



Synthesis and characterization of anti-body self-assembling monolayers on the surface of a quartz crystal microbalance for use as a biosensor
by Napawan Kositruangchai

A thesis submitted in partial fulfillment Of the requirements for the degree of Master of Science in
Chemical Engineering
Montana State University
© Copyright by Napawan Kositruangchai (2000)

Abstract:

A biosensor that could detect biological pathogens in contaminated drinking water would be very useful for preventing illnesses caused by these pathogens. The objective -of this work has been to bind anti-bodies for a bacterial pathogen to a Quartz Crystal Microbalance (QCM) in order, to make such a biosensor. A QCM is one of the most popular devices that can be used as a transducer for a biosensor because it is very small, inexpensive, portable, rapid, and has utility in a flow cell, and minimal plectrical and electronic requirements. The QCM is one type of acoustic wave sensor that can propagate in a specially cut crystal. This crystal will oscillate with changing frequency when the surface changes in an alternating electric potential. When the mass is increased, it will decrease the oscillation frequency so when molecules adsorb to the QCM surface, the frequency in the output signal will decrease.

In order to make an effective biosensor, a self-assembled monolayer was grown on the QCM surface. This SAM contains a thiol-terminated acid having an amide bond with an adipic dihydrazide terminus. The dihydrazide is then reacted with antibody to covalently bond the antibody to a gold surface.

X-ray photoelectron spectroscopy (XPS) and angle dependent XPS (20,40, 60 and 80 degrees) have been used to confirm the binding of Au-S in alkane and acid thiol, and the presence of amide group in adipic dihydrazide and antibody. XPS not only confirmed the binding to the surface, but also has been used to observe thickness and orientation of layers. In addition, hydrated and dehydrated QCM were observed for the orientation of antibodies.

XPS analysis, elution with tween20, and performance of the QCM sensor tests all confirm that the antibody is covalently bound to the acid thiol surface but not to the alkane thiol surface. The QCM, which were reacted with acid thiol, dihydrazide and antibody, showed a significant drop in frequency when exposed to solution containing the antigen. The results indicated that the method developed is effective for making a biosensor capable of detecting bacterial toxins in water.

SYNTHESIS AND CHARACTERIZATION OF ANTI-BODY SELF-ASSEMBLING
MONOLAYERS ON THE SURFACE OF A QUARTZ CRYSTAL MICROBALANCE
FOR USE AS A BIOSENSOR

by

Napawan Kositruangchai

A thesis submitted in partial fulfillment
Of the requirements for the degree

of

Master of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY – BOZEMAN
Bozeman, Montana

August 2000

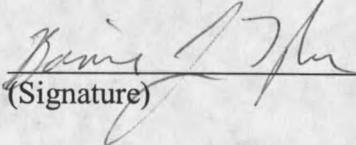
N378
K8467

APPROVAL

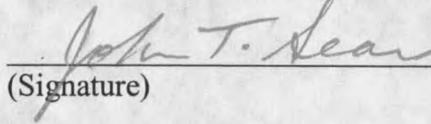
of a thesis submitted by

Napawan Kositruangchai

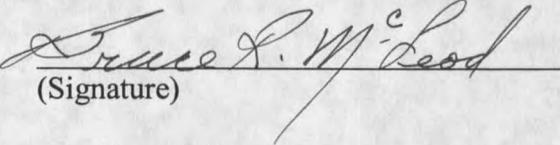
This thesis has been read by each member of the thesis committee, has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Bonnie J. Tyler  8/14/00
(Signature) Date

Approved for the Department of Chemical Engineering

Dr. John T. Sears  8/14/00
(Signature) Date

Approved for the College of Graduate Studies

Dr. Bruce McLeod  8-30-00
(Signature) Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for the master's degree at Montana State University-Bozeman, I agree that the library shall make it available to borrowers under the rules of the library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with the "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature Napaicon Kositruangchi

Date 8-14-00

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. Bonnie Tyler, my research and thesis advisor, for her invaluable advice, support, and encouragement. I would also like to thank my committee, Dr. Ron Larsen and Dr. John Mandell, for their guidance and kindness.

My special thanks go to Brenda Spangler for her help to prepare antibody, QCM test, and valuable suggestions, and Steve Hunt for his friendship and invaluable assistance with the experimental results and suggestion in writing this thesis. Thanks are also extended to Recep Avci and James Anderson of the Imaging and Chemical Analysis laboratory at Montana State University for their help with XPS work.

