the acceptor atoms. At the same time, the positive gate voltage pulls the electrons from the bulk of the substrate into the near-surface region. When enough electrons are pulled into the near-surface region, a channel is created with charge carriers (electrons), that is an n-type channel, that electrically bridges the source and drain electrodes. If a voltage is applied between the source and the drain when conducting channel has been created, a current will flow through this n channel via the mobile electrons. In the case of n-type semiconductors, applying a positive voltage accumulates carriers (electrons) and increases the conductance, whereas applying a negative voltage leads to the depletion of carriers and reduces the conductance. The electric field created by the applied voltage on the gate develops in the normal direction in the substrate. In most applications, the voltage applied between the source-drain and gate electrodes is constant for a BioFET sensor. Any change in the current can be then related to a change in the conductance of the biofunctionalized layer on the FET surface. When a charged molecule links to the biofunctionalized layer, an electric field is created near the surface and exerts an effect on the semiconductor channel. If the bond molecules carry the same charge as the carriers in the semiconductor, the carriers will deplete from the surface into the bulk, thus causing a decrease in the conductance. Thus if a negatively charged molecule such as 1-undecenoic acid was to be bound to an n-type silicon surface it caused electrons to be repelled, thus resulting in a decrease in conductance.
Figure 5.1: Hypothetical Schematic drawing of an n-type silicon BioFET device. A BioFET contains the following parts: a semiconductor transducer, a dielectric layer, a biofunctionalized surface, the analyte, and a reference electrode (the gate). The semiconductor transducer is realized by a conventional field-effect transistor. The dielectric layer is a self-assembled monolayer (SAM) performing two functions: one is to isolate the channel of the FET from the electrolyte and the other one is to bind the desired molecule with its functional receptor. The analyte is a solution which contains the dissolved sample molecules. The reference electrode allows adjusting the device so its sensitivity will be maximized. If the target molecules bind to the receptors, a change in the surface charge density occurs. This change alters the potential in the semiconductor and thus the conductivity in the channel of the FET.

The electrolyte-organic insulator-semiconductor (EOIS) structure, as presented in Figure 5.2, is similar to the metal-oxide-semiconductor (MOS) structure formed in semiconductor devices. In this study, the applied potential is defined relative to the electrolyte. When a DC bias potential is applied to an EOIS structure, the current will
move through the organic insulator region and then the space-charge region in the semiconductor. In an ideal EOIS structure, no bending of the energy bands occurs without an applied bias potential. In addition, the conductance of the organic insulator is zero; hence no current flows through the insulator under DC biasing conditions. Because the capacitive and conductive properties of the space-charge region in the semiconductor are DC bias potential-dependent, any change in the capacitance or conductance is derived from the space-charge region in the semiconductor.

5.1.2 Flat-band conditions under three different bias voltages

Flat-band is the condition where the energy band (E<sub>c</sub> and E<sub>v</sub>) of the substrate is flat at the organic insulator-semiconductor interface as shown in Figure 5.2. The numerical value depends on the doping of the semiconductor and any residual interface charge that may exist at the interface between the semiconductor and the organic insulator. When the band is flat in the semiconductor, the surface electric field in the substrate is zero. Therefore, according to the Gauss’s law, the electric field in the organic insulator is also zero. In fact, most systems cannot reach flat-band conditions at zero applied bias due to conductive defect sites in the organic insulator (i.e. pin-holes in the self-assembled monolayers (SAMs)).
Figure 5.2: Energy band diagram of the BioFET system at the flat-band condition. A voltage equal to $V_{fb}$ is applied between the gate and the n-Si substrate to achieve this condition.

**Surface accumulation**

When a positive voltage larger than the flat-band voltage is applied to the gate, it induces a positive charge on the gate and a negative charge in the semiconductor. The band diagram on the gate side is pushed downward\(^9\), as shown in Figure 5.3. The majority of carriers (electrons) in n-type silicon are accumulated in the narrow near-surface region of the silicon substrate. When the surface of the semiconductor is accumulated, the plot of the charge per unit area ($Q_N$) at the semiconductor-insulator interface as a function of the applied voltage ($V_g$) (Figure 5.4) is linear and the slope is the capacitance per unit area,\(^ {10}$ $C_{\text{insulator}}$, of the insulator which is given by

$$C_{\text{accumulation}} = C_{\text{max}} = C_{\text{insulator}} = \frac{\varepsilon_{\text{insulator}}}{d_{\text{insulator}}}$$

where $\varepsilon_{\text{insulator}}$ is the permittivity of the insulator, and $d_{\text{insulator}}$ is the thickness of the insulator.
Figure 5.3: The ISFET capacitor biased into accumulation. (a) types of charge present. + represents holes and – represents electrons, (b) equivalent circuit and (c) energy band diagram.  

Figure 5.4: Accumulation charge density as a function of the applied voltage. The slope of the line is the insulator capacitance per unit area, $C_{\text{insulator}}$.  

Surface depletion

When the voltage applied on the gate is smaller than the flat-band voltage, the interface between the electrolyte and organic insulator is negatively charged. This leads to a positive charge being induced at the interface between the organic insulator and semiconductor.  

At the positive potentials the energy bands in the semiconductor near the surface are bent upward and the carriers (electrons) are depleted from the
surface, leaving behind a positive space charge region (depletion region). The
depletion region behaves like a capacitor with a capacitance per unit area \( (C_d) \) that
depends on the gate voltage \( (V_g) \) and is defined as

\[
C_d(V_g) = \frac{\varepsilon_{Si} \varepsilon_o}{X_d(V_g)}
\]

where \( \varepsilon_{Si} \) is the permittivity of the silicon \( 12, \varepsilon_o \) is the permittivity of the free space
of air \( (= 8.854 \times 10^{-14} \text{ Fcm}^{-2}) \) and \( X_d \) is the depletion region thickness. The charge
distribution, equivalent circuit and energy band in the semiconductor are shown in
Figure 5.5 a-c.

