NOTE TO USERS

This reproduction is the best copy available.

UMI[®]

Stabilization of the Silicon – Organic Interface for Biosensing Applications

by

Trevor Mischki

A thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

Department of Chemistry

Carleton University
Ottawa, Ontario
February 2010

© Copyright
2010, Trevor Mischki

Library and Archives
Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file *Votre référence*
ISBN: 978-0-494-67882-4
Our file *Notre référence*
ISBN: 978-0-494-67882-4

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Abstract

Chemical modification of hydrogen terminated silicon is of interest both from a fundamental perspective and due to envisioned applications of hybrid organic – electronic devices, which include label free, electrical, chemical and biological sensors. Their successful development is contingent upon maintaining the highly sensitive electrical properties of the silicon during organic modification and on exposure to sensing environments. With this in mind, the objective of this thesis is to critically assess the potential for label-free biosensing on crystalline silicon.

The surface is least protected at the early stages of monolayer formation; therefore an understanding of these mechanisms is essential. A study of the differential reactivity of oxidized and hydrogen terminated silicon indicate Markovnikov addition of alkenes to silanol groups occurs at lower temperatures and that selectivity is reduced as the temperature increases. Subsequent studies compare the effect of reaction vessel (Pyrex versus PTFE) on the reaction. It is shown that the acidic silanol surface not only reacts with the alkene but also cracks the hydrocarbon reactant forming reactive species which initiate the reaction of alkenes with hydrogen terminated silicon.

Feasibility studies then assess firstly, the suitability of scanning-Kelvin probe to measure biological interactions and secondly, the stability of the silicon – monolayer interface under biologically relevant conditions. Initially the protein p19 was used however the electrical response is dominated by ionization of the nickel-nitrilotriacetate probe molecule. Non-ionic biotin terminated surfaces capture fluorescently labeled Streptavidin. The electrical and fluorescent measurements correlate providing confidence

in these results. These first generation monolayers oxidize on exposure to water. It is shown that branching via the Grignard reaction stabilizes the chemical and electrical properties of the interface.

The final challenge was to functionalize the branched surface. Several routes are investigated, oxidative conditions damage the substrate, and while olefin metathesis did not adversely affect the substrate the yield was not sufficient to enable specific capture of the protein. Ultimately an alcohol terminated layer functionalized with hexamethylene diisocyanate is shown to stabilize the electrical and chemical properties of the silicon monolayer interface and enable specific capture of the target.

Acknowledgements

I would like to thank my thesis advisor Dan Wayner for the opportunity and freedom to explore and grow as a scientist. His advice and perspective on the inevitable challenges and successes that accompanied this work over the past 5 years are appreciated.

I would like to express my thanks to Greg Lopinski for sharing his time and knowledge throughout this endeavour.

I was fortunate to have collaborated with John Pezacki and for being welcomed as an “honorary” member of the group. I would like to thank my colleagues and now friends at SIMS and Carleton for making this an enjoyable experience.

I am grateful to Carleton University, the National Research Council of Canada and the Natural Sciences and Engineering Research Council of Canada for financial support.

I would like to thank my friends and family for their enduring support and encouragement.

Lastly, I am grateful beyond words to my wife Corinne for her support patience and enthusiasm and our son Braeden who has brought me immeasurable joy.

Table of Contents

Abstract	i
Acknowledgements	iii
List of Figures	viii
List of Tables	x
List of Schemes	xi
List of Abbreviations	xii
Chapter 1 – Introduction	1
1.1 – Surface Modification	1
1.1.1 – Silicon	1
1.1.1.1 – Chemistry on Silicon Dioxide	7
1.1.1.2 – Chemistry on Hydrogen Terminated Silicon	8
1.1.1.2.1 – Etching	8
1.1.1.2.2 – Monolayer Formation by Thermal Initiation	11
1.1.1.2.3 – Grignard / Alkyl Lithium Derived Monolayers	13
1.1.1.2.4 – Photochemical Initiation of Monolayer Formation	15
1.1.1.2.5 – Other Methods for Forming Organic Layers on Silicon	19
1.1.1.3 – Functional Group Incorporation	19
1.1.1.4 – Inhibition of Non-specific Binding	22
1.1.1.5 – Silicon Stability	24
1.1.1.6 – Biosensing on Silicon	27
1.2 – Characterization Techniques	32
1.2.1 – Contact Angle	32

1.2.2 – Ellipsometry	34
1.2.3 – FTIR	35
1.2.4 – XPS	37
1.2.5 – Kelvin Probe and Surface Photovoltage	40
1.3 – Aims and Goals	44
Chapter 2 – Monolayer Formation	47
2.1 – Introduction	47
2.2 – Experimental	50
2.2.1 – Materials	50
2.2.2 – Surface Characterization	51
2.2.3 – Surface Preparation	52
2.3 – Results and Discussion	54
2.3.1 – Oxide Reactivity	54
2.3.1.1 – Time Dependence of the Physical Properties	54
2.3.1.2 – Process of Photooxidation	58
2.3.2 – Mechanism of Thermal Initiation	66
2.3.2.1 – Evidence of Hydrocarbon Degradation	67
2.3.3 – Electrical Properties	72
2.4 – Summary & Prospective	75
Chapter 3 – Feasibility Study	78
3.1 – Introduction	78
3.1.1 – p19-RNA	78
3.1.2 – Biotin - Streptavidin	81

3.1.3 – Stability	82
3.1.4 – Rationale	83
3.2 – Experimental Details	85
3.2.1 – Materials	85
3.2.2 – Surface Characterization (see Section 2.2.2)	86
3.2.3 – Synthesis	87
3.2.4 – Monolayer Preparation and Functionalization	90
3.2.5 – Protein Attachment	93
3.3 – Results and Discussion	93
3.3.1 – p19	93
3.3.2 – Biotin – Streptavidin	108
3.3.3 – Stability	115
3.4 – Summary and Prospective	123
Chapter 4 – Functionalization of Stable Surface	126
4.1 – Introduction	126
4.1.1 – Functional Groups Compatible with the Grignard Reaction	126
4.1.1.1 – Reactions of Alkenes	127
4.1.1.2 – Reactions of Alcohols	131
4.1.2 – Rationale	132
4.2 – Methods and Materials	133
4.2.1 – Materials	133
4.2.2 – Surface Characterization (see Section 2.2.2)	134
4.2.3 – Synthesis	135

4.2.4 – Monolayer Preparation and Functionalization	138
4.2.5 – Protein Attachment	143
4.3 – Results and Discussion	143
4.3.1 – Oxidative Conditions	143
4.3.1.1 – Pentenyl Branched Monolayer	143
4.3.1.2 – Undecylenyl Branched Monolayer	144
4.3.2 – Metathesis	149
4.3.3 – Alcohol Terminated Monolayers	157
4.4 – Summary and Conclusions	168
Chapter 5 – Overall Conclusions and Claims to Original Research	171
5.1 – Claims to Original Research	171
5.2 – Overall Conclusions	173
5.3 – Opportunities	174
References	176
Chapter 6 - Appendices	184
6.1 - Characterization	184

List of Figures

Figure 1.1 – Structure of crystalline silicon	2
Figure 1.2 – Fermi level of doped and undoped silicon.	3
Figure 1.3 – Field Effects	4
Figure 1.4 – Metal Oxide Semiconductor Field Effect Transistor (MOSFET)	6
Figure 1.5 – FTIR and STM Evidence of Ideal Si-H Termination of Si(111)	10
Figure 1.6 – STM Image Showing Radical Chain Reaction	18
Figure 1.7 – Origin of Protein Resistance by OEG Chains	23
Figure 1.8 – Penetration of solvent into (a) decyl and (b) UDA Modified Surfaces	25
Figure 1.9 – Overview of Label versus Label Free Approaches	28
Figure 1.10 – pH Dependence of Aminopropyl Silane Monolayer on Silicon Oxide	29
Figure 1.11 – Comparison of Biosensing on Silicon Oxide and Si-H Surfaces	30
Figure 1.12 – Protein Binding on Si-H Nanowire	32
Figure 1.13 – Illustration of Water Contact Angle	33
Figure 1.14 – ATR-FTIR Geometry	36
Figure 1.15 – ATR-FTIR Spectra of a Reaction Sequence	37
Figure 1.16 – Overview of XPS	38
Figure 1.17 – XPS Spectra Demonstrating Deprotection of Trifluoroethyl Ester	39
Figure 1.18 – Surface Photovoltage, Negative Charge on n-type Silicon	40
Figure 1.19 – Measurement of Protein Binding By Kelvin Probe	41
Figure 1.20 – Measurement of DNA Recognition by Surface Potential	43
Figure 2.1 – Picture of patterned surface exposed to water vapour	55
Figure 2.2 – Plot of contact angle versus time	56
Figure 2.3 – Plot of estimated thickness versus time	57
Figure 2.4 – Plot of integrated area of absorbance (2800 - 3000 cm^{-1})	58
Figure 2.5 – HREELS spectra showing effect of Si-H oxidative conditions	60
Figure 2.6 – XPS spectra of C1s region indicating decomposition of decene	62
Figure 2.7 – ATR-FTIR Spectra describing bonding orientation of alkene	64
Figure 2.8 – Pictures Demonstrating Recovery of Underlying Pattern	66
Figure 2.9 – UHV STM of Partially 1-decene reacted Si-H	67
Figure 2.10 – ATR-FTIR spectra of Si-H in Pyrex Schlenk tube	68
Figure 2.11 – ATR-FTIR Spectra of Si-H reacted with dodecane in PTFE vial	69
Figure 2.12 – SPV of Decene surfaces after Photochemical Reaction	73
Figure 2.13 – SPV of Decene Surface after Thermal Reaction	74
Figure 3.1 - Crystal Structure of p19	79
Figure 3.2 – Affinity Capture by NTA	81
Figure 3.3 – FTIR Absorbance Spectra of (A) UDA, (B) NHS and (C) NTA Surfaces	94
Figure 3.4 – SPV of surfaces before and after exposure to p19	97
Figure 3.5 – Survey XPS of Ni-NTA surface with inset of HR nickel region	98
Figure 3.6 – SPV of UDA surface exposed to base	98
Figure 3.7 – FTIR Spectra of (A) NHS, (B) NTA, and (C) Ni-NTA surfaces	100
Figure 3.8 – FTIR Difference Spectra Confirming Ni^{2+} complexation	102
Figure 3.9 – HR-XPS of Ni Region	103
Figure 3.10 – SPV of EG3OH and Ni-NTA Exposed to p19	106

Figure 3.11 - Fluorescence Image of Ni-NTA exposed to siRNA	107
Figure 3.12 - FTIR Spectra of NHS Exposed to Buffer (a) and Amine (b)	109
Figure 3.13 – SKP Images for Streptavidin Experiments	111
Figure 3.14 – Fluorescent Image after Exposure to Cy3 Labeled Streptavidin	112
Figure 3.15 – FTIR Spectra of Octadecene Monolayers	116
Figure 3.16 - FTIR Spectra of UDE and C-5 Branched Surfaces	119
Figure 3.17 – SPV changes on Aqueous Exposure	120
Figure 3.18 - HREELS Monitoring Surface Oxidation	122
Figure 4.1 – FTIR Spectra of KMnO_4 / NaIO_4 Oxidation	145
Figure 4.2 – FTIR Spectra of NHS and Amide Derived from KMnO_4 / NaIO_4 Oxidation, Inset is NHS ratioed to carboxylic acid terminated monolayer.	146
Figure 4.3 – HR-XPS Spectra of Si2p Region, Inset: SiO_2 Signal	147
Figure 4.4 – FTIR Spectra of NHS and Biotin Terminated Silicon Surface via Metathesis Route	151
Figure 4.5 - Surface Density versus Chain Length of OEG Groups	156
Figure 4.6 – FTIR Spectra of UDE and Alcohol Terminated Monolayer	159
Figure 4.7 – HR XPS of Si2P Region of Alcohol Terminated Monolayer	159
Figure 4.8 - FTIR Spectra of Succinic Anhydride Route to Modifying the Alcohol Terminated Surface	161
Figure 4.9 – FTIR Spectra of Isocyanate Route to Modified Alcohol Surfaces	162
Figure 4.10 – SKP and Fluorescence Image of Biotin Patterned Surface after Exposure to Streptavidin	166
Figure 4.11 – SKP and Fluorescence Images of Biotin Patterned Surface Exposed to Streptavidin using optimized spotting conditions	167
Figure 4.12 – SKP and Fluorescence Images of Patterned Silicon Sample Exposed to BSA and Streptavidin	168
Figure 5.1 – Model of Monolayer	173

List of Tables

Table 2.1 – Ratio of the antisymmetric to symmetric methyl stretch peak area	64
Table 2.2 – Effect of reactor material on Monolayer Properties	70
Table 3.1 – Thickness and Wettability data for NTA and p19 Surfaces	96
Table 3.2 – Thickness and Contact Angle using DMSO : DMF Route	102
Table 3.3 – Peak Data from HR-XPS of Ni-NTA Surface	105
Table 3.4 – Empirical XPS Sensitivity Factor	105
Table 3.5 – Thickness of EG3OH and Ni-NTA Exposed to p19	106
Table 3.6 – Properties of EG3OH and BioNH ₂ Surfaces on Exposure to Streptavidin	110
Table 3.7 - XPS Stability Study	114
Table 3.8 - Properties of Octadecene and C-5 Grig Surfaces	119
Table 4.1 – Integrated area of Saturated C-H Stretching Region for Pentenyl Modified Surfaces.	144
Table 4.2 – Integrated area of Saturated C-H Stretching Region for Undecylenyl Modified Surfaces.	146
Table 4.3 – XPS Analysis of KMnO ₄ / NaIO ₄ Oxidized Surface	148
Table 4.4 – XPS derived ML Equivalents of SiO ₂	152
Table 4.5 – Thickness Contact Angle and NHS Peak Area of Branched Surfaces after Metathesis and Amidation with BioNH ₂	155
Table 4.6 – Contact Angle Dependence on OEG Length	155
Table 4.7 – Comparison of Isocyanate Reacted in DCM and pH 8.0 Buffer	163
Table 4.8 – Optimization of Probe : OEG Ratio	164
Table 4.9 – Thickness Measurements of Selective Capture of Streptavidin versus BSA	167

List of Schemes

Scheme 1.1 – Formation of Silane Monolayer	8
Scheme 1.2 – Mechanism of Si(111) Etching by NH_4F	11
Scheme 1.3 – Mechanism of Radical Chain Growth on Hydrogen Terminated Si(111)	12
Scheme 1.4 – Preparation of Monolayer via Halogenation / Grignard Route ³⁵	14
Scheme 1.5 – Proposed Mechanism of Grignard Reaction	15
Scheme 1.6 – Mechanism of Exciton Mediated Hydrosilation on Porous Silicon	17
Scheme 1.7 – Proposed Mechanism for Visible Light Promoted Reaction on Si(111)-H	19
Scheme 3.1 – Preparation of 3	87
Scheme 3.2 – Preparation of 5	88
Scheme 3.3 – Preparation of 7	88
Scheme 3.4 – Preparation of 9	89
Scheme 3.5 – Preparation of NTA Modified Silicon Surface	94
Scheme 4.1 – Grignard Compatibility	127
Scheme 4.2 – Reactions of Alkenes	128
Scheme 4.3 – Mechanism of Epoxidation Reaction	128
Scheme 4.4 – Mechanism of Permanganate Oxidation under Acidic Conditions	129
Scheme 4.5 – Catalytic Cycle of Olefin Metathesis Using Grubbs Catalyst ¹⁸⁹	130
Scheme 4.6 – Possible Reactions of Alcohols	131
Scheme 4.7 – Syntheses of 12 and 13	135
Scheme 4.8 – Synthesis of 14	137
Scheme 4.9 – Synthesis of 15	137
Scheme 4.10 – Synthesis of 16	138
Scheme 4.11 – Preparation of Functionalized Surface via Metathesis	150
Scheme 4.12 – Potential Side Products in Metathesis Reaction	153
Scheme 4.13 – Route to Increased Density of Reactive Groups	157
Scheme 4.14 - Succinic Anhydride Route to Modified Alcohol Surfaces	160
Scheme 4.15 – Preparation Modified Surfaces via Isocyanate	162

List of Abbreviations

ATR – Attenuated total reflectance
BioNH₂ – Biotin amine
DNA – Deoxyribonucleic acid
EDC – N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride
FTIR – Fourier transform infrared spectroscopy
FWHM – Full width and half maximum
HR – High resolution
HREELS – High resolution electron energy loss spectroscopy
kDa – kilo Dalton
LPEI – Linear poly(ethyleneimine)
MIS – Metal Insulator Semiconductor
ML - Monolayer
MOSFET – Metal oxide semiconductor field effect transistor
Ni-NTA – Ni²⁺-Nitrilotriacetic acid complex
NHS – N-hydroxysuccinimide
NTA – (S)-N-(5-Amino-1-carboxypentyl)iminodiacetic acid
EG3OH – 2-[2-(2-aminoethoxy)ethoxy]ethanol modified silicon surface
OEG – oligoethylene glycol
p19 – tombusvirus suppressor of RNA silencing protein with apparent molecular mass of 19kDa
PBS – 1X phosphate buffered saline pH 7.4
pI – Isoelectric point
PTFE – poly-tetrafluoroethylene
RNA – Ribonucleic acid
SA – Streptavidin
Si-H – Hydrogen terminated silicon
siRNA – small interfering RNA
Si-UDA – silicon surface with undecylenic acid monolayer
Si-UDE – silicon surface with ethyl undecylenate monolayer
SPV – Surface photovoltage
TEMPO – 2,2,6,6-tetramethylpiperidine-1-oxy radical
TMS – Trimethylsilyl
UDA – Undecylenic acid
UHV – Ultra high vacuum
XPS – X-ray photoelectron spectroscopy

Chapter 1 – Introduction

1.1 – Surface Modification

The physical properties of an interface can greatly impact the function of the underlying material or structure, and can be modified by the addition of an adlayer or adlayers covalently or non-covalently bound to the substrate. Adlayers formed by organic molecules offer the potential to not only modify the physical properties of a surface but also impart novel functionality, for example a hydrophilic surface can be made hydrophobic¹, a metal can be modified to recognize a protein², and additionally this approach forms the basis of molecular electronics research³. Each of these examples represents a synergy obtained by combining the unique benefits of a substrate with the diversity afforded by organic chemistry.

The focus of this research has been the formation of covalent monolayers on hydrogen terminated silicon surfaces for applications in label-free biosensing. Two themes run through this thesis firstly, stabilizing the silicon interface (electrically and chemically) in biologically relevant conditions and secondly, forming layers that ensure specific recognition of the biological target. This chapter provides background on silicon chemistry including the rationale for choosing it as a biosensor platform and on the properties of biocompatible layers.

1.1.1 – Silicon

Silicon is a group IV element and, like carbon, exists as the diamond cubic lattice crystal structure (Figure 1.1a) with a spacing of 5.43 Å.⁴ The Aufbau principle indicates that a tetrahedral silicon atom contains four valence electrons and is sp³ hybridized.

Condensation of these atoms into a diamond structure leads to each atom being bonded to four others and surrounded by eight electrons. As a consequence of this condensation, the electrons are no longer confined to the discrete energy levels of the atoms but form occupied (valence) and unoccupied (conduction) bands due to energetic variations between the atoms. The space between the top of the valence band and the bottom of the conduction band is termed the band gap and is a region with no allowed states. In order for the material to conduct an electron must be excited from the valence band to the conduction band. Electrons can be excited either thermally or by photon absorption and the extent thermal excitation contributes to the conductivity depends on the energy gap. Excitation of an electron leaves behind a vacancy (hole) in the valence band which is also mobile. A term often used in discussions of semiconductors is the Fermi level, which is the energy level where the probability of occupancy by an electron is exactly one half. In the pure state, silicon forms a closed shell structure and it is an intrinsic semiconductor, meaning that the number of holes equals the number of electrons.

a) Diamond crystal structure of silicon lattice (adapted from: <http://www.msm.cam.ac.uk/phase-trans/2003/MP1.crystals/MP1.crystals.html>), electron configuration of the b) valence and c) conduction bands.

Figure 1.1 – Structure of crystalline silicon

The conductivity can be modified by introducing impurities from either group III (e.g. boron) or group V (e.g. phosphorous, nitrogen) which are called dopants. In the sp^3

hybridization state dopants from group III possess three valence electrons, when intercalated into the silicon lattice they donate three electrons and one empty orbital which is a hole. A group V dopant has five valence electrons and when intercalated into the silicon lattice donates four bonding electrons and one extra electron, which is mobile. In an energy level diagram the addition of electrons moves the Fermi level closer to the conduction band (Figure 1.2a) and addition of holes moves the Fermi level closer to the valence band. It follows that in an n-doped semiconductor current is carried in the conduction band by free electrons while in a p-doped semiconductor current is carried in the valence band by holes, and as the Fermi level gets closer to one of the bands (conduction or valence) the conductivity of the silicon increases.

Figure 1.2 – Fermi level of doped and undoped silicon.

Arguably one of the most significant technological developments of the twentieth century was the field-effect transistor which is the foundation of the information technology age. The first publication of a germanium transistor was in 1948 and the first silicon based device was produced by Texas Instruments in 1954, these were bipolar transistors; however it was not until 1960 that the first field effect MOS transistor

was invented by Dawon Kahng.^{5,6} The field effect transistor operates on the principles laid out in Figure 1.3, the example shown is for n-type silicon while the opposite effect would be observed on p-type. When no charge is present at the interface b) the carriers are distributed evenly throughout the material, the potential corresponding to this situation is called flat band. When a negative charge is applied to the surface a) the electric field of these charges penetrates into the silicon depleting the surface region of carriers (negative charges). The amount of energy required to remove an electron from the surface through a layer of negative charge is higher than when no charge is

Energy level diagrams for n-type silicon with a) negative charge at the interface, b) no charge at the interface and c) positive charge at the interface, the space charge region is noted in a).

Figure 1.3 – Field Effects

present at the interface, which is why the bands bend upwards. The distance into the material that the external electric field penetrates is called the space charge region and its width depends on the strength of the electric field and doping density. When positive charge is present at the interface c) the opposite effect is observed. The negatively

charged electrons accumulate near the surface and the energy required to remove an electron is decreased therefore the bands bend downwards.

The basic layout of a field-effect transistor is shown in Figure 1.4. The current between the source and drain can be modulated by the application of a voltage to an external (gate) electrode. In the diagram below the metallic source and drain are positioned above heavily p-doped (hole rich) regions and isolated from the silicon by an insulating oxide. The channel region between the source and drain is n-doped (electron rich) and this region is capped with an insulating oxide layer to isolate it from the metallic gate electrode. Application of a negative potential causes an increase in holes near the surface and opens up a channel, near the surface, for current to flow between the source and drain. It is apparent that any stray charge above the channel or impurities (other than explicitly added dopants) in the silicon body will affect the carrier density in the conducting channel, which in turn will affect the ability of the gate electrode to switch the device. Leakage current through the gate insulator also affects device performance. Over the past sixty years great strides have been made in shrinking device dimensions, as devices get smaller and the density increases the conducting channel consists of fewer and fewer carriers which puts heightened demand on fabrication plants. This incredible utility of silicon and germanium semiconductor devices did not go unnoticed by researchers in other fields and very quickly reports describing mechanical⁷, temperature⁸, magnetic⁹ and chemical¹⁰ sensors as well as the now ubiquitous photodiode¹¹ were published.

Figure 1.4 – Metal Oxide Semiconductor Field Effect Transistor (MOSFET)

Adapting the device shown in Figure 1.4 for chemical – biological sensing requires incorporation of an arbitrary recognition element. Removal of the metallic gate exposes the channel on which two surfaces are available for chemical modification: the silicon dioxide gate insulator or; alternatively, the oxide layer can be selectively etched revealing the underlying crystalline silicon body for modification. Chemistry is available to functionalize both surfaces, silanes¹² form organic layers on silicon dioxide and alkenes have been shown to covalently react with crystalline Si-H¹³. Development of chemistry is typically carried out on planar silicon wafers, because of the limited availability (cost) of devices and limited characterization techniques to validate the chemistry. Commercially available silicon wafers are terminated by an approximately 10 Å native oxide layer which forms on exposure to ambient. Prior to functionalization the wafer must be cleaned; the RCA process has been adopted as the standard method to remove organic and metal contaminants.¹⁴ The RCA clean involves three steps: firstly, the sample is cleaned in piranha solution (4 : 1 sulfuric acid : 30 % H₂O₂) it is then washed with deionized water, secondly, it is heated in 4 : 1 : 1 deionized water : NH₄OH : 30 % H₂O₂, after which it is immersed in dilute hydrofluoric acid to remove contaminants trapped in the oxide and thirdly heated in 4 : 1 : 1 deionized water : HCl : 30 % H₂O₂. The first two steps oxidize and remove any organic material deposited on the surface

(SC-I), as mentioned the HF etch removes the oxide and any trapped impurities and the final step (SC-II) dissolves any metal contamination and grows a new oxide layer. The resulting oxide surface presents a density of silanol groups¹⁵⁻¹⁸ of approximately $5 \times 10^{14} \text{ cm}^{-2}$ is then ready for functionalization directly with silanes or after a second etching step with alkenes. The chemistry, advantages and disadvantages of using the oxide and Si-H surfaces as a substrate are presented in the next sections.

1.1.1.1 – Chemistry on Silicon Dioxide

Silane layers can be formed by exposure of the surface to vapour¹⁶ or solutions^{12,16-18} of silane molecules, with vapour phase reactions limited to lower boiling reagents. An overview of the formation of silane layers is shown in Scheme 1.1.

Hydroxylated silicon surfaces are covered by $> 0.3 \text{ ML}$ of water at pressures above 10^{-3} Pa and temperatures above 210 K ; therefore under standard conditions the surface will be covered by a water layer.¹⁹ In the first step, the silane (alkoxy, or chloride) approaches the surface, and is hydrolyzed yielding a trisilanol. Once the leaving groups hydrolyze, the polar silanol head group can diffuse across the water layer and react with other silane molecules or with surface bound silanol groups. The observation that good quality layers were obtained on a hydrated gold surface¹⁶ supports this hypothesis since in this system no linkages to the substrate are possible. In order to ensure hydrolysis happens only at the interface anhydrous solvents or carrier gasses are employed.

Scheme 1.1 – Formation of Silane Monolayer

The length and nature of the side chain have a significant impact on the growth of the layer. Silanes with long alkyl side chains (e.g. octadecyl) display characteristics which led to them being termed self-assembled monolayers. The main requirement to fit the definition of self-assembly is that some equilibrium state is achieved which results in an increase in the order of the structure. A boundary or interface functions in a templating role. Typically, fast reactions lead to amorphous / disordered structures because molecules are unable to adjust their positions.^{20,21} Formation of an octadecyl silane layer is templated by the silicon surface, and the hydrolyzed polar head groups can diffuse in the water layer allowing the chains to order due to van der Waals forces. In contrast short hydrophilic (e.g. aminopropyl) side chains result in highly disordered and chemically unstable (multi)layers.²²⁻²⁴ Reduction in chain length reduces the van der Waals forces thereby reducing the thermodynamic driving force imparting order. Additionally, the shorter chains display reduced steric hindrance and increased hydrophilicity (e.g. amino terminated) which encourage multilayer growth and decrease the chemical stability of the layer.

1.1.1.2 – Chemistry on Hydrogen Terminated Silicon

1.1.1.2.1 – Etching

The silicon dioxide layer can be removed by etching in HF revealing the crystalline substrate. As described above, the crystal structure of silicon is diamond cubic. The most commonly encountered crystal planes (defined by their Miller Indices) are the Si(100) and Si(111) facets. After etching, the ideal Si(100) surface is terminated by a dihydride species (SiH_2) whereas the Si(111) surface is terminated by a monohydride (Si-H). Si(100) is currently the technologically more important surface whereas chemistry has been developed primarily on Si(111). A significant reason chemistry predominates on the Si(111) surface was the discovery by Chabal and coworkers that the etching was pH dependent. The authors noticed that the shape of the Si-H bands in FTIR changed when dilute HF was used instead of concentrated and postulated the difference was due to an increase in pH.¹⁷ A careful FTIR study of changes to the Si-H stretch as a function of pH led to the observation that a single sharp peak at 2083.7 cm^{-1} formed after etching in NH_4F . The sharp peak was observed with p-polarized infrared light (electric field perpendicular to the surface normal) but not with s-polarized infrared light (electric field is parallel to the surface normal) therefore, the Si-H stretch was perpendicular to the surface. This is characteristic of an ideal monohydride terminated Si(111) surface, which was later confirmed by scanning tunneling microscopy.^{18,25} (Figure 1.5) During this period there was debate in the literature as to the surface termination after fluoride etching. It was proposed that the surface was fluorine terminated owing to the strength of the Si-F versus Si-H bond ($\sim 580 \text{ kJ/mol}$ versus $\sim 340 \text{ kJ/mol}$ respectively).²⁶ In the following decade a great deal of knowledge was generated on the process of silicon etching.

Reprinted from¹⁷ with permission.
Copyright 1990 American Institute of
Physics

Reprinted from²⁵ with
permission. Copyright 2004
Elsevier.

FTIR spectra of Si(111) surface etched in pH modified HF (pH = 9 – 10) taken with A) p-polarized and B) S-polarized infrared light. UHV STM image of NH₄F etched Si(111) surface C) 150 × 150 nm² showing steps and terraces, D) 10 × 10 nm² with resolution of silicon atoms

Figure 1.5 – FTIR and STM Evidence of Ideal Si-H Termination of Si(111)

In particular the groups of Allongue^{18,27} and later Hines²⁸⁻³⁰ developed the now generally accepted “step flow” mechanism for etching of Si(111) by NH₄F. The first step of silicon etching is oxidative attack by a hydroxide ion with concomitant reduction of water to regenerate the hydroxide ion. In the second step, a fluorine atom is consumed releasing ammonium hydroxide, this cycle continues until all three silicon – silicon bonds are cleaved releasing ammonium hexafluorosilicate. The electronegative fluoride polarizes the silicon – silicon back-bond and the next hydroxide ion attacks the most electropositive silicon atom (the one already bonded to fluorine), the end result is a hydrogen terminated surface and released fluorosilicate ion.³¹ (Scheme 1.2) The

oxidative addition of hydroxide is the rate limiting step of this process; the observed order of reactivity is kink > point > vertical dihydride > horizontal monohydride >> terrace.³⁰ It is apparent that the most reactive sites are defects and the least reactive silicon atoms are found on atomically flat terraces; defects are rapidly removed exposing large flat hydrogen terminated terraces. An anisotropic chemical etch for Si(100) has not been identified and a recent paper from the Hines group³² suggests it is not possible due to steric hindrance. The silicon dihydride cannot be accommodated on a flat terrace without expansion of the underlying silicon lattice. The surface compensates by forming stripes with a characteristic 25 Å width.

Scheme 1.2 – Mechanism of Si(111) Etching by NH_4F

1.1.1.2.2 – Monolayer Formation by Thermal Initiation

In 1993 Linford and Chidsey published their pioneering work describing the direct modification of Si(111).¹³ In this initial report acyl peroxides were thermally decomposed in the presence of Si-H surfaces. The diacyl peroxide was expected to decompose to a carbon centred radical and carbon dioxide. Monolayer formation would involve one carbon radical abstracting a hydrogen atom with a second coming in to cap it forming a silicon-carbon bond. The results showed ~ 70 % Si-C (due to formation of organic radicals) and ~ 30 % Si-OC(O)C (due to addition of acyl peroxide) linkages.

This was followed up with a publication detailing the reaction of alkenes with Si-H with and without the presence of a radical initiator.³³ The diacylperoxide content was lowered from 100 % to 50 % to 10 % with no change in film properties, decreasing the

concentration down to 5 % resulted in approximately 10 % lower coverage. When fully deuterated diacylperoxide was employed the FTIR signal decreased by ~ 10 % demonstrating that the majority of surface bound molecules were derived from the alkene. This observation led to a proposed mechanism for the reaction of alkenes with the Si-H surface. In the first step a hydrogen atom is abstracted by a radical in solution creating a silicon dangling bond. In the second step the dangling bond reacts with the alkene and the radical is located on the β -carbon. In the chain propagating step the carbon centred radical removes a hydrogen atom from a neighbouring silicon atom regenerating a surface dangling bond. (Scheme 1.3). On the Si(111) surface (where each of the neighbouring Si-H sites are identical) chain growth is expected to follow a random walk.

Scheme 1.3 – Mechanism of Radical Chain Growth on Hydrogen Terminated Si(111)

Linford *et al.*³³ conducted a control reaction by heating neat alkene in the presence of a Si-H surface. Under standard conditions (100 °C, 1h) developed for reactions containing diacylperoxide, neat octadecene achieved approximately one third the coverage as measured by FTIR and ellipsometry. Increasing the reaction temperature

(200 °C) increased the coverage to approximately 70 % obtained in the presence of a radical initiator. It was noted that a shorter alkene (dodecene) achieved higher coverage than octadecene identifying a chain length dependence of the reaction. A route to initiating the chain reaction in the absence of diacylperoxide was not proposed.

Sieval *et al.* reported on the effect of diluting hexadecene in various solvents without a radical initiator.³⁴ Monolayer quality was only assessed by contact angle which limits the power of the study. A 10 % (v/v) solution of hexadecene in solvent was immersed in an oil bath at 210 °C for 2 hours. The solvents studied included the linear hydrocarbon decane and several aromatics including anisole, toluene, cumene and mesitylene. Of those solvents, only mesitylene produced layers with the same contact angle (advancing contact angle: 109 °) as neat hexadecene. The values were unchanged down to 2.5 % alkene. The ability to prepare good quality monolayers with dilute solution allows for the conservation of precious (either costly to purchase or time consuming to synthesize) reagents.

1.1.1.2.3 – Grignard / Alkyl Lithium Derived Monolayers

The use of Grignard chemistry to functionalize the silicon surface was reported by the Lewis group.³⁵ The sequence involves chlorination of the silicon surface via exposure to phosphorous pentachloride and a radical initiator such as benzoyl peroxide, followed by heating in the presence of a Grignard reagent such as methyl magnesium chloride to afford the methyl terminated surface. (Scheme 1.4). It has since been shown that chlorine terminated surfaces can be prepared via exposure of the Si-H surface to chlorine gas with illumination by a 350 nm lamp for 15 seconds³⁶ and even in the absence of a light source.²⁵ An advantage of the latter chlorination routes is that all the steps can

be carried out in a sealed environment which will reduce the chances of surface contamination. For example, Terry *et al.* seal a Si-H surface in a quartz cuvette, evacuate and then introduce chlorine gas. After illumination, the gas is pumped off and the cuvette backfilled with argon at which point the alkyl lithium was added via cannula.

Prompted by a report that hydrogen terminated porous silicon reacted with Grignard reagents without the necessity of chlorination³⁷, Boukherroub *et al.*³⁸ exposed a Si-H surface to decyl magnesium bromide and observed formation of a high quality monolayer with negligible oxygen content in the XPS. A mechanism for the grafting of Grignard reagents onto Si-H surfaces was proposed by Fellah *et al.*³⁹ and later by Nemanick *et al.*⁴⁰ The reaction rate was faster on n-type silicon than p-type implicating an electron transfer with the surface in the rate determining step. Additionally, the reaction was accelerated by adding alkyl halide to the Grignard reagent. Scheme 1.5(a) shows the net reaction of a Grignard reagent with alkyl halide, producing two equivalents of alkyl radical. The two reactions which occur are: oxidation of the Grignard “carbanion” and reduction of the halide derived “carbocation”. This is consistent with the observations provided the reactions are coupled and mediated by electron transfer with the silicon substrate. Once the alkyl radical is generated, monolayer formation is possible as described by Linford and Chidsey.¹³ Scheme 1.5(b) provides a pathway to a halogen terminated surface, in this case the halogen terminated surface forms *in situ* and does not need to be explicitly prepared. A critical point to take from this work is that

potentially numerous reaction pathways exist for what could be considered a “simple” reaction.