I would also like to thank Dr. John Sears for admittance to the master program at Chemical Engineering Department, and the staffs of the Chemical Engineering Department who played important roles in my graduate studies.

Finally, my parents and family deserve a special mention for providing support and encouragement throughout my life.

TABLE OF CONTENTS

LIST OF TABLES	VII
LIST OF FIGURES	IX
ABSTRACT	XI
1. INTRODUCTION	1
Motivation and Objectives	1
2. BACKGROUND	5
Antibodies	5
Structure of an Antibody (Immunoglobulin) Molecule	5
The Quartz Crystal Microbalance (QCM)	6
Self-Assembled Monolayers (SAMs)	8
Monolayers of Alkyl Thiol on Gold	9
X-Ray Photoelectron Spectroscopy	11
Principle of the Technique	11
General Uses.....	13
Common Applications.....	14
The Vacuum System	14
X-Ray Source	15
Analyzers.....	16
Data System.....	18
Accessories.....	19
Quantitation and Data Interpretation - Line Identification.....	19
Quantitation and Data Interpretation-Quantitative Analysis.....	20
Angle Dependent X-Ray Photoelectron Spectroscopy	22
Theory	24
3. SAMPLE PREPARATION	26
Cleaning Sample.....	26
Preparation of 16 – Mercapto Hexadecane ($\text{HS}(\text{CH}_2)_{15}\text{CH}_3$)	26
Preparation of 16 – Mercapto Hexadecanoic Acid ($\text{HS}(\text{CH}_2)_{15}\text{COOH}$)	27
Activation of Carboxylic-Acid-Terminated SAM with Dihydrazide.....	27
The QCM Sensor.....	27

TABLE OF CONTENTS (CONTINUED)

The QCM Flow Cell.....	28
Preparation of the Oxidized Antibody.....	28
Test for Presence of Aldehyde	29
Experimental Procedure.....	30
Techniques for Obtaining Spectra.....	30
 4. RESULTS AND DISCUSSION.....	 32
Cleaning Results.....	32
Results of Cleaned Gold Slides Linked with Alkane Thiol	34
Results of Alkane Thiol on the QCMs	36
Results of Acid Thiol on the QCMs.....	39
Results of Alkane Thiol +Adipic Dihydrazide.....	42
Results of Acid Thiol +Adipic dihydrazide	44
Results of Alkane Thiol + Adipic Dihydrazide + Antibody	48
Results of Acid Thiol + Adipic Dihydrazide + Antibody	50
Thickness Results.....	53
Thickness Results of Alkane Thiol on the QCMs.....	54
Thickness Results of Acid Thiol on the QCMs.....	55
Angle-Resolved XPS Results.....	56
Results of Hydrated and Dehydrated QCM	64
Biosensor Results	65
 5. CONCLUSIONS AND FUTURE WORK.....	 73
Conclusions.....	73
Future Work	75
 REFERENCES	 76
 APPENDIX A.....	 78
 MATLAB M-FILE	 78
RegExp M-file.....	79
SVDExp M-file	81
Plot2 M-file.....	83
Plot3a M-file	84

LIST OF TABLES

Table	Page
1. Adsorption of Terminally Functionalized Alkyl Chains from Ethanol onto Gold.	10
2. Energies and Widths of Some Characteristic X-Ray Lines (eV).....	16
3. Atomic Percent Components of Cleaning Results for the Gold Slides	32
4. Atomic Percent Components of Cleaning Results of Gold Slides Using Base Bath.....	33
5. Atomic Percent Components of Cleaning Results of QCM Using Base Bath + Nanopure Water + Hot Air Drying.....	33
6. Atomic Percent Components of Gold Slides Using Base Bath + Nanopure Water + Hot Air Drying + Mercapto Hexadecane (Alkane Thiol)	34
7. Atomic Percent Components of Gold Slides by Base Bath + Nanopure Water + Hot Air Drying + Mercapto Hexadecanoic Acid (Acid Thiol) + Dihydrazide ..	35
8. Atomic Percent Components of Alkane Thiol Results on QCM from XPS as a Function of Photoelectron Take-off Angle.....	36
9. Atomic Percent Components of Acid Thiol on QCM from XPS as a Function of Photoelectron Take-off Angle	39
10. Results of C1s Curve Fit for Acid Thiol on QCM.....	39
11. Atomic Percent Components of Alkane Thiol +Adipic Dihydrazide on QCM from XPS as a Function of Photoelectron Take-off Angle	42
12. Atomic Percent Components of Tween 20 Results of Alkane Thiol +Adipic Dihydrazide Compared with Alkane Thiol +Adipic Dihydrazide	42
13. Atomic Percent Components of Acid Thiol + Adipic Dihydrazide on QCM from XPS as a Function of Photoelectron Take-off Angle	44