![Figure 5.5: The ISFET capacitor biased into depletion. (a) types of charge present. + represents holes and – represent electrons, (b) equivalent circuit and (c) energy band diagram.](image)

Figure 5.5-b shows that the insulator capacitance connects with the depletion region
capacitance in series. Thus the total capacitance of the system is defined as

\[
\frac{1}{C_{tot}} = \frac{1}{C_{insulator}} + \frac{1}{C_d} = \frac{C_{insulator}C_d}{C_{insulator} + C_d}
\]
The silicon depletion layer thickness increases as the gate voltage decreases because more negative potentials at the interface between the electrolyte and organic insulator will push more electrons away from the surface, thereby leaving more positive ionized dopants in the space charge region and leading to a thicker depletion layer. The thicker the depletion layer in the semiconductor, the smaller the capacitance of the depletion layer will be. Hence, the total capacitance decreases as the gate voltage is decreased.

**Surface inversion**

If the applied gate voltage becomes increasingly more negative, this will bend the energy band further upward. At some gate voltage, the surface is no longer in depletion but at the threshold of inversion. The term inversion means that the semiconductor surface inverts its conduction type from n-type to p-type, or to hole-rich. The threshold is defined as the condition where the depletion region demarcates from the inversion region. At the threshold voltage the concentration of holes at the surface exceeds the concentration of electrons in the bulk. The number of holes at the surface increases as the applied voltage decreases. An additional increase in the applied gate voltage (hence increased bending of energy bands) leads to a linear increase in the charge per unit area of the inversion layer. The inversion layer is in series with the depletion layer at the interface between the organic insulator and semiconductor. It has been noted that the depletion layer thickness reaches the maximum when the inversion layer forms. When the applied gate voltage is equal to
the threshold voltage, the capacitance of the depletion layer reaches a minimum $C_{\text{min}}$ and likewise the total capacitance of the system also reaches its minimum. The capacitance is defined as

$$c_{\text{tot}} = c_{\text{min}} = \frac{c_{\text{insulator}}c_{\text{dmin}}}{c_{\text{insulator}} + c_{\text{dmin}}}$$

where

$$c_{d\text{min}} = \frac{\varepsilon_{\text{Si}}\varepsilon_0}{X_{d\text{max}}}$$

Where $\varepsilon_{\text{Si}}$ is the permittivity of the silicon is 12, $\varepsilon_0$ is the permittivity of the free space of air (\(= 8.854 \times 10^{-14} \text{ Fcm}^{-2}\)) and $X_{d\text{max}}$ is the maximum thickness of the depletion layer.

![Diagram of ISFET capacitor biased into inversion](image)

**Figure 5.6:** The ISFET capacitor biased into inversion. (a) types of charge present. + represents holes and – represent electrons, (b) equivalent circuit and (c) energy band diagram.\(^9\)

### 5.1.3 Capacitance as a function of applied voltage in the BioFET structure

The capacitance of the BioFET structure is a function of the applied gate voltage. The dependence is shown in Figure 5.7. It has been observed that the potential of the
depletion layer (surface potential: $\Psi_s$) has the opposite sign as the potential at the interface between the electrolyte and insulator (applied gate voltage: $V_g$), which is measured with respect to the solution, i.e. $V_g > 0$ for $\Psi_s < 0$. When $\Psi_s < 0$, the energy band bends downward as a result of the electrons accumulated at the surface of the n-type silicon. The capacitance of the accumulation region is very large due to its very small thickness. When $\Psi_s > 0$, the electrons are pushed away from the surface into the bulk and leave the ionized donors to form a depletion layer. At this condition, the capacitance increases as the thickness of the depletion layer decreases. When the inversion layer forms ($\Psi_s > 0$), holes accumulate near the surface and the inversion layer is thinner than the depletion layer, therefore the capacitance increases again. Deep depletion occurs when measuring the capacitance at the high frequency region while quickly sweeping the applied gate voltage. Under this condition, the applied gate voltage must be changed quickly enough so that the BioFET structure is not in thermal equilibrium. It has been observed that, when modifying the applied gate voltage from the flat-band point to the threshold point or beyond, the inversion layer does not form or is only partially formed. This results in the generation of holes, but not enough to form a full inversion layer. The depletion layer therefore keeps increasing, leading to a further decrease in the capacitance.
Figure 5.7: Theoretical capacitance vs. applied gate voltage (C-V) diagram of a BioFET capacitor. The flat-band voltage (V_{fb}) separates the accumulation region from the depletion region. The threshold voltage (V_T) separates the depletion region from the inversion region. C_{HF} is the high frequency capacitance at the deep depletion condition.

5.1.4 Conductance-control mechanism of the BioFETs

The operation of BioFETs can be described like that a potential difference (V_{DS}) drives a current (I_{DS}) between the drain (D) electrode and the source (S) electrode (Figure 5.1). In general, the conductance-control mechanism is based on an electric field generated by the potential (V_{RS}) between the reference electrode with an applied potential (V_R) and the source. In this study (n-type silicon), V_{RS} first created an accumulation condition, where the majority carriers (electrons) were attracted into the channel and the conductance in the silicon was high. As a reducing V_{RS}, a depletion...
layer was formed, where the electrons were repelled and thereby reduced the conductance in the channel. Further reducing $V_{RS}$ attracted minority carriers (holes) to a thinner region to form an inversion layer, which enhanced the conductance again. Thus it can be seen that the electric field controls the density of carriers in the channel, and it determines the conductivity of the channel. With these properties, the conductance-control mechanism of BioFETs can be used to detect the analyte of interest because the electric field generating from the binding of a charged biomolecule is analogous to applying a potential. In this chapter, we describe a series of experiments with electrical impedance spectroscopy (EIS) to monitor the changes in the space charge region of semiconductor wafers as a function of applied bias potentials, for various structures ranging from simple silicon dioxide layers, self-assembled monolayers (SAMs) and biomolecularly modified surfaces. Our results demonstrated that depletion or accumulation of carriers by change of electric field explored the possibility of being able to make a BioFET.