Scheme 1.5 – Proposed Mechanism of Grignard Reaction

1.1.1.2.4 – Photochemical Initiation of Monolayer Formation

The next development in monolayer formation on Si-H surfaces was reported by Chidsey and coworkers in 1997.^{41,42} They demonstrated the alkene reaction could be promoted by irradiation with UV light (185 and 253.7 nm pen lamp) for 2 hours. Effenberger *et al.* reported on a direct comparison of forming octadecyl monolayers under radical and photochemical promotion.⁴³ Photochemical initiation was found to produce monolayers of comparable coverage (by FTIR) as the radical initiated reactions. The authors also postulated that octadecanal (the ω -aldehyde) would react according to the radical chain reaction proposed for alkenes on Si-H; this was confirmed later by Wayner and coworkers⁴⁴. Effenberger⁴³ found that the aldehyde attained higher surface coverage and contact angle than the alkene and a maximum yield was obtained at 385 nm, which is insufficient energy to break a Si-H bond. The light intensity reaching the sample decreased continuously from 400 to 350 nm due to the intensity distribution of the Xenon lamp, the monochromator and the transmission properties of the cuvette; the maximum yield did not correspond to maximal photon energy or flux.

Cai *et al.* partially iodinated the Si-H surface and then initiated the reaction of alkenes and Si-H using a 514 nm light from an argon laser.⁴⁵ They selected iodine because molecular iodosilanes (e.g. R₃Si-I) were known to react with solvent under

illumination. Based on XPS, the Si-I surface was stable under illumination in inert solvent (benzene) and iodine loss was concomitant with monolayer formation.

Interestingly, they observe a strong doping dependence in the reaction, with increasing n-type doping corresponding to an increased rate of reaction. Based on this, they proposed a mechanism. Iodine termination induces band bending at the interface, photo-generated electrons will drift towards the bulk and holes will accumulate at the interface. The increased concentration of holes at the interface will make it more susceptible to nucleophilic attack by alkenes in solution, it was noted that there is no evidence monolayer formation proceeds via the radical chain reaction mechanism proposed by Linford *et al*³³. The involvement of surface localized holes is consistent with the work of Stewart and Buriak who demonstrated that monolayer formation on porous silicon is mediated by excitons.⁴⁶ During the formation of porous silicon, silicon nanoparticles are created and remain trapped in the layer. These nanoparticles are responsible for the photoluminescence of the material. The excitons are fairly long lived, under these conditions on the order of microseconds. Stewart and Buriak proposed that holes migrate to the surface where the alkene attacks the positive charge forming a pentavalent silicon atom. Hydride migration from the surface silicon, formally the electron generated with the exciton, neutralizes the carbocation. The hydrosilation reaction was halted in the presence of reagents which quench light emission supporting their proposed mechanism.

Scheme 1.6 – Mechanism of Exciton Mediated Hydrosilation on Porous Silicon

The group of Zuillhof and Sudhölter demonstrated the photochemical reaction of alkenes with Si-H could be initiated using 447 nm light.⁴⁷ The advantage of using higher wavelength light is that it is compatible with a greater variety of terminal groups.⁴⁸ An STM study⁴⁹ was conducted to determine whether long wavelength initiation led to the surface chain reaction pathway proposed by Linford and Chidsey³³ (Scheme 1.3) for radical and thermal initiated reactions on Si(111) or the hydride transfer mechanism proposed by Stewart and Buriak⁴⁶ (Scheme 1.6) for exciton mediated hydrosilation on porous silicon. Hydrogen terminated Si(111) wafers were irradiated with a 447 nm light source in a 0.2 M solution of 1-decene in mesitylene. Samples were removed, cleaned and rapidly transferred to the UHV chamber at 5, 15, 30 and 120 minute time points. The STM images (Figure 1.6) clearly show the reaction proceeds via the radical chain reaction mechanism.⁴⁹ It is interesting to note that chain growth is not random as predicted by theory.⁴¹ A preference for the growing chain to turn back on itself was observed, presumably due to van der Waals interactions between the alkyl chains.

Reprinted from⁴⁹ with permission. Copyright 2004
American Chemical Society.

Figure 1.6 – STM Image Showing Radical Chain Reaction

Based on the facts that 447 nm light is incapable of breaking Si-H bonds generating surface radicals, and monolayer formation proceeds via the radical chain reaction, Sun *et al.* proposed the mechanism shown in Scheme 1.7.⁵⁰ The mechanism is adapted from that of Stewart and Buriak⁴⁶ for porous silicon. Light absorption leads to charge separation and the surface undergoes nucleophilic attack by the alkene. Instead of hydride transfer the breaking of a silicon – silicon back bond allows for the initiation of the chain reaction. The authors support their mechanism with the observation of a doping dependence on the rate with n-type proceeding more quickly than p-type, which is consistent with observations on iodine terminated Si(111).⁴⁵ Several outstanding issues remain surrounding this mechanism. Cai *et al.*⁴⁵ indicate that accumulation of positive charge at the interface is due to the presence of electronegative iodine which induces band bending; there is no evidence for significant band bending on Si-H. Furthermore, Stewart and Buriak indicate the long lived excitons are due to the presence of silicon nanocrystals which are not present on flat crystalline Si(111). Finally, in contrast to the findings of Sun *et al.*, Chazalviel and coworkers³⁹ do not observe a doping dependence in

the photochemical reaction of alkenes with Si-H. While it is indisputable that the monolayer forms via the surface mediated radical chain reaction, like the thermal reactions, in the absence of radical sources, questions remain as to how the process is initiated.

Scheme 1.7 – Proposed Mechanism for Visible Light Promoted Reaction on Si(111)-H

1.1.1.2.5 – Other Methods for Forming Organic Layers on Silicon

Two less commonly encountered methods for forming organic layers on Si-H are Lewis acid catalyzed / mediated hydrosilation and diazonium based chemistry. Lewis acid catalyzed hydrosilation was first reported for porous silicon⁵¹ and later demonstrated to mediate monolayer formation on crystalline³⁸ Si-H. Likely due to the steric demands of the catalyst interacting at the interface, the coverage by FTIR was observed to be lower than either photochemical or Grignard based methods.³⁸ Allongue and coworkers⁵² first demonstrated the formation of organic layers on Si(111) based on reduction of diazonium salts. The limitation of the diazonium method is that it proceeds via solution based radicals which can undergo unwanted reactions in solution as well as forming multilayers. Because these methods are less commonly employed and were not used in this work so they will not be discussed further.

1.1.1.3 – Functional Group Incorporation

In order to introduce novel functionality onto the interface it is necessary to develop methods for sequential reactions on silicon surfaces. The first examples reported

by Wagner *et al.*⁵³ used highly reactive species to functionalize an octadecyl monolayer. One route made use of an NHS activated benzoic acid which also presented a photoreactive diazarene moiety. The photogenerated singlet carbene inserted into C-H bonds of the monolayer and the NHS group was available for binding DNA or peptide fragments. The second route involved exposing an octadecyl monolayer to a gaseous mixture of Cl₂ and SO₂ and illuminating with 351 nm light to afford sulfonyl chloride terminated surfaces, which were shown to be amine reactive as expected. Due to the lack of control in the incorporation level and highly reactive nature of the intermediates these routes did not gain favour in the community, when faced with the success of more traditional synthetic approaches.

Sudhölter and coworkers demonstrated that not only could ester, carboxylic acid and alcohol terminated monolayers be formed on Si-H but that they could undergo various hydrolysis, esterification, and saponification processes.⁵⁴ The reactions were promoted thermally at 200 °C in neat alkene. The preference for the Si-H surface reacting preferentially with alkene over acid has since been confirmed for photochemical initiation by Asanuma *et al.*⁵⁵ and Faucheux *et al.*⁵⁶ Bowden's group reported on the formation of carboxylic acid terminated layers mediated by a catalytic amount of a TEMPO containing alkene; the stable radical induced reaction at room temperature in 24 hours.⁵⁷

The formation and subsequent reactivity of ester terminated surfaces under UV illumination was reported by Boukherroub and Wayner.⁵⁸ The ester surface underwent hydrolysis yielding a carboxylic acid which could be amidated via a carbodiimide, reduced to an alcohol via NaBH₄, and react with alkyl Grignard reagents forming a

branched structure. They also showed that the ester density could be controlled by dilution with 1-decene forming mixed monolayers. A recent report by Yang *et al.*⁵⁹ describes the direct attachment of NHS activated esters to the silicon surface. Yu's group systematically studied the formation of mixed monolayers of ester and alkyl ω -alkenes.⁶⁰ They observed enrichment in the concentration of ester in the monolayer versus solution, an increased rate of ester hydrolysis when the alkyl chain was shorter than the ester and a pH dependence of the contact angle on ester hydrolysis.

Formation of amine terminated surfaces was demonstrated by Strother *et al.*⁶¹ The Si-H surface was reacted under photochemical conditions with *t*-boc protected 11-amino-1-undecene. The protecting group was removed under standard conditions, trifluoroacetic acid in methylene chloride. The amine was shown to react with a bifunctional linker containing an NHS and maleamide group; the maleamide was then reacted with thio-tagged DNA. Amines have also been introduced protected as a phthalamide.⁶²

Thermal grafting of OEG containing ω -alkenes was demonstrated by the groups of Hamers⁶³ and Gooding.^{64,65} Hamers' group studied the ability of thermally grafted OEG's to inhibit the non specific binding of proteins, while Gooding described the use of an epoxide terminus to react with thio-tagged DNA. The preparation of alkene,^{66,67} and alkyne^{68,69} terminated monolayers and subsequent metal catalyzed reactions have also been shown.

It is clear that a diversity of chemistry is compatible with crystalline silicon surfaces. Many of the functionalities introduced are commonly employed in either solid phase synthesis or microarray applications. A key point in these examples is that the

reactivity is altered by being tethered to a surface. In solution, molecules experience the bulk properties of the solution whereas on a surface the microenvironment and steric limitations may affect both rates of reaction and types of reactions available. The following sections give an overview of the remaining key concepts required to assemble a label free electrical biosensor.

1.1.1.4 – Inhibition of Non-specific Binding

Controlling the location and type of molecules which bind to the surface is essential for the success of a sensing device. The study of the interactions of monolayers with proteins was pioneered by the Whitesides group over 25 years ago.⁷⁰ They began their work with OEG based monolayers because OEGs are commonly incorporated into materials to increase their hydrophilicity and they are biocompatible.²⁰ In the first report, alkane thiol monolayers were bound to gold surfaces and exposed to buffered solutions of protein; the amount of protein which non-specifically bound was measured by ellipsometry.⁷⁰ They observed that maximum resistance to non-specific binding was afforded by hexaethylene glycol terminated layers. This work motivated research into determining the properties associated with protein resistance.

The ability to monitor non-specific binding *in situ* was demonstrated by Mrksich *et al.*;⁷¹ in this work protein binding was measured by surface plasmon resonance, a technique compatible with noble metal coated glass slides. This allowed for the rapid screening of surfaces, and a number of general properties of protein resistant monolayers were identified. Small molecule based (not including polymers such as dextran) protein resistant surfaces are hydrophilic, do not contain hydrogen bond donors but do contain hydrogen bond acceptors, and are overall electrically neutral (zwitterionic species are

acceptable).⁷²⁻⁷⁵ A notable exception to this rule are mannitol terminated monolayers which were found to be stable, protein resistant and contain hydrogen bond donors.⁷⁶

Over a number of publications, Grunze's group^{77,78} (including collaborations with Whitesides⁷⁹ at Harvard and Kreuzer⁸⁰ at Dalhousie) developed a thermodynamic model to identify the origin of the protein resistance of OEG layers. It was observed that OEG layers on gold exhibited protein resistance while those on silver did not. Structurally the chains are packed more tightly on silver, which makes the OEG chains pack more

Figure 1.7 – Origin of Protein Resistance by OEG Chains

densely and adopt an extended all trans conformation. In contrast the OEG chains on gold adopt a helical structure containing tightly bound water molecules. An overview is presented in Figure 1.7. The thermodynamic force driving a protein to non-specifically adsorb on a surface is the maximization of contact between the hydrophobic interior and a hydrophobic surface. In the case where water is tightly bound in the monolayer, the

energy required to expel the water is not provided by denaturation of the protein. In the case of monolayers on silver the tightly packed OEG chains cannot sustain an ordered water layer. Further requirements included the necessity of at least two ethylene glycol groups and while alcohol termination was preferred, methyl was acceptable but even the slightly more hydrophobic ethyl was not.

The close packing density on silver is unlikely to be replicated on the majority of substrates; therefore, it is perhaps unsurprising (and fortunate) that OEG incorporation was shown to impart protein resistance to oxide,⁸¹ silicon nitride,⁸² diamond^{63,83} and silicon^{63,84,85}. An interesting report by the Gooding group established a lower limit for monolayer density.⁸⁶ They prepared monolayers terminated in methoxy capped tri-OEG moieties and observed a lower density monolayer than on gold. These surfaces exhibited a decrease in protein resistance which was attributed to formation of a less strongly bound water layer. Clare *et al.*⁶³ prepared monolayers on the three substrates terminated with tri and hexa OEG and exposed them to fluorescently labeled avidin. The results indicated that < 3 % of a monolayer of avidin was bound in all cases, that hexa-OEG was slightly more efficient than tri-OEG and that the order of resistance was gold > silicon > diamond. They also studied the stability of the monolayers on exposure to water and found that monolayers on diamond were more stable than those on silicon or gold.

1.1.1.5 – Silicon Stability

There are many examples of organic monolayers on silicon surviving a variety of “torture tests”, starting with Linford and Chidsey’s³³ initial work and included boiling in organic solvents, water, acid and base. While demonstrating the robust nature of the monolayers, these extreme conditions do not represent standard biological conditions.

Additionally, the subtle changes in the silicon carbon interfacial properties may not be captured in such extreme studies. This section will provide an overview of stability studies carried out under biologically relevant conditions.

The decrease in protein resistance observed by Clare *et al.*⁶³ was attributed to oxidation of the silicon substrate leading to loss of monolayer based on XPS measurements. They observed the peak due to silicon oxide represented 6.6 % of the total peak area of silicon after 3 days in water and 8.4 % after 10 days. This increase in oxide content was associated with a loss of approximately 23 % of the organic monolayer. Gorostiza *et al.*⁸⁷ investigated the passivation of UDA modified surfaces. On storage in air for 7 and 25 days a broad peak grew in the silicon oxide region, the peak areas were not reported so it is not possible to compare directly with the OEG modified surfaces of Clare *et al.* A report by several of the same authors⁵⁶ developed a model to describe solvent penetration into the organic monolayer. (Figure 1.8). The hydrophilic head group is observed to facilitate the penetration of water a significant distance into the monolayer; therefore incorporation of a dense hydrophobic layer should inhibit solvent penetration and impede oxidation of the silicon substrate.

Reprinted from⁵⁶ with permission. Copyright
2006 American Chemical Society.

Figure 1.8 – Penetration of solvent into (a) decyl and (b) UDA Modified Surfaces

After their first report³⁵ of methyl modified silicon surfaces, Lewis's group investigated the stability of methyl terminated surfaces in aqueous electrolyte.⁸⁸ In this study Si-H and methyl modified surfaces were immersed in ferricyanide containing electrolyte for 10 minutes; XPS measurements were taken before and after treatment. The Si-H surface contained approximately 3ML of silicon oxide while the methyl modified surface contained <1ML. The experiment was driven by the application being pursued, the group is interested in developing silicon based solar cells which involves photo-excitation of an electron out of the silicon surface; therefore, a methyl modified surface represents the thinnest possible passivation layer and should maximize efficiency. There are reports indicating methyl groups cap each surface silicon, with measurements based on FTIR⁸⁹ and STM.⁹⁰ The STM images indicate the presence not only of step edges, but also etch pits and other defect sites which may limit the ability of methyl groups to passivate the surface. While methyl groups may be able to inhibit water from reaching the silicon on a flat terrace, these edge sites may serve as a location where oxidation is initiated.

The Haick group recently reported on the dependence of silicon oxidation on the hybridization state of the carbon – carbon bond nearest the silicon surface.^{91,92} Using the two step chlorination alkylation method developed by Bansal *et al.*³⁵ the following surfaces were prepared: Si-CH₃, Si-CH₂CH₂CH₃, Si-CH=CHCH₃ and Si-C≡CCH₃. The authors find that methyl, propenyl and propynyl react at all surface silicon sites, while propyl for steric reasons does not. XPS measurements demonstrate that only the propenyl surface is stable in water, the remaining three surfaces show substantial growth of silicon oxide. The methyl group was deemed too small to fully inhibit oxidation,

while the propyl failed because it was unable to cap all surface sites. The difference between propenyl and propynyl was attributed to the additional electron density of the alkyne bond. It was proposed that one set of π -bonds interacts with neighbouring groups while the other is available to donate electrons into the silicon surface making it more susceptible to oxidation. The propenyl surface was derivitized via the photoreactive diazirene first described by Wagner *et al.*⁵³ and as observed on octadecyl monolayers while chemically feasible the use of highly reactive species induced oxidation in the substrate. Further functionalization of this surface was not described.

1.1.1.6 – Biosensing on Silicon

Several examples of attaching biomolecules to silicon were mentioned in Section 1.1.1.3.^{53,65} The focus of this section is to highlight examples of “biosensing”, which involves modifying the silicon surface with a probe molecule that captures a target from solution by an affinity (non-covalent) interaction and the affinity capture is measured. Methods of measuring the interaction can be grouped into two categories the first are techniques which make use of a label and the other is label free. The middle image in Figure 1.9 shows the probe modified surface exposed to a solution containing both target and non-target molecules. The target molecules then bind to the surface, on the right they are identified by some exogenous label such as a fluorescent^{63,93} or electrochemical^{94,95} marker. The advantage of using a label is that only the target species is measured, which reduces interferences. The downside is that typically chemical reactions and purification steps are required to install the label which require time and money, and once installed the label may alter the function of the biomolecule or the recognition event. The label free approach is shown on the left and relies on measuring a physical property of the

analyte. The main challenge in label free sensing is to ensure the measured signal is due to the target molecule. Even when the interaction between probe and target is strong and will survive vigorous washing the signal due to the interference may be larger than the target. For example if the interference has a significantly higher charge to mass ratio the signal per molecule will be greater for a label free electrical sensor. These issues put increased demands on the surface chemistry and data interpretation.

Figure 1.9 – Overview of Label versus Label Free Approaches

Silicon is transparent to electromagnetic energy in the infrared⁹⁶ and telecommunication^{97,98} regions, which have been used to measure protein binding. Commonly, it is the electrical properties of silicon which are exploited for sensing. In order to produce an electrical response there must be a change in charge at the interface, which causes a change in the carrier distribution near the surface of the silicon. The electrical response can be measured in one of two general ways parallel with the surface, such as a FET⁸⁵ or perpendicular to the surface such as a Kelvin probe⁸⁴ or an electrochemical cell⁹⁹. Advantages of a FET layout include the ability to fabricate a very high density of sensors and perform measurements in parallel. However, substantial infrastructure is required to prepare integrated devices. Currently, there is a great deal of interest in silicon nanowires driven by the desire to reduce the number of target

molecules required to produce a signal and shrink overall device size.^{85,100} Analytical techniques to validate the surface chemistry on integrated devices and nanowires are limited therefore chemistry is developed and analyzed on wafers and then transferred to a device.⁸⁴ Because the Si(111) crystal plane can be exposed on devices and nanowires¹⁰¹; it is anticipated that chemistry developed on wafers will translate to these applications.

The simplest biosensing method available on silicon is to make use of the pH dependence of the silicon native oxide. A polymer can be used to bind an enzyme near the surface and if the product causes a change in pH this can be measured.¹⁰² Even when

Reprinted from¹⁰³ with permission.
Copyright 2001 AAAS.

Figure 1.10 – pH Dependence of Aminopropyl Silane Monolayer on Silicon Oxide Nanowires

the surface is reacted with silanes the pH dependence remains. Cui *et al.*¹⁰³ reacted oxide nanowires with 3-aminopropyl triethoxy silane and on increasing the pH from 2 to 9 observed first deprotonation of the amine group followed by ionization of the silanol groups on the surface. (Figure 1.10) In comparison, a docosyl monolayer formed on Si(111)-H was reported to contain less than 0.7 % the number of silanol groups of a

silicon oxide surface and displayed a pH dependence of approximately 25 mV per pH unit in the range of pH 4 – 7.¹⁰⁴ Based on the ideal case described by the Nernst equation (Equation 1.1) the pH dependence would be ~ 60 mV per pH unit. Where E_f and E_i are the final and initial voltages, z is the charge per molecule and $[H^+_f]$ and $[H^+_i]$ are the initial and final concentrations of protons.

$$E_f = E_i - (0.0592 \text{ V} / z) \times \log_{10}([H^+_f]/[H^+_i]) \quad (\text{Equation 1.1})$$

A comparison between oxide and hydrogen terminated silicon nanowires was conducted by the Heath group.¹⁰⁵ They fabricated p-type nanowires in a top down process from silicon on insulator. The oxide was functionalized with dimethylaminopropyl ethoxysilane. For the direct functionalization, the oxide was etched

Plot of resistance of probe / resistance control nanowire versus time for a modified A) oxide and B) Si-H nanowire. C) HR-XPS spectra of a Si(100) wafers: native oxide (black), Si-H (dark grey) and organic modified Si-H surface exposed to buffer for 10 hours (light grey)

Reprinted from¹⁰⁵ with permission. Copyright 2006 American Chemical Society.

Figure 1.11 – Comparison of Biosensing on Silicon Oxide and Si-H Surfaces

and the surface reacted with a protected amine ω-alkene under photochemical promotion, and after removal of the protecting group an amine terminated surface was obtained.

Probe single stranded DNA was electrostatically bound to the amine terminated surfaces and exposed to either complementary or noncomplementary DNA. The electrical responses are reported as differential measurements; the resistance of the probe nanowire is divided by the initial resistance. The resistance is observed to decrease on binding of the negatively charged DNA which is consistent with accumulation on a p-type device. It is evident by comparing Figure 1.11 A and B that a higher signal is obtained on the Si-H surface, despite the presence of silicon oxide as evident from the XPS spectra of a parallel wafer experiment shown in Figure 1.11 C.

Stern *et al.*⁸⁵ describe the functionalization of Si-H nanowires with a protected amino alkene in a similar process to that shown in Figure 1.11. After deprotection, the amine is reacted with biotin and shown to capture both Streptavidin and Avidin. (Figure 1.12) The vertical axis is source drain current and the surfaces are exposed to protein at time = 0. The blue trace indicates exposure to Avidin, and the red trace Streptavidin. The proteins are positively and negatively charged, respectively, under the neutral buffer conditions of the experiment and the changes in source – drain current are consistent for p-type nanowires. Two controls are shown, the green trace indicates Streptavidin pre-incubated with biotin does not alter the electrical properties and the black trace indicates no changes are observed on a PEG surface exposed to protein. The control experiments demonstrate that changes in the electrical properties of the nanowires only occur when a biotinylated surface is exposed to protein that has available binding sites, which is consistent with the conclusion that binding a positively charged protein (Avidin) induces a decrease in source – drain current while binding a negatively charged protein (Streptavidin) induces an increase in source – drain current on p-type nanowires. The

examples demonstrate that biomolecular binding can be measured on Si-H surfaces and the importance of fully characterizing the surfaces in order to assign measured signals to the interaction of interest.

Reprinted by permission from Macmillan Publishers Ltd: from⁸⁵, copyright 2007.

Figure 1.12 – Protein Binding on Si-H Nanowire

1.2 – Characterization Techniques

1.2.1 – Contact Angle

The shape of a drop of water placed on a surface is related to the free energy of the interface. There are three interfaces each with a unique surface tension (γ) with units of force per length (mN / m). The three surface tensions are γ_{SV} , γ_{SL} , and γ_{LV} referring to the solid – vapour, solid – liquid, and liquid – vapour interfaces. At equilibrium under ideal conditions they are related by Young's Equation, where θ is the contact angle at the intersection of the liquid, solid and vapour. (Equation 1.2) Based on Young's Equation,

$$\cos\theta = (\gamma_{SV} - \gamma_{SL}) / \gamma_{LV} \quad (\text{Equation 1.2})$$

when $\gamma_{SV} > \gamma_{SL}$ the liquid drop will wet the surface ($\theta < 90^\circ$, Figure 1.13A) and conversely when $\gamma_{SL} > \gamma_{SV}$ the drop is hydrophobic and will minimize interactions with the surface ($\theta > 90^\circ$, Figure 1.13B).^{106,107}

Figure 1.13 – Illustration of Water Contact Angle

Contact angle measurements have been used to assess the quality of an alkyl monolayer. A study by Sieval *et al.*³⁴ compared the contact angle obtained for the reaction of 1-hexadecene dissolved in different solvents. Because the product monolayer is methyl terminated the highest contact angle was associated with the optimal conditions. Yu's group demonstrated that contact angle could be used to obtain information about orientation of the bi-functional 11-undecylenic acid after reaction with the silicon surface.⁵⁵ In part by comparing contact angles obtained from an undecanoic modified surface reacted either directly with 11-undecylenic acid ($64 \pm 3^\circ$) or 11-ethyl undecylenate followed by ester hydrolysis ($52 \pm 5^\circ$) the authors were able to conclude 11-undecylenic acid was reacting both acid down and alkene down. The higher contact angle was attributed to a dilute concentration of alkenes being exposed, this result was consistent with previous work in the group which reported the relationship between contact angle and mole fraction acid and methyl in the monolayer as a function of pH.⁶⁰

1.2.2 – Ellipsometry

The estimation of film thickness is a very powerful yet technically straightforward measurement to perform. In the typical set up light from a He-Ne laser passes through a polarizer before reaching the surface at some angle. When polarized light interacts with a surface it is resolved into the parallel and perpendicular components, the amplitude and phase of the two components are affected by the film slightly differently. The result is that the light becomes elliptically polarized. The degree of polarization change is related to the properties of the film such as thickness and refractive index. In a single wavelength ellipsometer either the thickness or refractive index must be given and the other is calculated. In order for both thickness and refractive index to be measured the sample must be interrogated at multiple wavelengths and angles.¹⁰⁷ Wasserman *et al.*¹⁰⁸ studied silane monolayers on oxidized silicon comparing ellipsometric and X-ray reflectivity measurements and found agreement between the techniques when $\eta \sim 1.45$.

Linford and Chidsey³³ found using $\eta = 1.46$, which is also the refractive index of silicon oxide, the monolayer thickness for a given chain length could be predicted using Equation 1.3. Here d_{ML} is the predicted thickness, $1.86 \text{ \AA}$ is the Si – C bond distance, n is the number of carbons in the alkyl chain, $2.52 \text{ \AA}$ is the distance between carbon atoms in the all trans extended form and θ is the tilt angle of the chain. On films attaining saturation coverage the data was consistent with a tilt angle for the alkyl chains in the range of $28 - 36^\circ$ ³³ which is in the range found for gold $\sim 30^\circ$ ⁷⁹.

$$d_{ML} = 1.86 \text{ \AA} + 0.5 \times (n-1) \times 2.52 \text{ \AA} \times \cos\theta \quad (\text{Equation 1.3})$$

1.2.3 – FTIR

Fourier transform infrared spectroscopy is one of the most powerful techniques for analyzing monolayers on silicon. Information can be obtained from the location (frequency), intensity and polarization dependence of the peaks. In order for infrared radiation to interact with a bond, the dipole moment of the vibration must have an overlapping component with the incoming electric field. The degree of alignment between the electric field and the dipole moment determines the amount of energy that can be transferred to the vibration.^{106,107} In the case of a perfectly flat surface such as hydrogen terminated Si(111), the Si-H stretch is perpendicular to surface and absorbs p-polarized light but not s-polarized (see Figure 1.5).¹⁷

Porter et al.¹⁰⁹ extensively studied thiol monolayers on gold by FTIR. In the crystalline solid state the asymmetric methylene stretch (ν_a) of $\text{CH}_3(\text{CH}_2)_{21}\text{SH}$ was observed at 2918 cm^{-1} , versus 2924 cm^{-1} in the liquid state. As the number of methylene groups was decreased from 21 to 5, $-\text{CH}_2-$ ν_a increased from 2918 cm^{-1} to 2921 cm^{-1} . Cicero *et al.*⁴¹ observed a similar trend on silicon as the number of methylene groups in the alkyl chain decreased from 17 to 7 the $-\text{CH}_2-$ ν_a increased from 2917 cm^{-1} to 2920 cm^{-1} . In the crystalline state the alkyl chain adopts an all trans extended conformation with little rotation about the carbon-carbon bonds, whereas in the liquid or disordered state rotation by the methylene groups induces deformations termed “gauche defects”. The “gauche defects” cause the peak to inhomogeneously broaden, since the methylene groups experience a different environment and shift it to higher frequency. It should also be noted that for monolayers on both silicon and gold the peak area of the $-\text{CH}_2-$ ν_a

increased with the chain length. Therefore, the peak position gives information on degree of order and peak area on coverage.

Silicon is transparent in the infrared at frequencies larger than $\sim 1500\text{ cm}^{-1}$ which encompasses the diagnostic region, because of this FTIR spectra are often collected in the attenuated total reflectance geometry (ATR-FTIR). (Figure 1.14) When light enters the silicon crystal at an angle greater than the critical angle it undergoes total internal reflectance and propagates through the crystal. At each reflection the evanescent field interacts with the monolayer as the number of reflections increases the amount of energy absorbed by the monolayer increases, increasing the signal.¹⁰⁷ This increased sampling allows for fewer scans to be collected reducing the overall time per experiment.

Figure 1.14 – ATR-FTIR Geometry

The ability to validate chemistry at the interface is a critical application of ATR-FTIR. Figure 1.15 shows a reaction sequence⁹³, starting with a) the Si-H surface which has a sharp signal at 2084 cm^{-1} . After photochemical reaction with undecylenic acid b) the ATR-FTIR spectra has peaks in the alkyl region $2800 - 2980\text{ cm}^{-1}$ and a strong carbonyl stretch at 1715 cm^{-1} associated with the carboxylic acid. Upon activation with NHS / EDC c) the alkyl region is essentially unchanged while the carboxylic acid peak is gone and new peaks at 1815 , 1787 and 1744 cm^{-1} associated with the imide and ester stretches of NHS appear. In the final step the activated ester is reacted with tetraethylene glycol amine to form an amide d) which shows peaks at 3300 cm^{-1} assigned to N-H,

3100 cm^{-1} an overtone of the 1550 cm^{-1} peak, the alkyl region increases in size substantially after the reaction, the peaks associated with NHS are gone, replaced by signals at 1650 and 1550 cm^{-1} which are assigned to the carbonyl and the CNH vibration.

a) freshly etched Si-H, after reaction of previous with b) undecylenic acid, c) NHS / EDC and d) tetraethylene glycol amine

Reprinted from⁹³ with permission. Copyright 2006 American Chemical Society.

Figure 1.15 – ATR-FTIR Spectra of a Reaction Sequence

1.2.4 – XPS

X-ray photoelectron spectroscopy is a powerful and complementary technique which provides information on atomic identity, oxidation state, and concentration. The sample is irradiated with monochromatic X-rays which, on absorption by an atom, cause the ejection of a core level electron. (Figure 1.16) The energy of the ejected electron is measured and is equal to the kinetic energy of the X-ray minus the energy required to eject the electron from the atom, termed the binding energy. The binding energy

identifies the element and its chemical environment (oxidation state). The technique is surface sensitive because photoelectrons undergo inelastic scattering and the deeper into the material they originate the higher probability they are scattered. The typical escape depth is less than 5 nm depending on the material making it a very surface sensitive technique. The experiment is carried out under UHV conditions to eliminate any scattering of photoelectrons by gas molecules and to inhibit deposition of material from the atmosphere the surface.^{106,107}

Adapted from:

<http://www.hkbu.edu.hk/~csar/xps.html>

Figure 1.16 – Overview of XPS

A report by Strother *et al.*¹¹⁰ highlights the utility of XPS for the study of monolayers on silicon. (Figure 1.17) The high resolution fluorine spectra show the presence of a strong peak for the protected ester which is removed after saponification of the ester. The carbon spectra contains a large unshifted carbon signal due to the methylene groups of the alkyl chain and in Figure 1.17a three smaller peaks assigned to the methylene bound to oxygen and a trifluoromethyl group, the carbonyl carbon and the

most shifted is the trifluoromethyl carbon. After deprotection Figure 1.17b only the unshifted carbon peak and carbonyl signal remain. The peak size is also meaningful; the absorption cross section of carbon atoms is not significantly affected by oxidation state. Therefore, the peak areas should follow a ratio of 1 : 1 : 1 : 10 in a) and 1 : 10 in b). In ATR-FTIR this reaction could have been monitored by observing the loss of an ester signal and growth of a carboxylic acid peak; however, the silicon XPS region carries information not readily available in a traditional ATR-FTIR experiment. In Figure 1.17a only the main silicon signals are apparent. After saponification of the ester Figure 1.17b a shifted signal corresponding to oxidized silicon is present. The ability to observe elements or functional groups lacking an infrared signature and quantify silicon oxidation make XPS a very useful and complementary technique for the study of organic monolayers on silicon.

Reprinted from¹¹⁰ with permission. Copyright 2000 American Chemical Society.

Figure 1.17 – XPS Spectra Demonstrating Deprotection of Trifluoroethyl Ester

1.2.5 – Kelvin Probe and Surface Photovoltage

On a semiconductor such as silicon the difference between the surface potential measured in the dark and under illumination is termed the surface photovoltage. The SPV is a measure of band bending and amount of charge trapped at the interface. Figure 1.18 depicts the effect of a negative charge near the surface of n-type silicon. In the dark the bands are bent upwards (higher energy) by the negative charge at the interface. On illumination electron – hole pairs are generated which screen the charge at the interface and the bands flatten. The difference between the measurement in the dark and light is termed the surface photovoltage.^{111,112}

Figure 1.18 – Surface Photovoltage, Negative Charge on n-type Silicon

The property measured is the surface potential, which on a metal is called the work function. It refers to the energy required to move an electron from just inside the surface out to vacuum. If two materials are connected electrically electrons will flow from the lower work function material, to the higher work function material. This is the contact potential. Arranging the materials closely facing each other forms a parallel plate capacitor. Negative charge accumulates on the surface of the high work function material and positive charge balances it on the lower work function material. The contact potential is equal to the voltage required to neutralize this charge.^{106,111,112}

In a modern Kelvin Probe, one of the plates is formed by a tip of known work function (typically gold) and the other is the sample. The surface potential of the sample is measured relative to the tip therefore any changes, or contamination of the tip will induce changes in the measured surface potential. Off null techniques are also employed, instead of measuring the potential required to balance the charge. A vibrating tip is positioned near the sample and the voltage on the tip is measured throughout the oscillations. The peak to peak voltage of the tip is then plotted against different sample backing voltages, and the backing potential at zero peak to peak potential is equal to the contact potential.^{111,112}

Figure 1.19 – Measurement of Protein Binding By Kelvin Probe

The use of Kelvin probe measurements for biosensing was pioneered by the Thompson group. In a recent publication they describe the detection of a protein

Surface potential map of single stranded DNA probes a) and d), the surfaces were then exposed to complementary b) and non-complementary e) target DNA and line scans c) and f) through the surface potential maps

Reprinted by permission from Macmillan Publishers Ltd: from¹¹⁴, copyright 2007.