LIST OF TABLES (CONTINUED)

Table	Page
14. Atomic Percent Components of Compared between Tween 20 Results of Acid Thiol + Dihydrazide and Acid Thiol + Dihydrazide	44
15. Results of C1s Curve Fit for Acid Thiol + Dihydrazide.....	44
16. Atomic Percent Components of Alkane Thiol + Adipic Dihydrazide + Antibody on QCM from XPS as a Function of Photoelectron Take-off Angle	48
17. Atomic Percent Components of Compared between Tween 20 Results of Alkane Thiol + Dihydrazide + Antibody and Alkane Thiol + Dihydrazide + Antibody	48
18. Atomic Percent Components of Acid Thiol + Adipic Dihydrazide + Antibody on QCM from XPS as a Function of Photoelectron Take-off Angle	50
19. Atomic Percent Components of Compared between Tween 20 Results of Acid Thiol + Dihydrazide + Antibody and Acid Thiol + Dihydrazide + Antibody....	50
20. Results of C1s Curve Fit for Acid Thiol + Dihydrazide + Antibody.....	50
21. Thickness Results of Alkane Thiol on the QCMs	54
22. Thickness Results of Acid Thiol on the QCMs	55
23. Atomic Percent Components for Results of Dehydrated QCM from XPS as a Function of Photoelectron Take-off Angle.....	64
24. Atomic Percent Components for Results of Hydrated QCM from XPS as a Function of Photoelectron Take-off Angle.....	64

LIST OF FIGURES

Figure	Page
1. Reaction scheme for the functionalization and coupling of antibody to a self-assembled monolayer on a gold electrode surface. The antibody was oxidized with sodium periodate prior to addition.	4
2. The antibody molecule.....	6
3. A schematic view of the forces in a self-assembled monolayer	8
4. A self-assembled monolayer of alkanethiols on a gold surface.....	10
5. The basic XPS experiment.....	13
6. Cartoon illustrating the angle dependence on sampling depth	23
7. A schematic diagram of the PHI Model 5600 MultiTechnique system.....	31
8. Graph of XPS C1s photoemission line for alkane thiol on QCM.....	37
9. Graph of XPS S2p photoemission line for alkane thiol	38
10. Graph of XPS C1s photoemission line for acid thiol on QCM.....	40
11. Graph of XPS S2p photoemission line for acid thiol.....	41
12. Graph of XPS C1s photoemission line for tween 20 of alkane thiol + dihydrazide (lower peak) and alkane thiol + dihydrazide (upper peak)	43
13. Graph of XPS C1s photoemission line for tween 20 of acid thiol (upper peak) + dihydrazide and acid thiol + dihydrazide (lower peak)	46
14. Graph of XPS C1s photoemission line for acid thiol +dihydrazide.....	47
15. Graph of XPS C1s photoemission line for tween 20 results of alkane thiol + dihydrazide + antibody (lower peak) and alkane thiol + dihydrazide + antibody (upper peak).....	49

LIST OF FIGURES (CONTINUED)