5.2 Materials and methods

5.2.1 Materials

All materials were same as described in Chapter 2 and 3 except that the low doped silicon wafers (Single side polished and backside etched n-Si (111) wafers of 100 mm in diameter, 550 ± 25 µm in thickness, and resistivity of 10 ohm-cm) were used instead of highly doped silicon wafers.
5.2.2 Stepwise functionalization of low doped n-Si (111) surfaces

The procedures to obtain acid-terminated surfaces, NHS-ester surface and antibody immobilization were described in detail in Chapter 3 except that in case of a BioFET highly doped silicon wafers has been replaced with low doped silicon wafers to which the depletion region in the silicon can be investigated.

5.3 Results and discussion

A BioFET sensor usually contains three major electrical regions which contribute to its overall impedance and can induce a dispersion of the impedance with frequency. They are: (1) the ionic double layer formed in the solution (interface between the electrolyte and organic layer), (2) the organic layer (which cover the semiconductor surface) and (3) the space charge layer formed in the semiconductor (interface between the organic layer and semiconductor). The deposition of an organic layer on the semiconductor surface can induce a shift in the flat-band energy. The direction (upward or downward) and magnitude of this shift depend on the thickness, chemical composition and electrical properties of the organic layer. It is expected that the contributions from the space charge region will be highly dependent on the applied gate voltage, whereas contributions arising from the organic layer will be relatively constant. Thus, the impedance spectra of the electrolyte-organic layer-semiconductor structure were measured at different DC bias potentials. The structure in this study was based on relatively low doped n-type silicon (10 Ωm) functionalised with native silicon dioxide, 1-undecenoic acid monolayers (Si-C) and human IgG grafted on a
Si-C surface. The applied voltage was varied between -800 mV and 800 mV measured with respect to the silver/silver chloride electrode in the solution.

5.3.1 Electrical characterization of silicon dioxide on silicon surfaces

Figure 5.8a-d shows the impedance of a native silicon dioxide layer on a low doped n-type silicon (111) surface in 100 mM KCl at pH 7, measured at different DC potentials from -800 mV to 800 mV. The impedance spectra are alternatively presented as plots of the equivalent parallel capacitance and conductance spectra as a function of frequency. It would be expected that the capacitance of the silicon dioxide layer is voltage-independent. Therefore, the dispersion curves were attributed to the voltage-dependence of the space charge layer in the semiconductor. An applied positive gate voltage larger than the flat-band voltage induces a positive charge on the gate and a negative charge in the semiconductor. At this condition, the mobile majority carriers (in these case, electrons) would accumulate at the interface between the silicon and silicon dioxide. As presented in Figure 5.8a, this accumulated layer was clearly identified for gate voltages ≥ 400 mV. Examples of the impedance dispersion for the electrolyte-silicon dioxide-silicon structure show that the accumulation was revealed in the spectra recorded at 400 mV, 600 mV and 800 mV. These three spectra overlap and demonstrate that the system at the accumulated condition was voltage-independent. The contribution of the solution was observed at the high frequency region where the capacitance was close to zero and the conductance approached a constant value of ~350 mS/m² (Figure 5.8b). The properties
of the silicon dioxide layer dominated in the low frequency region where the capacitance approached a constant value of 23 mF m$^{-2}$ and the conductance value was 0.24 mSm$^{-2}$.

When the space charge layer in the semiconductor is in the accumulating state, its capacitance of is very large. The total interfacial capacitance $C_{\text{tot}}$ can be described as a series combination of the silicon dioxide capacitance $C_{\text{ox}}$ and the space charge layer capacitance $C_{\text{sc}}$.

$$\frac{1}{C_{\text{tot}}} = \frac{1}{C_{\text{ox}}} + \frac{1}{C_{\text{sc}}}$$

When $C_{\text{sc}} \gg 0$, then $\frac{1}{C_{\text{sc}}} \approx 0$

$$C_{\text{tot}} = C_{\text{ox}} = \frac{\varepsilon_{\text{ox}}\varepsilon_0}{d} \Rightarrow d = \frac{\varepsilon_{\text{ox}}\varepsilon_0}{C_{\text{ox}}}$$

$C_{\text{ox}}$ is constant for the silicon dioxide thickness $d$ and corresponds to the maximum capacitance of the system; $C_{\text{ox}}$ was obtained from the impedance spectra at $V_g \geq 400$ mV, $C_{\text{ox}} = 0.024$ Fm$^{-2}$. Assuming the dielectric constant of the silicon dioxide is $\varepsilon_{\text{ox}} = 4.4$, the thickness of the native silicon dioxide is 1.4 nm, which is in good agreement with previous measurements$^{11-13}$ of the thickness of silicon dioxide layer (1-2 nm).