Figure 1.20 – Measurement of DNA Recognition by Surface Potential

1.3 – Aims and Goals

This chapter introduced monolayer formation on silicon oxide and crystalline Si-H surfaces. Monolayers formed on crystalline silicon (Si-C bonded) were shown to be chemically more stable and lower concentrations of protein and DNA were measured on these surfaces. Additionally, removal of the insulating oxide gives access to the electronic structure of the silicon substrate which may be useful in certain applications. The overall goal of this research project is to develop an organic monolayer which functions as a platform for label-free electrical biosensing, and for the reasons described above crystalline silicon was selected as the substrate.

A great deal has been learned in the 17 years since Linford and Chidsey¹³ first reported the formation of Si-C linked monolayers and subsequently proposed the radical chain reaction mechanism³³ for the reaction of alkenes with Si-H surfaces. One unanswered question is: in the absence of radical initiator what is the origin of the dangling bond that nucleates chain growth? Chapter 2 describes efforts to address this question. The silicon surface is most susceptible to oxidation / degradation when it is hydrogen terminated. A better understanding of the mechanisms associated with monolayer formation is important and may lead to improved chemistry and yield of functional devices. The study comparing the reactivity of oxide and Si-H surfaces described in Chapter 2 adapted with permission from: Mischki, T. K.; Donkers, R. L.; Eves, B. J.; Lopinski, G. P.; Wayner, D. D. M. *Langmuir* 2006, 22, 8359-8365. Copyright 2006 American Chemical Society. The study describing the implication of impurities in the initiation of thermal reactions described in Chapter 2 is adapted with

permission from: Mischki, T. K.; Lopinski, G. P.; Wayner, D. D. M. *Langmuir* 2009, 25, 5626-5630. Copyright 2009 American Chemical Society.

Chapter 1 highlighted some of the advantages and disadvantages of both label free and labeled biosensing as well as several different approaches to measuring the interactions on silicon. The high sensitivity and technical ease of Kelvin probe measurements make this a very good option for detection of protein binding on silicon. Examples of measuring DNA and protein interactions via scanning work function measurements on gold were highlighted which demonstrate the validity of the approach.^{114,115} On silicon, the surface potential in the dark and light are different due to the band bending effects described above; therefore on silicon both surface potential and surface photovoltage data can be used to interpret binding events. The use of SPV to measure DNA binding on silicon was reported adding further confidence to this approach.⁸⁴ Chapter 3 describes scoping studies, which served to determine the feasibility and challenges of developing a label-free biosensing platform on crystalline silicon.

Chapter 3 demonstrates that protein binding can be measured by SKP on Si-H surfaces; however the substrate was found to be chemically and electrically unstable under these conditions. This was overcome by increasing the density of the organic monolayer improving both electrical and chemical stability of the silicon – carbon interface. Ambient Kelvin probe and HREELS measurements described in the stability study of Chapter 3 are adapted in part from Bin X., Mischki, T.K., Fan, C.Y., Lopinski, G.P. and Wayner, D.D.M. *Journal of Physical Chemistry C*, 2007, 111, 13547-13553. Copyright 2007 American Chemical Society. Chapter 4 brings together the lessons

learned in preceding sections of this thesis to describe the development an organic monolayer which passivates the chemical and electrical properties of the silicon – organic interface under biologically relevant conditions and supports the specific capture of a target protein. This thesis concludes with an overall summary and identifies several opportunities for further research.

Chapter 2 – Monolayer Formation

2.1 – Introduction

The development of sensors based on crystalline Si-H surfaces requires that the introduction of electrically active defects be minimized during preparation and once formed their growth must be inhibited. Despite the considerable number of publications concerned with organic functionalization of Si-H in the 15 years since the initial report, several important questions and challenges remain. While STM studies⁴⁹ have confirmed that photochemical reaction of alkenes with Si-H proceeds via a radical chain reaction (Figure 1.6), as first proposed by Chidsey and co-workers,³³ the mechanism by which this reaction is initiated is a matter of on-going debate. In the original report of monolayer formation by Chidsey and co-workers¹³ the reaction was initiated by the thermal decomposition of peroxy compounds. Subsequent reports revealed that alkenes would react in the absence of intentionally introduced initiator under both thermal^{33,34,38} and photochemical initiation^{36,41,43}. Monolayer formation begins at a surface dangling bond, and the fate of this surface radical at the end of the chain is unknown but may be quenched either by impurities in solution or water and oxygen once exposed to the atmosphere. Not only is the surface most susceptible to oxidation before and during monolayer formation but each island is derived from a highly reactive surface species, highlighting the importance of optimizing this process.

Previous work in the group identified a method for “reagentless” patterning of hydrogen terminated silicon,¹¹⁶ spatial control of the surface chemistry is required for

many device applications and has been reported by others as well.^{64,117,118} Briefly, our strategy involves irradiating a hydrogen terminated silicon wafer through a photomask with a UV pen lamp. The shaded regions remain hydrogen terminated while the illuminated regions are oxidized to form a thin layer of silicon oxide. An alkene then reacts in the hydrogen terminated regions forming a monolayer thick organic film. The oxidized portions are then etched with dilute hydrofluoric acid generating fresh hydrogen terminated regions ready to react with a second alkene resulting in a patterned bi-component monolayer. This strategy was applied using photochemical initiation for monolayer preparation, forming arrays of MIS diodes; the electrical measurements indicated a substantial quantity of electrically active surface defects.¹¹⁹ This motivated an investigation as to whether thermal initiation would improve the electrical properties of the devices.

Surprisingly, when the Si-H / SiO₂ patterned surface was reacted thermally, no contrast was observed between the oxidized and hydrogen-terminated regions based on the surface wetting properties. It was expected that the alkene would react exclusively in the hydrogen-terminated portion (as it appeared to in the photochemical reaction) but this result suggested the oxidized portion was also active under the conditions of the thermal reaction. A comparative study between the reaction of 1-decene with the hydrogen terminated and oxidized silicon surfaces under either thermal or photochemical initiation was conducted. Monolayer formation was followed by water contact angle, ellipsometry, and infrared spectroscopy. The results suggest the alkene reacts with silanol groups on the oxidized silicon surface under thermal promotion.

Dodecane, which lacks an alkene and was used as a control compound, reacted with both the Si-H and oxide surfaces at 150 °C. Data presented in this chapter will demonstrate that the acidic surface of the Pyrex Schlenk tube degrades the hydrocarbon, creating reactive species capable of both initiating the chain reaction on Si-H surfaces and covalently attaching to the oxide surface. Originally, it was suggested that the thermal and photochemical processes led directly to Si-H bond breaking, but this is inconsistent with existing data on the thermal and photochemical stability of the Si-H bond. The observation that visible light^{47,50,120} could be used to initiate the reaction further ruled out direct photocleavage of the Si-H as the sole operable mechanism as the photons lack sufficient energy to break the Si-H bond. Studies of gas phase photochemical reaction¹²¹ of alkenes with Si-H have implicated alkyl radicals produced via photolysis of the alkenes as the initiating species although this requires photons of wavelengths less than 200nm. In the case of thermal reactions, trace oxygen is a possible initiator as the abstraction of hydrogen by molecular oxygen to create a silyl radical has been observed in the molecule tris(trimethylsilyl)silane¹²². Additional mechanisms are required to account for recent reports of spontaneous reactions (at room temperature, in the dark) of alkynes with the H-terminated surface.^{123,124}

The ultimate goal of this work is to develop a label-free electrical biosensor, achieving this goal requires understanding how the chemistry affects the electrical properties of the silicon. Using conditions which ensured saturation coverage, the effect of reaction conditions (thermal and photochemical), and reagent purification on the electrical properties of the silicon substrate were studied by measuring the SPV.

This chapter is divided into four sections. The first reports on the unexpected reactivity of the oxide surface under thermal conditions. In the second part, this observation is used to provide insight into the initiation source of the radical chain reaction. The third section identifies how the SPV is affected under the various reaction conditions. The final section ties this work together and places it in the context of progressing towards a biosensor.

2.2 – Experimental

2.2.1 – Materials

ATR silicon crystals ($25 \times 4.5 \times 1 \text{ mm}^3$) were purchased from Harrick and the silicon wafers from Virginia Semiconductor. Sulfuric acid, 96% (H_2SO_4), ammonium fluoride, 40% (NH_4F), hydrochloric acid, 30% (HCl), ammonium hydroxide, 30% (NH_4OH) and hydrogen peroxide, 30% (H_2O_2) Finyte Grade were purchased from J. T. Baker, and hydrofluoric acid, 48% (HF) PPB grade was purchased from Arch. Milli-Q water ($18 \text{ M}\Omega$) was used for all experiments. 1-Decene (94%) and dodecane (99%) purchased from Aldrich were used directly from the bottle, vacuum distilled (1-decene at $38\text{-}40^\circ\text{C}$, dodecane at 80°C), or purified by filtration through a pad of Florisil (100-200 mesh from Aldrich) and then vacuum distilled (1-decene at $38\text{-}40^\circ\text{C}$, dodecane at 80°C) as indicated. Silicon shards and ATR elements were cleaned after reactions in a Soxhlet Extractor under argon with continuously refluxing 1,1,1-trichloroethane (TCE) purchased from Aldrich.

2.2.2 – Surface Characterization

Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). ATR-FTIR spectra were recorded using a Nicolet MAGNA-IR 860 spectrometer at 4cm^{-1} resolution. The ATR crystals were mounted in a purged sample chamber with the light focused normal to one of the 45° bevels. Background spectra were obtained using an oxidized surface.

Surface Wettability Measurements. Surface wetting properties were measured with a contact angle goniometer (model: Cam-Micro, Tantec Inc.) under ambient conditions ($18\text{-}22^\circ\text{C}$, 50-60% relative humidity) using a collimated horizontal light beam to illuminate the liquid droplet.

High-Resolution Electron Energy Loss Spectroscopy (HREELS). HREELS was carried out under ultrahigh vacuum conditions with a LK3000 (LK Technologies) spectrometer. The nominal system resolution was set to 36 cm^{-1} . Spectra were acquired in the specular geometry at an incident energy of 6eV.

X-Ray Photoelectron Spectroscopy (XPS). XPS spectra were recorded on a PHI 5500 instrument, using monochromated Al $K\alpha$ (1486eV) radiation with detection on the surface normal. The pressure during analysis was $\sim 5 \times 10^{-8}$ Torr. Spectra were fitted with Gaussian profiles using standard procedures. The positions of all peaks were normalized to C 1s at 285.0 eV.

Thickness Measurements. The thickness of organic monolayers and silicon oxide were estimated using a Gaertner model L116S single wavelength (633nm) ellipsometer at an angle of incidence of 70° . The thickness was obtained using a two-layer model with n

= 1.46 as the refractive index of the monolayer and silicon oxide, and the silicon substrate described by $n = 3.85$ and $k = 0.02$.

Surface Photovoltage Measurements (SPV). A scanning Kelvin probe (KP Technology Ltd, Wick, Scotland) was used to measure the SPV, which provides a direct measure of band-bending (the difference in surface potential in the dark and under illumination). A white light fiber optic source was used to illuminate the surface; the light was determined to be of sufficient intensity to saturate the photovoltage. A motorized stage was used to scan the sample allowing measurement of the SPV at 16 spatially distinct points to be collected per sample.

2.2.3 – Surface Preparation

Cleaning and Hydrogen termination. Single sided silicon crystals were used once and discarded; ATR elements were cleaned and reused. All crystals were cleaned with piranha solution (3:1 H_2SO_4 , 96% : H_2O_2 , 30%) at 120 °C for 30 minutes, then rinsed with Milli-Q water. (*Warning: Piranha solutions should be handled with care and kept isolated from organic materials*). ATR elements were then cleaned with 4:1:1 Milli-Q water : NH_4OH , 30% : H_2O_2 , 30% at 80 °C for 15 minutes, rinsed with Milli-Q water and heated in 4:1:1 Milli-Q water : HCl , 30% : H_2O_2 , 30% at 80 °C for 15 minutes then rinsed with Milli-Q water. Samples were hydrogen terminated by etching in degassed ammonium fluoride for 15 minutes followed by a brief rinse in degassed Milli-Q water.

Surface Oxidation. Patterned surfaces were prepared according to our previous work.¹¹⁶ Irradiation in ambient air of a hydrogen terminated surface through a photomask (100 μm open squares separated by 50 μm lines; the mask from Adtek is chromium on quartz with an anti-scratch coating) with UV light (Hg(Ar) pen-lamp, Oriel Model 6035)

for 30min led to the formation of a surface comprised of 50 μm Si-H lines and 100 μm oxide squares. The ultra-violet radiation produced by the lamp was dominated by the 254 nm line ($\sim 95\%$), but also found to contain a weaker 185 nm line ($\sim 5\%$). It is this weaker 185nm line that has been shown to be responsible for the photochemical oxidation.¹²⁵ Un-patterned surfaces were prepared either by irradiation for 30 min from a distance of 2 cm (this has been reported as sufficient to ensure complete oxidation¹¹⁹) or by heating in piranha solution for 30 minutes at 120 °C. Ellipsometry indicated the thickness of the photooxide to be $12 \pm 1 \text{ \AA}$ while that of the chemical oxide was $10 \pm 1 \text{ \AA}$.

Monolayer Formation – Standard Thermal and Photochemical. After etching samples were briefly dried using a stream of nitrogen then immersed in 5 – 8 mL of deoxygenated 1-decene in a Pyrex Schlenk tube under continuously bubbling argon. The reaction was initiated either via irradiation at 300 nm in a Rayonette photoreactor or thermally at 150 °C for the desired time. The ambient temperature during irradiation was typically 35°C. The samples were removed from the reaction vessel, washed with TCE in a Soxhlet for 20 minutes dried under a stream of nitrogen and measured. For time course studies single sided wafers were prepared for each time-point while ATR elements were returned to the reaction vessel. Each FTIR time-point represents the average of at least 3 ATR elements, thickness and contact angle measurements represent an average of at least five points on each crystal and a minimum of two crystals were prepared.

Monolayer Formation – Comparison of Reaction Vessel. Samples were cleaned and etched then dried using a stream of nitrogen and immersed in a deoxygenated solution of either 1-decene or dodecane in a Pyrex Schlenk tube or PTFE screw cap vial (equipped with PTFE inlet and outlet tubing through the cap) under continuously

bubbling argon. The Schlenk tube and PTFE vials were 2 cm outer diameter and contained 4 mL of liquid, after bubbling at room temperature for 10 minutes the reaction vessels were placed in an aluminum heating block at 150°C for the desired time. The vials were cooled to room temperature and the samples were removed from the reaction vessel, washed with TCE in a Soxhlet for 20 minutes dried under a stream of nitrogen.

2.3 – Results and Discussion

2.3.1 – Oxide Reactivity

2.3.1.1 – Time Dependence of the Physical Properties

UV-irradiation of a hydrogen-terminated silicon surface through a photomask (50 μm lines spaced 100 μm apart) results in regio-specific oxidation in the exposed regions; after 30 minutes irradiation the pattern can be visualized by exposure to water vapour. The water droplets are centered on the hydrophilic oxide squares (Figure 2.1A). Thermal reaction of this surface with 1-decene at 150 °C for 90 minutes results in a loss of contrast between the lines and squares (Figure 2.1B), indicating that the decene reacts with the oxidized portion of the surface. Photochemical initiation did not result in pattern loss,¹¹⁶ suggesting different pathways are available under thermal reaction conditions. In an effort to elucidate the cause of pattern loss monolayer formation was studied on the photochemically oxidized and hydrogen terminated silicon surfaces (i.e. not patterned) initiated both thermally and photochemically. It is important to note that the material reacted on the oxidized portion was sensitive to etching by 2 % HF and therefore did not affect the ability to form bi-component monolayers.

Si-H surface A) immediately following photo-patterning and B) after reaction with 1-decene for 90 minutes at 150 °C. Photo-mask is 100 μm lines separated by 100 μm .

Figure 2.1 – Picture of patterned surface exposed to water vapour

The surface wetting properties of H-terminated surfaces reacted with decene were comparable whether prepared via the thermal (Figure 2.2A) or photochemical (Figure 2.2B) reaction and achieved the maximum value (102°) within 15 minutes. On the other hand, the wettability of the photooxidized surfaces was highly dependent on reaction conditions, with the thermal reaction (Figure 2.2C) producing significantly more hydrophobic surfaces, 91° , than the photochemical reaction (Figure 2.2D), 59° . The minimal difference ($\sim 10^\circ$) between the thermally reacted oxidized surface and the decene terminated surface is consistent with loss of contrast in the pattern when tested by exposure to water vapor. An oxidized surface immersed in decene in the dark for 16 hours was noticeably more hydrophilic than the photochemically reacted surface. However, the measured contact angle (33°) was greater than the initially prepared surface (0°) suggesting some very low inherent reactivity of the oxide surface towards decene.

The thickness of the oxide and hydrogen terminated surfaces were measured at the start of the experiment and subtracted as a background, allowing for a comparison of the alkyl layer thickness. This assumes that the optical properties of the oxide do not

change on addition of an alkyl monolayer. The apparent thickness of the monolayer formed on hydrogen terminated silicon increases more rapidly for the thermal reaction (Figure 2.3A) and attains a slightly higher maximum value of 13.5 ± 0.9 Å compared to

Figure 2.2 – Plot of contact angle versus time

the photochemical reaction of 11.4 ± 0.5 (Figure 2.3B). On the oxidized surface the thermal reaction (Figure 2.3C) attains higher coverage, 9 ± 1 Å, and a faster rate than the photochemical reaction (Figure 2.3D), 5 ± 1 Å. Immersion of an oxidized silicon crystal in 1-decene in the dark for 16 hours resulted in an apparent thickness of 2.2 ± 0.2 Å.

Figure 2.3 – Plot of estimated thickness versus time

During the course of monolayer formation on hydrogen terminated silicon the antisymmetric CH_2 stretching frequency was observed at $2922 \pm 1 \text{ cm}^{-1}$ throughout the reaction regardless of initiation procedure. As evidence of the lower coverage the antisymmetric methylene stretching frequency of monolayers formed on the oxidized surface was observed at $2927 \pm 2 \text{ cm}^{-1}$. Thermal initiation results in a faster rate of reaction on both hydrogen terminated and oxidized surfaces; after only 10 minutes both surfaces reached 80 % of the maximum absorbance observed (Figure 2.4A and 2.4C). After 90 minutes the photochemical reaction on the hydrogen terminated surface has reached about 90 percent of its maximum absorbance. The photochemical reaction on the oxide requires a much longer time to reach its maximum (the reaction was stopped after 16 h although it was not clear that the reaction was complete). The quantity of organic

material observed on the oxide surfaces was quite high, after 16 hours at 150 °C the coverage is approximately 40 percent that on the hydrogen terminated surface and after 16 hours in the photo-reactor the coverage is 25 percent. Both resulted in more material being deposited than found by immersion of an oxidized ATR crystal in degassed 1-decene for 16 hours in the dark at room temperature (22°C) which resulted in accumulation equivalent to 4 percent the amount on the hydrogen terminated surface (data not shown).

Si-H: (A) thermal, (B) photochemical

Photo-oxide: (C) thermal, (D) photochemical

Figure 2.4 – Plot of integrated area of absorbance (2800 - 3000 cm^{-1})

2.3.1.2 – Process of Photooxidation

It is interesting to contrast the more controlled vacuum photochemical oxidation process¹²⁶ with surfaces photooxidized in lab air or thermally oxidized in solution, which are in fact the relevant oxidized surfaces whose reactivity is the subject of the current study. HREELS spectra of silicon surfaces oxidized with piranha solution (120 °C for 20

minutes, Figure 2.5A) or nitric acid (80°C, Figure 2.5B) exhibit rather similar spectra, characterized by strong peaks at 450 cm^{-1} and 1180 cm^{-1} . These spectra are similar to that previously reported for thin SiO_2 films.¹²⁷ The presence of surface silanol groups is clearly indicated by the appearance of O-H stretch modes at 3720 cm^{-1} . The shoulder at $\sim 850\text{ cm}^{-1}$ reflects contributions from the Si-O-H stretch mode as well as modes of the oxide. There is no evidence of a Si-H peak, the weak feature at 2360 cm^{-1} can be assigned to the overtone of the 1180 cm^{-1} mode. For a surface that was photooxidized in air for 30 minutes (Figure 2.5C), the spectrum is somewhat different. The main oxide peaks are still present although the lower frequency peak is shifted up to 470 cm^{-1} . Two additional modes at 225 and 760 cm^{-1} , are observed which are not apparent on the chemically oxidized samples. Another noticeable difference is the reduced intensity of the 3720 cm^{-1} mode, indicating a decreased number of silanol groups relative to the samples prepared in solution. A weak peak at 2230 cm^{-1} can be assigned to Si-H with oxygen inserted into all three silicon-silicon back bonds ($\text{O}_3\text{-Si-H}$) suggesting that, in contrast to the chemical oxidation processes, not all Si-H bonds have been removed. In summary, the HREELS spectra indicate that the photo and chemically oxidized surfaces have a similar structure, although the latter exhibits some residual Si-H groups and correspondingly fewer hydroxyl moieties. Comparing the spectra after air oxidation versus that under oxygen in vacuum¹²⁶ it is apparent that the former is considerably more rapid, perhaps suggesting a role for water in accelerating the photooxidation reaction.

Si-H surface oxidized by (A) piranha solution, (B) nitric acid, and (C) irradiated in air. For (C) the region between 2000 – 2450 cm^{-1} has been magnified by a factor of 5 with arrow indicating the weak peak at 2230 cm^{-1} attributed to residual Si-H bonds. The feature at 2360 cm^{-1} is an overtone of the 1180 cm^{-1} peak.

Figure 2.5 – HREELS spectra showing effect of Si-H oxidative conditions

On confirming the unexpected loss of pattern after thermal initiation was due to decene reacting with the oxide surface, a model was developed centred on inhomogeneous oxidation of the hydrogen terminated silicon surface. It was initially hypothesized that after photooxidation in ambient the surface resulted in incomplete oxidation and was composed of a mixture of $\text{O}_n\text{Si-H}$ and O_3SiOH regions since the HREELS indicated the presence of unshifted Si-H after exposure to oxygen in vacuum. On such a surface decene was expected to react under both photochemical and thermal initiation with the unoxidized regions. The additional material reacted under thermal initiation was postulated to be derived from reaction on the partially oxidized region.

This model was consistent with wettability data, ellipsometric thickness, and FTIR measurements. However, in comparing the HREELS spectra of surfaces after chemical or ambient photooxidation it was apparent that they were not very different (apart from the presence of a small number of $\text{O}_3\text{-Si-H}$ moieties on the photooxidized surface). In order to determine whether these groups were responsible for the attachment of decene, the reactivity of chemically oxidized surfaces were studied. Piranha oxidized silicon wafers were found to thermally react with decene at $150\text{ }^\circ\text{C}$ in a manner similar to that observed on the photooxidized surfaces. Even piranha cleaned glass microscope slides were observed to react with 1-decene; after 16 hours at $150\text{ }^\circ\text{C}$ the surface was observed to be hydrophobic with a contact angle of 87° .

The final characterization performed was XPS on a freshly photooxidized silicon sample and oxide samples reacted thermally and photochemically for 90 minutes. The C1s region on the oxide reference sample shows un-shifted carbon at 285 eV, an ether linkage at 286.5 eV, and a small amount of acid / ester at 289 eV (Figure 2.6A). Potential sources of this adventitious hydrocarbon contamination include deposition during photooxidation which was conducted in ambient with a UV pen lamp and generated ozone which in turn may have induced the formation of reactive intermediates that may have deposited on the surface. The photochemically reacted surface shows only a small accumulation of organic material, (Figure 2.6B) consistent with the contact angle, ellipsometry and FTIR results. The increase in the ether feature is consistent with hydrocarbons attached via a Si-O-C link, although the ratio of ether to unshifted carbon is much larger than expected based on decyl chains attaching to the surface. In contrast, the surface reacted thermally (Figure 2.6C, 90 minutes at $150\text{ }^\circ\text{C}$) indicates a much larger

amount of hydrocarbon on the surface. Decomposing the observed spectrum into the various components yields an ether to methylene carbon ratio of $\sim 1/10$, close to that expected for decyl chains bound to the surface via a Si-O-C link. The spectrum also indicates the presence of a significant amount of carbon in higher oxidation states including the acid/ester at 289.5eV and a weak ketone related feature at 288eV.

Figure 2.6 – XPS spectra of C1s region indicating decomposition of decene

The presence of oxidized hydrocarbon is unique to the oxide surfaces as 1-decene reacted with the hydrogen terminated results in a single C1s peak in the XPS.³⁸ These experiments confirmed that the reactivity of the alkene with the photooxidized surface was not due to residual Si-H modes but rather a more general property of SiO₂ surfaces.

Coupled with the XPS data which shows evidence for ether linkages, this implicates the silanol groups as likely reactive sites.

Further analysis of the FTIR spectra sheds light on the mechanism of alkene reaction with the oxidized surface. It is important to note that the ratio of methyl to methylene absorbance (e.g. comparing the integrated absorbance of the anti-symmetric modes) is greater from the reactions with the oxide surface compared to the hydrogen terminated surface (Table 1). This could be consistent with Markovnikov addition since the FTIR spectra would show an increase in methyl groups relative to methylene groups. A report of the addition of 2,4,4-trimethyl-1-pentene to a silica-alumina surface under UHV conditions at $-63\text{ }^{\circ}\text{C}$ provides support for this suggestion. In this case, addition to the silanol occurred exclusively at the 2-position of the alkene.¹²⁸ The more acidic zeolite surface presumably allows the reaction to proceed at lower temperature. Radical mediated addition of an alkene to the hydrogen terminated surface is known to result in addition at the 1-position, while addition to the silanol was expected to occur at both the 1 and 2 positions. Indeed, the peak area of the anti-symmetric methyl stretch (2960 cm^{-1}) relative to the anti-symmetric methylene stretch (2922 cm^{-1}) was higher on the oxidized surface (Table 2.1). The ratio was highest when the reaction was performed at lower temperature (thermally at $80\text{ }^{\circ}\text{C}$ or at room temperature photochemically), and is consistent with a thermally activated process favoring Markovnikoff addition at lower temperatures. The ratio did not change during the course of the reaction and after 16 hours at $80\text{ }^{\circ}\text{C}$ the total coverage was 18% of the maximum coverage achieved on the hydrogen terminated surface.

Decene reacted with (A) photo-oxide and (B) Si-H surface, C-H region expanded and normalized to absorbance maximum.

Figure 2.7 – ATR-FTIR Spectra describing bonding orientation of alkene

Table 2.1 – Ratio of the antisymmetric to symmetric methyl stretch peak area

Surface Termination	Thermal (150°C)	Thermal (80°C)	Photochemical (300 nm)
Si-H	0.20 ± 0.01		0.20 ± 0.02
Si-O	0.32 ± 0.02	0.37 ± 0.05	0.39 ± 0.05

It is clear at this time that photochemical initiation of the reaction results in the addition of more organic material to the surface than when left in the dark at room temperature (25 versus 4% relative to a complete decyl terminated monolayer) and approximately the same as when the reaction is conducted thermally at 80°C (18% of a complete monolayer). The reason behind the enhanced reactivity is not understood at this time but is likely a result of an acid catalyzed process by the silanol groups at the surface. The presence of oxidized hydrocarbon species attached to the surface, detected by XPS,

suggest the surface may be catalytically active; a process known for more acidic substrates such as metal doped silica or silica – alumina.^{129,130} A greater understanding of this process is required in order to limit unwanted side products that may have an impact on the electrical performance of a device.

This provides a more complete understanding of the original observations.¹¹⁶ At that time it was reported that scanning auger electron spectroscopy of a patterned surface after reaction with 1-decene photochemically showed contrast between oxidized and alkyl regions. However, in the oxidized region the carbon signal indicated the presence of a low level of carbonaceous material. This was initially attributed to adventitious, physisorbed carbon contamination. Based on current results it is likely 1-decene covalently bonded to the oxidized surface. This is supported by the XP spectrum in Figure 2.6B as well as the thickness and FTIR data in Figures 2.2 and 2.3. Although the total amount of hydrocarbon by XPS is much lower on the “photochemically initiated” reactions, there is evidence for SiO-C bonding and a much smaller amount of carbonyl compared to Figure 2.6C. The much slower reaction under the photochemical conditions is consistent not with a photoactivated process but a slow thermally activated process (35°C).

Since the organic material is bound to the oxidized region via SiO-C bonds, it is chemically sensitive to HF and can be selectively removed leaving newly formed hydrogen terminated silicon. Figure 2.8B shows the pattern partially restored after immersion in 2% HF following thermal reaction of the oxide patterned surface with 1-decene. A second brief photooxidation fully restores the pattern fidelity (Figure 2.8C). As an additional note it is expected that in order to derivatize the oxide using alkoxy /

chloro silane chemistry the initial oxide will have to be etched to remove accumulated alkyl material and re-oxidized to provide a surface free from organic contamination.

Patterned surface after A) reaction with 1-decene for 18 hours at 150 °C
B) immersion in 2 % HF for 2 minutes and C) UV oxidation in ambient.

Figure 2.8 – Pictures Demonstrating Recovery of Underlying Pattern

2.3.2 – Mechanism of Thermal Initiation

Figure 2.9 shows STM images taken at the early stages of the thermal reaction of decene with H-terminated Si(111). Clearly visible in Figure 2.9A are several monoatomic height steps, separated by atomically flat terraces. On these flat terraces, several irregularly shaped islands with nominal heights of 4-5 Å are observed. HREEL spectra on this sample (not shown) confirm the presence of a submonolayer coverage of alkyl chains on the H-Si(111) surface, allowing the islands to be attributed to assemblies of decyl chains. Figure 2.9B shows a smaller area scan of a different region on the same sample showing a close up view of one of these island structures. The observation of the growth of irregularly shaped islands is consistent with the reaction proceeding via a propagating radical chain mechanism, as seen previously for photochemical reactions under solution or gas phase conditions.^{49,121} On the Si-H surface, this propagating chain reaction proceeds via a pseudo random walk process.^{41,49}

A) 26nm² image obtained at 43pA with a sample bias of -2.6V, B) 12nm², 43pA, -3.2V.

Figure 2.9 – UHV STM of Partially 1-decene reacted Si-H

2.3.2.1 – Evidence of Hydrocarbon Degradation

To investigate the feasibility of an oxide induced degradation of alkyl chains, the stability of H-terminated Si(111) in the “inert” solvent dodecane was studied. The surface is expected to be unreactive in this solvent in the absence of a degradation pathway. Figure 2.10 shows the ATR-FTIR spectrum of the H-terminated surface upon exposure to deaerated dodecane in a Pyrex Schlenk tube at 150°C. The freshly etched sample (Fig. 2.10A) shows a sharp peak at 2083 cm⁻¹ corresponding to the terrace Si-H monohydride stretch, along with a small amount of hydrocarbon contamination indicated by the C-H stretches observed in the 2800-3000 cm⁻¹ range. However, after reaction with dodecane for 1 hour at 150°C (Fig. 2.10B), the Si-H peak has been completely eliminated and the growth of significant peaks in the C-H stretch region is observed. The spectra clearly indicate the alkylation of the surface under these conditions, presumably from the dodecane solvent. In contrast to the alkyl monolayers formed from the reaction of alkenes with Si-H, the alkylated surface from the dodecane reaction is unstable to etching in hydrofluoric acid (HF). After 1 minute in 2% HF (Figure 2.10C), the hydrocarbon signal is significantly reduced and Si-H modes are once again observed in the region from 2080 to 2150cm⁻¹. The removal of the hydrocarbon in HF is consistent with the

alkyl chains being attached to the surface via an Si-O-C link while the presence of multiple modes in the Si-H region (indicative of the formation of dihydride species) indicates that the initial reaction had also led to a significant amount of oxygen insertion into Si-Si back bonds. For the alkane to react with the Si-H surface, the organic must have been degraded elsewhere in the reaction vessel and then subsequently reacted with the surface. Given the propensity for hydroxyl groups to crack hydrocarbons noted above, the surface of the Pyrex Schlenk tube was implicated as the likely source of the dodecane degradation. GC analysis of the solvent following the reaction did not show any changes in chemical composition. However, it should be noted that the exceedingly small concentrations of reactive intermediates are sufficient to degrade pristine Si-H surfaces.

(A) Freshly etched Si-H, then (B) 60 min in dodecane at 150°C, then (C) 2% HF 2 minutes.

Figure 2.10 – ATR-FTIR spectra of Si-H in Pyrex Schlenk tube

To test the idea that it was the Pyrex surface that was responsible for the reaction on the silicon surface, the dodecane experiment was repeated in a Teflon (PTFE) vial. Figure 2.11 shows representative ATR-FTIR spectra for the reaction of Si-H surface in dodecane using a PTFE vial. While the starting freshly etched Si-H surface exhibits the same characteristic 2083cm^{-1} peak, the spectrum after reaction is substantially different. Even after heating for 1 hour in dodecane, the peak remains at approximately 70% the intensity of a freshly etched sample and only minimal accumulation of hydrocarbon is apparent. This provides additional evidence that silanol groups on the surface of Pyrex can lead to the degradation of the hydrocarbon and initiation of a surface reaction.

(A) Freshly etched Si-H, then (B) 60 min in dodecane at 150°C.

Figure 2.11 – ATR-FTIR Spectra of Si-H reacted with dodecane in PTFE vial

Given these unexpected results, it was important to investigate the impact of reaction vessel on the reaction of alkenes with the Si-H surface. The properties of monolayers formed via thermal reaction of 1-decene for 1 hour at 150°C in both Pyrex and PTFE were compared. As for the dodecane reactions, 1-decene was filtered through Florisil then distilled under vacuum, the purified reactant was then separated into two portions with one going into a Pyrex Schlenk tube and the other into a PTFE vial. The data in Table 2.2 represents the average of three separate experiments. Based on the integrated IR absorption intensity over the entire C-H stretching region, it is evident that the reaction in Pyrex led to an increased amount of hydrocarbons grafted to the surface. The film thicknesses determined by ellipsometry were also consistent with this trend although the observed differences were within the experimental error. While the thermal reaction in Pyrex was essentially complete after 1 hr., the IR integrated intensity and contact angle in PTFE continued to increase for longer reaction times.

Table 2.2 – Effect of reactor material on Monolayer Properties

Reactor	Thickness (Å)	Contact Angle (°)	IR C-H Peak Area (AU)
Pyrex Schlenk (1 h)	13.6±1.3	107.6±1.0	0.61±0.02
PTFE Vial (1 h)	12.2±1.0	109.1±0.8	0.46±0.01
PTFE Vial (2 h)	13.2±0.8	110.7±0.6	0.55±0.02

Although the STM images confirm that the reaction proceeds via the radical chain mechanism, the question remains as to how the reaction is initiated (i.e. the origin of the initial dangling bond that starts the chain process). Although initially it was suggested that this could occur by thermally induced Si-H bond cleavage,^{33,131} this scenario is unlikely in view of existing data on the thermal stability of this bond. Heating an H-terminated silicon surface in the absence of impurities will result in desorption of molecular hydrogen (generating pairs of dangling bonds). Based on the measured

activation energy for this process ($\sim 2.6\text{eV}$)^{132,133} we can estimate that generation of dangling bonds at 150°C is negligible. Therefore, it is highly likely that other mechanisms for silicon radical generation are responsible for initiating the reaction. As discussed in the introduction, molecular oxygen has been suggested as a possible initiator.^{134,135}

The observation that thermal reactions proceed more rapidly in Pyrex is consistent with the reaction being initiated by reactive intermediates generated by the thermally induced degradation of reactants/solvent on the surface of the reaction vessel. It is perhaps interesting to note that although the reaction in Pyrex appears to increase the rate of monolayer formation, the contact angle for these samples was slightly lower, indicative of a poorer quality monolayer.