Figure	Page
16. Graph of XPS C1s photoemission line for acid thiol + dihydrazide + antibody	51
17. Graph of XPS C1s photoemission line for tween 20 results (upper peak) of acid thiol + dihydrazide + antibody and acid thiol + dihydrazide + antibody	52
18. Concentration depth profiles for alkane thiol on QCM calculated from ARXPS results using the regularization and SVD algorithm.....	58
19. Concentration depth profiles for alkane thiol + dihydrazide on QCM calculated from ARXPS results using the regularization and SVD algorithm.....	59
20. Concentration depth profiles for alkane thiol + dihydrazide + antibody on QCM calculated from ARXPS results using the regularization and SVD algorithm... ..	60
21. Concentration depth profiles for acid thiol on QCM calculated from ARXPS results using the regularization and SVD algorithm.....	61
22. Concentration depth profiles for acid thiol + dihydrazide on QCM calculated from ARXPS results using the regularization and SVD algorithm.....	62
23. Concentration depth profiles for acid thiol + dihydrazide + antibody on QCM calculated from ARXPS results using the regularization and SVD algorithm... ..	63
24. Response of QCM with acid thiol layer when exposed to PBS buffer.....	66
25. Response of QCM with acid thiol + dihydrazide + antibody layer when exposed to PBS buffer	67
26. Response of QCM with alkane thiol + dihydrazide + antibody layer when exposed to PBS buffer	68
27. Response of QCM with acid thiol layer when exposed to 100 μ g antigen-LT... ..	69
28. Response of QCM with alkane thiol + dihydrazide + antibody when exposed to 50 μ g antigen-LT.....	70
29. Response of QCM with acid thiol + dihydrazide + antibody when exposed to 50 μ g antigen-LT.....	71
30. Response of QCM with acid thiol + dihydrazide + antibody when exposed to 100 μ g antigen-LT.....	72

ABSTRACT

A biosensor that could detect biological pathogens in contaminated drinking water would be very useful for preventing illnesses caused by these pathogens. The objective of this work has been to bind anti-bodies for a bacterial pathogen to a Quartz Crystal Microbalance (QCM) in order to make such a biosensor. A QCM is one of the most popular devices that can be used as a transducer for a biosensor because it is very small, inexpensive, portable, rapid, and has utility in a flow cell, and minimal electrical and electronic requirements. The QCM is one type of acoustic wave sensor that can propagate in a specially cut crystal. This crystal will oscillate with changing frequency when the surface changes in an alternating electric potential. When the mass is increased, it will decrease the oscillation frequency so when molecules adsorb to the QCM surface, the frequency in the output signal will decrease.

In order to make an effective biosensor, a self-assembled monolayer was grown on the QCM surface. This SAM contains a thiol-terminated acid having an amide bond with an adipic dihydrazide terminus. The dihydrazide is then reacted with antibody to covalently bond the antibody to a gold surface.

X-ray photoelectron spectroscopy (XPS) and angle dependent XPS (20, 40, 60 and 80 degrees) have been used to confirm the binding of Au-S in alkane and acid thiol, and the presence of amide group in adipic dihydrazide and antibody. XPS not only confirmed the binding to the surface, but also has been used to observe thickness and orientation of layers. In addition, hydrated and dehydrated QCM were observed for the orientation of antibodies.

XPS analysis, elution with tween20, and performance of the QCM sensor tests all confirm that the antibody is covalently bound to the acid thiol surface but not to the alkane thiol surface. The QCM, which were reacted with acid thiol, dihydrazide and antibody, showed a significant drop in frequency when exposed to solution containing the antigen. The results indicated that the method developed is effective for making a biosensor capable of detecting bacterial toxins in water.

CHAPTER 1

INTRODUCTION

Motivation and Objectives

In the environment, there are a multitude of diseases that are transmitted by contaminated water. These diseases can cause illness in many people. Food-borne and water-borne enteric pathogens[1] are a main cause of illness in the US and in the world. Devices used to measure for microbial contaminants are difficult to use and normally unavailable. This thesis is part of an ongoing project to develop a portable rapid biosensor that could be used for on-site detection of water-borne and food-borne microbial pathogens in water.

A biosensor is a tool that can measure concentrations of biologically important chemicals and also uses a biological material to detect the analyte [2]. Typically, a biosensor consists of two parts: a receptor and a transducer. The receptor selectively reacts with a particular analyte of interest. The transducer can convert the interaction of the receptor and the analyte to a quantifiable signal.

The biosensor considered in this work is based on a Quartz Crystal Microbalance (QCM). The QCM is one of the most popular tools in mass-sensitive detection [3] that can be used as a transducer for biosensors. The advantages of the QCM are that it is very small, inexpensive, portable, rapid, efficient, has minimal electrical and electronic

requirement, can be used in a flow cell, and provides for sequential refinement of positive signals. QCM is one type of acoustic wave sensor that can propagate in a specially cut crystal. The crystal will oscillate when the surface changes under an alternating electric potential with changing frequency. When the mass is increased, the crystal will decrease the oscillation frequency as described in the Sauerbrey equation (equation 1) [4].

$$\Delta f = -\frac{f_R}{\rho_Q d A} \Delta m = -\frac{f_R^2}{N A} \Delta m \quad (1)$$

Where Δf is the frequency change, f_R is the resonant frequency, ρ_Q is the density of the crystal, d is the thickness of the crystal, A is the area of the electrodes, Δm is the mass change, and N is the frequency constant of the quartz.