It is known that when the gate voltage decreases below the flat-band voltage, the majority carriers (electrons) are drawn away from the interface between the silicon dioxide and silicon. On decreasing the voltage, the capacitance would decrease until the depletion layer becomes stable, but the applied voltage supplies additional
minority carriers (holes) to the inversion layer. In Figure 5.8a, a significant change in the dispersion curve was observed when the gate voltage was decreased from 400 mV to 200 mV. Therefore, we assume that at the gate voltage at 400 mV the system was in the flat-band. Gradual changes in the dispersion curves were obtained for gate voltages in the range from 200 mV to zero. These changes were characterised by a decrease in the conductance at high frequencies larger than 1 kHz, indicating that the measurements at these frequencies are no longer completely assigned to the solution. The changes in the depletion layer induced a pronounced dispersion in the capacitance and conductance in the mid range of frequencies. Based on our results, the depletion layer formed at a gate voltage of approximately 400 mV and the dispersion attributed to the space charge condition was observed at relatively high frequencies (1 kHz < f < 10 kHz). By further decreasing the gate voltage (i.e. to 200 mV and 150 mV), the dispersion moved to lower frequencies (i.e. 100 Hz < f < 1 kHz for 200 mV). When the gate voltage was reduced to zero, the depletion layer seemed to reach its maximum thickness (1 Hz < f < 10 Hz). It was noted that before the depletion condition (accumulation), the capacitance at the low frequency region was independent of frequency and we attributed this to that of the silicon dioxide layer. The formation of the depletion layer in the silicon induces a shift in the capacitance spectrum from the high frequency region to the low frequency region. Therefore, when the depletion layer reaches its maximum thickness, the real capacitance values attributed to the silicon dioxide move to the extra low frequency region which was beyond the range of our measurements. At the condition shown in Figure 5.8a, the
capacitances at low frequencies for gate voltages between zero and 400 mV were partially attributed to the depletion layer.

It should be noted that, once the inversion layer is formed, the depletion layer thickness reaches its maximum. Therefore, the region of the capacitance curves attributed to the depletion layer (1 Hz < $f$ < 10 Hz) remains almost constant. The region of the capacitance curves arising from the charge in the inversion layer as a function of the gate voltage was measured at the frequencies smaller than 1 Hz. Continuously decreasing the gate voltage makes the semiconductor surface invert its conductive type from n-type to p-type. At this condition, the concentration of holes at the surface exceeds the concentration of electrons in the bulk. The number of holes at the surface increases as the gate voltage decreases. In Figure 5.8c, the dispersion arising from the space charge moves back to higher frequencies, which contributed to the onset of inversion ($V_g = -100$ mV). The capacitances at low frequencies ($f < 1$ Hz) returned to the original values attributed to the silicon dioxide; we assume this point represents the threshold voltage. A decrease in the gate voltage led to an increase in the charge of the inversion layer and the interfacial region became more conductive, as shown in Figure 5.8d.
Figure 5.8: Equivalent parallel capacitance and conductance dispersion of an electrolyte-silicon dioxide-silicon structure at various gate voltages: (a) and (b) from 800 mV to zero, (c) and (b) from zero to -200 mV. The accumulation case was observed for $V_g \geq 400$ mV. The depletion layer was detected when the gate voltage was decreased to 200 mV and the depletion layer thickness reaches a maximum when the gate voltage was reduced to zero. Continuously decreasing the gate voltage to -100 mV led to the detection of the inversion layer at low frequencies.
5.3.2 C-V characteristics of the electrolyte-silicon dioxide-silicon structure

Figure 5.9 shows the C-V characteristics of the electrolyte-silicon dioxide-silicon structure at selected frequencies from 0.0279 Hz to 28.6 Hz. Since the capacitance for the silicon space charge layer $C_{sc}$ and the silicon dioxide capacitance $C_{ox}$ are in series, the total capacitance of the structure can be represented by \( \frac{1}{C_{tot}} = \frac{1}{C_{ox}} + \frac{1}{C_{sc}} \).

At sufficiently positive gate voltages the space charge layer near the silicon surface would be at the accumulation condition, leading to a minimal $\frac{1}{C_{sc}}$; the total capacitance would then be more affected by the capacitance of the silicon dioxide for all frequencies ($f \geq 28.6$ Hz) as presented in Figure 5.9. The capacitance of the silicon dioxide at low frequencies was independent of frequency. Therefore, it was observed that the capacitances for all frequencies at accumulation conditions show insignificant changes.

When the gate voltage was decreased from 400 mV to 200 mV, the capacitance dropped rapidly due to the formation of the depletion layer which is in series with the silicon dioxide and thereby reduced the total capacitance. When the gate voltage was further decreased to zero, the capacitance of the depletion layer reached its maximum thickness as a result of the minimum capacitance.

Continuously decreasing the gate voltage inverted the conductive type of the silicon surface from n-type to p-type. The minority carriers (holes) in the n-type silicon
accumulated at the surface and thus increased the capacitance. It was simply that at low frequencies the impedance was dominated by the capacitance of the silicon dioxide layer and the capacitance of the space-charge region was not detectable because its conductance was much larger than that of the silicon dioxide layer. These two regions really formed a simple Maxwell-Wager system with two layers. At low frequencies one saw the layer with the low time constant and at high frequencies one saw the capacitances of the two layers in series. Thus the inversion layer capacitance at low frequencies is given by $C_{\text{inversion}} = C_{\text{max}} = \frac{\varepsilon_{\text{ox}} \varepsilon_0}{d_{\text{ox}}}$.

Examples of this behavior were observed for the C-V curves at 0.0279 and 0.447 Hz where the overall capacitance returned to the value for silicon dioxide at the inversion condition.