A recent trend in organic functionalization reactions of hydrogen terminated silicon surfaces, expressed in several publications, has been a push to “milder” reaction conditions. For photoinitiated reactions, this has involved the use of visible rather than UV light whereas in the case of thermal reactions it has meant investigation of lower temperatures to initiate the reaction. For example, de Smet *et al*¹³⁴ have reported thermal reaction of H-terminated porous silicon with 1-undecene at $90\text{--}110^\circ\text{C}$, considerably lower than the temperatures $>150^\circ\text{C}$ that are typically employed. When efforts were made to exclude oxygen and peroxides from the system minimal reaction was observed. This is consistent with the present finding that at 80°C in an oxygen free environment the Si-H peak is stable for extended periods of time in dodecane or toluene and the reaction of 1-decene was inhibited (data not shown). These observations provide evidence for trace oxygen as a source of silyl radicals. Abstraction of hydrogen by molecular oxygen has

been observed in the molecule tris(trimethylsilyl)silane¹²², a molecular analog of the H/Si(111) surface. However, the activation energy for this process was estimated to be ~ 130 kJ/mol,¹³⁵ implying that this pathway can only account for reactions at temperatures above $\sim 100^\circ\text{C}$. Recently this trend to mild reaction conditions has been extended further with two of reports of room temperature reactions of alkynes with H-terminated silicon.^{123,124} Although these reactions have been attributed to the molecular oxygen initiation mechanism discussed above, this is difficult to rationalize in view of the activation energy required for this process unless the chain length of the ensuing reaction is exceedingly long.

2.3.3 – Electrical Properties

The impact of the reaction vessel and reagent purification on the electrical properties of the silicon was investigated by measuring the surface photo-voltage (SPV) by a Kelvin probe. Briefly, the surface potential is first measured in the dark and then under illumination with the difference representing the degree of band-bending associated with charge trapped in electrically active defects at the organic/silicon interface.^{80,81} Ideally, alkylation is expected to retain the extremely low density of electrically active defects and minimal band bending observed on H-terminated silicon surfaces.

Figure 2.12 shows the SPV of surfaces obtained from a 90 minute photochemical reaction, and compares the effect of purification of the 1-decene. Decene was used as received from the bottle, passed through a column of florisil⁵⁶, or distilled under vacuum just prior to use; three independent experiments were conducted. The results indicate reagent treatment has a slight impact on the SPV of the resulting samples which was

observed to be about -70mV. While the error bars are larger when surfaces were prepared using untreated 1-decene the photovoltage is not significantly greater.

Figure 2.12 – SPV of Decene surfaces after Photochemical Reaction

Figure 2.13 reports the SPV of surfaces obtained after thermal reaction at 150°C for 1 hour. It compares untreated 1-decene reacted in a glass Schlenk tube, and 1-decene passed through a florisil column then distilled and reacted in a glass Schlenk tube or PTFE vial; 3 independent experiments were conducted. The photovoltages from these experiments were also approximately -70mV.

This moderate amount of band-bending is likely due to oxidation which occurred during monolayer formation. The reactions are not carried out in a glove box which could reduce water and oxygen levels. SPV is a measure of occupied defect sites and does not measure unoccupied defects; however a recent study from the group shows good correlation between total surface state density measured electrochemically in solution and

Figure 2.13 – SPV of Decene Surface after Thermal Reaction

occupied states measured by Kelvin probe, on the order of $1 \times 10^{11} \text{ cm}^{-2}$.¹³⁶ As to whether a defect is associated with each island, it has been reported that each island contains approximately 30 decene molecules⁴², the area of each Si-H atom is $12.77 \text{ \AA}^2$ ($\sim 7.8 \times 10^{14}$ atoms/ cm^2) and monolayer coverage is 30-50% of Si-H atoms.^{33,54,137} At 40% coverage there are 3.1×10^{14} alkyl chains / cm^2 and 1×10^{13} islands / cm^2 . Therefore, if the surface states are associated with monolayer formation only 1 in 100 islands introduces an electrically occupied defect. Furthermore, the expected impurities in 1-decene are homologs, regioisomers (no effect) and oxygenated compounds (may or may not have an effect) and the chain growth mechanism favours reaction with a terminal alkene. When an alkene reacts with a surface dangling bond island growth occurs and another surface dangling bond is created propagating the reaction on the surface. Conversely, if an oxygenated hydrocarbon reacts with the same surface radical it may be capped by a hydrogen atom of the impurity (halting island growth but not introducing a defect) or a Si-O-C link may be formed (halting island growth but not necessarily introducing an

electrically active defect). Therefore unless an impurity is charged or makes up a significant proportion of the solution there may be little impact on the electrical properties of the surface. It is more likely that defects form in the 50-70% of the surface not covered by a monolayer and are in fact due to trace water and oxygen present during monolayer formation. This is also consistent with there being little correlation between reagent purity, reaction conditions and SPV.

2.4 – Summary & Prospective

In summary, it was shown that photooxidation of Si-H (111) in ambient lab conditions leads to the formation of a SiO₂ surface layer, spectroscopically and functionally similar to a chemically oxidized surface. The oxide surface reacts thermally (150 °C) and photochemically (Rayonette photoreactor) with 1-decene to afford a partial alkyl monolayer. This is consistent with the addition of alkene to the silanol surface via Markovnikoff addition to the silanol in an acid catalyzed process. The increased methyl to methylene ratio at 150°C compared to 35°C is consistent with both a competing anti-Markovnikoff addition at higher temperatures and the reaction of catalytically degraded hydrocarbon fragments. For example, X-ray photoelectron spectroscopy indicates the presence of oxidized hydrocarbon on the surface after thermal reaction indicating other reaction pathways lead to the oxidative degradation of the films and the “inert” solvent dodecane was observed to react with the Si-H surface at 150°C.

Thermal reactions of alkenes with H-terminated silicon were observed to proceed via the radical chain mechanism established for photochemically initiated reactions. Furthermore, evidence was provided that these reactions can be initiated by the degradation of hydrocarbons catalyzed by the silanol groups on the surface of glass

vessels typically used for these reactions. The Si-H surface in dodecane at 150°C was found to be substantially more stable in PTFE vials versus Pyrex Schlenk tubes. The reaction of decene with Si-H proceeded considerably more slowly in Teflon vessels (relative to Pyrex), although the resulting monolayers were of higher quality. These observations illustrate the impact of what is typically considered to be an inert reaction vessel on reactions of H-terminated silicon. The results also indicate the importance of minor degradation pathways and traces of impurities in the initiation of surface reactions of H-terminated silicon, providing alternative (more plausible) mechanisms for the initiation of these reactions. In addition to molecular oxygen, impurities and reactive intermediates generated during the thermal reaction (and by implication the photochemical reactions) are a source of initiators of the solution phase reaction of alkenes with Si-H surfaces.

The electrical properties of decyl monolayers were studied by SPV and little correlation with either reagent treatment or reaction conditions was observed. Under all conditions the SPV was approximately -70mV, corresponding to 1 electrically occupied defect per 100 islands formed, and it is quite possible that the interface states are introduced in regions between islands where there is free Si-H. There being no clear method to ranking the purification processes and reaction conditions, the general protocol adopted in following experiments was to distill the reagent and initiate the reaction photochemically.

In closing this chapter identifies several issues which are of importance to the silicon surface chemistry community. It is imperative to consider all of the materials present during the reaction. In the case of functionalizing an integrated device, metals or

metal oxides exposed to the reagent could impact the chemistry on the active region.

Reactions are commonly carried out as solutions in an “inert” solvent (for example 0.2M alkene in mesitylene) and care must be taken to ensure that only the desired alkene attaches to the surface and not degradation products of the solvent. The following chapter studies the feasibility of measuring biological interactions using SPV and identifies challenges associated with ensuring the chemical and electronic stability of the surfaces.

Chapter 3 – Feasibility Study

3.1 – Introduction

The goals of this chapter are to determine whether biomolecule binding can be detected by Kelvin probe and to ensure the chemical and electrical stability of the surface is retained in an aqueous environment. The proteins studied were p19¹³⁸ (a viral protein) and streptavidin. The p19 was modified with a poly-histidine tag as a handle for surface attachment via interaction with a surface bound Ni-NTA complex, while the streptavidin was captured by a surface modified with biotin. The stability of the electrical properties was studied by monitoring changes in the SPV on extended exposure to water or buffer.

3.1.1 – p19-RNA

RNA interference is a system used by cells to help control the activity and level of activity of genes. Additionally, when exposed to foreign DNA or RNA (e.g. viral) many organisms initiate a highly specific response to silence the invading genetic material in order to prevent its incorporation into the host's genome.¹³⁹ In a 1995 paper, Guo and coworkers described gene silencing in *C. elegans* induced by dosing with short strands of complementary RNA (antisense).¹⁴⁰ The surprising result was that treatment with the sense strand (same sequence as the target messenger RNA) as a control also induced gene silencing. This was resolved three years later when Fire and coworkers demonstrated that RNA interference was induced by small double stranded RNA fragments 21-23 base pairs long and that the effects could be passed through generations and across tissues within an organism.^{141,142} This work identified the RNA silencing pathway and its importance was recognized by the awarding of the 2006 Nobel Prize in Physiology and Medicine to Andrew Fire and Craig Mello.

The basic process involves cleavage of double stranded RNA into 19-25 base pair siRNA fragments. The siRNA complexes with a protein, the double stranded RNA is unwound and the RNA protein complex then binds complementary messenger RNA inducing its cleavage and thereby silencing gene transcription.¹³⁹ Interference systems have been identified in plants, animals and fungi suggesting its ancient origin.^{143,144} It is therefore not surprising that viruses have developed countermeasures to overcome this cellular defense mechanism. A 19kDa protein isolated from tombusviruses, was found to inhibit RNA silencing in plants in a size but not sequence specific manner.^{145,146} Crystal structures of p19 have been obtained of both the protein and the protein-RNA complex, Figure 3.1.^{147,148} The crystal structures indicate the active structure is a homodimer and the binding pocket is rich in polar and basic residues which contact the phosphate groups of the siRNA. It binds specifically siRNA because of two tryptophan residues which act as caps; the binding of a 23 base pair siRNA is 20 times weaker than a 21 base pair siRNA.¹⁴⁹ For this reason p19 has been termed a “molecular caliper”.¹⁴⁷

Reprinted from¹⁴⁷ with permission.
Copyright 2003 Elsevier.

Reprinted by permission from Macmillan
Publishers Ltd: from¹⁴⁸, copyright 2003.

A) Surface potential map of p19 with siRNA bound, B) ribbon structure of p19 showing homodimer and N and C-termini

Figure 3.1 - Crystal Structure of p19

Since p19 does not show significant sequence specificity it could prove a useful tool for studying siRNA *in vitro* and *in vivo*. Koukietolo and coworkers have incorporated a poly-histidine tag onto the carbon-terminus of the protein and used this handle to bind the protein to a Ni-NTA coated 96 well plate.¹⁴⁶ The protein was then exposed to Cy3 labeled siRNA and, after washing, fluorescence was measured to characterize binding. The major limitation of this approach is the requirement of labeled siRNA; in order to study siRNA in cells it would be necessary to specifically label the molecules of interest. Therefore, a label free method to monitor binding of siRNA to p19 is of great interest. Because both the protein and nucleic acid are charged, this system is a good candidate for electrical detection. It was envisioned that a carboxylic acid terminated silicon surface could be activated with NHS and reacted to give a NTA terminated monolayer. After complexation with Ni^{2+} , the surface would bind the hexa-histidine tagged p19.

The advantage of attaching p19 using the hexa-histidine affinity tag is that the protein can be specifically oriented ensuring the binding pocket is available to capture siRNA. Additionally, the protein is not covalently bound and can be washed off regenerating the surface. In these experiments, the histidine tag is attached to the C-terminus¹⁴⁶ and based on the crystal structure¹⁴⁸ it will be located on the bottom of the protein (Figure 3.1). Affinity tags are short peptide sequences added to the coding sequence of a protein to enable the selective capture of the desired gene product from the cellular contents.¹⁵⁰ As shown in Figure 3.2, NTA occupies four coordination sites of the Ni^{2+} cation leaving the remaining two available for interaction with the hexa-histidine tag. An added benefit of developing this chemistry is that the histidine tag is a common

tool in biochemistry therefore it is envisioned that this will represent a general method for the attachment of proteins to the silicon surface.

A) NTA, B) Ni-NTA, C) Histidine tagged protein interacting with Ni-NTA

Figure 3.2 – Affinity Capture by NTA

3.1.2 – Biotin - Streptavidin

In contrast to the NTA affinity tag, which is a general method for binding biomolecules, biotin (Vitamin H) is the specific target of the proteins avidin and streptavidin. They are tetrameric proteins composed of 4 identical subunits. While the avidin monomer displays an equilibrium binding constant of $\sim 10^7 \text{M}^{-1}$ the tetramer is significantly higher $\sim 10^{15} \text{M}^{-1}$, while tetrameric streptavidin shows slightly weaker binding $\sim 10^{13} \text{M}^{-1}$.^{151,152} The main physical differences are that avidin is glycosylated, while streptavidin is not and the pI of avidin is about 10 and streptavidin is about 6.¹⁵³ At neutral / biologically relevant pH, avidin will be positively charged and streptavidin will be negatively charged. The strong binding and robust nature of the biotin – protein complex has led to a wide range of applications: it is used as an affinity tag, a linking layer in sandwich assays, and for DNA assays.¹⁵⁴⁻¹⁵⁶ Biotin is an uncharged molecule

and based on crystallographic and computational studies the interaction between biotin and streptavidin and avidin is driven by hydrogen bonding to the urea^{151,152} functionality of biotin (Scheme 3.3, Compound 6) and does not involve ionic interactions like NTA. The crystal structure of the avidin – biotin complex indicates the protein is roughly rectangular ($40 \times 50 \times 50$ Å) with two binding sites on each side separated by about 30 Å.¹⁵⁷ Furthermore, the binding of biotin induces changes to streptavidin and avidin which enhance its stability; the thermally induced denaturation temperature increases from 75 and 85°C to 112 and 117°C respectively when all four binding sites are occupied,¹⁵⁸ since the biotin groups on the surface are likely less than 30 Å apart (allowing for both binding sites to be occupied) the protein stability could be enhanced on binding to the surface. These properties make biotin – streptavidin / avidin an ideal test case for the development of novel sensors.^{98,105,159}

3.1.3 – Stability

Once the detection of a protein by Kelvin probe on silicon is demonstrated it is imperative to study the stability of the surface. Two reports, from the labs of Heath (silicon nanowires) and Hamers (planar silicon), provide XPS data indicating substantial oxidation of the silicon for the functionalized monolayers; Hamers also demonstrates that the surface continues to oxidize after 3 and 7 days in water.^{63,105} Though not stated it is reasonable to infer that oxidation of the substrate contributes to the low device yield (~2%) in Reed's nanowire array.⁸⁵ Changes in the electrical properties of Si-H in air¹⁶⁰ and organic modified Si-H in aqueous¹³⁶ indicate that oxidation leads to charge trapping at the interface which will effectively increase the resistance in the gate of a BioFET decreasing its performance.

As described in the previous chapter the coverage of Si-H groups with alkyl chains is on the order of 30 – 50 %.^{33,54,137} It is limited by the mismatch in diameter between the surface silicon atoms ($12.8 \text{ \AA}^2$) and the alkyl chains ($15.3 \text{ \AA}^2$) as only 83 % of the Si-H sites can be capped by an alkyl chain; therefore, this coverage of Si-H sites represents 35 – 60 % of a closed packed hydrocarbon monolayer. Effectively this means that 40 – 65 % of the silicon surface remains unreacted and is available for attack by water or oxygen. It was hypothesized that increasing the density of the hydrophobic hydrocarbon layer would decrease the oxidation of the substrate. Two approaches were investigated. In the first a longer hydrocarbon chain formed from 1-octadecene was prepared; the second approach formed branched chains via the Grignard reaction of an ester terminated monolayer.

3.1.4 – Rationale

Developing a label free method to detect siRNA would clearly be a valuable tool for biochemists in the study of RNA interference and from an electrical biosensor development standpoint it is also an interesting system to study. Each event can cause a change in charge at the interface: coordination of Ni^{2+} by NTA, binding of the positively charged protein and finally capture of the negatively charged double stranded RNA.

A significant negative photovoltage was observed on forming the Ni-NTA complex and this was attributed to deprotonation of the carboxylic acid groups of the NTA. After exposure to the protein, the photovoltage became much less negative and at higher concentrations of p19, the photovoltage became positive corresponding to an accumulation of positive charge at the interface. XPS analysis of these initial monolayers however indicated Ni^{2+} was below the level of detection and suggested NTA had not in

fact been incorporated. Control experiments showed that acid terminated surfaces ionize on exposure to base and the SPV can be cycled by subsequent exposure to acid and base. The chemistry was optimized to ensure Ni-NTA surfaces were successfully prepared; the Ni^{2+} levels were quantified as approximately 1:1 with respect to the nitrogen core level by XPS. It was interesting to note that the same changes were observed in SPV, coordination of Ni^{2+} led to a large negative SPV which shifted towards positive after the protein was bound. Efforts to bind fluorescently labeled RNA were ultimately unsuccessful as it was found the protein denatured on binding to the surface. These experiments identified several key issues: charged groups in the monolayer should be avoided, the chemistry should allow the probe to be spotted on the surface and an external technique such as fluorescence is required to validate binding.

These issues were addressed with the study of biotin-streptavidin. Biotin was covalently bound to an OEG linker affording aqueous solubility allowing it to be spotted onto the silicon surface. The remainder of the monolayer was capped with hydroxyl terminated OEG to inhibit non-specific binding. After exposure to Cy-3 labeled streptavidin, contrast was observed in the scanning Kelvin probe and it correlated with the fluorescence image. This confirmed that the protein was localized in the regions containing biotin and it induced measurable changes in the electrical properties of the silicon. The signal corresponded to an accumulation of negative charge, this could be the result of the protein being negatively charged or oxidation of the silicon. The stability of the biotin surface in aqueous solution was studied by XPS which indicated that the silicon was being oxidized on exposure to water.

The stability of organic monolayers was then studied by SPV, HREELS, and XPS. It was found that by increasing the density of the layer using either longer chain alkenes or branching of an ester terminated monolayer via the Grignard reaction the SPV could be stabilized in aqueous solution for over 16 hours. HREELS and XPS confirmed that oxidation of the silicon had been inhibited. This left the final challenge of incorporating functionality into these stabilized monolayers which is the topic of the following chapter.

3.2 – Experimental Details

3.2.1 – Materials

ATR silicon crystals ($25 \times 4.5 \times 1 \text{ mm}^3$) were purchased from Harrick and the silicon wafers from Virginia Semiconductor. Sulfuric acid, 96% (H_2SO_4), ammonium fluoride, 40% (NH_4F), hydrochloric acid, 30% (HCl), ammonium hydroxide, 30% (NH_4OH) and hydrogen peroxide, 30% (H_2O_2) Finyte Grade were purchased from J. T. Baker, and hydrofluoric acid, 48% (HF) PPB grade was purchased from Arch. 10-Undecylenic acid 98% was purchased from Aldrich and distilled in a Kugelrohr apparatus under vacuum at $\sim 160^\circ\text{C}$. Ethyl-undecylenate 97% was purchased from Aldrich and distilled under vacuum at 134°C . 1-Octadecene 90% was purchased from Aldrich and distilled in a Kugelrohr apparatus under vacuum at $\sim 150^\circ\text{C}$. N-hydroxysuccinimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride, methylmagnesium bromide 3.0 M in diethyl ether, pentyl magnesium bromide 2.0 M in diethyl ether and synthetic reagents were purchased from Aldrich at $>95\%$ purity and used as received. (S)-N-(5-Amino-1-carboxypentyl)iminodiacetic acid $>97\%$ was purchased from Dojindo

Molecular Technologies and used as received. Unless otherwise specified, solvents were obtained from a purification system.

3.2.2 – Surface Characterization (see Section 2.2.2)

Estimated Thickness. Unless otherwise indicated reported numbers represent an average of five points on each sample and a minimum of two independent experiments. The error represents the arithmetic mean of the standard deviation from each experiment.

Contact Angle. Unless otherwise indicated reported numbers represent an average of three points on each sample and a minimum of two independent experiments. The error represents the arithmetic mean of the standard deviation from each experiment.

SPV. Unless otherwise indicated reported values represent an average of sixteen points in the dark and sixteen points collected in the light from a representative experiment.

Fluorescence Measurements. Silicon wafers were affixed to a glass slide and scanned in a BioRAD VersArray Chip Reader with 5 μm resolution using 635 and 532 nm excitation.

X-Ray Photoelectron Spectroscopy (XPS). XPS spectra were recorded on a PHI 5500 instrument, using monochromated Al K α (1486eV) radiation with detection at 45° to the surface normal. The pressure during analysis was $\sim 5 \times 10^{-8}$ Torr. Spectra were fitted with Gaussian profiles and Shirley background correction. The positions of all peaks were normalized to C 1s at 284.8 eV.

3.2.3 – Synthesis

Unless otherwise indicated the references provided correspond to data used for characterization purposes.

Scheme 3.1 – Preparation of 3

2-[2-(2-azidoethoxy)ethoxy]ethanol (2). *2-[2-(2-chloroethoxy)ethoxy]ethanol (1)* (10g, 59.3mmol) was dissolved in 95% ethanol (40mL) in a 250mL round bottom flask equipped with a magnetic stir bar. To this was added KI (3g, 18mmol) and sodium azide (5g, 78mmol) the reaction was then heated at 80°C over night. The solution was allowed to cool to room temperature, filtered and concentrated to afford a clear yellow oil. The crude was purified by silica gel column chromatography to afford a clear oil (7.3g, 71% yield). ¹H NMR (400 MHz, CDCl₃) δ ppm 3.73 (t, *J*=4.88 Hz, 2 H), 3.68 (m, 6 H), 3.61 (t, *J*=4.88 Hz, 2 H), 3.40 (t, *J*=4.89 Hz, 2 H), 2.64 (s, 1 H).¹⁶¹

2-[2-(2-aminoethoxy)ethoxy]ethanol (3). *2-[2-(2-azidoethoxy)ethoxy]ethanol* (2g, 11.4mmol) was dissolved in dry tetrahydrofuran (40mL) in a 250mL round bottom flask, to this was added triphenylphosphine (3.3g, 12.6mmol). The reaction was sealed under a nitrogen balloon and stirred at room temperature over night. The reaction was opened and water (821μL, 45.6mmol) was added and stirred for four hours. The reaction was concentrated, suspended in water (20mL) and washed with toluene (5 × 20mL). The water was concentrated to a clear yellow oil which was dissolved in dichloromethane,

dried with MgSO_4 , filtered concentrated and flashed eluting with 10-25% methanol :

chloroform : 1-2.5% NH_4OH to yield a pale yellow oil (1.5g, 88% yield). ^1H NMR (400 MHz, CDCl_3) δ ppm 3.69 (m, 2 H), 3.62 (m, 4 H), 3.57 (m, 2 H), 3.53 (t, $J=5.14$ Hz, 2 H), 2.96 (s, 3 H), 2.86 (t, $J=5.06$ Hz, 2 H).¹⁶² ES-MS: calc 149.1, found MH^+ 150.1.

Scheme 3.2 – Preparation of **5**

tert-butyl 3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propylcarbamate (**5**). 3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}-1-propanamine (**4**) (8.8mL, 40mmol) was dissolved in dichloromethane (150mL) in a 250mL round bottom flask. To this was added di-*tert*-butyl dicarbonate (2.2g, 10mmol) in four portions over 20 minutes. The reaction was stirred overnight, concentrated and purified by silica gel column chromatography eluting with 20% methanol : chloroform : 2% NH_4OH to yield a pale yellow oil (2.2g, 68% yield). ^1H NMR (400 MHz, CDCl_3) δ ppm 5.15 (s, 1 H), 3.63 (m, 4 H), 3.56 (m, 8 H), 3.21 (m, 2 H), 2.82 (t, $J=6.66$ Hz, 2 H), 2.12 (s, 2 H), 1.74 (p, $J=6.44$, Hz, 4 H), 1.42 (s, 9 H).¹⁶³ ES-MS: calc 341.1, found MH^+ 342.3.

Scheme 3.3 – Preparation of **7**

(+)-Biotin *N*-hydroxysuccinimide ester (**7**). (D)-biotin (**6**) (1.01g, 4.14mmol) was suspended in dry chloroform (6mL), a drop of *N,N*-dimethylformamide was added and the reaction sealed under nitrogen. Thionyl chloride (4mL, 55mmol) was added drop-wise and the reaction stirred at room temperature for 1 hour. The solvent and thionyl chloride were evaporated under reduced pressure and the residue taken up in dry chloroform (10mL) this was drop-wise added to a stirring suspension of *N*-hydroxysuccinimide (952mg, 8.28mmol) and triethylamine (1.45mL, 10.3mmol) in chloroform (30mL) under nitrogen. The reaction was stirred 3 hours then concentrated and the residue purified by silica gel column chromatography eluting with 10% methanol : chloroform (1.1g, 77% yield). ¹H NMR (400 MHz, DMSO-d₆) δ ppm 6.43 (s, 1 H), 6.37 (s, 1 H), 4.31 (s, 1 H), 4.15 (s, 1 H), 3.11 (dd, *J*=6.97, 11.46 Hz, 1 H), 2.84 (s, 5 H), 2.68 (t, *J*=7.35 Hz, 2 H), 2.59 (d, *J*=12.43 Hz, 1 H), 1.65 (m, 3 H), 1.48 (m, 3 H).¹⁶⁴

Scheme 3.4 – Preparation of **9**

tert-butyl 3-{2-[2-(3-biotin amidopropoxy)ethoxy]ethoxy}propylcarbamate (**8**).

tert-butyl 3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propylcarbamate (**5**) (1.1g, 3.43mmol) and triethylamine (482μL, 3.43mmol) were dissolved in dichloromethane (10mL) and added to a stirring suspension of (+)-Biotin *N*-hydroxysuccinimide ester (**7**)

in dichloromethane (10mL) the reaction was stirred at room temperature over night. It was then concentrated and purified by silica gel column chromatography eluting with 1-5% methanol : chloroform (1.2g, 93% yield) as a pale yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 6.59 (s, 1 H), 6.25 (s, 1 H), 5.46 (s, 1 H), 5.06 (s, 1 H), 4.52 (dd, $J=5.01$, 7.58 Hz, 1 H), 4.33 (dd, $J=5.08$, 7.15 Hz, 1 H), 3.61 (s, 12 H), 3.36 (dd, $J=6.00$, 12.08 Hz, 2 H), 3.23 (dd, $J=5.83$, 11.83 Hz, 2 H), 3.16 (dt, $J=4.78$, 7.30 Hz, 1 H), 2.92 (dd, $J=4.92$, 12.81 Hz, 1 H), 2.76 (d, $J=12.77$ Hz, 1 H), 2.21 (t, $J=7.47$ Hz, 2 H), 1.73 (s, 8 H), 1.47 (m, 11 H).

3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}-1-biotinyl-propanamide (**9**). tert-butyl 3-{2-[2-(3-biotin amidopropoxy)ethoxy]ethoxy}propylcarbamate (**8**) (1.2g, 2.12mmol) was dissolved in dichloromethane (10mL) and trifluoroacetic acid (2mL) and stirred at room temperature for 2 hours. The reaction was concentrated then dissolved in methanol (20mL) added potassium carbonate powder and stirred for 5 minutes then filtered and concentrated. The pale yellow oil was taken up in dichloromethane, dried with MgSO_4 filtered and concentrated to give a pale yellow oil (830 mg, 88% yield). ^1H NMR (400 MHz, CDCl_3) ppm 7.04 (s, 1 H), 6.62 (s, 1 H), 4.50 (dd, $J=4.86$, 7.26 Hz, 1 H), 4.31 (dd, $J=4.50$, 7.47 Hz, 1 H), 4.11 (s, 1 H), 3.58 (m, 14 H), 3.32 (m, 1 H), 3.14 (dd, $J=7.20$, 11.69 Hz, 1 H), 2.86 (m, 2 H), 2.74 (d, $J=12.72$ Hz, 1 H), 2.47 (m, 1 H), 2.20 (t, $J=7.05$ Hz, 1 H), 1.72 (m, 8 H), 1.43 (td, $J=8.17$, 16.33 Hz, 2 H).¹⁶⁵ ES-MS: calc 446.3, found MH^+ 447.3.

3.2.4 – Monolayer Preparation and Functionalization

Cleaning and Hydrogen termination. Single sided silicon crystals were used once and discarded; ATR elements were cleaned and reused. All crystals were cleaned with

piranha solution (3:1 H₂SO₄, 96% : H₂O₂, 30%) at 120 °C for 30 minutes, then rinsed with Milli-Q water. (*Warning: Piranha solutions should be handled with care and kept isolated from organic materials*). ATR elements were then cleaned with 4:1:1 Milli-Q water : NH₄OH, 30% : H₂O₂, 30% at 80 °C for 15 minutes, rinsed with Milli-Q water and heated in 4:1:1 Milli-Q water : HCl, 30% : H₂O₂, 30% at 80 °C for 15 minutes then rinsed with Milli-Q water. Samples were hydrogen terminated by etching in degassed ammonium fluoride for 15 minutes followed by a brief rinse in degassed Milli-Q water.

Standard Monolayer Formation. Distilled alkene was transferred to a Pyrex Schenk tube and degassed with argon for at least 10 minutes. The silicon samples were added and degassed a further 5 minutes then placed in a Rayonette photoreactor for 1.5 – 3 hours. After the reaction the samples were removed and washed with either continuously refluxing 1,1,1-trichloroethane in a soxhlet or with copious volumes of chloroform and ethanol at room temperature.

Octadecyl Monolayer Formation. Freshly distilled octadecene was transferred to a Pyrex Schenk tube and degassed with argon for at least 10 minutes. The silicon samples were added and degassed a further 5 minutes then heated at 110°C for 18 hours. After the reaction the samples were cooled to room temperature, removed and washed with either continuously refluxing 1,1,1-trichloroethane in a soxhlet or with copious volumes of chloroform and ethanol at room temperature.

NHS Ester Formation. EDC (10mg / mL, 52mM) and NHS (6mg/mL, 52mM) were dissolved in Milli-Q water and the silicon samples immersed for 1 hour. The samples were removed rinsed with Milli-Q water, and ethanol then blown dry with a stream of nitrogen.

Amide Formation.

Standard aqueous conditions. The amine (20mM) is dissolved in buffer (PBS at pH 7.4 or 0.2M carbonate at pH 8.0) and either the NHS activated sample is immersed for 3 hours or a 1-2 μ L drop is placed on the silicon surface for 1 hour to allow the probe molecule to react followed by immersion in a 20mM solution of the OEG amine for 3 hours. The sample was washed with water, ethanol and blown dry under a stream of nitrogen.

Standard organic conditions. The amine (20mM) is dissolved in dimethylformamide and the sample is immersed for 1 hour. The sample was washed with dimethylformamide, water, ethanol and blown dry under a stream of nitrogen.

NTA (2.62mg) was dissolved in dimethylsulfoxide (2mL) and added to a solution of diisopropylethylamine (50 μ L) in dimethylformamide (3mL), providing a final concentration of 2mM NTA and 50mM diisopropylethylamine. The NHS activated sample was immersed for 1 hour then washed with dimethylsulfoxide, water, ethanol and blown dry under a stream of nitrogen.

Ni²⁺ complexation. NTA terminated surface was placed in 20mM NaOH for 1 minute rinsed briefly with Milli-Q water and immersed in 50mM NiCl₂ for 1 hour. The sample was removed and rinsed thoroughly with Milli-Q water, ethanol and then dried under a stream of nitrogen.

Grignard Reaction. Ester terminated surface was placed inside a clean oven dried test tube and sealed under an argon atmosphere. The Grignard reagent was introduced by syringe and slowly bubbled with argon for 90 minutes at room temperature. At this time

the reaction was quenched by successive dilutions with THF and finally acetic acid. The sample was removed washed with Milli-Q water, ethanol and blown dry under a stream of nitrogen.

3.2.5 – Protein Attachment

Protein solutions (unless otherwise specified, 0.6 or 0.1mg/mL for p19 and streptavidin respectively) in PBS were exposed to the silicon surface for 1 hour in a humid chamber. The protein was rinsed off and the samples were shaken in a vial containing 0.1% Tween-20-PBS (3×1min) rinsed with Milli-Q water and blown dry under a stream of nitrogen.

3.3 – Results and Discussion

3.3.1 – p19

Affinity tags are short peptide sequences attached to proteins to facilitate their selective isolation from cellular contents and hexahistidine-NTA is one of the most common.¹⁵⁰ A further advantage of affinity tags is that they allow the protein to be captured in a defined orientation, ensuring the binding pocket is exposed. For these reasons the NTA – hexahistidine interaction is widely used for purification and quantification via fluorescence and SPR.^{2,146} The synthetic route is shown in Scheme 1, an acid terminated monolayer is formed by irradiation of the Si-H surface in UDA. The acid is then activated with EDC – NHS and the NHS ester reacted with NTA amine.

Scheme 3.5 – Preparation of NTA Modified Silicon Surface

Figure 3.3 shows the FTIR spectra of this synthetic sequence. The diagnostic features of these spectra are in the carbonyl region. Starting with UDA the acid stretch is observed at 1714 cm^{-1} . After reaction with EDC-NHS it is replaced by peaks at 1743 cm^{-1} for the activated ester carbonyl and 1816 and 1784 cm^{-1} for the imide. The sample was

Figure 3.3 – FTIR Absorbance Spectra of (A) UDA, (B) NHS and (C) NTA Surfaces

then placed in a PBS solution of NTA for 12 hours and the ester peaks have decreased leaving a broad acid band at 1714cm^{-1} and amide bands at 1650 and 1556 cm^{-1} . It was initially expected that the NTA surface containing three acid groups would result in a substantially larger acid stretch than UDA; however, hydrogen bonding between the neighbouring acid groups can inhomogeneously broaden the peak as it is clearly not as sharp.

Estimated thickness and contact angle data for these surfaces is shown in Table 3.1 and correspond with previously reported data on UDA and NHS modified surfaces.¹⁶⁶ The thickness and contact angle are lower than what was observed for decyl modified surfaces (Table 2.2). While the difference in thickness is not significantly lower numerically, the UDA monolayer (versus decyl) is one carbon longer plus an acid functional group suggesting a lower density of molecules on the surface. The contact angle of the NTA surface is lower than both the UDA and NHS surfaces. This may be due to the increased density of acid groups at the surface or if the NTA exists as a zwitterion, since carboxylates are known to possess a lower contact angle than acid terminated silicon monolayers.⁶⁰ Significant changes were observed after exposure to the protein p19. It was applied to the UDA surface as a control, and after 30 minutes exposure washed off using 1% sodium dodecylsulfate in PBS, followed by Milli-Q water to remove any surfactant and salts, resulting in an increase in thickness of approximately 6 Å. After exposing Ni^{2+} -NTA surfaces for 30 minutes and 2 hours the observed increases were approximately 20 Å and 30 Å respectively. This suggests that the histidine tagged protein is indeed being captured by the Ni^{2+} -NTA modified silicon surface.