In order to make the QCM an effective biosensor, the QCM has to be coated with suitable capture agents. These capture agents must have a high degree of specificity and affinity for the targets [1]. After they are linked to the QCM, these capture agents must remain stable and tightly associated with the electrode during sample analysis.

The objective of this thesis has been to couple an antibody to the gold surface of a QCM so that it can be used as a receptor for the sensor. For this experiment, Anti-LT (anti-*Escherichia coli* heat – labile enterotoxin) gift of W. Ceiplak (Corixa Corp.) was used as a capture agent. For the improved biosensor, we have used a method for organizing a derivatized self-assembled monolayer (SAM) on the gold surface of the QCM. The SAM contains a thiol-terminated alkane having an amide bonded with adipic dihydrazide terminus. This dihydrazide is then reacted with the antibody to covalently bond the antibody to the gold surface [1]. The system of SAM is shown in the cartoon of

Figure 1. XPS (x-ray photoelectron spectroscopy) and angle dependent XPS were used to examine the surface.

The objectives of this experiment were to: (i) optimize reaction conditions to obtain high density and reactivity of the antibody on the surface, (ii) characterize the thickness and orientation of antibody layer, and (iii) determine whether antibody is covalently bound to the adipic dihydrazide.

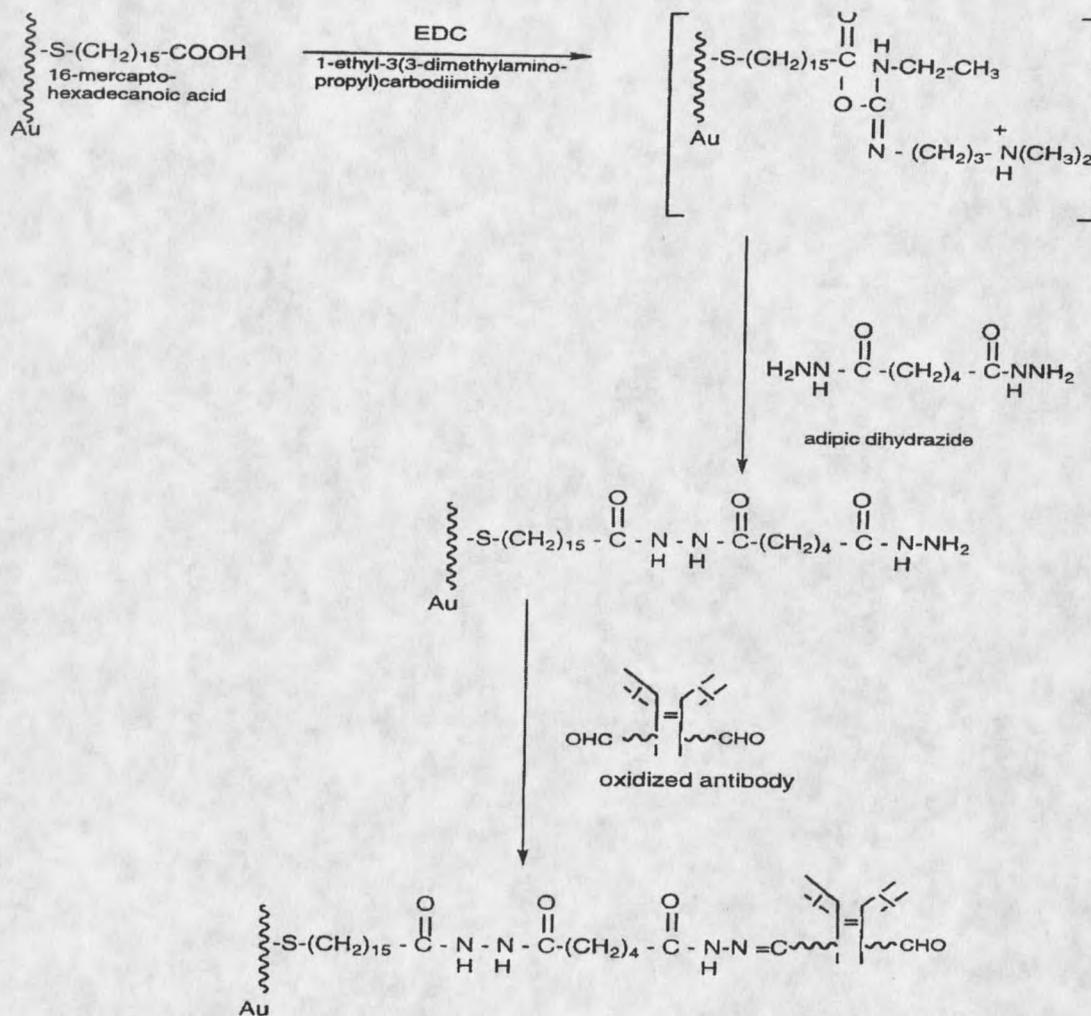


Figure 1. Reaction scheme for the functionalization and coupling of antibody to a self-assembled monolayer on a gold electrode surface. The antibody was oxidized with sodium periodate prior to addition.

CHAPTER 2

BACKGROUND

Antibodies