Compared to the low frequency measurements, the generation rate at high frequencies was not fast enough to allow the formation of a hole charge density at the interface between the silicon dioxide and silicon. Therefore, the capacitance did not return to the value for the silicon dioxide but instead approached a constant minimum value ($C_{\text{min}}$). In this case, the depletion layer thickness in the silicon is still at its maximum value ($d_{\text{max}}$) and the corresponding inversion layer capacitance at a high frequency is $C_{\text{min}}$ and is given by $C_{\text{inversion}} = C_{\text{min}} = C_{\text{D min}} = \frac{C_{\text{ox}} C_{\text{D min}}}{C_{\text{ox}} + C_{\text{D min}}}$. The typical C-V curves in which the capacitance remains at $C_{\text{min}}$ after the onset of the inversion condition were observed at $f > 14.3$ Hz.
5.3.3 Theoretical calculations

The flat-band voltage is equal to the difference between the gate work function, $\Phi_g$, and the semiconductor work function, $\Phi_s$, if there is no charge present in the silicon dioxide or the silicon dioxide-silicon interface.

$$V_{FB} = \Phi_g - \Phi_s$$  \hspace{1cm} (5.1)

The work function is the voltage required to extract an electron from the Femi energy to the vacuum level.$^{14}$ The work function of a semiconductor is dependent on the doping type and doping concentration. This work function equals the sum of the electron affinity in the semiconductor, $X$, and the difference between the conduction band energy and the intrinsic energy divided by the electronic charge in addition to
the bulk potential. It is expressed in Equation 5.1 as

\[ V_{FB} = \phi_g - \phi_s = \phi_s - X \frac{E_s}{2q} + V_t \ln \left( \frac{N_a}{n_i} \right) \] (5.2)

where \( N_a \) is the donor impurity concentration of the n-type silicon substrate (in this case, \( 5.67 \times 10^{-14} \text{ cm}^{-3} \)), \( V_t \) equals to \( \frac{kT}{q} \) (in this case, 0.026 V), work function of the gate, \( \Phi_g \), is 4.8 V and the electron affinity, \( \chi \), is 4.05 V. The theoretical flat-band voltage is then equal to 470 mV, which is close to our experimental measurement of the flat-band voltage of \( V_g \approx 400 \text{ mV} \).

For an ideal n-type substrate, the threshold voltage is expressed as

\[ V_t = V_{FB} - \phi_s - \frac{\sqrt{qN_a \varepsilon_o \varepsilon_{Si} \phi_s}}{C_{ox}} \] (5.3)

\[ \phi_s = -2\phi_F \] (5.4)

\[ \phi_F = V_t \ln \frac{N_a}{n_i} \] (5.5)

where \( C_{ox} \) is the capacitance of the silicon dioxide layer and is equal to \( \frac{\varepsilon_o \varepsilon_{Si}}{d_{ox}} \approx 0.027 \text{ F m}^{-2} \). \( \varepsilon_{ox} \) is 4.4, \( \varepsilon_{Si} \) is 12 and \( \varepsilon_0 \) is \( 8.85 \times 10^{-14} \text{ F m}^{-1} \). The threshold voltage for an ideal structure would then be about -153 mV, which is close to our experimental result of -100 mV.

The capacitance of an electrolyte-silicon dioxide-silicon structure is treated as a series connection of two capacitors: the capacitance of the silicon dioxide and the capacitance of the depletion layer.
In accumulation, there is no depletion layer. Thus, the capacitance of the system is equal to the capacitance of the silicon dioxide.

\[ C_{\text{tot}} = C_{ox} = \frac{\varepsilon_0 \varepsilon_{\text{ox}}}{d_{\text{ox}}} \]  \hspace{1cm} (5.5)

In depletion, the capacitance is obtained from the series connection of the silicon dioxide capacitance and the depletion layer, which can be expressed as:

\[ C_{\text{tot}} = \frac{1}{\frac{1}{C_{\text{ox}}} + \frac{x_d}{\varepsilon_0 \varepsilon_{\text{SI}}}} \text{ for } V_{FB} \leq V_g \leq V_T \]  \hspace{1cm} (5.6)

where \( x_d \) is the variable depletion layer thickness calculated from

\[ x_d = \sqrt{\frac{2 \varepsilon_0 \varepsilon_{\text{SI}} + \varepsilon_0}{qN_a}} \]  \hspace{1cm} (5.7)

In inversion, the low frequency capacitance equals the silicon dioxide capacitance since the charge is added to and removed from the inversion layer. The high frequency capacitance is obtained from the series connection of the silicon dioxide capacitance and the capacitance of the depletion layer having maximum thickness, \( x_{d}^{\text{max}} \), and can be expressed as:

\[ C_{L} = C_{ox} \text{ and } C_{H} = \frac{1}{\frac{1}{C_{\text{ox}}} + \frac{x_{d}^{\text{max}}}{\varepsilon_0 \varepsilon_{\text{SI}}}} \text{ for } V_g \geq V_T \]  \hspace{1cm} (5.8)

Using the Equations 5.5 - 5.8, the capacitance of the silicon dioxide was calculated to be 2655 nFcm\(^{-2}\), the flat-band capacitance, 60 nFcm\(^{-2}\), the low frequency capacitance in inversion is 2655 nFcm\(^{-2}\), the high frequency capacitance in inversion, 7.55 nFcm\(^{-2}\) and the maximum thickness of the depletion layer, 1390 nm.

It should be noted that our experimental measurements of the flat-band voltage and
threshold voltage were slightly different from the theoretical values. We assume that the differences are due to non-ideal effects in the electrolyte–silicon dioxide-silicon structure, mobile charge and charge in the surface states.

The fixed charge in the silicon dioxide simply shifts the dispersion curve. It is known that silicon dioxide at pH 7 is negatively charged.\textsuperscript{15, 16} Consequently, the positive fixed charge forms at the silicon dioxide layer and the silicon interface and shifts the flat-band voltage to a lower position. The amount of the shift would be equal to the charge divided by the silicon dioxide capacitance. A fixed charge is caused by ions, which are incorporated in the silicon dioxide during growth. A mobile charge shifts the flat-band voltage in a manner similar to that of a fixed charge. However, the dispersion curves differ when the curve is shifted toward the applied voltage. A charge due to electrons occupying surface states also induces a shift in the flat-band voltage. However as the gate voltage is varied, the Femi energy at the silicon dioxide-silicon interface changes and affects the occupancy of the surface states. These non-ideal effects are not part of this research and therefore are not included in the analyses.