Table 3.1 – Thickness and Wettability data for NTA and p19 Surfaces

Surface Termination	Thickness (Å)	Contact Angle (°)
UDA	11.9 ± 0.9	63 ± 1
NHS	15 ± 1	62 ± 3
NTA	16 ± 1	53 ± 1
Ni-NTA	17 ± 1	52 ± 1
UDA p19 30min	18 ± 2	46 ± 2
Ni-NTA p19 30min	37 ± 1	<30
NTA p19 2h	48 ± 2	<30

The SPV of these surfaces is shown in Figure 3.4. The UDA surface displays a SPV of -150mV; acid terminated surfaces typically show a higher SPV than decyl terminated layers. The SPV increases after the surface is activated and reacted with NTA, this shift is consistent with oxidation due to immersion in aqueous solvent during modification. On exposure to NiCl_2 the SPV becomes very negative which indicates an accumulation of negative charge at the interface (on n-type silicon). An explanation is obtained by looking at the structure of the complex (Figure 3.2) which is expected to display a net negative charge. It was at first surprising to observe what appeared to be an unscreened charge however this notion is supported by the work of McCarty¹⁶⁷ and coworkers who studied the electrostatic charging of silane modified silica beads and demonstrated the cause was due to loss of the counterion. Further evidence for counterion loss or exchange with a proton can be gained from XPS spectra of DNA modified silicon surfaces in which no counterion is observed even though there is a poly-ionic phosphate backbone.^{93,168} After exposure to p19 the UDA surface is relatively unchanged displaying a slightly more negative SPV which could be attributed to oxidation. In contrast the Ni^{2+} -NTA surfaces show a dramatic change. After 30 minutes exposure the SPV has shifted towards the positive by 400mV and after 2 hours by 500mV; further exposure was not investigated. This indicates a significant increase in

positive charge at the interface, and was attributed to the protein for three reasons.

Firstly, changes in SPV are consistent with estimated thickness, secondly, the change is in the opposite direction to oxidation so it is unlikely an artifact and thirdly, the protein is positively charged at neutral pH.

Figure 3.4 – SPV of surfaces before and after exposure to p19

The final piece of data collected was XPS to validate the surface chemistry; surprisingly, it indicated that nickel was not present above the detection limit. Additionally, the nitrogen peak is not observed above the noise in the survey spectrum. These data indicate nickel and NTA incorporation occurred below the detection limit of the XPS experiment or not at all. However, since p19 bound specifically to Ni-NTA the reaction sequence altered the surfaces, two possible explanations are the presence of residual NHS esters groups leading to covalent binding of the protein or low levels of Ni-NTA.

Figure 3.5 – Survey XPS of Ni-NTA surface with inset of HR nickel region

Additional control reactions with UDA showed that the large negative SPV associated with nickel complexation could be replicated by exposing to basic solution

Figure 3.6 – SPV of UDA surface exposed to base

forming a carboxylate. Immersing the sample in 2% trifluoroacetic acid : chloroform protonated the acid and shifted the photovoltage back (Figure 3.6). The same sample could be cycled multiple times. Despite this setback it was clear that measurable changes in SPV were concomitant with protein being present on the surface. The challenge was to improve the chemistry to ensure a Ni-NTA moiety was unequivocally present to capture p19.

The first success came from a reaction in a saturated solution of NTA in dimethylformamide with excess triethylamine; the saturated solution was prepared by heating, allowing the solution to cool and filtering through a syringe filter to remove insoluble NTA. NTA was found to be soluble in DMSO but the reaction rate was greatly reduced, a mixture of 3:2 DMF : DMSO was found to provide acceptable solubility and reaction rate, 2mM NTA and 3 hour reaction time. The FTIR spectra are shown in Figure 3.7. The peaks at 2362 and 2336 cm^{-1} are due to carbon dioxide and the levels vary due to the efficiency of purging prior to collecting the spectra. In this optimized synthesis the NHS surface again displays characteristic imide peaks at 1814 and 1780 cm^{-1} and ester stretch at 1741 cm^{-1} . Also noted are the bands at 1546 and 1648 cm^{-1} , these represent contamination likely from the urea byproduct of EDC. Extensive rinsing with water and DMF did not completely eliminate these peaks. This is consistent with the findings of Moraillon *et al*¹⁶⁹, who recently reported a reduction in surface contamination using lower concentrations of EDC (10mM) and NHS (1mM), even under these optimized conditions a peak is visible at 1550 cm^{-1} attributed to CN-H presumably due to the urea of EDC. After reaction with NTA in the DMSO : DMF solvent mixture, significantly

Figure 3.7 – FTIR Spectra of (A) NHS, (B) NTA, and (C) Ni-NTA surfaces

larger changes (than using the PBS conditions) are observed at 1708, 1637 and 1548 cm^{-1} and can be assigned to carboxylic acid, amide I ($\text{C}=\text{O}$) and overlapping deprotonated carboxylic acid and amide II ($\text{C}=\text{N}$ coupled to $\text{N}-\text{H}$) respectively.¹⁷⁰ The possibility that the carboxylic acid stretch at 1708 cm^{-1} results from hydrolyzed NHS cannot be excluded. NTA has been studied by FTIR on gold surfaces in aqueous solution, in these cases the amide I and II are observed at 1658 and 1572 cm^{-1} respectively with concomitant loss of the NHS signals.^{114,115} In another study NTA was coupled to silanized germanium surfaces and the major absorbance at 1625 cm^{-1} was assigned to carboxylate and amide I.¹⁷¹ The apparent discrepancy in peak assignment is likely a combination of differences in experimental conditions (solution versus dry measurements) and more significantly the broadness of the peak, in Figure 3.7 the full width at half-maximum is 60 cm^{-1} . Once the surface NTA complexes nickel (Figure 3.7C) the carboxylic acid peak at 1708 cm^{-1} is diminished and the peak at 1641 cm^{-1}

grows; a similar effect was observed on both gold and silane monolayers and is attributed to carboxylate groups complexing nickel ions.¹¹⁴⁻¹¹⁶ The difference spectra shown in Figure 3.8 confirm specific changes associated with exposure to Ni^{2+} ions. Spectrum (A) shows the NTA surface after exposure to 5mM NaOH for 5 minutes, referenced to the NTA surface. Spectrum (B) shows the same surface after exposure to 20 mM NiCl_2 for 60 minutes also referenced to the NTA surface. Exposure to NaOH does not induce any significant change; there is a slight minimum observed at 1729 cm^{-1} which could be a combination of hydrolysis of some unreacted NHS groups (1743 cm^{-1}) and deprotonation of acid groups (1715 cm^{-1}). After exposure to NiCl_2 there is a negative peak at 1718 cm^{-1} and a positive peak at 1596 cm^{-1} , these confirm a loss of carboxylic acid and increase in carboxylate chelating Ni^{2+} ; this result shows good agreement with literature values for the complex formation, 1581 cm^{-1} and 1594 cm^{-1} .^{115,117,118}

The estimated thickness of NTA surfaces prepared using these conditions was slightly larger (compare Tables 3.1 and 3.2) at $19 \pm 1\text{ \AA}$ versus $16 \pm 1\text{ \AA}$ than when reacted in a PBS solution for 16 hours. The increase over the NHS surface was now $4\text{ \AA}$ compared with $1\text{ \AA}$ consistent with a reaction occurring. It is possible that the increase observed in PBS is due to growth of silicon dioxide in the substrate. No change in thickness was observed on complexing nickel ions indicating the changes observed in the FTIR spectra were not caused by precipitation of large amounts of nickel on the surface. The contact angle of NTA surfaces prepared in organic solvent is slightly more

Figure 3.8 – FTIR Difference Spectra Confirming Ni^{2+} complexation

hydrophilic $50 \pm 1^\circ$ versus $53 \pm 1^\circ$ than that prepared in PBS and is also unchanged after complexing nickel ions. Taken together, the FTIR, ellipsometric and contact angle measurements provide greater confidence that NTA surfaces were indeed prepared and that they do capture nickel; however the final confirmation came from XPS.

Table 3.2 – Thickness and Contact Angle using DMSO : DMF Route

Surface Termination	Thickness (Å)	Contact Angle ($^\circ$)
UDA	11.9 ± 0.9	63 ± 1
NHS	15 ± 1	62 ± 3
NTA	19 ± 1	50 ± 1
Ni-NTA	19 ± 1	51 ± 1

A significant peak due to Ni^{2+} was observed in the survey scan and confirmed in a HR scan of the region (Figure 3.9). The peak shape was consistent with literature data, consisting of a main Ni^{2+} peak with a binding energy of around 857 eV and adjacent satellite peaks approximately 5 eV higher in binding energy.¹⁷² Two XPS spectra of Ni-NTA on gold surfaces were found in the literature^{173,174}, which are consistent with the

spectra collected for this work however a detailed analysis of the peak is not provided. The binding energy was referenced to the unshifted carbon peak at 284.8 eV and fitting was accomplished using CasaXPS software. The main Ni^{2+} 2p_{3/2} was fit with two components (856.0 and 857.4 eV), the peaks were identified as $\text{Ni}(\text{OH})_2$ and Ni-NTA respectively, based on comparison with the National Institute of Science and Technology XPS Database.¹⁵⁷ Reported binding energies for $\text{Ni}(\text{OH})_2$, range from 855.3 – 856.6 eV; therefore the component at 856.0 eV is attributed to $\text{Ni}(\text{OH})_2$. The source of $\text{Ni}(\text{OH})_2$ is likely precipitation after exposure of the sample to 5mM NaOH, due to the very small $K_{\text{sp}} = 5.5 \times 10^{-16}$.¹⁷⁵ The database does not contain an entry for Ni-NTA or the ethylenediamine tetraacetic acid chelate, however nickel (II) acetate has a reported

Figure 3.9 – HR-XPS of Ni Region

binding energy of 856.6 eV, and the observed peak has a binding energy of 857.4 eV. The NTA complex is slightly different than nickel (II) acetate and based on the 1.3 eV range reported for $\text{Ni}(\text{OH})_2$ exact agreement is not expected. The other possible source for the peak is NiCl_2 which has a reported binding energy of 856.3 – 856.7 eV, while this

cannot be unequivocally ruled out no chloride peak was observed in the XPS survey spectrum in this case and if NiCl_2 had a propensity to bind to the surface it would have been observed on the monolayers previously described.

Table 3.3 presents the peak positions and areas of the components in the HR-XPS of the carbon, nitrogen and silicon regions (spectra for C, N, and Si and survey are located in the appendix). The number of ML equivalents were calculated based on a method reported by Chidsey and coworkers.⁴¹ The surface coverage is obtained by first determining the number of silicon monolayers the total signal of the silicon region represents (Equation 3.1). The monolayers of silicon represented by the peak area is given by I_{Si} , d_{Si} is the vertical distance between silicon atoms (1.36 Å), λ_{Si} is the attenuation of silicon photoelectrons by silicon (16 Å), θ is the take-off angle (45°), t_{c} is the thickness of the hydrocarbon layer (Table 3.2) and λ_{SiC} is the attenuation of silicon photoelectrons by the hydrocarbon layer (41.5 Å). In the work by Chidsey the coverage is obtained by integration versus analytically in this case. Using the measured thickness of 19 Å for the Ni-NTA and NTA surfaces the number of monolayers of silicon contributing to the measured peak areas is 4.6.

$$I_{\text{Si}} := \sum_{j=1}^{100} \exp \left[- (j-1) \cdot \frac{d_{\text{Si}}}{\lambda_{\text{Si}} \cdot \sin(\theta)} \right] \cdot \left(\exp \left(\frac{-t_{\text{c}}}{\lambda_{\text{SiC}} \cdot \sin(\theta)} \right) \right)$$

Equation 3.1 – Monolayer Equivalents of Silicon

The ratio of nitrogen and nickel to silicon could then be calculated taking into account the sensitivity factors of the elements and is presented in Table 3.3.¹⁷⁶ There are 1.07 monolayers of $\text{Ni}(\text{OH})_2$, 0.28 monolayers of Ni-NTA and 0.43 monolayers of nitrogen and two nitrogen atoms per NTA molecule therefore 0.22 monolayers of NTA.

The modeled monolayer coverage of all nickel species is consistent with no observable change in estimated thickness and the ratio of Ni-NTA to nitrogen. The coverage of NTA based on the nitrogen signal (0.22 monolayers) is less than the estimated coverage of alkyl chains 0.3 – 0.4 monolayers^{33,177} this could be due to an error in the model, underestimating the attenuation of the silicon signal through the monolayer or the reaction of the NHS surface with the NTA amine may not be complete. While this may not provide a quantitative measure of monolayer density the ratio of nickel to nitrogen is meaningful. There was slightly more nickel complexed to NTA than nitrogen (0.28 versus 0.22 monolayers); however, it is expected that the nitrogen signal will be slightly underestimated because one of the two nitrogen atoms is attenuated by six carbon atoms. There could be additional error in the determination of peak areas as the Ni-NTA peak is under the larger Ni(OH)₂ signal and the signal to noise ratio of the nitrogen spectrum is lower than for the nickel spectra. Considering these potential errors the agreement in coverage of nickel and nitrogen gives further confidence that the Ni-NTA surface was successfully prepared.

Table 3.3 – Peak Data from HR-XPS of Ni-NTA Surface

	Position (eV)	Area	FWHM	ML equivalents (vs. Si)
Ni(OH) ₂	856.0	14787	1.89	1.07
Ni-NTA	857.4	3912	1.78	0.28
N	400.1	829	1.70	0.43
Unshifted Si	99.5	2761	0.46	4.6
Unshifted Si	99.4	2769	0.69	-
SiO ₂	102.4	189	1.04	-

Table 3.4 – Empirical XPS Sensitivity Factor

	C-1s	Si-2p	N-1s	Ni-2p3/2	O-1s
Sensitivity Factor ¹⁷⁶	0.25	0.27	0.42	3	0.66

With the chemistry validated the binding of p19 to the Ni-NTA surface was investigated; an EG3OH surface was used as a control. The estimated thickness of the EG3OH and Ni-NTA surfaces before and after exposure to 0.6 mg/mL p19 for one hour washing in 0.1% Tween-20 : PBS and Milli-Q water. Approximately 8 Å of protein accumulated on the EG3OH surface, versus 30 Å on the Ni-NTA surface. These data are similar to that observed on UDA and the unsuccessful Ni-NTA surfaces (7 and 31 Å increase respectively). This indicates p19 does not have a significant propensity to non-

Table 3.5 – Thickness of EG3OH and Ni-NTA Exposed to p19

	EG3OH	EG3OH-p19	Ni-NTA	Ni-NTA p19
Thickness (Å)	19±1	27±3	19±1	49±2

Figure 3.10 – SPV of EG3OH and Ni-NTA Exposed to p19

specifically bind, and it further suggests that either residual NHS groups remained on the original Ni-NTA surface or there was a low (below detection limit) concentration of Ni-NTA. The SPV of the EG3OH surface was -83 mV and after exposure to p19 decreased to -172 mV, suggesting surface oxidation is the dominant process whereas the Ni-NTA

surface displayed a SPV of -384 mV which increased to 13 mV after exposure to the protein confirming an accumulation of positive charge at the interface (Figure 3.10).

Fluorescence imaging provided a measure of protein binding and whether it maintained an active conformation. Three samples were prepared in these experiments, fluorescently labeled siRNA (the target of p19) was incubated with the protein in solution and then exposed to the Ni-NTA surface (Figure 3.11 A), p19 was bound to the Ni-NTA surface and then exposed to siRNA (Figure 3.11 B) and in the third sample an EG3OH surface was exposed to siRNA (Figure 3.11 C). Pre-complexing the siRNA and p19 in solution then binding to the surface is a positive control, and results in a homogeneous fluorescence signal. In the second sample the entire Ni-NTA surface is covered in p19 then washed and exposed to siRNA, the surface is kept wet through the process to ensure drying does not denature the protein. The result is a lower fluorescence signal which is inhomogeneous and not significantly different from the EG3OH surface exposed to siRNA. An unambiguous conclusion cannot be drawn from these data since it cannot be determined whether the siRNA in Figure 3.11 B is non-specifically bound or captured by the p19.

Figure 3.11 - Fluorescence Image of Ni-NTA exposed to siRNA

3.3.2 – Biotin – Streptavidin

The work with p19 to this point raised critical issues which could be addressed in the following set of experiments, using biotin – Streptavidin as the recognition couple. The probe molecule (biotin) is uncharged; thereby removing one source of complexity. The probe molecules were spotted and then the surface was backfilled with EG3OH, allowing for non-specific binding to be observed on the same sample using SKP and fluorescence. These correlated measurements should improve the ability to draw firm conclusions on the potential for direct electrical detection of biomolecules.

In order to make use of the NHS activated silicon surface, biotin was coupled to compound **5** providing a terminal amine for reaction with the surface and installing a triethylene glycol linker. The hydrophilic linker should enable the biotin probe to extend slightly beyond the EG3OH background molecules and impart substantial aqueous solubility to biotin. To validate the chemistry, NHS activated silicon wafers and ATR elements were prepared and reacted with 20mM BioNH₂ and EG3OH in 0.2M, pH 8.0 carbonate buffer and the reaction was followed by IR until the NHS signal was gone (Figure 3.12). The rate of hydrolysis in buffer was also tracked and found to be pH dependant. At pH 8.0 approximately 45% of the ester peak area was gone after 1h, 75%

Figure 3.12 - FTIR Spectra of NHS Exposed to Buffer (a) and Amine (b)

at three hours and it was completely gone after 18 hours. In contrast, in pH 7.0 PBS there was a significant amount of NHS remaining after 18 hours. In the presence of amine (EG3OH or BioNH₂) the NHS ester peak is reduced by >85% in 1 hour and not visible

after 3 hours; therefore at three hours the surface will contain approximately 2 : 1 amide : carboxylic acid.

The estimated thickness of the EG3OH surface as reported in Table 3.5 is 19 ± 1 Å, and BioNH₂ is 22 ± 1 Å confirming that the BioNH₂ is slightly longer than the EG3OH background. The EG3OH surface however is more hydrophobic than the BioNH₂ surface the contact angle is $45 \pm 2^\circ$ versus $52 \pm 2^\circ$. After exposure to 0.1 mg/mL streptavidin for 60 minutes and washing with 0.1 % Tween-20 : PBS the thickness of the EG3OH layer did not substantially change (21 ± 1 Å) whereas a significant increase was observed on the biotin samples (45 ± 2 Å), the contact angle of the biotin surface also decreased substantially ($<30^\circ$). Based on the dimensions of streptavidin ($40 \times 50 \times 50$ Å)¹⁵⁷ the 24 Å increase represents nearly 50 % of a closed packed monolayer, assuming it binds via the smaller 40×50 Å side. The thickness estimates use a one layer model and refractive index of 1.44 and assumes the index of the protein layer and organic are the same which is reasonable considering published indices of protein. In PBS solution, avidin multilayers were found by spectral ellipsometry to have a refractive index of 1.38 compared with a dry sample 1.45; films of four other proteins (fibrinogen, γ -immunoglobulin, albumin and lysozyme) were found to display refractive indices ranging from 1.36 – 1.55 depending on protein and buffer.¹¹³⁻¹¹⁵

Table 3.6 – Properties of EG3OH and BioNH₂ Surfaces on Exposure to Streptavidin

	EG3OH	EG3OH-SA	BioNH ₂	BioNH ₂ -SA
Thickness (Å)	19 ± 1	21 ± 2	22 ± 1	45 ± 2
Contact Angle ($^\circ$)	45 ± 2	45 ± 2	52 ± 2	<30

In order to prepare a patterned surface for SKP and fluorescence measurements, the relative hydrophobicity of the NHS surface was exploited. Using a micropipette 1-2

μL drops of the 20 mM BioNH₂ solution were placed on the NHS activated silicon wafer and covered with an inverted crystallizing dish. In order to ensure the water did not evaporate, a vial of Milli-Q water was also present under the crystallizing dish to maintain the humidity. After 60 minutes the BioNH₂ was washed off and the surface covered in 20 mM EG3OH in either DMF or pH 8.0 carbonate. DMF was preferred

Figure 3.13 – SKP Images for Streptavidin Experiments

because it would wet the surface completely (requiring less EG3OH), the reaction was faster (1 hour versus 3 hours) and hydrolysis of NHS was not an issue. Based on the

FTIR spectra after 1 hour exposure to BioNH₂ in pH 8.0 carbonate, approximately 60 % of the NHS groups will have reacted, leaving the remaining 40 % EG3OH terminated which is expected to improve the ratio of specific to non-specific binding.⁶³ The SKP image of the surface indicates a higher surface potential in regions containing BioNH₂ than the EG3OH background (Figure 3.13). In this image the surface potential in the dark is above the scan in the light, giving a negative photovoltage which is consistent with depletion on n-type silicon. Subtracting the light from the dark measurement does not show contrast indicating that while the surface potential of BioNH₂ and EG3OH regions is different there is no difference in band bending between the regions. After exposure to streptavidin for 1 hour the scan is remarkably different in the region containing biotin. The surface potential of the EG3OH region is essentially unchanged whereas the BioNH₂ region shifts by approximately 300 mV. The SPV map shows significant contrast with a peak SPV of -300 mV.

The Streptavidin used in these experiments was fluorescently labeled with Cy3 dye. A fluorescent image of this sample after the SKP measurements were complete shows a similar pattern to the electrical measurements. The fluorescent image (Figure 3.14) also confirms the ellipsometry measurements which indicated the protein was specific to biotin presenting surfaces.

Figure 3.14 – Fluorescent Image after Exposure to Cy3 Labeled Streptavidin

The correlation of three measurement techniques (SKP, ellipsometry and fluorescence) give confidence that the Streptavidin was captured by the biotinylated silicon surface. The electrical measurements indicate localizing Streptavidin on the surface causes an increase in negative charge at the interface; there are at least three plausible interpretations of the data. The first possibility is that the protein is negatively charged at neutral pH and retains this negative charge after rinsing in Milli-Q water and drying, this interpretation would be consistent with solution measurements.^{63,105} Secondly, the protein may induce changes in the monolayer, such as deprotonating acid groups which form as NHS is hydrolyzed during attachment of BioNH₂. A third possibility is that the protein induces oxidation of the silicon surface. It should be noted that the EG3OH backfill for the data presented above was carried out in pH 8.0 carbonate buffer; therefore it is expected that there should not be a significant difference in the concentration of carboxylic acid groups inside and outside the BioNH₂ spots. In this scenario the hydrolyzed carboxylic acid groups can be considered a “label”; the electrical signal measured is not from the target itself but from its effect on a pH sensitive group in the monolayer. Of these three possibilities the most straightforward to address is the stability of the silicon – monolayer interface because oxidation of the substrate can be monitored by XPS.

In order to assess the stability of the silicon – monolayer interface X-ray photoelectron spectra were collected of the UDA, NHS, BioNH₂ as prepared and stored in water for 18 hours. Changes in the relative amount of SiO₂ indicate an unprotected surface. The number of monolayers of silicon represented by the observed area was calculated using Equation 3.1. The estimated thicknesses of the UDA surface was $11.9 \pm$

0.9 Å and results in 5.88 monolayers of silicon being apparent, 5.29 monolayers are calculated to be visible for the 15 ± 1 Å NHS terminated monolayer, for the BioNH₂ the apparent thickness of 22 ± 1 Å gives 4.17 monolayers of silicon. At submonolayer coverage the SiO₂ signal is only attenuated by the hydrocarbon monolayer, assuming it grows in at the interface, and it can be calculated using the constant component of Equation 3.1 related to the attenuation of silicon by a hydrocarbon layer. The signal remaining after attenuation is calculated to be 0.667 for UDA, 0.60 for NHS and 0.472 for BioNH₂; Table 3.7 shows both the observed area of the SiO₂ signal and the area

$$\% \text{ SiO}_2 = \frac{(\text{SiO}_2 \text{ Corrected Peak Area})}{(\text{Total Peak Area Si region / ML Silicon})} \times 100$$

Equation 3.2 - Ratio of Si to SiO₂

Table 3.7 - XPS Stability Study

Sample	Peak	Position (eV)	Area (corrected)	FWHM	ML equivalents
UDA	Unshifted Si	99.1 / 99.7	8668	0.48 / 0.57	5.88
	SiO ₂	102.8	61 (91)	1.06	0.06
NHS	Unshifted Si	99.2 / 99.8	7191	0.48 / 0.58	5.29
	SiO ₂	102.5	107 (178)	1.50	0.13
BioNH ₂	Unshifted Si	98.9 / 99.5	6051	0.48 / 0.58	4.17
	SiO ₂	102.4	114 (239)	1.45	0.16
BioNH ₂ -H ₂ O	Unshifted Si	98.9 / 99.6	6397	0.48 / 0.57	4.17
	SiO ₂	102.5	166 (352)	1.42	0.22

corrected for attenuation. The ratio of Si : SiO₂ at the interface can be expressed using Equation 3.2. The total peak area of the Si region (including SiO₂) is divided by the number of monolayers of silicon apparent giving a peak area per monolayer of silicon which is then divided into the corrected area of the SiO₂ peak. The correction for monolayer attenuation of total peak area of the silicon region (denominator Equation 3.2)

is taken into account by dividing by the number of monolayers of silicon. After the photochemical reaction to form the UDA surface approximately 6 % of the surface silicon atoms are SiO_2 . It increases quite rapidly, after 1 h in the aqueous EDC / NHS solution there is 13 % oxide and 16 % after a further 3 hours forming the BioNH_2 surface. The growth rate decreases as the oxide peak represents 22 % of the surface silicon atoms after 18 hours in Milli-Q water.

This stability study indicates that the silicon interface is not only unstable on storage in water but carrying out chemical reactions in aqueous solutions induces substantial growth of silicon oxide. This calculation represents a lower bound of oxidation because the lower oxides of silicon are likely buried in the main peak. It was therefore a goal to identify a monolayer which can inhibit growth of oxide during exposure to biologically relevant buffers.

3.3.3 – Stability

The most straightforward method to increase the thickness of the hydrocarbon monolayer is to increase the chain length from the standard ten carbons to eighteen. The octadecyl monolayer was prepared by irradiating a silicon sample immersed in a freshly distilled alkene solution for ninety minutes. The FTIR spectrum, displays the expected C-H stretching bands and no other distinguishing features are associated with the monolayer. The integrated peak area of the C-H stretching region is 1.11 AU compared with 0.60 AU for decene which based on linear scaling from ten to eighteen carbons is consistent. These surfaces were found to be stable to room temperature washings with ethanol and TCE however when washed in a TCE Soxhlet the majority of the monolayer washed off. Self assembled monolayers of thiols on gold are well known to pack more

densely and with higher order as the chain length is increased due to greater van der Waals forces.¹⁰⁹ It was hypothesized that the intramolecular interactions between

Octadecene: A) Photochemical reaction 90 mins, DCM rinse, sonicate 2 minutes, B) sample A after TCE Soxhlet 15 minutes, C) 110oC 16 hours, TCE Soxhlet 15 minutes, D) sample C after 2% HF 2 minutes and TCE Soxhlet 15 minutes.

Figure 3.15 – FTIR Spectra of Octadecene Monolayers

octadecene molecules was sufficient to inhibit transport of impurities to the surface thereby slowing the reaction. A reason that impurities were invoked is that if the reaction was exclusively initiated by photochemical interactions with the silicon (e.g. excitons) after washing only 50 % of the layer should have been removed, because 50 % of the chains should have presented an alkene to the surface. In fact <20 % of the monolayer remained after washing (0.16 AU) (Figure 3.15).

Taking into account what was learned from the study of 1-decene reactivity in PTFE and glass at 150 °C, the reaction temperature was lowered to 110 °C and the reaction time increased to 18 hours. The temperature was reduced from 150 °C in an effort to decrease the degradation of the hydrocarbon as evidenced by the lower contact angle obtained from reactions carried out in glass and oxidized hydrocarbon present in the XPS. It was found that at 80 °C the Si-H surface was stable in degassed toluene over night but that the reaction with 1-octadecene was incomplete. The temperature was increased and 110 °C was found to provide a complete reaction after 18 hours with an integrated peak area of 1.1 AU the same observed for the physically absorbed monolayer and consistent with scaling the number of groups covalently attached to the surface from decene. These surfaces were stable to washing in the TCE Soxhlet and immersion in 2% HF followed by a second 15 minute wash in the TCE Soxhlet.

The peak position of the asymmetric methylene stretch was 2020 cm^{-1} for the octadecyl surface the same as for 1-decene and it was unchanged between a physically adsorbed or covalently bound octadecyl layer. However after the photochemical reaction was washed in refluxing TCE the remaining covalently bound material showed a shift to 2023 cm^{-1} , after the physically adsorbed material was removed. The thickness of the optimized layer was $21 \pm 1 \text{ \AA}$ versus $11.4 \pm 0.5 \text{ \AA}$ for a decyl layer, within error of the predicted value based on linear scaling. The contact angle was found to be $112 \pm 1^\circ$, higher than the $108 \pm 1^\circ$ obtained for decyl layers. These data indicate the chemistry was optimized to produce a thicker hydrocarbon layer which is also more hydrophobic than decyl layers. The second approach to increasing the hydrocarbon content of the

monolayer was based on branching an ester terminated surface using the Grignard reaction⁵⁸.

The Grignard reaction was chosen because it will directly functionalize the Si-H surface³⁹ filling in any large holes in the monolayer, and reacting with an ester at the end of a flexible chain divorces the monolayer density from the silicon lattice spacing allowing for potentially higher packing densities. The FTIR spectra after photochemical reaction in freshly distilled UDE for 90 minutes is shown in Figure 3.16, the characteristic features are peaks associated with C-H stretching notably the asymmetric methyl stretch at 2982 cm^{-1} , asymmetric methylene stretch at 2921 cm^{-1} and carbonyl stretch at 1743 cm^{-1} . After reaction with pentyl magnesium bromide at room temperature for 90 minutes, an acetic acid quench and thorough washing the spectra obtained shows an increase in intensity in the C-H stretching region and a decrease in the carbonyl region. The changes observed are a shift in the asymmetric methyl stretch, now at 2959 cm^{-1} , the same observed on octadecene and decene, a decrease in the ester carbonyl stretch at 1743 cm^{-1} and a new peak at 1717 cm^{-1} , attributed to ketone. The yield of this reaction can be calculated using changes in C-H stretching intensity, because partially reacted chains are present as evidenced by the ketone stretch, measuring a loss of the ester peak will give an overestimate. The C-H peak area increases from 0.59 to 0.88 AU, again assuming linear scaling from 12 C-H groups in UDE to 20 for the branched surface gives a yield of $> 88\%$. With the initial UDE surface in the range of $45 - 55\%$ of a closed packed hydrocarbon monolayer this means after the Grignard reaction it is $> 85\%$ of a closed packed layer which is a significant improvement. The estimated thickness increases from $13 \pm 1\text{ \AA}$ to $17 \pm 1\text{ \AA}$ which is consistent for an increase of three carbons

in total length, but low if the measurement represents total hydrocarbon on the surface.

As expected the surface becomes substantially more hydrophobic and is within error of the octadecene surface at $111 \pm 1^\circ$.

Figure 3.16 - FTIR Spectra of UDE and C-5 Branched Surfaces

Table 3.8 - Properties of Octadecene and C-5 Grig Surfaces

Sample	$-\text{CH}_3 / -\text{CH}_2-$ (cm^{-1})	C-H area (AU)	Thickness ($\text{\AA}$)	Contact Angle ($^\circ$)
Decene	2958 / 2920	0.60	11.4 ± 0.5	108 ± 1
Octadecene	2958 / 2920	1.10	21 ± 1	112 ± 1
UDE	2982 / 2921	0.58	13 ± 1	83 ± 2
C-5 Grig	2959 / 2921	0.88	17 ± 1	111 ± 1

The electrical properties of the surfaces were studied by measuring the SPV after preparation and then immersing in water for 16 hours. The as prepared SPV's were around -80 mV, as reported for decene in the previous chapter. The SPV of decyl and UDE surfaces approximately doubled after incubation in water, -80 ± 12 mV to -160 ± 17

mV and -75 ± 10 mV to -141 ± 17 mV for decyl and UDE surfaces respectively. The dimethyl Grignard surfaces were less stable than the unbranched surfaces, increasing from -71 ± 24 mV to -332 ± 33 mV. Increasing the monolayer thickness either by branching or using a longer chain alkene stabilizes the SPV in water. Of these two approaches the SPV of octadecyl surfaces nearly double increasing from -43 ± 20 mV to -85 ± 15 mV whereas the C-5 Grig surfaces remain well within the error bars changing from -56 ± 12 mV to -62 ± 17 mV.

Figure 3.17 – SPV changes on Aqueous Exposure

The similarity between decyl and UDE surfaces is not surprising as the monolayer thickness and integrated FTIR intensity were within error. The ester terminal group makes UDE surfaces more hydrophilic and it might be expected that the hydrophobic region which prevents penetration of water to the silicon – monolayer interface is essentially the same. The stability of dimethyl surfaces was very poor compared with the

UDE precursor and especially compared with the longer C-5 Grig surfaces. The instability may be due to the methyl groups opening up the terminus of the monolayer; however the cause of the instability is not as important as the fact it demonstrates a structure – property relationship. Short branches decrease stability and longer branches increase stability. This gives confidence that the stability of the C-5 Grig surfaces is not due to salt deposits or trapping of positively charged magnesium ions in the layer. The C-5 surfaces displayed a slightly higher (though within error) SPV than the octadecyl surfaces however the SPV of C-5 Grig surfaces remained within error while the octadecyl increased beyond the range of the error bars. Based on these data the branching approach was deemed superior.

The stability of the silicon substrate was studied by HREELS. HREELS measurements of decyl, UDE and Si Branched modified surfaces are shown in Figure 3.18 and c respectively. Black curves are HREELS of freshly made samples; grey curves represent HREELS of samples after 16 hours in water. The vibration signals are nearly the same for three modified surfaces, only extra C=O stretches from the Si-UDE surface. The peaks in Figure 3.18 are assigned as following: 2920 cm^{-1} = C-H asymmetric and symmetric stretching (due to the resolution limitation, these two modes are merged), 2080 cm^{-1} = Si-H stretching, 1700 cm^{-1} = C=O stretching, 1400 cm^{-1} = symmetric and asymmetric C-H bending overlapping, 1070 cm^{-1} = Si-O-Si stretching. For the purpose of this work, only Si-O-Si stretches were discussed qualitatively in order to monitor the stability of three modified surfaces. As shown in Figure 3.18, the intensity of Si-O-Si peak of decyl surface increased significantly after being in water for 16 hours. This implied that water and/or oxygen molecules penetrate the monolayer and oxidize the

silicon surface. In the case of the UDE surface, although the existence of C-O stretching in this region overlaps with the Si-O peak, it is reasonable to assume peak intensity of the C-O stretch does not change during the whole process; the net increase of peak intensity

(A) Si-C10, (B) Si-UDE and (C) Si-B surfaces. Black Freshly made surface; grey after 16 hours in water.

Figure 3.18 - HREELS Monitoring Surface Oxidation

only comes from the growth of silicon oxidation. Compared to the decyl surface, the oxidation of UDE is slower in water. For the C-5 Grig surface, the Si-O peak grows remarkably slower in water compared to other two modified surfaces. HREELS measurements of these three surfaces after 16 hours in PBS solution (the samples have already been in water for 16 hours) have been performed and show no significant changes compared with those after 16 hours in water. The above results show the branched alkyl surface is the most oxidation resistant surface, which is consistent with results of the above Kelvin probe measurements.

3.4 – Summary and Prospective

The objectives of this chapter were to determine whether biomolecular binding on crystalline silicon could be measured by Kelvin probe and to identify challenges that would need to be overcome in order to develop a label free biosensor. The first system studied was the capture of his-tagged p19 by a Ni-NTA modified surface. Initial ellipsometry results suggested that the protein was selectively captured by Ni-NTA surfaces, and induced changes in SPV consistent with the positively charged protein. Characterization of these surfaces by XPS indicated the NTA reaction was unsuccessful and nickel was not present on the surface at a level above the detection limit. The chemistry was optimized by changing the solvent from PBS to a DMF : DMSO mixture and Ni-NTA surfaces were successfully prepared and validated with XPS and FTIR. The results of protein binding experiments were quite interesting as the same changes were observed on the surfaces presenting Ni-NTA as those that did not. This highlighted a significant challenge in developing a label free biosensor, which is the signal, is derived from a physical property of the analyte and in order to obtain reliable binding data the

interactions at the interface must be well understood. In this case, the response is due to charge at the interface and it was shown that deprotonation of carboxylic terminated surfaces cause the same changes as chelation of Ni^{2+} by NTA. It was also shown that the same changes in SPV are observed whether the protein binds to Ni-NTA or carboxylic acid surfaces. Fluorescently labeled siRNA, the target of p19 was found not to specifically bind to the protein and it is likely that the protein denatured on binding to the surface. Due to these challenges a more robust interaction was investigated: biotin – streptavidin.