Antibodies are host proteins produced in reaction to the antigens that are the foreign molecules in the body [5]. Antibodies are produced by plasma cells and the precursor-B-lymphocytes, then circulate through the body blood and lymph. An antibody binds specifically with an antigen. Then the antigen-antibody complexes are removed from circulation by macrophages through phagocytosis. Because of this specific antibody-antigen binding, antibodies are very important reagents to use in immunological research and clinical diagnostics.

Structure of an Antibody (Immunoglobulin) Molecule

Antibody (or immunoglobulin) molecules are glycoproteins composed of one or more units, each unit containing four polypeptide chains [5] two identical light chains (L) and the other two identical heavy chains (H) in Figure 2. The difference of the amino-terminal end of each polypeptide chain shows variation in amino acid composition and is referred to as the variable (V) regions. The light chain (L) consists of a variable domain (V_L) and a constant domain (C_L), and the heavy chain (H) consists of a variable domain (V_H) and three constant domains (C_H^1 , C_H^2 , and C_H^3). The amount of amino

acids, along with the molecular weight of each heavy chain, is approximately twice that of each light chain. The chains are bound by a combination of noncovalent interactions and in most antibodies, by covalent interchain disulfide bonds that form a bilaterally symmetric structure. The V regions of H and L chains include the antigen

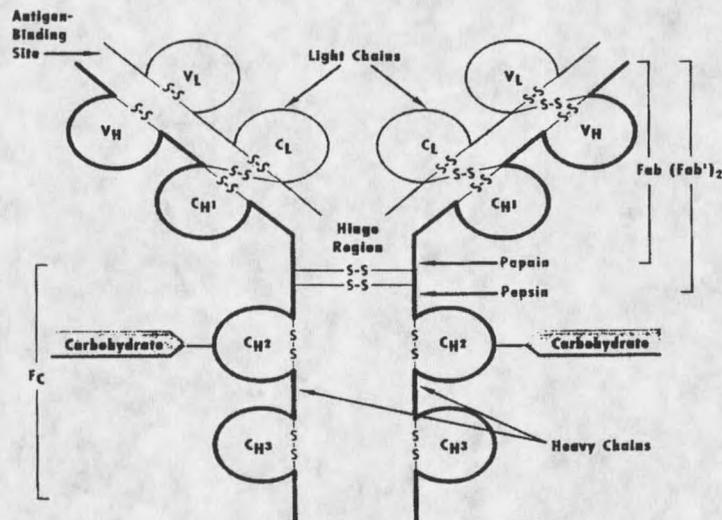


Figure 2. The antibody molecule.

binding sites of the immunoglobulin (Ig) molecules, and each Ig monomer chain has two antigen binding sites so the molecule is bivalent. The hinge region is the area of the heavy chains between the first and second C region domains, and is bonded together by disulfide bonds. The length of hinge region is flexible so the distance between the two antigen binding sites can vary.

