Assembling Surface Linker Chemistry and Minimization of Biosensor Non-Specific Adsorption

by

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Abstract

The development of a preparation method for biosensing interface on hydroxylated surfaces is presented in this manuscript. This method involves the use of thiosulfonate-based linkers to construct robust and durable SAMs (Self-Assembling Monolayers) onto hydroxylated surfaces. The resulting SAMs have the ability to chemoselectively immobilize thiol-containing molecules under aqueous condition in a single, straightforward, reliable and coupling-free manner. This method is then implemented in the construction of biosensing interfaces for EMPAS (ElectroMagnetic Piezoelectric Acoustic Sensor) dedicated to the detection of avidin. Efforts were devoted to improving the performance of the biosensing interface by reducing/preventing non-specific adsorption. Improvements were systematically observed with the incorporation of an OEG (Oligo(Ethylene Glycol)) backbone and a diluent (i. e. SAM molecules without a head functional group). The hydroxyl head function and short length of the diluent were crucial in preventing non-specific adsorption. This showed that both
enthalpy-driven and entropy-driven mechanisms work in conjunction in order for the surface to achieve high resistance in non-specific adsorption. Expansions of this method to silicon nitride (Si₃N₄), aluminium nitride (AlN) and gold (Au) were pursued. Lastly, a previously undocumented EMPAS coil circuit design was reported and discussed.
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Jack Sheng
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# Table of Contents

Abstract ii
Acknowledgment iv
Table of Contents v
List of Abbreviations ix
List of Figures xi

## I. Introduction

I.1. Biosensors 1

I. 2. Interactions between the Biosensing Interface and the Sample 3

I. 3. Self-Assembling Monolayer Chemistry 4
   I. 3. 1. Background 4
   I. 3. 2. Tail Function of a Linker 5
      I. 3. 2. 1. Trichlorosilyl Tail Function 5
      I. 3. 2. 2. Thiol Tail Function 7
   I. 3. 3. Head Function of a Linker 8
      I. 3. 3. 1. Immobilization of Biosensing Element onto SAMs 8
      I. 3. 3. 2. Covalent Immobilization 9
   I. 3. 4. Backbone of a Linker 11
   I. 3. 5. Linker/Diluent Mixed SAMs 12

I. 4. Surface Characterization Techniques 13
   I. 4. 1. Contact Angle Measurements (CAM) 13
   I. 4. 2. X-ray Photoelectron Spectroscopy (XPS) 15

I. 5. Detection of Biochemical Interactions 18
   I. 5. 1. Acoustic Wave Devices 18
   I. 5. 2. Thickness Shear Mode Acoustic Wave Sensor (TSM) 20
   I. 5. 3. ElectroMagnetic Piezoelectric Acoustic Sensor (EMPAS) 22

I. 6. Thesis Project 25
I. 6. 1. Background
I. 6. 2 Thiosulfonate Head Function
I. 6. 3. Thesis Objective and Methods

II. Experimental
II. 1. General Remarks
II. 2. Chemical Synthesis
   II. 2. 1. Synthesis of TUBTS
   II. 2. 2. Synthesis of OEG-TUBTS
   II. 2. 3. Synthesis of 9-OEG
   II. 2. 4. Synthesis of 10-OEG-TFA
   II. 2. 5. Synthesis of 7-OEG-TFA
   II. 2. 6. Synthesis of 13-OEG-TFA
   II. 2. 7. Synthesis of DNBTS
   II. 2. 8. Synthesis of Biotin thiol
II. 3. Surface Modification
   II. 3. 1. Preparation of Cleaned Substrates
   II. 3. 2. Silanization Procedure
   II. 3. 3. Formation of DNBTS SAMs on Gold
   II. 3. 4. Biotin thiol Immobilization Procedure
   II. 3. 5. Cleavage of TFA Head Function Group
II. 4. Surface Analysis Techniques
   II. 4. 1. Contact Angle Measurement (CAM)
   II. 4. 2. X-ray Photoelectron Spectroscopy (XPS)
   II. 4. 3. ElectroMagnetic Piezoelectric Acoustic Sensor (EMPAS)

III. Results and Discussion
III. 1. Thiosulfonate-Based Linker: TUBTS
   III. 1. 1. Synthesis of TUBTS
III. 1. 2. SAM Formation 55
III. 1. 3. Biotin Immobilization 58
III. 1. 4. EMPAS Measurements 61

III. 2. OEG Backbone Thiosulfonate-Based Linker: OEG-TUBTS 63
   III. 2. 1. Synthesis of OEG-TUBTS 63
   III. 2. 2. Preparation of a Biosensing Interface 65
   III. 2. 3. EMPAS Measurements 68