Biotin was coupled to a diamino-OEG linker which enabled spotting of the probe molecule onto NHS activated surfaces; the remaining NHS groups were quenched by EG3OH. This format allowed for a direct measurement of binding specificity, by exposing the entire surface to protein the difference between biotin and EGOH regions could be directly observed on the same sample. Additionally, because streptavidin is so commonly used, the protein was fluorescently labeled and allowed for direct comparison between electrical and fluorescence imaging. These measurements confirmed that protein was localized specifically in the biotin regions of the surface and significant contrast was observed by both optical and SKP measurements indicating protein binding could be measured by SKP on silicon. On binding the protein, the SPV became more negative where the protein was bound and was essentially unchanged in the EG3OH regions. The increased negative SPV may be the result of the negatively charged protein, deprotonation of carboxylic acids in the monolayer by the protein, or oxidation of the silicon substrate. Substrate oxidation was monitored using XPS, the results indicated that the majority of the increase in SiO_2 content occurs during preparation of the surface. The

oxide increased 4 fold on conversion of UDA to BioNH₂ whereas exposure of the BioNH₂ surface to water for 18 hours caused a further increase of 37 %; therefore, the substantial difference in SPV in protein containing regions is likely due to either the ionization of acid groups in the monolayer or the negative charge of the protein.

While biotin – streptavidin interactions can be measured, reducing or inhibiting oxidation of the substrate should improve reproducibility and the sensitivity to target molecules. Use of a long chain alkene, octadecyl versus decyl improved the stability however the SPV increased by a significant amount on exposure to water. Formation of high quality octadecyl layers was found to require a thermal reaction at 110 °C for 16 hours. It was also demonstrated that the SPV could be stabilized by branching the surface via the Grignard reaction. The monolayer after this process is greater than 85 % that of a closed packed hydrocarbon layer and oxidation of the substrate was demonstrated to be significantly reduced by HREELS.

The objectives of this chapter were achieved: the specific recognition of a biomolecule was observed using SKP and oxidation of the substrate was inhibited. Additionally the necessity to unambiguously characterize the surface and correlate optical and electrical measurements was demonstrated. The electrical signal is consistent with formation of carboxylate ions lacking a counterion in the monolayer as demonstrated for polystyrene beads.¹⁶⁷ The current limitation of the stabilized surfaces is that they are methyl terminated and as such further modification is impaired. The goal for the following chapter is to incorporate a synthetic handle into a monolayer which inhibits oxidation of the silicon while presenting a layer that ensures the specific interaction of probe and target.

Chapter 4 – Functionalization of Stable Surface

4.1 – Introduction

The goal of this chapter is to apply the information gained during the scoping studies and develop a monolayer on silicon which is chemically and electrically stable under biologically relevant conditions and detects the binding of streptavidin. In Chapter 3 it was demonstrated that Streptavidin can be selectively captured on a biotin presenting surface and detected using a SKP. It was also shown that the chemical and electrical properties of the silicon substrate are stabilized in water for extended periods of time by increasing the density of the monolayer via the Grignard reaction of an ethyl ester terminated monolayer. Bearing this in mind, a strategy was developed to incorporate an amine reactive synthetic handle onto the terminus of the branched monolayer.

Chemically, several routes were found to functionalize the branched surface, two were identified which did not damage the silicon substrate and one of those was found to satisfy all requirements. This monolayer was synthesized by performing a Grignard reaction using TMS protected 11-bromoundecan-1-ol to afford an alcohol terminated layer. The alcohol was activated with hexamethylene diisocyanate which was subsequently functionalized with biotin and found to support the capture and identification of Streptavidin.

4.1.1 – Functional Groups Compatible with the Grignard Reaction

Surviving in the presence of the nucleophilic carbanion requires that the synthetic handle be protected or masked; Scheme 4.1 shows potential Grignard reagents.

Biomolecules tend not to contain electrophiles as their presence would lead to inter and intra-molecular reactions. Furthermore, it was desirable to make use of the existing

amine terminated BioNH₂ and EG3OH molecules. For these reasons an electrophile terminated monolayer was considered most general and the azide, disilyl amine, and alkyne routes were not investigated. The acetal was not pursued due to the potential complications of aldol reactions in an aldehyde terminated layer.

Scheme 4.1 – Grignard Compatibility

4.1.1.1 – Reactions of Alkenes

The alkene was explored first because it represented minimal structural change. A high density branched layer was found necessary for stabilizing the surface and it was expected that an alkene should be able to achieve a similar density to methyl.

Scheme 4.2 shows the reactions considered for an alkene terminated surface. Epoxide coated glass slides are used as a substrate in DNA microarray printing. The nucleic acids can have an amine specifically added for reaction with the surface or nitrogen containing bases such as guanine inherently react with the surface.^{118,119} Omega-alkene epoxides have been covalently bound to silicon and observed to react with amines; however in this case the monolayer is unbranched and is not expected to be chemically stable.⁶⁵ Epoxidation of an alkene with meta-chloroperoxybenzoic acid is a well known process in organic chemistry.¹⁷⁸ The mechanism of this reaction is shown in Scheme

4.3.¹⁷⁹ It is believed to proceed via a concerted process; the electrophilic oxygen coordinates the π -system of the alkene resulting in formation of the carboxylic acid and epoxide.

Scheme 4.2 – Reactions of Alkenes

Scheme 4.3 – Mechanism of Epoxidation Reaction

Oxidation with potassium permanganate under acidic conditions directly affords the carboxylic acid.¹⁸⁰ The first step of the reaction is the formation of a cyclic manganate ester, this process is rate limiting and involves a nucleophilic attack of the permanganate on the alkene.¹⁸¹ The following steps are pH dependant. In the presence of acid and excess permanganate, the carbon-carbon bond is cleaved and two equivalents of carboxylic acid are generated. The reason for the pH dependence of the products lies in the reduction potential of potassium permanganate, under acid conditions it is 1.68 V and under basic conditions it is 0.59 V.¹⁸² Wasserman *et al.*¹⁸³ prepared an acid

terminated silane monolayer on oxidized silicon using a periodate-permanganate oxidation first reported by Lemieux and von Rudolff¹⁸⁴. This reaction is carried out under slightly basic conditions (pH 7.5) using catalytic potassium permanganate, with sodium periodate serving to re-oxidize the permanganate.

Scheme 4.4 – Mechanism of Permanganate Oxidation under Acidic Conditions

Two routes were considered and not pursued. Starting at the top centre (Scheme 4.2) the cyclopropyl group could be introduced via photochemical decomposition of the diazarene precursor to afford a reactive carbene.^{53,185} Secondly, radical mediated addition of a di-thiol has been demonstrated on a Si-H surface¹⁸⁶ promoted by 254 nm light and is known in solution to proceed in the presence of a carboxylic acid¹⁸⁷ when initiated by azobisisobutyronitrile. These two routes involve the generation of highly reactive intermediates (carbene and radical) in solution which in turn could not only react with the alkene but may also damage the silicon surface either by direct reaction or by the generation of reactive impurities. Despite rigorous efforts to purify the solvent and reagent, Wagner *et al.*⁵³ observe significant oxidation of the silicon surface on an octadecyl terminated monolayer. Due to this inherent liability these routes were deemed lower priority, as will be shown, the oxidative conditions described above damage the silicon surface. Gas phase exposure of organic monolayers on silicon to carbenes is an interesting alternative as the flux and purity of reactive intermediate can be more tightly controlled, however this was not pursued in this work.

The 2005 Nobel Prize in chemistry was awarded to Yves Chauvin, Robert Grubbs and Richard Schrock for the development of olefin metathesis as a synthetic method. Continued development of this methodology has led to catalysts which can effect cross metathesis on a protein.¹⁸⁸ In that study, the solvent system was aqueous buffer with 30% *t*-butanol to improve the catalyst solubility; importantly it demonstrates the robust nature of the catalyst and reaction. The active species enters the catalytic cycle (Scheme 4.5) upon dissociation of a phosphine ligand, (1st generation catalyst is symmetric and L = tricyclohexyl phosphine, 2nd generation catalyst L = N-heterocyclic carbene). The next step is co-ordination of the alkene, followed by a cycloaddition generating a cyclobutane containing the metal. Cycloreversion affords the product alkene co-ordinated to the metal centre which is then ejected, regenerating the active species.¹⁸⁹ Each step of the catalytic cycle is reversible and the overall product distribution is an equilibrium mixture, limiting its utility in cross-metathesis. However, in the case of surface functionalization

Scheme 4.5 – Catalytic Cycle of Olefin Metathesis Using Grubbs Catalyst¹⁸⁹

the alkene in solution is in large excess to the surface species favouring the cross-metathesis product. Two reports of metathesis on crystalline silicon surfaces were found. Using Grubbs' 1st generation catalyst the yield of the metathesis reactions were reported

to be ~30% and ~50% respectively.^{66,67} Both reports carry out cross metathesis between a vinyl terminated surface and a fluorine containing alkene in solution; the yield is determined using the fluorine peak in the XPS spectra. This approach avoids the use of reactive species however the increased density of the branched surface may sterically hinder the bulky catalyst.

4.1.1.2 – Reactions of Alcohols

As shown in Scheme 4.1 an alcohol terminated monolayer can be prepared by protection of the alcohol during the Grignard reaction. A silyl protecting group was

Scheme 4.6 – Possible Reactions of Alcohols

chosen because silyl ethers are cleaved by fluoride ions which have been shown to improve the electrical properties of the interface by removing any silicon oxides formed during monolayer formation.¹⁹⁰ An alcohol terminated monolayer opens up many

options for functionalization (Scheme 4.6), and because it does not ionize under biologically relevant conditions, unreacted groups will not result in an electrical signal. Two desirable routes were activation with a di-isocyanate, as isocyanates readily react with amines under neutral conditions, and reaction with succinic anhydride to produce a carboxylic acid terminated monolayer. These two routes make use of pure addition reactions meaning all the atoms are present in the product¹⁹¹ and reduces the likelihood of creating reactive impurities.

4.1.2 – Rationale

In the previous chapter it was shown that the protein streptavidin could be selectively bound to a crystalline silicon surface and the binding measured by SKP. The binding was independently verified using fluorescence. Unfortunately, this first generation monolayer did not passivate the silicon substrate. It was however shown that branched methyl terminated monolayers did stabilize the chemical and electrical properties of the silicon substrate. The focus of this final chapter is to functionalize the branched surface and measure the binding of streptavidin by SKP. The optimization algorithm followed in this chapter is to validate the chemistry, and ensure the reaction product can be characterized. Next, investigate the chemical stability of the surface first by FTIR, then XPS. During this process the electrical stability is determined. Finally, suitability as a biosensing platform is addressed.

Work began with the alkene terminated monolayer because it was deemed the smallest structural perturbation, it did not require protecting group manipulation, and a number of derivatization routes were available. Epoxidation with 3-chloroperoxybenzoic acid was first attempted in an effort to maximize overlap with the known literature.

Epoxide coated glass slides are the standard substrate in DNA microarray analysis. A carboxylic acid terminated monolayer was also desired as it would allow for direct transfer of the chemistry developed in the previous chapter. It became clear that despite the increased density of the branched monolayer and its ability to inhibit oxidation due to oxygen and water it was still sensitive to strong oxidizing agents.

Olefin metathesis was found to functionalize the alkene surface without damaging the substrate chemically or electrically. Additionally the coupling partner was pre-activated with NHS therefore it avoided surface contamination during the activation step (described in the previous chapter). The limitation of this route was found to be in the yield. Presumably due to steric hindrance, the NHS density of this surface was approximately 10 – 20 % that obtained from an un-branched surface. The length of the branch, ratio of alkene to methyl termination, and catalyst were investigated unsuccessfully to try and improve the yield. Initial efforts were made to increase the density of reactive groups by coupling a polymer to the surface, but this work was halted when functionalization of the alcohol surface showed potential.

Conditions were optimized to enable a hexamethylene diisocyanate modified surface to support the specific capture and detection of streptavidin, even after exposure to BSA. These surfaces were found to be the most hydrophilic prepared in this work and the alcohol terminated surface remained stable on exposure to aqueous.

4.2 – Methods and Materials

4.2.1 – Materials

ATR silicon crystals ($25 \times 4.5 \times 1 \text{ mm}^3$) were purchased from Harrick and the silicon wafers from Virginia Semiconductor. Sulfuric acid, 96% (H_2SO_4), ammonium

fluoride, 40% (NH_4F), hydrochloric acid, 30% (HCl), ammonium hydroxide, 30% (NH_4OH) and hydrogen peroxide, 30% (H_2O_2) Finyte Grade were purchased from J. T. Baker, and hydrofluoric acid, 48% (HF) PPB grade was purchased from Arch. Ethylundecylenate 97%, 11-brom-1-undecene 95%, and 1,3,5 benzenetricarbonyl trichloride 98%, were purchased from Aldrich and distilled under vacuum at 134°C . 11-bromo-1-undecanol 98%, pentylmagnesium chloride 2M in tetrahydrofuran, Grubbs Catalyst 1st and 2nd Generation Catalysts, Grubbs-Hoveyda 2nd Generation Catalyst, 4-pentenoic acid 97%, N-hydroxysuccinimide, chlorotrimethylsilane >99% N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1,6-hexamethylene diisocyanate 97%, succinic anhydride >99%, 3-chloroperoxybenzoic acid 77%, potassium permanganate 99%, sodium periodate 99%, 4-dimethylaminopyridine 99%, PEG 1000, hexaethylene glycol, p-toluenesulphonyl chloride >99%, sodium azide >99.5%, triphenylphosphine 99% and synthetic reagents were purchased from Aldrich at >98% purity or highest available and used as received. BSA AlexaFluor 647 conjugate and streptavidin AlexaFluor 555 conjugate were purchased from Invitrogen and avidin Cy-5 conjugate was purchased from Rockland Immunochemicals. Unless otherwise specified, solvents were obtained from a purification system.

4.2.2 – Surface Characterization (see Section 2.2.2)

Estimated Thickness. Unless otherwise indicated reported numbers represent an average of five points on each sample and a minimum of two independent experiments. The error represents the arithmetic mean of the standard deviation from each experiment.

Contact Angle. Unless otherwise indicated reported numbers represent an average of three points on each sample and a minimum of two independent experiments. The error represents the arithmetic mean of the standard deviation from each experiment.

SPV. Unless otherwise indicated reported values represent an average of sixteen points in the dark and sixteen points collected in the light from a representative experiment.

Fluorescence Measurements. Silicon wafers were affixed to a glass slide and scanned in a BioRAD VersArray Chip Reader with 5 μm resolution using 635 and 532 nm excitation.

4.2.3 – Synthesis

Scheme 4.7 – Syntheses of **12** and **13**

1-amino-hexaethylene glycol (12). Hexaethyleneglycol (5g, 17.1 mmol) was dissolved in dichloromethane (30mL) to this was added triethylamine (2.75 mL, 19.5 mmol) followed by p-toluenesulfonyl chloride (3.38g, 17.71 mmol). The reaction was stirred at room temperature under nitrogen 8h. The solvent was evaporated and the residue dissolved in ethanol (50 mL) to this was added sodium azide (1.73 g, 26.6 mmol) and the reaction heated at 80 °C over night. The reaction was concentrated and flashed through a pad of silica gel eluting with 10% methanol : ethyl acetate to afford **10** as a pale yellow impure oil (822 mg). The oil (822 mg, 2.8 mmol) was dissolved in tetrahydrofuran (10mL), triphenylphosphine (900 mg, 3.4 mmol) was added and the reaction sealed under nitrogen for 6 hours. The reaction was quenched by the addition of

water (2 mL) and stirred for 1 hour, then concentrated. The residue was suspended in water and the triphenylphosphine oxide extracted with toluene $\times$ 3. The aqueous was concentrated dissolved in dichloromethane dried (MgSO_4) filtered, concentrated and flashed eluting with 10 % methanol : chloroform : 1% NH_4OH to yield 550mg (11 % yield) of **12** as a clear pale yellow oil. ^1H NMR (400 MHz, CDCl_3) ppm 3.62 (t, J = 5.2Hz, 2 H), 3.56 (m, 16 H), 3.50 (s, 2 H), 3.44 (t, J = 5.2Hz, 2 H), 2.77 (m, 5 H).¹⁹² ES-MS: calc 281.2, found MH^+ 282.3.

1-amino-PEG 1000 (13). PEG1000 (16g, 16 mmol) was dissolved in dichloromethane (30mL) to this was added triethylamine (1.4 mL, 10 mmol) followed by p-toluenesulfonyl chloride (1.6 g, 8 mmol). The reaction was stirred at room temperature under nitrogen 8h. The solvent was evaporated and the residue dissolved in ethanol (25 mL) to this was added sodium azide (650 mg, 10 mmol) and the reaction heated at 80 °C over night. The reaction was concentrated and flashed through a pad of silica gel eluting with 10% methanol : ethyl acetate to afford **11** as a pale yellow impure oil (4.2 g). The oil (4.2 g, 4.2 mmol) was dissolved in tetrahydrofuran (20mL), triphenylphosphine (1.1 g, 4.2 mmol) was added and the reaction sealed under nitrogen for 6 hours. The reaction was quenched by the addition of water (2 mL) and stirred for 1 hour, then concentrated. The residue was suspended in water and the triphenylphosphine oxide extracted with toluene $\times$ 3. The aqueous was concentrated dissolved in dichloromethane dried (MgSO_4) filtered, concentrated and flashed eluting with 10 % methanol : chloroform : 1% NH_4OH to yield 1.25 mg (16 % yield) of **12** as a clear pale yellow wax. ES-MS: calc 1029.7 $\pm$ 44, found MH^+ 1030.6 $\pm$ 44.

Scheme 4.8 – Synthesis of **14**

N-hydroxysuccinimide 4-pentenoate (**14**). 4-pentenoic acid (5g, 50 mmol) was dissolved in dichloromethane (100 mL), to this was added N,N-dimethylformamide (drop) and thionyl chloride (5 mL) under nitrogen. The reaction was stirred at room temperature for 90 minutes then concentrated. The oil was dissolved in dichloromethane (10 mL) and added over 10 minutes to a stirring solution of triethylamine (6.9 mL, 50 mmol) and N-hydroxysuccinimide (5.7 g, 50 mmol). The reaction was stirred for 2 hours then concentrated and flashed eluting with 5 % hexanes : chloroform to afford 5.5 g (56 % yield) as a clear wax. ^1H NMR (400 MHz, CDCl_3) ppm 5.85 (m, 1 H), 5.12 (m, 2 H), 2.84 (s, 4 H), 2.72 (t, $J=7.43$, 2 H), 2.50 (m, 2 H).¹⁹³

Scheme 4.9 – Synthesis of **15**

Trimesic NHS (**15**). Freshly distilled 1,3,5-benzenetricarbonyl trichloride (2 g, 7.53 mmol), was dissolved in chloroform (10 mL) and added via syringe to a stirring solution of N-hydroxysuccinimide (3.47g, 30 mmol) and triethylamine (4.2 mL, 30 mmol) in chloroform (140 mL) under nitrogen. The reaction was stirred over night at room temperature, then washed with water (4×50 mL), dried (MgSO_4) and concentrated to a foam. The foam was dissolved in 25 mL dichloromethane and to this was added 25 mL of ethyl acetate. After about 1 minute a white solid formed, this was isolated by

vacuum filtration to afford 782 mg (20% yield) of **15** as a white solid. ^1H NMR (400 MHz, CDCl_3) ppm 9.16 (s, 3 H), 2.96 (s, 12 H).¹⁹⁴

Scheme 4.10 – Synthesis of **16**

11-bromo-1-trimethylsilyloxyundecane (**16**). 11-bromoundecan-1-ol (3.83g, 15.2mmol) was dissolved in tetrahydrofuran (100mL), triethylamine (2.4mL, 17.2mmol) was added and the reaction sealed under nitrogen. Trimethylsilyl chloride (2.02mL, 15.8mmol) was added dropwise and the reaction stirred at room temperature over night. The solution was filtered, concentrated, taken up in hexanes, filtered a second time then concentrated and purified by silica gel column chromatography eluting with 5% ethyl acetate : hexanes to afford 4.13g (84% yield) as a clear oil. ^1H NMR (400 MHz, CDCl_3) ppm 3.58 (t, $J=6.73$, 6.73 Hz, 2 H), 3.42 (t, $J=6.87$, 6.87 Hz, 2 H), 1.87 (m, 1 H), 1.53 (dd, $J=6.75$, 13.52 Hz, 2 H), 1.43 (dd, $J=6.97$, 14.35 Hz, 2 H), 1.30 (m, 14 H), 0.13 (s, 9 H).¹⁹⁵

4.2.4 – Monolayer Preparation and Functionalization

Cleaning and Hydrogen termination. Single sided silicon crystals were used once and discarded; ATR elements were cleaned and reused. All crystals were cleaned with piranha solution (3:1 H_2SO_4 , 96% : H_2O_2 , 30%) at 120 °C for 30 minutes, then rinsed with Milli-Q water. (*Warning: Piranha solutions should be handled with care and kept isolated from organic materials*). ATR elements were then cleaned with 4:1:1 Milli-Q water : NH_4OH , 30% : H_2O_2 , 30% at 80 °C for 15 minutes, rinsed with Milli-Q water and heated in 4:1:1 Milli-Q water : HCl , 30% : H_2O_2 , 30% at 80 °C for 15 minutes then rinsed

with Milli-Q water. Samples were hydrogen terminated by etching in degassed ammonium fluoride for 15 minutes followed by a brief rinse in degassed Milli-Q water.

Monolayer Formation. Distilled ethyl undecylenate was transferred to a Pyrex Schenk tube and degassed with argon for at least 10 minutes. The silicon samples were added and degassed a further 5 minutes then placed in a Rayonette photoreactor for 1.5 – 16 hours, until the peak area of the C-H stretching region reached a maximum 0.55 – 0.63 AU. After the reaction the samples were removed and washed with either continuously refluxing 1,1,1-trichloroethane in a soxhlet or with copious volumes of chloroform and ethanol at room temperature.

Grignard reaction – Alkene Terminated Monolayers. Magnesium turnings (350mg, 14.6mmol) were added to a round bottom flask and sealed under an atmosphere of nitrogen with a condenser. Dry tetrahydrofuran (9mL) was added via syringe and the reaction heated to 80 °C. The magnesium was activated by 1,2-dibromoethane (200 μ L) for 15 minutes and then either 5-bromo-1-pentene (1.55 mL, 13 mmol) or (11-bromo-1-undecene (2.8 mL, 13 mmol) were added via syringe. The reaction was heated a further 1 hour at 80 °C. Just prior to introduction to the reaction vessel, the silicon sample can be exposed to 2% HF solution for 2 minutes in order to remove any silicon oxide which may have formed during the hydrosilation reaction. The silicon sample was sealed under an atmosphere of nitrogen in a clean, dry test tube with a silicone septum and the Grignard reagent was introduced via syringe. The sample was allowed to react at 80 °C for 90 minutes. At this point the Grignard reaction was stopped by successive dilution with dry tetrahydrofuran ensuring the sample remained covered in solvent. After 3 exchanges, 2 mL of glacial acetic acid was introduced to quench remaining Grignard

reagent. The sample was washed with glacial acetic acid, Milli-Q water, ethanol and dried under a stream of nitrogen.

Epoxide formation. A solution of 3-chloroperoxybenzoic acid (0.2 M, 45 mg/mL) in dichloromethane was prepared and cooled in an ice bath under nitrogen. An alkene terminated silicon sample was introduced and reacted for 2 hours. At this point it was removed rinsed with TCE and placed in the Soxhlet for 20 minutes then blown dry under a stream of nitrogen.

Permanganate Oxidation. Dissolved KMnO_4 (500 mg, 3.2 mmol), in Milli-Q water (19 mL) then added acetic acid (1 mL). The silicon samples were immersed in this oxidizing solution for 5 minutes, then rinsed with water, ethanol and blown dry under a stream of nitrogen.

Permanganate – Periodate Oxidation. An oxidizing solution consisting of 0.5 mM KMnO_4 , 19.5 mM NaIO_4 , and 1.8 mM K_2CO_3 , at a pH of 7.5 was prepared in Milli-Q water. The silicon sample was immersed in this solution for 30 minutes and then washed with 0.3 M NaHSO_3 , Milli-Q water, 0.1 N HCl, Milli-Q water, ethanol and blown dry under a stream of nitrogen.

NHS Ester Formation. EDC (10 mg / mL, 52mM) and NHS (6mg/mL, 52mM) were dissolved in Milli-Q water and the silicon samples immersed for 1 hour. The samples were removed rinsed with Milli-Q water, and ethanol then blown dry with a stream of nitrogen.

Metathesis reaction. Compound **14** (100 mg, 0.51 mmol) was dissolved in dry methylene chloride 5mL in a 25 mL sealed tube an alkene terminated silicon sample was

added and the mixture degassed with bubbling argon for 5 minutes. To this was added catalyst (5 mol %) and the sealed tube closed under an argon atmosphere. The reaction was heated at 50 °C over night, then opened and the sample washed with methylene chloride, ethyl alcohol and blown dry under a stream of nitrogen.

Amide formation. A NHS activated silicon ATR element was immersed in a solution of compound **3** (20 mM) in pH 8.0 carbonate buffer (0.2 M) for 3 hours. The sample was washed with Milli-Q water, ethanol and dried under a stream of nitrogen.

Standard aqueous conditions. The amine (20mM) is dissolved in buffer (PBS at pH 7.4 or 0.2M carbonate at pH 8.0) and either the NHS activated sample is immersed for 1 hour or a 1-2 μ L drop is placed on the silicon surface for 3 hours. The sample was then washed with water, ethanol and blown dry under a stream of nitrogen.

Grignard reaction – Alcohol Terminated Monolayers. Magnesium turnings (340 mg, 14 mmol) were added to a round bottom flask and sealed under an atmosphere of nitrogen with a condenser. Dry tetrahydrofuran (10 mL) was added via syringe and the reaction heated to 80 °C. The magnesium was activated by 1,2-dibromoethane (200 μ L) for 15 minutes and then 11-bromo-1-trimethylsilyloxyundecane (3.1 g, 9.6 mmol) was added via syringe. The reaction was heated a further 1 hour at 80 °C. Just prior to introduction to the reaction vessel the silicon sample can be exposed to 2% HF solution for 2 minutes in order to remove any silicon oxide which may have formed during the hydrosilation reaction. The silicon sample was sealed under an atmosphere of nitrogen in a clean, dry test tube with a silicone septum and the Grignard reagent was introduced via syringe. The sample was allowed to react at 80 °C for 16 hours. At this point the Grignard reaction was stopped by successive dilution with dry tetrahydrofuran ensuring

the sample remained covered in solvent. After 3 exchanges 2 mL of glacial acetic acid was introduced to quench remaining Grignard reagent. The sample was washed with glacial acetic acid, Milli-Q water, ethanol and dried under a stream of nitrogen.

Isocyanate Terminated Monolayer. An alcohol terminated silicon sample was introduced into an argon purged solution of neat hexamethylene diisocyanate, sealed under nitrogen and heated at 60 °C for 90 minutes. The sample was removed washed with methylene chloride and dried under a stream of nitrogen.

Carbamate Formation. The isocyanate surfaces (ATR crystal or wafer) were immersed a solution of 20 mM amine in pH 8.0 carbonate buffer (0.2 M) for 1 hour. They were washed with water, ethanol and methylene chloride then dried under a stream of nitrogen.

Carbamate formation – Silicon wafer mixed monolayer. An isocyanate activated silicon wafer was manually spotted with 2 μ L drops of 20 mM amine in pH 8.0 carbonate buffer (0.2 M) and placed in a humid chamber for 1 hour. The sample was rinsed in Milli-Q water and the entire surface covered in compound 3 (20 mM) in pH 8.0 carbonate buffer (0.2 M) for 1 hours.

Carboxylic Acid Terminated Monolayer. An alcohol terminated silicon sample was introduced into an argon purged solution of 1 M succinic anhydride, 0.05M N,N-dimethylaminopyridine in dimethylformamide, sealed under nitrogen and heated at 90 °C for 18 hours. The sample was removed washed with dimethylformamide, ethanol, and dried under a stream of nitrogen. NHS ester and amide formation were carried out as above.

4.2.5 – Protein Attachment

Protein solutions 0.1mg/mL in PBS were exposed to the silicon surface for 1 hour in a humid chamber. The protein was rinsed off and the samples were shaken in a vial containing 0.1% Tween-20-PBS (3×1min) rinsed with Milli-Q water and blown dry under a stream of nitrogen.

4.3 – Results and Discussion

4.3.1 – Oxidative Conditions

4.3.1.1 – Pentenyl Branched Monolayer

Based on the success obtained in stabilizing the chemical and electrical properties of the silicon surface using a branched monolayer, this was chosen as the foundation on which a biocompatible layer would be bound. The first attempts at functionalizing a branched surface involved a Grignard reaction with pentenyl magnesium bromide and a UDE surface followed by epoxidation with 3-chloroperoxybenzoic acid. The FTIR (see Appendix) spectra indicate the yield of the Grignard reaction is comparable to pentyl magnesium bromide, and an alkene peak is present at 3080 cm^{-1} . The peak area increases on average from $0.51 \pm 0.01\text{ AU}$ to $0.64 \pm 0.05\text{ AU}$ (see Table 4.1). The number of methyl and methylene units increases by one third (from 12 to 16) giving a predicted peak area of 0.68 AU, 93 % yield. The presence of remaining carbonyl in the IR spectrum is consistent with a reaction that did not go to completion. After oxidation with 3-chloroperoxybenzoic acid the peak area of the C-H stretching region was observed to significantly decrease indicating oxidation of the silicon surface and removal of the monolayer. Because the epoxide does not contain a diagnostic signal it was not possible to unequivocally determine the success of the reaction. In comparison, the permanganate

oxidation clearly shows a broad acid peak at 1714 cm^{-1} upon exposure to the acidic KMnO_4 solution; however it was concomitant with significant loss of hydrocarbon. While it is useful to have a characteristic signal (increase of COOH stretch), damage to the silicon surface must be reduced.

Table 4.1 – Integrated area of Saturated C-H Stretching Region for Pentenyl Modified Surfaces.

	Integrated Area (AU)
UDE	0.51 ± 0.01
Pentenyl Branch	0.64 ± 0.05
3 – chloroperoxybenzoic acid	0.48 ± 0.08
KMnO_4 / AcOH	0.51 ± 0.01

4.3.1.2 – Undecylenyl Branched Monolayer

It was hypothesized that a longer branch would better protect the silicon surface from the oxidizing agent by increasing the thickness of the layer. A Grignard reagent was prepared from 11-bromo-1-undecene and reacted with UDE modified silicon. The reaction was performed at elevated temperature to increase the solubility of 11-undecylenyl magnesium bromide. The FTIR spectrum of the UDE surface shows the presence of a methyl stretch at 2982 cm^{-1} , methylene stretches at 2922 and 2952 cm^{-1} , and the carbonyl stretch of the ester group is observed at 1741 cm^{-1} . For this series of experiments the integrated area of the of the C-H stretching region was $0.62 \pm 0.08\text{ AU}$. After branching the FTIR spectrum shows the presence of an alkene C-H stretch at 3079 cm^{-1} , methylene stretches at 2924 and 2952 cm^{-1} , the remaining carbonyl stretch of the ester group is observed at 1738 cm^{-1} and ketone from the partially reacted ester at 1718 cm^{-1} . The peak area increased to $1.06 \pm 0.15\text{ AU}$ indicating a yield of approximately 73 %. While the yield is lower than the pentenyl surface, likely due to increased steric hindrance from the longer alkene and higher surface coverage of UDE, the total quantity

of hydrocarbon is greater. After oxidation with $\text{KMnO}_4 / \text{AcOH}$ the peak area of the C-H stretching region decreased significantly while the $\text{KMnO}_4 / \text{NaIO}_4$ oxidized surface shows negligible loss of monolayer. The FTIR spectrum indicates absence of the alkene signal at 3079 cm^{-1} , methylene stretches at 2925 and 2953 cm^{-1} , remaining ester carbonyl at 1743 cm^{-1} , and ketone and carboxylic acid stretches overlapping at 1718 cm^{-1} . (Table 4.2, Figure 4.1). On exposure to 2% HF the peak area of the C-H stretching region was observed to decrease by approximately 10 % in two experiments, which is on the order of the error observed in the experiments. It does however suggest that the surface is not completely passivated.

Figure 4.1 – FTIR Spectra of $\text{KMnO}_4 / \text{NaIO}_4$ Oxidation

Table 4.2 – Integrated area of Saturated C-H Stretching Region for Undecylenyl Modified Surfaces.

	Integrated Area (AU)
UDE	0.62 ± 0.08
Undecylenyl Branch	1.06 ± 0.15
KMnO ₄ / AcOH	0.83 ± 0.08
KMnO ₄ / NaIO ₄	1.1 ± 0.1

On activation of the KMnO₄ / NaIO₄ oxidized surface with NHS the FTIR indicates methylene stretches at 2924 and 2952 cm⁻¹, imide peaks at 1813 and 1785 cm⁻¹ and ester carbonyl at 1738 cm⁻¹ ketone at 1718 cm⁻¹. The inset of Figure 4.2 shows the NHS ester ratioed to the carboxylic acid terminated monolayer. After reaction with EG3OH in pH 8.0 carbonate buffer methylene stretches are observed at 2924 and 2952 cm⁻¹, and remaining ethyl ester carbonyl is observed at 1743 cm⁻¹. As described in the previous chapter under the aqueous amide forming conditions the NHS ester hydrolyzes

Figure 4.2 – FTIR Spectra of NHS and Amide Derived from KMnO₄ / NaIO₄ Oxidation, Inset is NHS ratioed to carboxylic acid terminated monolayer.

to some extent during the amidation reaction, therefore the peak at 1720 cm^{-1} is most likely a combination of acid and ketone carbonyl, with the amide I and II bands at 1650 and 1538 cm^{-1} respectively. The spectrum of the NHS surface shows a peak at 1646 cm^{-1} due to deposition of EDC urea, but the amide I peak is significantly larger after reaction with the amine and the amide II band grows in, adding confidence to the spectral assignment. (Figure 4.2)

Figure 4.3 – HR-XPS Spectra of Si₂p Region, Inset: SiO₂ Signal

Because the FTIR studies indicated the monolayer was not being removed during the KMnO₄ / NaIO₄ oxidation, XPS spectra were collected to determine whether the silicon substrate was affected to an extent below the level of FTIR quantification.

(Figure 4.3) The spectra were analyzed as described in section 3.3.2 in order to determine the amount of oxidation introduced in monolayer equivalents (Table 4.3). The spectra in Figure 4.3 are normalized to the peak area of the unshifted silicon signal in 4.3-B, the areas reported in Table 4.3 are the as recorded values. Despite initial concern about manganese dioxide deposition on the silicon surface during the reaction it was not observed in the XPS survey spectrum.