The Quartz Crystal Microbalance (QCM)

The quartz crystal microbalance is a simple tool and is increasing in use for mass-sensitive detection due to improvements in experimental procedures [3]. QCMs are used increasingly in liquid phase especially in bioaffinity measurements for analytical or

research purposes. A QCM is a piezoelectric device that can be used to measure specific analytes in contaminated water or in water used to wash contaminated food [1]. The advantages of a QCM are very small size, utility in flow cell, sequential refinement of positive signal, minimal electrical and electronic requirement, adaptability to microfluidic techniques, and inexpensive fabrication [1]. The QCM is a type of acoustic wave sensor. The acoustic wave can propagate in specially cut crystals that mechanically oscillate when subjected to an alternating electric potential. When a proper alternating electrical potential is directed to gold electrodes on opposite sides of the piezoelectric crystal, this crystal will oscillate at a characteristic frequency. The primary theory describing the relationship between frequency change and mass change is described by the Sauerbrey (equation 1). The limit of this equation is the assumption that the mass deposition forms a rigid and thin film and the mass sensitivity is uniform across the full surface.

The QCM can be used as an effective biosensor when the surface of the QCM has been coated with appropriate capture agents, and the capture agents must be stable and tightly associated with the electrode for use in the flow cell. The QCM surface must not react with other substances in the sample. The capture agents must have a high degree of specificity and affinity for the target [1]. Sensitivity and detection limits have to be adequately high to be biologically relevant. For this work, we have used a derivatized self-assembled monolayer (SAM) on the gold surface of the QCM.

Self-Assembled Monolayers (SAMs)

Self-assembled monolayers are continuing to provoke widespread interest in recent years because of their utility in technological applications[6-8]. The areas of current interest include lubrication, adhesion promotion/resistance, corrosion inhibition, lithographic patterning, microelectronic fabrication, wetting adhesion, and for making structures and materials for biological interfaces, membranes and electrochemistry.

Self-assembled monolayers are molecular assemblies that are linked spontaneously to surface phases by the immersion of an appropriate substrate in a solution of an active surfactant in an organic solvent [6]. There are many types of SAM methods including alkanethiols on gold, silver and copper; dialkyl sulfides on gold, dialkyl disulfides on gold; carboxylic acids on aluminum oxide, and silver and silane on quartz and silicon.

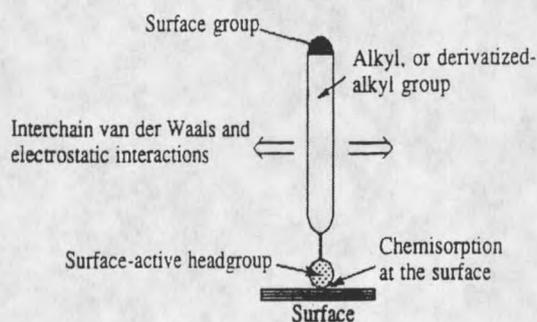


Figure 3. A schematic view of the forces in a self-assembled monolayer

A self assembling surfactant molecule can be separated into three parts in

Figure 3. The first part is the head group that provides the most exothermic process: chemisorption. The strong molecular substrate interactions result in pinning of the head group. This bond can be a covalent bond such as Au-S bond in the case of alkanethiols on gold or ionic $\text{CO}_2^- \text{Ag}^+$ bond in case of carboxylic acids on AgO/Ag. The energy associated with this bond, for example thiolate on gold, is about 40-45 kcal/mol [6]. The second part is the alkyl chain. Van der Waals interactions are the main forces between simple alkyl chains ($\text{C}_n\text{H}_{2n+1}$), on the other hand in some case when a polar group is substituted into the alkyl chain, the electrostatic interactions are energetically more important than the van der Waals force. The last part is the chain terminal group. The structure and orientation of the chain terminating functionality (x) will dictate the surface chemistry of the organic thin film. To provide a simple alkyl chain, the methyl (CH_3) group is widely studied.

Monolayers of Alkane Thiol on Gold

In 1983, Nuzzo and Allara published the first paper in this area. The formation of well-defined organic surface phases by the immersion of a clean gold substrate in a dilute solution of a long chain, ω -substituted dialkyldisulfide showed that dialkyldisulfides (RS-SR) form oriented monolayers on gold surface. Later it was found that sulfur compounds link strongly to gold, silver and copper surfaces as reported in (Table1) [6]. Gold is commonly used because it is reasonably inert which allows substrates to be manipulated in air without concern for contamination. Gold does not have a stable oxide so it can be used in ambient conditions. The reason that SAMs consisting of n-alkane thiol adsorbed on gold are popular is because this system had already proven to be thermally stable,