III. 3. OEG-Based Diluents 71
   III. 3. 1. Head Function of OEG-Based Diluents 71
   III. 3. 1. 1. Synthesis of 9-OEG 71
   III. 3. 1. 2. Synthesis of 10-OEG-TFA 72
   III. 3. 1. 3. SAM Formation 74
   III. 3. 1. 4. EMPAS Measurements 78
   III. 3. 2. Length of OEG-Based Diluents 80
   III. 3. 2. 1. Synthesis of 7-OEG-TFA 80
   III. 3. 2. 2. Synthesis of 13-OEG-TFA 81
   III. 3. 2. 3. SAM Formation 83
   III. 3. 2. 4. EMPAS Measurements 88

III. 4. OEG-TUBTS/7-OEG: Towards Elimination of Non-Specific Adsorption 91
   III. 4. 1. Preparation of Biosensing Interface 91
   III. 4. 2. EMPAS Measurements 95
   III. 4. 3. EMPAS Calibration Study 97
   III. 4. 4. EMPAS Measurements with Complex Samples 99

III. 5. Expanding the Chemistry of the Thiosulfonate-Base Linker 104
   III. 5. 1. TUBTS on Si$_3$N$_4$ and AlN 105
   III. 5. 2. DNBTS on Gold 112
III. 5. 2. 1. DNBTS Synthesis  
III. 5. 2. 2. DNBTS SAM Preparation  
III. 6. EMPAS Coil Circuit  
   III. 6. 1. Purpose  
   III. 6. 2. Design  
   III. 6. 3. Possible Improvements  
IV. Conclusion  
V. Future Work  
References  
Appendix  
   A. $^1$H NMR of TUBTS  
   B. XPS survey spectra for TUBTS SAM on quartz crystal  
   C. Example of EMPAS measurement profiles for TUBTS system  
   D. $^1$H NMR of OEG-TUBTS  
   E. XPS survey spectra for OEG-TUBTS SAM on quartz crystal  
   F. Example of EMPAS profiles for OEG-TUBTS system  
   G. $^1$H NMR of Diluents  
   H. XPS survey spectra for diluent SAM on quartz crystal  
   I. Example of EMPAS profiles for pure diluent systems  
   J. XPS survey spectra for OEG-TUBTS/7-OEG SAM on quartz crystal  
   K. Example of EMPAS profiles for OEG-TUBTS/7-OEG system  
   L. Example of EMPAS profiles for complex sample studies  
   M. XPS survey spectra for TUBTS SAM on Si$_3$N$_4$  
   N. XPS survey spectra for TUBTS SAM on AlN  
   O. $^1$H NMR of DNBTS  
   P. XPS survey spectra for DNBTS SAM on gold-plated TSM crystals  
   Q. $^1$H NMR of Biotinethiol
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AC</td>
<td>Alternating Current</td>
</tr>
<tr>
<td>AM</td>
<td>Amplitude Modulation</td>
</tr>
<tr>
<td>ARXPS</td>
<td>Angle-Resolved X-ray Photoelectron Spectroscopy</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated Total Reflectance</td>
</tr>
<tr>
<td>BAW</td>
<td>Bulk Acoustic Wave</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>CAM</td>
<td>Contact Angle Measurement</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>DilsoButylAluminum Hydride</td>
</tr>
<tr>
<td>DNA</td>
<td>DeoxyriboNucleic Acid</td>
</tr>
<tr>
<td>4-DMAP</td>
<td>4-DiMethylAmino-Pyridine</td>
</tr>
<tr>
<td>EDC</td>
<td>N-Ethyl-N’-(3-Dimethylaminopropyl)-Carbodiimide</td>
</tr>
<tr>
<td>EMPAS</td>
<td>ElectroMagnetic Piezoelectric Acoustic Sensor</td>
</tr>
<tr>
<td>ESI</td>
<td>ElectroSpray Ionization</td>
</tr>
<tr>
<td>FET</td>
<td>Field Effect Transistor</td>
</tr>
<tr>
<td>FPW</td>
<td>Flexural Plate Wave</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IR</td>
<td>InfraRed (spectroscopy)</td>
</tr>
<tr>
<td>ISFET</td>
<td>Ion-Selective Field Effect Transistor</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium Aluminum Hydride</td>
</tr>
<tr>
<td>LC</td>
<td>Inductor- Capacitor</td>
</tr>
<tr>
<td>LoD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>MARS</td>
<td>Magnetic Acoustic Resonance Sensor</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular Weight</td>
</tr>
</tbody>
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NHS: N-Hydroxy-Succinimide
NMR: Nuclear Magnetic Resonance
NTA: NitriloTriAcetate
OEG: Oligo(Ethylene Glycol)
OEG-TUBTS: S-(2-(2-(2-(3-trichlorosilyl-propyloxy)-ethoxy)-ethoxy)-ethyl)
7-OEG: 2-(3-trichlorosilyl-propyloxy)-ethyl trifluoroacetate
PEG: Poly(Ethylene Glycol)
PBS: Phosphate Buffered Saline
ppm: parts per million
QCM: Quartz Crystal Microbalance
R:\: Retardation Factor
RLC: Resistor-Inductor- Capacitor
RSD: Relative Standard Deviation
rt: room temperature
SAM: Self-Assembling Monolayer
SAW: Surface Acoustic Wave
THF: Tetrahydrofuran
TSM: Thickness Shear Mode
TTU: 1-(Thiotrifluoroacetato)-11-(Trichlorosilyl)-Undecane
TUBTS: S-(11-Trichlorosilyl-Undecenyl) BenzeneThioSulfonate
v/v: volume by volume
XPS: X-ray Photoelectron Spectroscopy
List of Figures