Table 4.3 – XPS Analysis of KMnO_4 / NaIO_4 Oxidized Surface

Sample	Peak	Position (eV)	Area (corrected)	FWHM	ML equivalents
Undecylenyl (as prepared)	Unshifted Si	98.9 / 99.5	989	0.43 / 0.61	4.17
	SiO_2	102.9	10 (21)	0.82	0.09
Undecylenyl (18 h water)	Unshifted Si	98.9 / 99.5	1912	0.43 / 0.61	4.17
	SiO_2	102.5	7 (15)	0.55	0.03
Oxidized (as prepared)	Unshifted Si	98.9 / 99.5	1697	0.47 / 0.55	4.17
	SiO_2	102.3	46 (97)	0.97	0.24
Oxidized (18 h water)	Unshifted Si	99.0 / 99.6	2009	0.49 / 0.60	4.17
	SiO_2	102.5	82 (174)	1.16	0.36

Formation of the branched undecylenyl surface does not significantly oxidize the surface. The 0.09 ML equivalents of SiO_2 reported in Table 4.3 should be considered an upper bound. The overall signal (counts) obtained for this sample was approximately half the other samples; therefore the noise was higher and with such low values any change in the baseline or peak height could have a significant effect on the area of the SiO_2 peak and hence the output of the model. After immersion in water for 18 hours 0.03 ML equivalents of SiO_2 were observed, a reasonable conclusion is that the surface is stabilized in water at <0.05 ML equivalents of SiO_2 . In contrast after oxidation with

KMnO₄ / NaIO₄, 0.23 ML equivalents of SiO₂ were observed which then increased to 0.36 ML equivalents after exposure to water for 18 hours.

Despite the differing reaction conditions all three oxidative approaches investigated damaged the silicon surface. The 3-chloroperoxy benzoic acid reaction was carried out in organic solvent which would solvate the monolayer, opening it up and allowing for greater penetration of the reagent. The solvent for the acidic KMnO₄ oxidation contained 5 % acetic acid which may serve a similar purpose whereas the strictly aqueous KMnO₄ / NaIO₄ oxidized the surface to a lesser extent. Monolayer solvation is likely a minor effect compared to the higher oxidation potential of permanganate under acidic conditions.¹⁸²

4.3.2 – Metathesis

An alternate methodology available to take advantage of the alkene terminated surface is olefin metathesis. This approach had the advantage of using “inert” reagents and produced an activated surface in one step, avoiding exposure of the surface to EDC, which so far has been observed as a trace impurity in most FTIR spectra. It was unknown whether the ruthenium catalyst would contaminate the surface; this was investigated in the XPS survey spectra and not observed. The synthetic approach is detailed in Scheme 4.11. An alkene terminated branched surface is prepared as described in Section 4.3.1, it was then added to a dry, degassed dichloromethane solution of Grubbs 2nd generation catalyst (5 mol %) and compound **14** and sealed in a pressure tube under argon. The pressure tube was then heated for 18 hours at 50 °C to afford the NHS activated silicon surface, which was reacted under standard amidation conditions.

Scheme 4.11 – Preparation of Functionalized Surface via Metathesis

The FTIR spectra after the metathesis reaction and amidation are shown in Figure 4.4, spectra of a UDE and alkene surface can be found in Figure 4.1. The FTIR spectrum of the metathesis product shows methylene stretches at 2924 and 2952 cm^{-1} , imide peaks at 1818 and 1787 cm^{-1} , the ester carbonyl stretch is observed at 1743 cm^{-1} , it has increased due to the presence of the N-hydroxysuccinimide ester, and ketone from the partially reacted ester at 1718 cm^{-1} . The inset of Figure 4.4 shows an expansion of the carbonyl region of the metathesis spectrum ratioed to the alkene starting surface. The signals due to NHS are thereby separated from carbonyls related to the underlying branched monolayer. The peak height of a NHS terminated UDA layer is about 0.1 AU (Figure 3.12) suggesting the yield of the metathesis reaction is low. The stability of the surface was studied by XPS because if this route stabilized the silicon substrate it would be worthwhile exploring routes to improve the yield.

Figure 4.4 – FTIR Spectra of NHS and Biotin Terminated Silicon Surface via Metathesis Route

The number of ML equivalents determined by XPS was calculated for the different surfaces in this study and presented in Table 4.4. Where error bars are given the value represents an average of at least two independent experiments. After monolayer formation approximately 4 % of the surface silicon atoms are in the Si^{IV} oxidation state; after the branched surface is formed the average amount of oxide does not increase however the variability increases. Approximately half of the samples show a decrease in oxide content and the other half show an increase. Prior to the Grignard reaction the UDE surface is immersed in 2 % HF solution, which removes any underlying oxide. A possible explanation for this result lies in the work of Ozanam and coworkers³⁹ who demonstrated that the presence of alkyl halide in the Grignard reagent increases the halogenation of the surface. If the Si-X does not react with a Grignard molecule it is hydrolyzed by water during the quenching step. The Grignard reagents used in this work

were prepared just prior to use and there may have been some variability in the halide content, which contributed to the variability observed. After the metathesis reaction and amide formation the amount of oxide approximately doubles and it does not change after exposure to water. Like the epoxidation (3-chloroperoxybenzoic acid) reaction this step is done in organic solvent. The reaction is carried out at elevated temperature (50 °C) for 18 hours, allowing time for any impurities to react with the surface. The SPV does not indicate any significant changes throughout the synthetic sequence. The uncertainties of the measurements are higher after processing but the values remain within the error bars.

Table 4.4 – XPS derived ML Equivalents of SiO₂

Surface	ML Equivalents SiO ₂	SPV (mV)
UDE	0.04 ± 0.01	-75 ± 10
UDE water 18 hours	0.13	-141 ± 10
Undecylenyl Branch	0.03 ± 0.02	-88 ± 24
Undecylenyl Branch water 18 hours	0.03 ± 0.03	-92 ± 28
Amide	0.08 ± 0.01	-112 ± 29
Amide water 18 hours	0.08 ± 0.01	-78 ± 21

As described above, after the metathesis reaction the peak area of the NHS signal was approximately 10 – 20 % the area observed when a UDA surface is NHS activated. This did not allow sufficient BioNH₂ or EG3OH to be grafted to the surface to enable detection of Streptavidin by SKP. Several approaches to increasing NHS density were pursued. Because this route provided stable surfaces (electrical and chemical) additional efforts were made to improve the yield versus concluding the sterics of the catalyst / monolayer interaction were insurmountable.

The effect of branch length and concentration of alkene to methyl groups was investigated. The desired reaction pathway (Scheme 4.12) is on the right. In this case,

cross metathesis between the alkene terminated surface and compound **14** is predominant.

In the pathway on the left side of the scheme inter and intra chain metathesis products

Scheme 4.12 – Potential Side Products in Metathesis Reaction

predominate. Because the metathesis reaction is an equilibrium process it is not surprising that the higher local concentration of neighbouring alkenes in the monolayer may shift the product distribution to favour this route. It is possible to shift the equilibrium by greatly increasing the concentration of alkene and catalyst in solution; however this rapidly becomes very expensive (due to catalyst cost) and was not investigated.

To separate the alkenes, mixed monolayers (methyl and alkene terminated) were prepared using different alkyl chain lengths (five and eleven). In a closed packed monolayer a ratio of alkene to methyl of 1 : 9 means that no more than one alkyl chain separates the alkene. By inference once the alkenes are spaced far enough apart to not interact the yield may increase but the NHS density may not. Dutta and coworkers⁶⁷ observed a doubling in the amount of fluorine incorporated in the monolayer as the alkene concentration was decreased from 100 % to 50 %, the alkene was three methylene units longer than the methyl chains. To prepare these surfaces the Grignard reagents were mixed and added via syringe to an argon purged tube containing the silicon wafer

and ATR crystal. The ratio represents the solution mixture and the surface ratio may be different. The first row is for a full monolayer of undecene branches, the second row a mixture of 2 : 1 pentyl magnesium bromide : undecylenyl magnesium bromide. The next two entries are 2 : 1 and 1 : 2 pentyl magnesium bromide : pentenyl magnesium bromide and the final two entries demonstrate the effect of changing the catalyst on a fully undecene terminated layer.

The estimated thickness and contact angle are given in Table 4.5 for the biotin terminated surfaces (after metathesis and amidation). In order to compare the yield of the metathesis reactions the FTIR spectrum of the product was ratioed to the starting alkene surface, to eliminate any contributions from the branched surface, these data are also found in Table 4.5. The estimated thickness of all the layers ranged from 18 – 22 Å. On exchanging two thirds of the undecene branches for pentyl branches the thickness decreased by 4 Å, and the FTIR peak area of the NHS (Table 4.5) and C – H (not shown) regions were lower. The thickness of the exclusively five carbon derived surfaces was 20 Å, slightly higher than the mixed layer, the NHS peak area was also higher while the C-H peak area was lower. This discrepancy may be due to increased growth of oxide. Recall the pentenyl monolayer was more heavily oxidized by KMnO_4 (Section 4.3.1). Changing the catalyst did not have a significant effect. The contact angle of these surfaces remained high (78 – 80 °). Because no significant differences were observed and the exclusively undecene modified surface was found to be chemically and electrically stable, remaining optimization was done on this monolayer using Grubbs 2nd generation catalyst.

Table 4.5 – Thickness Contact Angle and NHS Peak Area of Branched Surfaces after Metathesis and Amidation with BioNH₂

BioNH ₂ derivative	Thickness (± 2 Å)	Contact Angle (± 2 °)	NHS Integrated Area (AU)
undecene	22	80	0.035 ± 0.004
2 : 1 pentane : undecene	18	80	0.028 ± 0.005
2 : 1 pentane : pentene	20	78	0.035 ± 0.003
1 : 2 pentane : pentene	20	80	0.034 ± 0.004
Undecene Grubbs G-I	21	80	0.035 ± 0.06
Undecene Grubbs – Hoveyda GII	22	79	0.038 ± 0.05

Inhibition of non specific protein binding is associated with a hydrophilic interface.^{77,80} Increasing the length of the OEG's was investigated as a route to increasing the hydrophobicity of the surface; it was hypothesized that a longer molecule would compensate for a lower density of molecules on the surface. The three amino terminated OEG compounds investigated were EG3OH, and compounds **12** (containing 6 ethylene glycol units) and **13** (a mixture of lengths centred around 24 ethylene glycol units). These molecules were reacted with the NHS surface and the contact angles are given in Table 4.6.

Table 4.6 – Contact Angle Dependence on OEG Length

	EG3	EG6	EG24
Contact Angle (± 2 °)	80	76	78

No difference in contact angle was observed on incorporation of the longer ethylene glycol chains, indicating the energy required to dehydrate the monolayer is not affected. In the previous chapter unbranched surfaces (Table 3.6) modified with EG3OH had a contact angle of 45 ± 2 °. Based on FTIR the metathesis reaction produces a NHS density approximately 10 % that of the unbranched surface. Compound **13** introduces eight times more ethylene glycol groups per molecule than EG3OH, which means the surfaces should have a similar number of ethylene glycol units attached to them just in a

different orientation (Figure 4.5). The unbranched surface will have short chains relatively close packed versus long chains spaced by some distance; the contact angle measurements indicate changes in orientation result in substantially different wetting properties and by inference ability to inhibit non-specific binding.

Figure 4.5 - Surface Density versus Chain Length of OEG Groups

These experiments indicated it was necessary to increase the density of reactive groups on the surface; having failed to improve the yield of the metathesis reaction a second strategy was explored. The concept was to bind a polymer such as linear poly(ethyleneimine) to the NHS activated surface and then activate this surface with tri-functional **15**. Reaction with LPEI increased the thickness by 27 ± 2 Å and the surface became significantly more hydrophobic (contact angle 48°), which is similar to results reported on gold (thickness ~ 30 Å and contact angle 37°).¹⁹⁶ Upon reaction with **15** very little NHS was observed in the FTIR spectra, due to multiple reactions with the polymer layer.

While linkage to a polymer is a viable option, focus shifted to the alcohol terminated monolayer for several reasons. The polymer layer was relatively thick, total monolayer thickness was 56 ± 2 Å before addition of the biological interface and thicker layers may adversely affect the sensitivity of the electrical measurements. Additionally, the polymer is highly ionizable which may confound electrical measurements. Exploratory work was done with dextran polymers, which are non-ionic and known to inhibit non-specific binding.¹⁹⁷ However, optimization of a dextran interface was expected to be a highly involved process and so full attention was given to optimizing the alcohol terminated surface.

Scheme 4.13 – Route to Increased Density of Reactive Groups

4.3.3 – Alcohol Terminated Monolayers

The first step was to characterize the alcohol terminated layer and ensure its chemical and electrical stability on exposure to water. The Grignard reaction was found to require a longer reaction time (12 – 18 hours) presumably due to increased bulk. As the layer forms it is sterically hindered by the alkyl chains and trimethyl silyl protecting groups. Additionally, this Grignard reagent was also found to be insoluble in THF at

room temperature at the desired concentration 1 M; therefore once formed it was kept at 70 °C and used immediately. The FTIR spectra (Figure 4.6) show the characteristic bands of UDE: a methyl stretch at 2982 cm^{-1} , methylene stretches at 2922 and 2952 cm^{-1} , the carbonyl stretch of the ester group is observed at 1741 cm^{-1} . After the Grignard reaction the alcohol terminated monolayer shows the presence of methylene stretches at 2924 and 2952 cm^{-1} , the remaining carbonyl stretch of the ester group is observed at 1737 cm^{-1} and ketone from the partially reacted ester at 1716 cm^{-1} . There is also a broad peak between $3500 - 3200\text{ cm}^{-1}$ which could be due the alcohol. It should be noted that the trimethylsilyl group was never observed by IR (methyl stretches), because it is sensitive to acid the bond was likely cleaved by the neat acetic acid used in the quench of the Grignard reaction. The thickness was found to increase from $12 \pm 1\text{ \AA}$ to $21 \pm 2\text{ \AA}$ and the contact angle to decrease from 84° to 74° during the transformation from ester to branched alcohol. The SPV of the alcohol surface on average increased but remained within the error bars before and after immersion in water for 18 h ($-90 \pm 22\text{ mV}$ as prepared versus $-131 \pm 26\text{ mV}$ after exposure to water). The HR XPS of the silicon region (Figure 4.7) confirms this branched surface is stable on exposure to water, the number of ML equivalents of SiO_2 were observed to be non detectable as prepared and 0.03 after immersion in water. Close inspection of the SiO_2 region (inset) does show a small peak in the “as prepared” sample however it was difficult to quantify being just above the noise.

Figure 4.6 – FTIR Spectra of UDE and Alcohol Terminated Monolayer

Figure 4.7 – HR XPS of Si2P Region of Alcohol Terminated Monolayer

After optimization, the alcohol surface was found to react with succinic anhydride providing an acid terminated surface (Figure 4.8, Scheme 4.14). The reaction was performed in *N,N*-dimethylformamide with 1 M succinic anhydride and 0.1 M 4-*N,N*-dimethylamino pyridine as catalyst; it was heated at 80 °C for 18 hours. The FTIR spectra displays methylene stretches at 2924 and 2952 cm^{-1} , ester carbonyls (product plus unreacted ethyl ester) at 1737 cm^{-1} , and a carboxylic acid with underlying ketone at 1716

cm^{-1} . Upon activation with NHS, the methylene stretches do not shift, there are imide stretches at 1814 and 1781 cm^{-1} , larger ester carbonyls at 1740 cm^{-1} , and remaining ketone at 1717 cm^{-1} . The peak area of the NHS is $0.15 \pm 0.04 \text{ AU}$ approximately three times larger than the metathesis and about 60 % the peak area of an unbranched surface. After reaction with BioNH_2 , the methylene bands shifted slightly to 2920 and 2950 cm^{-1} , the imide peaks are no longer present, the ester has decreased and the carbonyl is observed at 1735 cm^{-1} , there is ketone at 1717 cm^{-1} , and amide bands at 1656 and 1568 cm^{-1} . This route was put on hold with the success of the isocyanate route because the reaction of succinic anhydride with the alcohol terminated surface requires heating for 18 hours which may lead to oxidation of the substrate, it requires activation on the surface, which leads to deposition of EDC urea, and unreacted acids are ionizable.

Scheme 4.14 - Succinic Anhydride Route to Modified Alcohol Surfaces

Alcohol terminated surface after (A) succinic anhydride, (B) activation with NHS/EDC and (C) reaction with BioNH₂

Figure 4.8 - FTIR Spectra of Succinic Anhydride Route to Modifying the Alcohol Terminated Surface

Immersion of the alcohol terminated monolayer in a neat solution of hexamethylene diisocyanate at 60 °C for ninety minutes was found to cleanly activate the surface. Use of a solution of isocyanate in organic solvent (THF, DCM, or N,N-dimethylformamide) resulted in a much lower yield of free isocyanate. The neat solution favours reaction with an isocyanate in solution versus with one on a neighbouring chain. The FTIR spectra of the isocyanate surface is shown in Figure 4.9, methylene stretches are observed at 2925 and 2952 cm⁻¹, the isocyanate carbonyl at 2272 cm⁻¹, and carbamate stretches at 1699 cm⁻¹ and 1537 cm⁻¹. The breadth and size of the carbamate peak overwhelm any unreacted ester and ketone signals in these samples. It is important to keep this reaction dry as water will hydrolyze the isocyanate revealing an amine which in turn will react with an isocyanate group. In fact, it was hoped that once the isocyanate layer formed if any hydrolyzed the amine would be rapidly reacted to form a non-ionic,

chemically stable urea which could be considered a cross-linking agent in the monolayer. The FTIR spectrum suggests multi-layers have not formed; the isocyanate and carbamate peaks are approximately the same size, and there is no urea signal. After reaction with BioNH₂ the methylene stretches are unshifted at 2924 and 2954 cm⁻¹, the isocyanate is gone and carbamate and urea carbonyl stretches are observed at 1699, 1647 and 1548 cm⁻¹. The broad peak 3200 – 3500 cm⁻¹ is likely a combination of water trapped in the hydrophilic film, and amine N-H stretches of the urea and carbamate functional groups.

Figure 4.9 – FTIR Spectra of Isocyanate Route to Modified Alcohol Surfaces

Scheme 4.15 – Preparation Modified Surfaces via Isocyanate

Reaction of the isocyanate surface with amine was investigated in both DCM and pH 8.0 carbonate buffer to determine how effectively hydrolysis would compete with carbamate formation. Isocyanate terminated wafers were immersed in a 20 mM solution of **12** for 1 hour then rinsed and measured. The samples were then exposed to 0.1 mg/mL of BSA for 1 hour, washed with 2 % Tween-20 in pH 7.4 PBS three times, finally Milli-Q water and then measured again. Two results stand out, firstly the contact angle of these surfaces is $40 \pm 2^\circ$ more hydrophilic than any of the surfaces prepared to date and secondly the thickness does not change on exposure to BSA.

Table 4.7 – Comparison of Isocyanate Reacted in DCM and pH 8.0 Buffer

	Thickness $\pm 3 \text{ \AA}$	Contact Angle $\pm 2^\circ$
DCM	34	40
DCM then BSA	34	39
Carbonate	34	40
Carbonate then BSA	34	40

The next step was to ensure Streptavidin could be captured on a biotin presenting surface. A study was conducted varying the ratio of BioNH₂ and either EG3OH (EG3) or **12** (EG6), Table 4.8. The surfaces containing EG3OH were approximately 5 Å lower in thickness and had a contact angle which was about 12 – 14° higher. Compound **12** is nine atoms longer, considering this an increase of 5 Å is reasonable, the improved wettability could be due to a combination of a longer ethylene glycol chain and increased shielding of the biotin. The BioNH₂ molecule is longer than the EG3OH molecule and approximately the same length as compound **12**; therefore the hydrophobic biotin head group will be more exposed on the EG3OH surface. This is consistent with the observation that more Streptavidin was captured on the EG3OH surface versus the longer hexaethylene glycol containing surface. As the ratio of biotin in the monolayer increased

from 10 to 33 %, a significant increase in the amount of Streptavidin captured was observed, with minimal change in the range of 33 – 66 % BioNH₂. On all of the surfaces which capture > 20 Å of streptavidin the contact angle decreases to less than 20 ° below this point values are not reliably measureable on the instrument.

Table 4.8 – Optimization of Probe : OEG Ratio

	As Prepared		Streptavidin		Δ Thickness (± 4 Å)
	Thickness ± 2 Å	Contact Angle $\pm 2^\circ$	Thickness ± 3 Å	Contact Angle $\pm 2^\circ$	
EG3:Bio 9:1	32	54	49	38	17
EG3:Bio 2:1	31	52	60	< 20	29
EG3:Bio 1:2	31	53	64	< 20	33
EG6:Bio 9:1	36	42	43	36	6
EG6:Bio 2:1	36	41	56	< 20	20
EG6:Bio 1:1	36	40	59	<20	23
EG6:Bio 1:2	36	48	58	< 20	22
BioNH ₂	34	55	65	<20	32

Due to their superior wetting properties, BioNH₂ – EG6 solutions were spotted on a silicon wafer for 1 hour, rinsed off, and the entire sample blocked with compound **12**. Next, the sample was exposed to Cy-3 tagged Streptavidin for one hour, washed with 0.2 % Tween-20, Milli-Q water and blown dry under a stream of nitrogen. The sample was measured in the SKP followed by fluorescence (Figure 4.10), which confirms the conclusions drawn from the thickness measurements. The amount of Streptavidin captured increases with increasing levels of biotin in the spots, and maximum capture is achieved between 33 and 66 % biotin. The fluorescence scanner was not working at this time, however it was noted during collection of the SKP measurement that the surface

potential in the dark was different at each end of the sample. When dipped in water and blown dry the surface potential would drift upwards and stabilize after about 30 minutes; it was hypothesized that this extended drying time was due to the strong interaction of water with the protein. It has been reported that physisorbed water has a significant effect on the conductivity of silicon-on insulator¹⁹⁸ and so drying of the monolayer is likely to cause measurable changes in surface potential. The sample was stored in a nitrogen purge box until the scanner was repaired and indeed protein was observed outside of the spots. The streptavidin outside the spots could be due to biotin reacting with the surface during rinsing, or non-specifically bound protein. The former is more probable, since in later experiments the drop was removed by carefully touching the top of the drop with a filter paper to remove most of the solution, this resulted in lower observable levels of binding outside the spots. Furthermore, thickness measurements consistently showed that non-specific binding of streptavidin was not a problem and the amount of protein captured adjacent to the spots is correlated with the concentration of biotin inside the spot. Options do exist for the optimization of spotting conditions for example microfluidic patterning has been shown to improve the quality and reproducibility of microarrays but was not employed in this work.¹⁹⁹ The important conclusion is that the protein is measurable by SKP and the electrical response is correlated with coverage confirmed by fluorescence and thickness measurements.

Figure 4.10 – SKP and Fluorescence Image of Biotin Patterned Surface after Exposure to Streptavidin

After optimizing the spotting conditions, the surfaces were spotted with 1 : 1 biotin : compound 12 and after 1 hour the spots were removed using a filter paper rinsed with water and then blocked with compound 12. The biotin spots are visible in the SKP image as slight protrusions which become significantly larger on exposure to streptavidin (Figure 4.11 A, C). The fluorescence image indicates the protein is localized on top of the biotin, the sample cracked while being attached to the microarray slide which is why the top half of one spot is missing (Figure 4.11 E). It is important to note that, unlike the unbranched monolayers, there is no pattern in the SPV. It was postulated that the large SPV observed underneath the protein were due to ionization of hydrolyzed carboxylic

acid groups in the monolayer, these data support that conclusion. It also argues for ensuring any future strategies favour a synthetic approach which avoids ionizable groups.

Figure 4.11 – SKP and Fluorescence Images of Biotin Patterned Surface Exposed to Streptavidin using optimized spotting conditions

An important test was to determine whether streptavidin could selectively be measured after exposure of the surface to interferences. The thickness measurements indicate minimal change after exposure to BSA followed by an increase of 20 Å after incubation in streptavidin. (Table 4.9)

Table 4.9 – Thickness Measurements of Selective Capture of Streptavidin versus BSA

	As prepared ± 2 Å	BSA ± 4 Å	BSA $\rightarrow$ SA ± 4 Å	SA ± 4 Å	SA $\rightarrow$ BSA ± 4 Å
EG6:Bio 1 : 1	36	37	57	59	58

A 1 : 1 EG6OH : BioNH₂ patterned surface was prepared as described for Figure 4.10, then exposed first to BSA and then streptavidin. The fluorescence images indicate BSA bound essentially without discrimination across the surface (Figure 4.12 C), whereas streptavidin was localized in the biotin containing regions (Figure 4.12 E). The SKP measurements (Figure 4.12 A,B) confirmed that streptavidin could be detected after initial exposure to BSA; however, comparing the SKP images in Figures 4.11 and 4.12 BSA reduces the signal due to streptavidin. Based on the observation that the SKP response is related to the amount of streptavidin captured (Figure 4.10, Table 4.8) it is likely that non-specifically bound BSA inhibits access to the underlying BioNH₂ groups.

Figure 4.12 – SKP and Fluorescence Images of Patterned Silicon Sample Exposed to BSA and Streptavidin

4.4 – Summary and Conclusions

In this chapter three approaches to modifying the branched surface were explored. For reasons of synthetic expediency and to maximize overlap with standard microarray

chemistry, epoxidation of an alkene terminated surface was investigated. The conditions utilized caused significant damage to the silicon substrate evidenced by loss of C-H stretching intensity in the FTIR spectra, additionally the lack of a characteristic IR signature makes it challenging to determine the success or failure of this reaction. Therefore, oxidation of the alkene to a carboxylic acid was explored. The first method used an acidic, aqueous solution of KMnO_4 which also cleaved substantial quantities of the monolayer even when the chain length of the branches was increased. It was however possible to validate the chemistry using FTIR. A milder oxidation, at neutral pH and with catalytic KMnO_4 , did not remove the monolayer by FTIR, but did oxidize the surface according to XPS measurements.

In contrast, olefin metathesis was found to functionalize the surface without substantially oxidizing the silicon substrate. An increase in oxide content during the metathesis reaction was attributed to extended reaction times in organic solvent. The hydrophobic monolayer may prevent penetration of water but it will be solvated by organic solvent and this may allow impurities to reach the surface since both the alkene and functionalized surfaces are stable in water. Unfortunately, the yield of this reaction was too low to support the binding of streptavidin. Unsuccessful efforts were made to increase first the hydrophilicity, by synthesizing and using longer chain OEG molecules. Next, increasing the number of reactive groups was investigated by binding linear poly(ethyleneimine), the approach was analogous to growing a dendrimer. At each subsequent step, the number of reactive groups would increase; however when it was found that a tri-NHS bound multiple times to the polymer versus being available for

derivitization and considering each step increases the number of ionizable groups work in this area was halted.

Success was found with an alcohol terminated monolayer. It was found to be stable both chemically and electrically, and sufficient reactive groups could be attached to form the most hydrophilic surface obtained in this work. Chemically, the alcohol terminated layer was found to react with both succinic anhydride and hexamethylene diisocyanate, with the latter being the preferred route due to the fact it did not require subsequent activation and a higher density of reactive groups was obtained. The electrical response was found to be sensitive to the amount of biotin captured and the capture of streptavidin reached maximum with a 1 : 1 ratio of biotin to ethylene glycol. When surfaces were pretreated with BSA they were able to subsequently bind streptavidin however the electrical response appears attenuated due to the underlying BSA.

The conclusions are that while chemically many options are available to functionalize the surface, those which satisfy the stringent requirements of a sensor based on crystalline silicon are limited. Additionally, the chemistry must support a biointerface which allows for the specific capture of a target, further limiting the available options. It was shown that branched surfaces are stable for extended periods in aqueous solutions, and that alcohol terminated monolayers functionalized with a diisocyanate support a biological interface which enables specific capture of streptavidin.

Chapter 5 – Overall Conclusions and Claims to Original Research

5.1 – Claims to Original Research

In the 25 years since Linford and Chidsey reported the functionalization of Si-H surfaces a significant amount of knowledge has been acquired describing various approaches to forming Si-C bound monolayers and their derivatization. A major driver for this field is the desire to combine the ability to tailor the physical – chemical properties of the interface afforded by organic chemistry with the highly sensitive semi-conducting properties of silicon to form hybrid devices. In order to move from the lab to real-world applications each step of the process must be fully understood and the surfaces must be able to withstand the environmental conditions in which they will operate, which was the underlying theme of this research project. I collected and analyzed all data with the exception of STM and HREELS experiments which were performed by Brian Eves, Chaoyang Fan and Greg Lopinski.

The unexpected reaction of alkenes with oxidized silicon was described. It was shown that alkenes react with the silanol groups of oxidized silicon, providing the thermodynamic (Markovnikov) product at lower temperature and the kinetic (anti-Markovnikov) addition product at higher temperature. A comparison of the reactivity of alkenes with hydrogen terminated and oxidized silicon was published in *Langmuir*.¹²⁶

This line of research was continued based on the observation that an alkane, used as a control molecule, reacted with the Si-H surface. The reactivity was attributed to cracking of the hydrocarbon on oxidized silicon surfaces. Generation of these reactive intermediates was found to accelerate the reaction of alkenes with the Si-H surface.

Monolayers formed in Pyrex Schlenk tubes were more hydrophilic than those formed in PTFE suggesting hydrocarbon oxidation. This work has been published in *Langmuir*.²⁰⁰

Having gained an understanding of the reaction mechanism, the stability of the interface was studied under biologically relevant conditions. It was found that increasing the density of the hydrophilic hydrocarbon layer by forming a first generation dendrimer stabilized the chemical and electrical properties of the silicon – carbon interface. The ambient measurements were published in the *Journal of Physical Chemistry C* as part of a detailed electrochemical study conducted by Xiaomin Bin.¹³⁶

The final step was to modify the branched surface to enable capture and measurement of a biological interaction. A number of approaches were investigated. On stable alkene terminated branched surfaces, oxidative conditions damaged the chemical and electrical properties of the silicon substrate, and while olefin metathesis did not degrade the interface the yield was low and protein could not be specifically captured. Derivatization of an alcohol terminated branched surface with a diisocyanate produced a monolayer which stabilized the interface and supported the specific recognition of protein. A U.S. patent application covering this work has been filed.²⁰¹

While not included in this thesis, silane chemistry was developed to modify silicon oxide surfaces with biotin in order to monitor the Streptavidin binding using novel photonic waveguide evanescent field sensors. My contribution was to provide a protocol for chemical modification of the sensors. This involved studying the chemical stability, interfacial physical properties and an estimate of the amount of Streptavidin captured. The chemistry was optimized on silicon wafers and then transferred to the optical devices and is published in *Optics Express*⁹⁷ and *Optics Letters*⁹⁸.

5.2 – Overall Conclusions

A model describing the design criteria of an ideal monolayer for electrical biosensing on silicon was built based on the concepts explored in this thesis. (Figure 5.1). The silicon substrate requires protection from oxygen, water and other contaminants in order to stabilize the physical and electrical properties of the interface. A dense hydrophobic hydrocarbon layer accomplishes this. Branching via the Grignard reaction divorces the packing density from the lattice spacing of the silicon allowing a higher density alkyl layer. On top of this, a region must be grafted which enables the specific interaction of surface bound probes with their target molecule. A sufficiently dense OEG layer serves this purpose. The underlying challenge is to accomplish these goals while minimizing (preferably eliminating) the presence of ionizable groups (such as amines, or acids) as they have been shown to contribute substantially to the electrical response.

Figure 5.1 – Model of Monolayer

The stringent criteria laid out above are met by the branched monolayer described in this thesis. The alcohol terminated layer stabilizes the electrical properties and inhibits silicon oxidation after extended exposure to water. After modification with biotin,

capture of the target protein Streptavidin was measurable even when the surface was pre-exposed to BSA, demonstrating specificity to the target. Most convincingly, the electrical response correlated with thickness and optical measurements. In conclusion, the goals of the research project were met – a methodology for the development of biosensing platforms on hydrogen terminated silicon was identified.

5.3 – Opportunities

Demonstrating the detection of Streptavidin on a surface which is chemically and electrically stable lays a solid foundation for future work. The critical properties of an ideal monolayer and the analytical techniques to validate them have been identified. Owing to its stability and versatility the alcohol terminated layer is a good starting point for optimization. The functionalization could be generalized by replacing the diisocyanate linker with an activating group that has enhanced stability in water and hydrolyzes back to the alcohol, for example, nitrophenylcarbamate.

This thesis has demonstrated that silicon is well suited to the measurement of ionizable groups and small-molecule protein interactions. Two applications where label-free electrical sensing can have significant impact are kinase profiling and small-molecule microarray analysis. Kinases are a family of proteins which play a role in intracellular signaling. They function by installing a phosphate group onto substrates such as peptides, amino acids, sugars and nucleotides. Electrical detection is ideally suited to measure the incorporation of this charged group. Additionally, Mrksich and coworkers measured phosphorylation of surface bound peptides using mass-spectrometry demonstrating surface compatibility.²⁰² Stuart Schreiber's group pioneered the study of proteins with small-molecule microarrays.^{203,204} In this work, the microarrays are

fabricated on glass with detection carried out via fluorescence. The commonality in these applications is a small neutral probe interacting with a charged target.

References

- (1) Brandriss, S.; Margel, S. *Langmuir* **1993**, *9*, 1232-1240.
- (2) Sigal, G. B.; Bamdad, C.; Barberis, A.; Strominger, J.; Whitesides, G. M. *Analytical Chemistry* **1996**, *68*, 490-497.
- (3) Piva, P. G.; DiLabio, G. A.; Pitters, J. L.; Zikovsky, J.; Rezeq, M.; Dogel, S.; Hofer, W. A.; Wolkow, R. A. *Nature* **2005**, *435*, 658-661.
- (4) O'Mara, W. C.; Herring, R. B.; Hunt, L. P. *Handbook of Semiconductor Silicon Technology*; William Andrew Publishing/Noyes, 1990.
- (5) Bardeen, J.; Brattain, W. H. *Physical Review* **1948**, *74*, 230-231.
- (6) Riorden, M.; Hoddeson, L. *Crystal Fire: The Invention of the Transistor and the Birth of the Information Age*; Norton & Company: New York, 1997.
- (7) Smith, C. S. *Physical Review* **1954**, *94*, 42-49.
- (8) McNamara, A. G. *Review of Scientific Instruments* **1962**, *33*, 330-333.
- (9) Pearson, G. L. *Review of Scientific Instruments* **1948**, *19*, 263-265.
- (10) Bergveld, P. *IEEE Transactions on Biomedical Engineering* **1970**, *BM17*, 70-71.
- (11) Chapin, D. M.; Fuller, C. S.; Pearson, G. L. *Journal of Applied Physics* **1954**, *25*, 676-677.
- (12) Sagiv, J. *Journal of the American Chemical Society* **1980**, *102*, 92-98.
- (13) Linford, M. R.; Chidsey, C. E. D. *Journal of the American Chemical Society* **1993**, *115*, 12631-12632.
- (14) Kern, W.; Puotinen, D. A. *Rca Review* **1970**, *31*, 187-206.
- (15) Zhuravlev, L. T. *Langmuir* **1987**, *3*, 316-318.
- (16) Allara, D. L.; Parikh, A. N.; Rondelez, F. *Langmuir* **1995**, *11*, 2357-2360.
- (17) Higashi, G. S.; Chabal, Y. J.; Trucks, G. W.; Raghavachari, K. *Applied Physics Letters* **1990**, *56*, 656-658.
- (18) Allongue, P.; Kasparian, J. *Microscopy Microanalysis Microstructures* **1994**, *5*, 257-267.
- (19) Sneh, O.; Cameron, M. A.; George, S. M. *Surface Science* **1996**, *364*, 61-78.
- (20) Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *Journal of the American Chemical Society* **1991**, *113*, 12-20.
- (21) Lehn, J. M. *Science* **2002**, *295*, 2400-2403.
- (22) Smith, E. A.; Chen, W. *Langmuir* **2008**, *24*, 12405-12409.
- (23) Pasternack, R. M.; Amy, S. R.; Chabal, Y. J. *Langmuir* **2008**, *24*, 12963-12971.
- (24) Krumpfer, J. W.; Fadeev, A. Y. *Langmuir* **2006**, *22*, 8271-8272.
- (25) Eves, B. J.; Lopinski, G. P. *Surface Science* **2005**, *579*, L89-L96.
- (26) Weinberger, B. R.; Peterson, G. G.; Eschrich, T. C.; Krasinski, H. A. *Journal of Applied Physics* **1986**, *60*, 3232-3234.
- (27) Allongue, P.; de Villeneuve, C. H.; Morin, S.; Boukherroub, R.; Wayner, D. D. M. *Electrochimica Acta* **2000**, *45*, 4591-4598.
- (28) Huang, Y. C.; Flidr, J.; Newton, T. A.; Hines, M. A. *Journal of Chemical Physics* **1998**, *109*, 5025-5035.