5.3.4 Electrical characterization of undecanoic acid monolayers on silicon surfaces

Figure 5.10a-d shows the capacitance and conductance spectrum of the electrolyte-undecanoic acid monolayer-silicon structure at various gate voltages ranging from -200 mV to 800 mV. The trend of the dispersion curves arising from the
The undecanoic acid monolayer was very similar to those of the silicon dioxide layer shown in Figure 5.8a. At positive gate voltages above the flat-band voltage of the modified silicon, majority carriers (electrons) accumulate in the interfacial region between the silicon and the undecanoic acid monolayer. Under the accumulation condition, the dispersion curves were dominated by the capacitance of the undecanoic acid monolayer. The electric properties of the organic monolayer are independent of the gate voltage. Therefore, the dispersion curves did not show significant differences in accumulation. The accumulation was clearly identified for gate voltages $V_g \geq 600$ mV. Compared to the accumulation in the silicon dioxide system at gate voltages $V_g \geq 400$ mV (Figure 5.8a), the undecanoic acid monolayer required a higher gate voltage to obtain surface accumulation. It is suggested that this was due to the fixed (-COO-) charges present on the undecanoic acid monolayer. An example of the impedance dispersion curves, in accumulation, for the capacitance and conductance recorded at $V_g = 800$ mV is shown in Figure 5.10. Similar dispersion curves were obtained at $V_g = 600$ mV. The properties of the electrolyte were observed at high frequencies as the capacitance approached zero (Figure 5.10a) and the conductance reached a constant value of ~ 350 S m$^{-2}$ (Figure 5.10b), which is the same as the measurements for the electrolyte-silicon dioxide-silicon structure (Figure 5.8a-d).

When the gate voltage was decreased to 400 mV, significant changes in the dispersion of both the capacitance and conductance spectra were observed. These changes contributed to the formation of a depletion layer in the silicon. It is noted that the
thickness of the depletion layer increased as the decrease in the gate voltage shifted the dispersion curves from high frequencies to lower frequencies. This shift stopped until the depletion layer in the silicon reached its maximum thickness with the lowest capacitance value. For the results shown, the depletion layer reached to its maximum thickness at the gate voltage $V_g = 0$ mV.

By further decreasing the gate voltages ($V_g \leq -100$ mV), the dispersion curves moved back to higher frequencies due to the onset of the formation of the inversion layer. The thickness of the monolayer was calculated for the system under the accumulation condition and this yielded a dielectric thickness of 1.3 nm assuming that the dielectric constant of the alkyl monolayer is 2.1.\textsuperscript{17} This estimate of the thickness is in good agreement with the geometric length of the undecenoic acid molecules. The capacitance at low frequencies attributed to the alkyl monolayer at accumulation was constant. However, the depletion layer that formed in the silicon induced a shift in the capacitance curve from high frequencies to lower frequencies.
a.

![Capacitance vs Frequency graph](image)

b.

![Conductance vs Frequency graph](image)
Figure 5.10: Equivalent parallel capacitance and conductance dispersion of an electrolyte-undecanoic acid monolayer-silicon structure at various gate voltages (a) and (b) from 800 mV to zero, (c) and (b) from zero to -200 mV. The accumulation case was observed for $V_g \geq 400$ mV. The depletion layer was detected when the gate voltage was decreased to 200 mV and the depletion layer thickness reached a maximum when the gate voltage was reduced to zero. Further decreasing the gate voltage to -100 mV led to the detection of the inversion layer at low frequencies.
5.3.5 Electrical characterization of proteins grafted on Si-C linked alkyl monolayers

Figure 5.11a shows that, after human IgG was immobilized on the undecanoic acid modified silicon surface, the accumulation condition was found at a gate voltage of \( V_g \geq 700 \text{ mV} \). A similar dispersion curve was observed at a gate voltage of 800 mV. It is known that, at the accumulation condition, the capacitance of the system is dominated by layers on the surface. Our results show a decrease in the accumulation capacitance after antibody immobilization. This is expected since the additional layer formed on the silicon surface would act as a dielectric in series leading to a decrease in capacitance.

A significant change in the dispersion curve attributed to the space charge region was observed when the gate voltage decreased to 600 mV, which represented the onset of the depletion layer formation. Therefore, we assume that the gate voltage of \( \sim 700 \text{ mV} \) was the flat-band voltage after antibody immobilization. The conductance spectrum at gate voltages higher than 700 mV in Figure 5.11b indicates that the dispersion curves at high frequencies were attributed to the solution.

A pronounced dispersion in the capacitance and conductance was observed when the gate voltage was decreased to 500 mV. The decrease in capacitance with the decrease in gate voltage indicated the growth of the depletion layer; i.e. the capacitance decreased from \( 2.71 \times 10^{-3} \text{ F m}^{-2} \) to \( 5.33 \times 10^{-4} \text{ F m}^{-2} \) at 1 Hz when the gate voltage
was decreased from 600 mV to 200 mV. The growth of the depletion layer induced a shift in the dispersion curves from high frequencies to low frequencies and this shift stopped until the depletion layer reached its maximum thickness at gate voltage \( V_g = 200 \) mV. By further decreasing the gate voltage ( \( V_g \leq 100 \) mV, see Figure 5.11c), the dispersion arising from the silicon space charge region moved back to higher frequencies, which is attributed to the onset of inversion. When n-type silicon is inverted to p-type silicon at the inversion condition, holes are generated at the silicon surface and enhance the conductance of the interfacial region (see Figure 5.11d).