- (29) Flidr, J.; Huang, Y. C.; Hines, M. A. *Journal of Chemical Physics* **1999**, *111*, 6970-6981.
- (30) Flidr, J.; Huang, Y. C.; Newton, T. A.; Hines, M. A. *Chemical Physics Letters* **1999**, *302*, 85-90.
- (31) Trucks, G. W.; Raghavachari, K.; Higashi, G. S.; Chabal, Y. J. *Physical Review Letters* **1990**, *65*, 504-507.
- (32) Faggini, M. F.; Green, S. K.; Clark, I. T.; Queeney, K. T.; Hines, M. A. *Journal of the American Chemical Society* **2006**, *128*, 11455-11462.
- (33) Linford, M. R.; Fenter, P.; Eisenberger, P. M.; Chidsey, C. E. D. *Journal of the American Chemical Society* **1995**, *117*, 3145-3155.
- (34) Sieval, A. B.; Vleeming, V.; Zuilhof, H.; Sudholter, E. J. R. *Langmuir* **1999**, *15*, 8288-8291.
- (35) Bansal, A.; Li, X. L.; Lauermann, I.; Lewis, N. S.; Yi, S. I.; Weinberg, W. H. *Journal of the American Chemical Society* **1996**, *118*, 7225-7226.
- (36) Terry, J.; Linford, M. R.; Wigren, C.; Cao, R. Y.; Pianetta, P.; Chidsey, C. E. D. *Applied Physics Letters* **1997**, *71*, 1056-1058.
- (37) Kim, N. Y.; Laibinis, P. E. *Journal of the American Chemical Society* **1998**, *120*, 4516-4517.
- (38) Boukherroub, R.; Morin, S.; Bensebaa, F.; Wayner, D. D. M. *Langmuir* **1999**, *15*, 3831-3835.
- (39) Fellah, S.; Boukherroub, R.; Ozanam, F.; Chazalviel, J. N. *Langmuir* **2004**, *20*, 6359-6364.
- (40) Nemanick, E. J.; Hurley, P. T.; Brunschwig, B. S.; Lewis, N. S. *Journal of Physical Chemistry B* **2006**, *110*, 14800-14808.
- (41) Cicero, R. L.; Linford, M. R.; Chidsey, C. E. D. *Langmuir* **2000**, *16*, 5688-5695.
- (42) Terry, J.; Linford, M. R.; Wigren, C.; Cao, R. Y.; Pianetta, P.; Chidsey, C. E. D. *Journal of Applied Physics* **1999**, *85*, 213-221.
- (43) Effenberger, F.; Gotz, G.; Bidlingmaier, B.; Wezstein, M. *Angewandte Chemie-International Edition* **1998**, *37*, 2462-2464.
- (44) Boukherroub, R.; Morin, S.; Sharpe, P.; Wayner, D. D. M.; Allongue, P. *Langmuir* **2000**, *16*, 7429-7434.
- (45) Cai, W.; Lin, Z.; Strother, T.; Smith, L. M.; Hamers, R. J. *Journal of Physical Chemistry B* **2002**, *106*, 2656-2664.
- (46) Stewart, M. P.; Buriak, J. M. *Journal of the American Chemical Society* **2001**, *123*, 7821-7830.
- (47) Sun, Q. Y.; de Smet, L.; van Lagen, B.; Wright, A.; Zuilhof, H.; Sudholter, E. J. R. *Angewandte Chemie-International Edition* **2004**, *43*, 1352-1355.
- (48) de Smet, L.; Pukin, A. V.; Stork, G. A.; de Vos, C. H. R.; Visser, G. M.; Zuilhof, H.; Sudholter, E. J. R. *Carbohydrate Research* **2004**, *339*, 2599-2605.
- (49) Eves, B. J.; Sun, Q. Y.; Lopinski, G. P.; Zuilhof, H. *Journal of the American Chemical Society* **2004**, *126*, 14318-14319.
- (50) Sun, Q. Y.; de Smet, L.; van Lagen, B.; Giesbers, M.; Thune, P. C.; van Engelenburg, J.; de Wolf, F. A.; Zuilhof, H.; Sudholter, E. J. R. *Journal of the American Chemical Society* **2005**, *127*, 2514-2523.
- (51) Buriak, J. M.; Allen, M. J. *Journal of the American Chemical Society* **1998**, *120*, 1339-1340.

- (52) Allongue, P.; de Villeneuve, C. H.; Pinson, J. *Electrochimica Acta* **2000**, *45*, 3241-3248.
- (53) Wagner, P.; Nock, S.; Spudich, J. A.; Volkmuth, W. D.; Chu, S.; Cicero, R. L.; Wade, C. P.; Linford, M. R.; Chidsey, C. E. D. *Journal of Structural Biology* **1997**, *119*, 189-201.
- (54) Sieval, A. B.; Demirel, A. L.; Nissink, J. W. M.; Linford, M. R.; van der Maas, J. H.; de Jeu, W. H.; Zuilhof, H.; Sudholter, E. J. R. *Langmuir* **1998**, *14*, 1759-1768.
- (55) Asanuma, H.; Lopinski, G. P.; Yu, H. Z. *Langmuir* **2005**, *21*, 5013-5018.
- (56) Faucheux, A.; Gouget-Laemmel, A. C.; de Villeneuve, C. H.; Boukherroub, R.; Ozanam, F.; Allongue, P.; Chazalviel, J. N. *Langmuir* **2006**, *22*, 153-162.
- (57) Perring, M.; Dutta, S.; Arafat, S.; Mitchell, M.; Kenis, P. J. A.; Bowden, N. B. *Langmuir* **2005**, *21*, 10537-10544.
- (58) Boukherroub, R.; Wayner, D. D. M. *Journal of the American Chemical Society* **1999**, *121*, 11513-11515.
- (59) Yang, M.; Teeuwen, R. L. M.; Giesbers, M.; Baggerman, J.; Arafat, A.; de Wolf, F. A.; van Hest, J. C. M.; Zuilhof, H. *Langmuir* **2008**, *24*, 7931-7938.
- (60) Liu, Y. J.; Navasero, N. M.; Yu, H. Z. *Langmuir* **2004**, *20*, 4039-4050.
- (61) Strother, T.; Hamers, R. J.; Smith, L. M. *Nucleic Acids Research* **2000**, *28*, 3535-3541.
- (62) Ara, M.; Tsuji, M.; Tada, H. *Surface Science* **2007**, *601*, 5098-5102.
- (63) Clare, T. L.; Clare, B. H.; Nichols, B. M.; Abbott, N. L.; Hamers, R. J. *Langmuir* **2005**, *21*, 6344-6355.
- (64) Bocking, T.; Kilian, K. A.; Hanley, T.; Ilyas, S.; Gaus, K.; Gal, M.; Gooding, J. J. *Langmuir* **2005**, *21*, 10522-10529.
- (65) Bocking, T.; Killan, K. A.; Gaus, K.; Gooding, J. J. *Langmuir* **2006**, *22*, 3494-3496.
- (66) Plass, K. E.; Liu, X. L.; Brunschwig, B. S.; Lewis, N. S. *Chemistry of Materials* **2008**, *20*, 2228-2233.
- (67) Dutta, S.; Perring, M.; Barrett, S.; Mitchell, M.; Kenis, P. J. A.; Bowden, N. B. *Langmuir* **2006**, *22*, 2146-2155.
- (68) Hurley, P. T.; Nemanick, E. J.; Brunschwig, B. S.; Lewis, N. S. *Journal of the American Chemical Society* **2006**, *128*, 9990-9991.
- (69) Ciampi, S.; Bocking, T.; Kilian, K. A.; James, M.; Harper, J. B.; Gooding, J. J. *Langmuir* **2007**, *23*, 9320-9329.
- (70) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, 1164-1167.
- (71) Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*, 4383-4385.
- (72) Ostuni, E.; Chapman, R. G.; Liang, M. N.; Meluleni, G.; Pier, G.; Ingber, D. E.; Whitesides, G. M. *Langmuir* **2001**, *17*, 6336-6343.
- (73) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605-5620.
- (74) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M. *Journal of the American Chemical Society* **2000**, *122*, 8303-8304.
- (75) Chapman, R. G.; Ostuni, E.; Yan, L.; Whitesides, G. M. *Langmuir* **2000**, *16*, 6927-6936.
- (76) Luk, Y. Y.; Kato, M.; Mrksich, M. *Langmuir* **2000**, *16*, 9604-9608.

- (77) Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. *Journal of the American Chemical Society* **2003**, *125*, 9359-9366.
- (78) Feldman, K.; Hahner, G.; Spencer, N. D.; Harder, P.; Grunze, M. *Journal of the American Chemical Society* **1999**, *121*, 10134-10141.
- (79) Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis, P. E. *Journal of Physical Chemistry B* **1998**, *102*, 426-436.
- (80) Wang, R. L. C.; Kreuzer, H. J.; Grunze, M. *Journal of Physical Chemistry B* **1997**, *101*, 9767-9773.
- (81) Hoffmann, C.; Tovar, G. E. M. *Journal of Colloid and Interface Science* **2006**, *295*, 427-435.
- (82) Cerruti, M.; Fissolo, S.; Carraro, C.; Ricciardi, C.; Majumdar, A.; Maboudian, R. *Langmuir* **2008**, *24*, 10646-10653.
- (83) Lasseter, T. L.; Clare, B. H.; Abbott, N. L.; Hamers, R. J. *Journal of the American Chemical Society* **2004**, *126*, 10220-10221.
- (84) Li, Z.; Chen, Y.; Li, X.; Kamins, T. I.; Nauka, K.; Williams, R. S. *Nano Letters* **2004**, *4*, 245-247.
- (85) Stern, E.; Klemic, J. F.; Routenberg, D. A.; Wyrembak, P. N.; Turner-Evans, D. B.; Hamilton, A. D.; LaVan, D. A.; Fahmy, T. M.; Reed, M. A. *Nature* **2007**, *445*, 519-522.
- (86) Bocking, T.; Gal, M.; Gaus, K.; Gooding, J. J. *Aust. J. Chem.* **2005**, *58*, 660-663.
- (87) Gorostiza, P.; de Villeneuve, C. H.; Sun, Q. Y.; Sanz, F.; Wallart, X.; Boukherroub, R.; Allongue, P. *Journal of Physical Chemistry B* **2006**, *110*, 5576-5585.
- (88) Bansal, A.; Lewis, N. S. *Journal of Physical Chemistry B* **1998**, *102*, 4058-4060.
- (89) Fidelis, A.; Ozanam, F.; Chazalviel, J. N. *Surface Science* **2000**, *444*, L7-L10.
- (90) Yu, H. B.; Webb, L. J.; Ries, R. S.; Solares, S. D.; Goddard, W. A.; Heath, J. R.; Lewis, N. S. *Journal of Physical Chemistry B* **2005**, *109*, 671-674.
- (91) Puniredd, S. R.; Assad, O.; Haick, H. *Journal of the American Chemical Society* **2008**, *130*, 13727-13734.
- (92) Puniredd, S. R.; Assad, P. O.; Haick, H. *Journal of the American Chemical Society* **2008**, *130*, 9184-9185.
- (93) Voicu, R.; Boukherroub, R.; Bartzoka, V.; Ward, T.; Wojtyk, J. T. C.; Wayner, D. D. M. *Langmuir* **2004**, *20*, 11713-11720.
- (94) Lie, L. H.; Mirkin, M. V.; Hakkarainen, S.; Houlton, A.; Horrocks, B. R. *Journal of Electroanalytical Chemistry* **2007**, *603*, 67-80.
- (95) Pike, A. R.; Ryder, L. C.; Horrocks, B. R.; Clegg, W.; Connolly, B. A.; Houlton, A. *Chemistry-a European Journal* **2005**, *11*, 344-353.
- (96) Hirano-Iwata, A.; Yamaguchi, R.; Miyamoto, K.; Kimura, Y.; Niwano, M. *Journal of Applied Physics* **2009**, *105*, 11.
- (97) Xu, D. X.; Densmore, A.; Delage, A.; Waldron, P.; McKinnon, R.; Janz, S.; Lapointe, J.; Lopinski, G.; Mischki, T.; Post, E.; Cheben, P.; Schmid, J. H. *Optics Express* **2008**, *16*, 15137-15148.
- (98) Densmore, A.; Xu, D. X.; Janz, S.; Waldron, P.; Mischki, T.; Lopinski, G.; Delage, A.; Lapointe, J.; Cheben, P.; Lamontagne, B.; Schmid, J. H. *Optics Letters* **2008**, *33*, 596-598.
- (99) Cai, W.; Peck, J. R.; van der Weide, D. W.; Hamers, R. J. *Biosensors & Bioelectronics* **2004**, *19*, 1013-1019.

- (100) Stern, E.; Vacic, A.; Reed, M. A. *IEEE Transactions on Electron Devices* **2008**, 55, 3119-3130.
- (101) Chen, S. Y.; Bommer, J. G.; van der Wiel, W. G.; Carlen, E. T.; van den Berg, A. *Acs Nano* **2009**, 3, 3485-3492.
- (102) Dzyadevych, S. V.; Soldatkin, A. P.; Korpan, Y. I.; Arkhypova, V. N.; El'skaya, A. V.; Chovelon, J. M.; Martelet, C.; Jaffrezic-Renault, N. *Analytical and Bioanalytical Chemistry* **2003**, 377, 496-506.
- (103) Cui, Y.; Wei, Q. Q.; Park, H. K.; Lieber, C. M. *Science* **2001**, 293, 1289-1292.
- (104) Faber, E. J.; Sparreboom, W.; Groeneveld, W.; de Smet, L.; Bommer, J.; Olthuis, W.; Zuilhof, H.; Sudholter, E. J. R.; Bergveld, P.; van den Berg, A. *Chemphyschem* **2007**, 8, 101-112.
- (105) Bunimovich, Y. L.; Shin, Y. S.; Yeo, W. S.; Amori, M.; Kwong, G.; Heath, J. R. *Journal of the American Chemical Society* **2006**, 128, 16323-16331.
- (106) Atkins, P. W. *Physical Chemistry*; 5 ed.; W.H. Freeman and Co.: New York, 1994.
- (107) Ulman, A. *An Introduction to Ultrathin Organic Films From Langmuir-Blodgett to Self-Assembly*; Academic Press Inc.: San Diego, 1991.
- (108) Wasserman, S. R.; Whitesides, G. M.; Tidswell, I. M.; Ocko, B. M.; Pershan, P. S.; Axe, J. D. *Journal of the American Chemical Society* **1989**, 111, 5852-5861.
- (109) Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. E. D. *Journal of the American Chemical Society* **1987**, 109, 3559-3568.
- (110) Strother, T.; Cai, W.; Zhao, X. S.; Hamers, R. J.; Smith, L. M. *Journal of the American Chemical Society* **2000**, 122, 1205-1209.
- (111) <http://www.kelvinprobe.info/>.
- (112) Nauka, K.; Li, Z.; Kamins, T. I. *Materials Science-Poland* **2005**, 23, 653-661.
- (113) Thompson, M.; Cheran, L. E.; Zhang, M. Q.; Chacko, M.; Huo, H.; Sadeghi, S. *Biosensors & Bioelectronics* **2005**, 20, 1471-1481.
- (114) Sinensky, A. K.; Belcher, A. M. *Nature Nanotechnology* **2007**, 2, 653-659.
- (115) Thompson, M.; Cheran, L. E.; Zhang, M. Q.; Chacko, M.; Huo, H.; Sadeghi, S. **2005**, p 1471-1481.
- (116) Wojtyk, J. T. C.; Tomietto, M.; Boukherroub, R.; Wayner, D. D. M. *Journal of the American Chemical Society* **2001**, 123, 1535-1536.
- (117) Aizawa, M.; Buriak, J. M. *Journal of the American Chemical Society* **2005**, 127, 8932-8933.
- (118) Yang, L.; Lua, Y. Y.; Lee, M. V.; Linford, M. R. *Accounts of Chemical Research* **2005**, 38, 933-942.
- (119) Lopinski, G. P.; Fabre, B.; Wayner, D. D. M. *Submitted to Journal of Physical Chemistry B*.
- (120) de Smet, L.; Pukin, A. V.; Sun, Q. Y.; Eves, B. J.; Lopinski, G. P.; Visser, G. M.; Zuilhof, H.; Sudholter, E. J. R. *Appl. Surf. Sci.* **2005**, 252, 24-30.
- (121) Eves, B. J.; Lopinski, G. P. *Langmuir* **2006**, 22, 3180-3185.
- (122) Chatgililoglu, C.; Guarini, A.; Guerrini, A.; Seconi, G. *Journal of Organic Chemistry* **1992**, 57, 2207-2208.
- (123) Liu, Y.; Yamazaki, S.; Yamabe, S.; Nakato, Y. *Journal of Materials Chemistry* **2005**, 15, 4906-4913.
- (124) Scheres, L.; Arafat, A.; Zuilhof, H. *Langmuir* **2007**, 23, 8343-8346.

- (125) Mitchell, S. A. *Journal of Physical Chemistry B* **2003**, *107*, 9388-9396.
- (126) Mischki, T. K.; Donkers, R. L.; Eves, B. J.; Lopinski, G. P.; Wayner, D. D. M. *Langmuir* **2006**, *22*, 8359-8365.
- (127) Wang, S. D.; Dong, X.; Lee, C. S.; Lee, S. T. *Journal of Physical Chemistry B* **2005**, *109*, 9892-9896.
- (128) Ishikawa, H.; Yoda, E.; Kondo, J. N.; Wakabayashi, F.; Domen, K. *Journal of Physical Chemistry B* **1999**, *103*, 5681-5686.
- (129) Babitz, S. M.; Williams, B. A.; Miller, J. T.; Snurr, R. Q.; Haag, W. O.; Kung, H. H. *Applied Catalysis a-General* **1999**, *179*, 71-86.
- (130) Narbeshuber, T. F.; Brait, A.; Seshan, K.; Lercher, J. A. *Journal of Catalysis* **1997**, *172*, 127-136.
- (131) Buriak, J. M. *Chemical Reviews* **2002**, *102*, 1271-1308.
- (132) Koehler, B. G.; Mak, C. H.; Arthur, D. A.; Coon, P. A.; George, S. M. *Journal of Chemical Physics* **1988**, *89*, 1709-1718.
- (133) Schulze, G.; Henzler, M. *Surface Science* **1983**, *124*, 336-350.
- (134) de Smet, L.; Zuilhof, H.; Sudholter, E. J. R.; Lie, L. H.; Houlton, A.; Horrocks, B. R. *Journal of Physical Chemistry B* **2005**, *109*, 12020-12031.
- (135) Woods, M.; Carlsson, S.; Hong, Q.; Patole, S. N.; Lie, L. H.; Houlton, A.; Horrocks, B. R. *Journal of Physical Chemistry B* **2005**, *109*, 24035-24045.
- (136) Bin, X. M.; Mischki, T. K.; Fan, C. Y.; Lopinski, G. P.; Wayner, D. D. M. *J. Phys. Chem. C* **2007**, *111*, 13547-13553.
- (137) Sieval, A. B.; van den Hout, B.; Zuilhof, H.; Sudholter, E. J. R. *Langmuir* **2001**, *17*, 2172-2181.
- (138) Lakatos, L.; Szitty, G.; Silhavy, D.; Burgyan, J. *Embo Journal* **2004**, *23*, 876-884.
- (139) Mello, C. C.; Conte, D. *Nature* **2004**, *431*, 338-342.
- (140) Guo, S.; Kempfues, K. J. *Cell* **1995**, *81*, 611-620.
- (141) Fire, A.; Xu, S. Q.; Montgomery, M. K.; Kostas, S. A.; Driver, S. E.; Mello, C. C. *Nature* **1998**, *391*, 806-811.
- (142) Grishok, A.; Tabara, H.; Mello, C. C. *Science* **2000**, *287*, 2494-2497.
- (143) Voinnet, O. *Trends in Genetics* **2001**, *17*, 449-459.
- (144) Zamore, P. D. *Nature Structural Biology* **2001**, *8*, 746-750.
- (145) Sagan, S. M.; Koukiekolo, R.; Rodgers, E.; Goto, N. K.; Pezacki, J. P. *Angewandte Chemie-International Edition* **2007**, *46*, 2005-2009.
- (146) Koukiekolo, R.; Sagan, S. M.; Pezacki, J. P. *Febs Letters* **2007**, *581*, 3051-3056.
- (147) Vargason, J. M.; Szitty, G.; Burgyan, J.; Hall, T. M. T. *Cell* **2003**, *115*, 799-811.
- (148) Ye, K. Q.; Malinina, L.; Patel, D. J. *Nature* **2003**, *426*, 874-878.
- (149) Zamore, P. D. *Current Biology* **2004**, *14*, R198-R200.
- (150) Lichty, J. J.; Malecki, J. L.; Agnew, H. D.; Michelson-Horowitz, D. J.; Tan, S. *Protein Expression and Purification* **2005**, *41*, 98-105.
- (151) DeChancie, J.; Houk, K. N. *Journal of the American Chemical Society* **2007**, *129*, 5419-5429.
- (152) Wilchek, M.; Bayer, E. A.; Livnah, O. *Immunology Letters* **2006**, *103*, 27-32.
- (153) Leckband, D. E.; Schmitt, F. J.; Israelachvili, J. N.; Knoll, W. *Biochemistry* **1994**, *33*, 4611-4624.
- (154) Lahiri, J.; Ostuni, E.; Whitesides, G. M. *Langmuir* **1999**, *15*, 2055-2060.

- (155) Butler, J. E.; Ni, L.; Nessler, R.; Joshi, K. S.; Suter, M.; Rosenberg, B.; Chang, J.; Brown, W. R.; Cantarero, L. A. *Journal of Immunological Methods* **1992**, *150*, 77-90.
- (156) Li, Y. C.; Ou, L. M. L.; Yu, H. Z. *Analytical Chemistry* **2008**, *80*, 8216-8223.
- (157) Pugliese, L.; Coda, A.; Malcovati, M.; Bolognesi, M. *Journal of Molecular Biology* **1993**, *231*, 698-710.
- (158) Gonzalez, M.; Argarana, C. E.; Fidelio, G. D. *Biomolecular Engineering* **1999**, *16*, 67-72.
- (159) Fischer, L. M.; Wright, V. A.; Guthy, C.; Yang, N.; McDermott, M. T.; Buriak, J. M.; Evoy, S. *Sensors and Actuators B-Chemical* **2008**, *134*, 613-617.
- (160) Dubey, G.; Lopinski, G. P.; Rosei, F. *Applied Physics Letters* **2007**, *91*, 3.
- (161) Fernandez-Megia, E.; Correa, J.; Rodriguez-Meizoso, I.; Riguera, R. *Macromolecules* **2006**, *39*, 2113-2120.
- (162) Sato, H.; Hayashi, E.; Yamada, N.; Yatagai, M.; Takahara, Y. *Bioconjugate Chemistry* **2001**, *12*, 701-710.
- (163) Zhou, M.; Nakatani, E.; Gronenberg, L. S.; Tokimoto, T.; Wirth, M. J.; Hruby, V. J.; Roberts, A.; Lynch, R. M.; Ghosh, I. *Bioconjugate Chemistry* **2007**, *18*, 323-332.
- (164) Gotz, M. G.; James, K. E.; Hansell, E.; Dvorak, J.; Seshadri, A.; Sojka, D.; Kopacek, P.; McKerrow, J. H.; Caffrey, C. R.; Powers, J. C. *Journal of Medicinal Chemistry* **2008**, *51*, 2816-2832.
- (165) Huang, Z. H.; Park, J. I.; Watson, D. S.; Hwang, P.; Szoka, F. C. *Bioconjugate Chemistry* **2006**, *17*, 1592-1600.
- (166) Mitchell, S. A.; Ward, T. R.; Wayner, D. D. M.; Lopinski, G. P. *Journal of Physical Chemistry B* **2002**, *106*, 9873-9882.
- (167) McCarty, L. S.; Winkleman, A.; Whitesides, G. M. *Journal of the American Chemical Society* **2007**, *129*, 4075-4088.
- (168) Zhang, X. C.; Kumar, S.; Chen, J. H.; Teplyakov, A. V. *Surface Science* **2009**, *603*, 2445-2457.
- (169) Moraillon, A.; Gouget-Laemmel, A. C.; Ozanam, F.; Chazalviel, J. N. *Journal of Physical Chemistry C* **2008**, *112*, 7158-7167.
- (170) Venyaminov, S. Y.; Kalnin, N. N. *Biopolymers* **1990**, *30*, 1243-1257.
- (171) Rigler, P.; Ulrich, W. P.; Hoffmann, P.; Mayer, M.; Vogel, H. *Chemphyschem* **2003**, *4*, 268-275.
- (172) Grosvenor, A. P.; Biesinger, M. C.; Smart, R. S.; McIntyre, N. S. *Surface Science* **2006**, *600*, 1771-1779.
- (173) Du Roure, O.; Debiemme-Chouvy, C.; Malthete, J.; Silberzan, P. *Langmuir* **2003**, *19*, 4138-4143.
- (174) Lee, J. K.; Kim, Y. G.; Chi, Y. S.; Yun, W. S.; Choi, I. S. *Journal of Physical Chemistry B* **2004**, *108*, 7665-7673.
- (175) Ji, J.; Cooper, W. C.; Dreisinger, D. B.; Peters, E. *Journal of Applied Electrochemistry* **1995**, *25*, 642-650.
- (176) Wagner, C. D.; Davis, L. E.; Zeller, M. V.; Taylor, J. A.; Raymond, R. H.; Gale, L. H. *Surface and Interface Analysis* **1981**, *3*, 211-225.
- (177) Jin, H.; Kinser, C. R.; Bertin, P. A.; Kramer, D. E.; Libera, J. A.; Hersam, M. C.; Nguyen, S. T.; Bedzyk, M. J. *Langmuir* **2004**, *20*, 6252-6258.
- (178) Vyvyan, J. R.; Meyer, J. A.; Meyer, K. D. *Journal of Organic Chemistry* **2003**, *68*, 9144-9147.

- (179) Smith, M. B. *Organic Synthesis*; McGraw-Hill Inc.: New York., 1994.
- (180) Pasquinet, E.; Rocca, P.; Richalot, S.; Gueritte, F.; Guenard, D.; Godard, A.; Marsais, F.; Queguiner, G. *Journal of Organic Chemistry* **2001**, *66*, 2654-2661.
- (181) Nelson, D. J.; Henley, R. L. *Tetrahedron Letters* **1995**, *36*, 6375-6378.
- (182) Smith, M. B. *Organic Synthesis*; McGraw-Hill Inc.: New York, 1994.
- (183) Wasserman, S. R.; Tao, Y. T.; Whitesides, G. M. *Langmuir* **1989**, *5*, 1074-1087.
- (184) Lemieux, R. U.; Vonrudloff, E. *Canadian Journal of Chemistry-Revue Canadienne De Chimie* **1955**, *33*, 1701-1709.
- (185) Assad, O.; Puniredd, S. R.; Stelzner, T.; Christiansen, S.; Haick, H. *Journal of the American Chemical Society* **2008**, *130*, 17670-+.
- (186) Mizuno, H.; Buriak, J. M. *Journal of the American Chemical Society* **2008**, *130*, 17656-17657.
- (187) Fairfull-Smith, K.; Redon, P. M. J.; Haycock, J. W.; Williams, N. H. *Tetrahedron Letters* **2007**, *48*, 1317-1319.
- (188) Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G. *Journal of the American Chemical Society* **2008**, *130*, 9642-+.
- (189) Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117-7140.
- (190) Bin, X. M.; Mischki, T. K.; Fan, C. Y.; Lopinski, G. P.; Wayner, D. D. M. *Journal of physical Chemistry C* **2007**, *111*, 13547-13553.
- (191) Trost, B. M. *Accounts of Chemical Research* **2002**, *35*, 695-705.
- (192) Kilian, K. A.; Bocking, T.; Ilyas, S.; Gaus, K.; Jessup, W.; Gal, M.; Gooding, J. J. *Advanced Functional Materials* **2007**, *17*, 2884-2890.
- (193) Gress, A.; Volkel, A.; Schlaad, H. *Macromolecules* **2007**, *40*, 7928-7933.
- (194) Platzek, J. G., K.D.; WO, Ed.; Schering Aktiengesellschaft: Germany, 2005, p 28.
- (195) Yasuda, M.; Shimizu, K.; Yamasaki, S.; Baba, A. *Organic & Biomolecular Chemistry* **2008**, *6*, 2790-2795.
- (196) Yan, L.; Huck, W. T. S.; Zhao, X. M.; Whitesides, G. M. *Langmuir* **1999**, *15*, 1208-1214.
- (197) Monchaux, E.; Vermette, P. *Langmuir* **2007**, *23*, 3290-3297.
- (198) Dubey, G.; Lopinskia, G. P.; Rosei, F. *Applied Physics Letters* **2007**, *91*, 3.
- (199) Eddings, M. A.; Miles, A. R.; Eckman, J. W.; Kim, J.; Rich, R. L.; Gale, B. K.; Myszka, D. G. *Analytical Biochemistry* **2008**, *382*, 55-59.
- (200) Mischki, T. K.; Lopinski, G. P.; Wayner, D. D. M. *Langmuir* **2009**, *25*, 5626-5630.
- (201) Mischki, T. K.; Lopinski, G. P.; Wayner, D. D. M. *U.S. Patent Pending* **2009**.
- (202) Houseman, B. T.; Huh, J. H.; Kron, S. J.; Mrksich, M. *Nature Biotechnology* **2002**, *20*, 270-274.
- (203) Bradner, J. E.; McPherson, O. M.; Mazitschek, R.; Barnes-Seeman, D.; Shen, J. P.; Dhaliwal, J.; Stevenson, K. E.; Duffner, J. L.; Park, S. B.; Neuberg, D. S.; Nghiem, P.; Schreiber, S. L.; Koehler, A. N. *Chemistry & Biology* **2006**, *13*, 493-504.
- (204) MacBeath, G.; Schreiber, S. L. *Science* **2000**, *289*, 1760-1763.

tert-butyl 3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propylcarbamate (5)

(+)-Biotin N-hydroxysuccinimide ester (7)

Date: 23 Jun 2009
 Document's Title: TM04-036 biotin
 Spectrum Title: TM04-036-B, dmsd-d6, 1H, 24Feb09
 Frequency (MHz): (f1) 400.132
 Original Points Count: (f1) 16384
 Actual Points Count: (f1) 32768
 Acquisition Time (sec): (f1) 1.9923
 Spectral Width (ppm): (f1) 20.552
 Pulse Program: ZG30
 Temperature: 296.16
 Number of Scans: 32
 Acq. Date: Tue Feb 24 07:36:35 AM

tert-butyl 3-{2-[2-(3-biotin amidopropoxy)ethoxy]ethoxy}propylcarbamate (8)

Date: 23 Jun 2009
Document's Title: TM02-128 boc EG3 biotin-2
Spectrum Title: TM02-128, CDCl₃, 1H, 29Jan07
Frequency (MHz): (f1) 400.132
Original Points Count: (f1) 16384
Actual Points Count: (f1) 32768
Acquisition Time (sec): (f1) 1.9923
Spectral Width (ppm): (f1) 20.552
Pulse Program: ZG30
Temperature: 300
Number of Scans: 32
Acq. Date: Mon Jan 29 12:44:04 AM

3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}-1-biotinyl-propanamide (9)

Date: 23 Jun 2009
Document's Title: TM04-042 Biotin EG3 NH2-1
Spectrum Title: TM04-042, CDCl₃, 1H, 2Mar09
Frequency (MHz): (f1) 400.132
Original Points Count: (f1) 16384
Actual Points Count: (f1) 32768
Acquisition Time (sec): (f1) 1.9923
Spectral Width (ppm): (f1) 20.552
Pulse Program: ZG30
Temperature: 299.16
Number of Scans: 32
Acq. Date: Mon Mar 02 08:29:12 AM

HR-XPS Spectra and fitting for Ni-NTA, Table 3.3.

HR-XPS of N1s region of Ni-NTA Surface

HR-XPS of C1s region of Ni-NTA Surface

HR-XPS and Fitting for Silicon Region of Surfaces used in Stability Study Table 3.7

l-amino-PEG 1000 (13). *N*-hydroxysuccinimide 4-pentenoate (14).

Date:
16 Jul 2009

Document's Title:
TM03-168 N-HS pentene

Spectrum Title:
TM03-173, CDCl₃, 1H, 5Mar09

Frequency (MHz):
(f1) 400.132

Original Points Count:
(f1) 16394

Actual Points Count:
(f1) 32768

Acquisition Time (sec):
(f1) 1.9923

Spectral Width (ppm):
(f1) 20.552

Pulse Program:
ZG30

Temperature:
297.16

Number of Scans:
32

Acq. Date:
Thu Mar 05 08:50:07 AM

Trimesic NHS (15).

Date: 15 Jul 2009
 Document's Title: TMD3-150 Trimesyl NHS.mrc
 Spectrum Title: TMD3-150 C1, CDCl₃, 1H, 14July08
 Frequency (MHz): (f1) 400.132
 Original Points Count: (f1) 16384
 Actual Points Count: (f1) 32768
 Acquisition Time (sec): (f1) 1.9923
 Spectral Width (ppm): (f1) 20.552
 Pulse Program: ZG30
 Temperature: 683.16
 Number of Scans: 32
 Acq. Date: Mon Jul 14 08:25:16 AM

ppm

11-bromo-1-trimethylsilyloxyundecane (16).

Date: 15 Jul 2009
 Document's Title: TMD3-187 TMSundecand.mrc
 Spectrum Title: TMD3-187, CDCl₃, 1H, 26Sept08
 Frequency (MHz): (f1) 400.132
 Original Points Count: (f1) 16384
 Actual Points Count: (f1) 32768
 Acquisition Time (sec): (f1) 1.9923
 Spectral Width (ppm): (f1) 20.552
 Pulse Program: ZG30
 Temperature: 683.16
 Number of Scans: 32
 Acq. Date: Fri Sep 26 08:00:38 AM

ppm

FTIR Spectra showing preparation of epoxide terminated silicon surface

FTIR Spectra showing preparation of carboxylic acid terminated surface via KMnO_4 / AcOH on pentenyl modified UDE surface.

FTIR Spectra showing preparation of carboxylic acid terminated surface via KMnO_4 / AcOH on undecylenyl modified UDE surface.

Thickness, Contact angle and Silicon Oxide Content for Alcohol Terminated Monolayer

	Th ± 1 Å	CA $\pm 2^\circ$	SPV (mV)	ML SiO ₂
UDE	12	84	-91 $\pm$ 16	
ROH	20	74	-90 $\pm$ 22	n.d.
18h H ₂ O			131 $\pm$ 26	0.04

HR XPS of C1s Region for Alcohol Terminated Monolayer

A) Alcohol as prepared

B) Alcohol as prepared