An important phenomenon was observed after antibodies were immobilized on Si-C linked alkyl monolayers; the gate voltage to attain the accumulation region changed from ~ 600 mV to ~ 700 mV and the gate voltage at the maximum thickness of the depletion layer changed from ~ 0 mV to ~ 200 mV. These changes are probably due to contributions to the total fixed charge from the antibodies. As negatively-charged undecenoic acid molecules approach the interface, electrons drift into the bulk and holes drift toward the surface, thus leading to upward band-bending. Since on n-type silicon the majority carriers are electrons, the upward band-bending represents a decrease in the density of majority carriers in the semiconductor space charge region. Most antibodies are negatively-charged at pH 7. Thus, in this case, the binding of the negatively-charged antibodies would repel the electrons in the adjacent semiconductor and accentuates the upward band-bending. On n-type silicon this would lead to a further decrease in the density of majority carriers. Consequently, a more positive gate
voltage is required to detect the regions of inversion, depletion and accumulation (Figure 5.12).
Figure 5.11: Equivalent parallel capacitance and conductance dispersion of antibody grafted on Si-C linked alkyl monolayers at various gate voltages (a) and (b) from 800 to 200 mV, (c) and (b) from 200 to zero mV. The accumulation case was observed for $V_g \geq 700$ mV. The depletion layer was detected when the gate voltage was decreased to 600 mV and the depletion layer thickness reached a maximum when the gate voltage was reduced to 200 mV. Further decreasing the gate voltage to 100 mV led to the detection of the inversion layer at low frequencies.
Figure 5.12: Schematic depiction of the changes in band-bending induced by exposure to antibodies. The negatively-charged antibody immobilization induces upward band-bending.

5.4 Conclusion

In this chapter, our results demonstrated that under appropriate applied gate voltages, the binding of antibodies to the functionalized surface led to a change in the depletion layer. Therefore if this formed a part of the BioFET then the binding of the antibodies could in principle be detected by a change in the source-drain current. For instance, the current measured at fixed potential for both source-drain and gate electrodes is constant over time for an n type silicon device. Any change in the current can be related to a change in conductance of the silicon wafer. When a negatively charged analyte molecule binds to the receptor on the silicon surface, this leads to depletion of main carriers (electrons) beneath the bound analyte, causing a depletion region to expand in width (Figure 5.13a). We could assume that if the depletion region expands to completely close the channel between source and drain electrodes, the resistance of the channel becomes large, and the BioFET is effectively turned off like a switch (Figure 5.13b). On the other hand, when a positively charged analyte molecule links to the silicon surface, it absorbs holes in the region near the silicon surface. This attraction causes carriers to accumulate, leading to narrow the width of the depletion
region and resulting in an enhancement of the current between source and drain electrodes (Figure 5.13c). This would therefore allow the construction of a direct read-out BioFET that could be turn on and turn off by antigen-antibody interactions. This would not require expensive instrumentation such as ellipsometry or impedance and highly skilled personnel.

![Diagram](image)

**Figure 5.13:** Mechanism to operate an n type BioFET. (a) When a negatively charged molecule is captured, it repels the carriers (electrons) in the silicon causing a decrease in conductance. (b) If the depletion region expands to completely close the channel, BioFET is turned off. (c) When a positively charged molecule binds to the receptor, it attracts negative electron carriers in the silicon. This attraction causes to enhance the current flow.

When the system was in accumulation, the capacitance of the space charge region was very large and the total capacitance was dominated by the capacitance of the layers on the surface. Therefore, the capacitance curves in accumulation did not show any significant differences. In the case of n-type silicon, when the gate voltage decreases below the flat-band voltage, the majority carriers (electrons) are drawn away from the interface, thus leading to upward band-bending. The capacitance of the space charge
layer decrease with the growth of the depletion layer and the dispersion curves shift from high frequencies to low frequencies. Once the depletion layer reached its maximum thickness, the gate voltage would supply additional minority carriers (holes) to the inversion layer. The growth of the depletion layer induced by the changes in the gate voltage shifted the dispersion curves of capacitance from high frequencies to low frequencies. The fixed charge of the antibodies can induce changes in both the flat-band and threshold voltages. The direction of the changes is controlled by the charge of the antibodies.


5.5 References


Chapter 6

Summary and future directions

6.1 Summary

The aim of the research in this thesis was to a BioFET and towards this end the properties of organic and biological materials bound to silicon substrates were investigated. In the first instance this was done using highly doped silicon wafers in order to avoid complication arising from depletion layers in the silicon at the surface. The investigations of the electric properties of layers on highly doped silicon surfaces including:

1) Self-assembled monolayers (SAMs) directly immobilized on atomically flat silicon surfaces.

2) Biological molecules grafted on silicon surface via the functional groups of SAMs.

First, a hydride-terminated silicon surface was modified by the attachment of alkyl monolayers with and without functional groups. The quality and properties of these monolayers were studied by electrical impedance spectroscopy (EIS) and the results were confirmed by x-ray photoelectron spectroscopy (XPS), ellipsometry and water contact angle measurements. Self-assembled monolayers directly immobilized on the silicon surfaces were obtained by reacting the hydride-terminated silicon surfaces with 1-alkene solution in a UV mediated hydrosilylation reaction. The quality of the monolayers was highly dependent on the methods of preparation. Rinsing the silicon samples with water after 40% ammonium fluoride etching was able to eliminate the
contamination from nitrogen and fluorine ions and quickly rinsing them did not induce the re-oxidation of Si-H surfaces. Photochemical hydrosilylation reactions of alkenes must occur under inert gases, such as nitrogen or argon, because the combination of UV light and oxygen not only re-oxidizes the hydrogen-terminated silicon surfaces, but also degrades the alkyl monolayers. The doping effect analysis using both XPS and EIS demonstrated that well-ordered monolayers can easily be obtained by irradiating an n-type Si (111)-H surface with UV (254 nm) light in the presence of alkenes solution, but not on a p-type Si (111)-H surface. This can be explained by the excitation mechanism. Under UV irradiation, band-bending near the n-type silicon surface exhibits an upward movement that drives electrons into the bulk of the silicon and accumulates holes near the surface, resulting in a more positively charged surface that is more susceptible to nucleophilic attack by alkenes. Conversely, the downward band-bending of the p-type silicon surface leads to an accumulation of electrons near the surface that can repel nucleophilic attack. The stepwise functionalization of the silicon indicated that EIS could detect the binding of antibodies and antigen-antibody interactions quickly via the influence of bond charges on conductance and capacitance at both low and high frequencies. In this study, an antibody was immobilized on a silicon surface via the NHS-ester activated monolayer. At the high frequency region which is sensitive to antigen-antibody interactions, we found that the capacitance decreases as additional biomolecular layers are immobilized. We observed increases as well as decreases in the capacitance based on the various frequency regions. Therefore, we conclude that the change in capacitance
after additional biomolecular layer immobilization depends on details of the surface structure, the quality of the molecular layers and the sensitivity to frequency regions.

For an ultimate BioFET, the depletion layer was a crucial element in its function and hence the effects of the organic and biomolecular layers on low doped silicon were also investigated. EIS studies of the stepwise functionalization of low doped silicon surfaces revealed that the contributions arising from the organic layer, the space charge region in the silicon and the electrolyte could be identified separately. The dispersion curves at various gate voltages were attributed to the different regions of the structure. When the system was in accumulation, the capacitance of the space charge region was very large and the total capacitance was dominated by the capacitance of the layers on the surface. In the case of n-type silicon, when the gate voltage decreased below the flat-band voltage, the majority carriers (electrons) were drawn away from the interface, thus leading to upward band-bending. The capacitance of the space charge layer decreased with the growth of the depletion layer and the dispersion curves shifted from high frequencies to low frequencies. Once the depletion layer reached its maximum thickness, the gate voltage supplied additional minority carriers (holes) to the inversion layer, which converted the n-type silicon to p-type silicon. Therefore, the space charge conditions in the silicon characterised by changing the gate voltages have a great potential to directly sense protein attachment.

In addition, silicon surfaces coated with two different polymerisation conditions
(nitrogen or nitrogen + argon) as the alternatives of SAMs were also investigated. The addition of nitrogen to the acetylene modified the chemical content and the intrinsic stress, while the argon altered the energetic ion bombardment occurring during polymerisation. The capacitance of the nitrogen-containing plasma polymer (NPP) was characterized by frequency-independence in the low frequency region as a result of a homogeneous surface. Inversely, the capacitance of nitrogen + argon-containing plasma polymer (NArPP) on a silicon surface as a function of frequency showed a dispersion at low frequencies, indicating a heterogeneous surface. However, compared with 2-4 nm antibodies, the thickness of the plasma polymer (usually greater than 75 nm) displayed a large background signal, which is outside of the sensitivity of EIS detection. Therefore, we used ellipsometry and XPS to study the properties of antigen-antibody interactions on the NPP. XPS and QCM-D were used to confirm the covalent bounding nature of the NPP surface before and after the freeze-drying treatment. The antigen-antibody interactions on the NPP biosensors were comprehensively reconfirmed after freeze drying using the XPS technique. Three different pairs of antigens-antibodies, together with protein-A to accommodate different antibodies, were tested to demonstrate the new biosensor methodology. The high sensitivity of the biosensors using the antigen-antibody interactions was consistently demonstrated for cases with and without the freeze-drying treatment. Protein-A remained functional after covalent immobilization for the following cases: protein-A only with or without freeze-drying treatment, and antibodies on protein-A with the freeze-drying treatment. BSA blocking showed very little change in the
sensor readings, compared to the antigen-antibody interactions, indicating the high density of covalently immobilized functional proteins for all cases with or without the freeze-drying treatment. The ellipsometry Psi signal ratios of antibody to antigen were consistent with dense layers of antibody or antigen that have been captured or immobilized.

6.2 Future directions

We believe that BioFETs have great potential for detecting antibodies/antigens DNA, enzymes and even cells when suitably functionalised. However, BioFETs at this stage would present many difficulties in manufacturing in addition to the instability of the self-assembled monolayers or plasma polymers on the substrate surface and impurities in the semiconductor. To overcome these problems, future experiments investigating the properties of organic layers on silicon are required. Thus,

- Further EIS studies to investigate the antigen-antibody interactions on low doped silicon substrate in more detail.
- An actual experimental BioFET should be constructed based on a MOSFET or an ISFET. A hypothetical design was described in Figure 5.1.
- In this research, the monolayers with a terminal methyl group provided a more hydrophobic surface, which prevented oxidation. However, the SAMs require a functional terminal group that can react with other chemical moieties to attach proteins etc. In the present study, carboxylic acid groups were used as the terminal functional group on the SAMs and these were converted to
NHS-ester activated surfaces that could react with the primary amine groups in the proteins. However, this kind of monolayer did not provide protection of re-oxidation of the silicon substrate surface. Therefore, the formation of mixed monolayers at an optimal ratio of carboxylic acid to methyl group terminal might be required to produce more stable but still functional surfaces. This will require extensive exploration.

- The pre-treatment process was too time consuming, especially for the SAMs directly grafted on the silicon substrate. There is a scope for optimization of the procedure

- Experiments should be conducted using plasma coating of low doped silicon to explore the effect of protein binding in such systems on the depletion layer at the silicon surface. If this works as well as that obtained with the grafted SAMs, then this would provide a simpler method to manufacture BioFETs.