Figure 1. Schematic representation of a biosensor. 1
Figure 2. Schematic of a SAM and subsequent immobilization of the biosensing element. 4
Figure 3. Schematic representation of the silanization process: hydrolysis of the trichlorosilyl group, hydrogen bonding formation and condensation with surface hydroxyl groups. 6
Figure 4. Schematic representation of the cross-linkage of silanols forming siloxanes. 6
Figure 5. General chemical structure of dithiols tail function for forming SAM on gold. 8
Figure 6. Schematic representation EDC/NHS coupling chemistry. 10
Figure 7. Schematic representation of the contact angle ($\theta$) at the solid (s), liquid (l), and vapour (v) interface. 14
Figure 8. Schematic representation of a XPS instrument. 15
Figure 9. X-ray photoelectron spectroscopy excitation of a core electron. 16
Figure 10. Illustration of ARXPS analysis: a) at lower angles relative to the surface, greater surface (S) sensitivity is observed; b) at higher angles relative to the surface, greater bulk (B) sensitivity is obtained. 18
Figure 11. Illustration of an AT-cut quartz crystal, cut in a quartz wafer at $+35^\circ 15'$ angle relative to the optical axis. 19
Figure 12. Different types of acoustic wave devices. 20
Figure 13. Schematic representation of an electrode plated AT-cut quartz crystal subjected to a perpendicular electrical field ($E$) applied along the z-axis, the resulting thickness shear mode acoustic wave ($1^{st}$ harmonic), and the associated mechanical displacements along the x-axis ($u_x$). 21
Figure 14. Schematic representation of the EMPAS system. 23
Figure 15. a) Schematic representation of an AT-cut quartz crystal subjected to an oscillating electromagnetic field which induces a secondary electric field within the crystal; b) quartz crystals used for EMPAS (13.5 mm in diameter, and a thickness of 83 $\mu$m); c) hand-wound spiral coil (5 mm in diameter). 24
Figure 16. Chemical structures of TTU. 26
Figure 17. Chemical structures of different SAM linkers with various head function.

Figure 18. General mechanism of the reaction between a thiosulfonate and a thiol.

Figure 19. Chemical structures of TUBTS, OEG-TUBTS, 7-OEG-TFA, 9-OEG, 10-OEG-TFA, 13-OEG-TFA, DNBTS and biotinthiol.

Figure 20. Synthesis of biotinthiol.

Figure 21. Synthesis of TUBTS.

Figure 22. Sections of $^1$H NMR spectra for the TUBTS precursor: a) with the impurity; b) without the impurity.

Figure 23. Illustration of the formation of a TUBTS SAM onto a cleaned quartz crystal.

Figure 24. Contact angle measurements obtained with cleaned quartz crystal and TUBTS SAM on quartz crystal.

Figure 25. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon and sulfur (20° angle relative to the surface) obtained with TUBTS SAM and cleaned quartz crystal.

Figure 26. Illustration of the immobilization of biotinthiol onto a TUBTS SAM.

Figure 27. Contact angle measurements obtained with TUBTS SAM and biotinthiol functionalized TUBTS SAM.

Figure 28. XPS narrow scans and relative atomic percentages for nitrogen and sulfur (20° angle relative to the surface) obtained with TUBTS SAM and biotinthiol functionalized TUBTS SAM.

Figure 29. EMPAS non-specific and specific adsorption frequency shifts respectively measured with TUBTS and biotinylated TUBTS SAMs, using 0.1 mg mL$^{-1}$ BSA, IgG and avidin solutions in PBS.

Figure 30. Synthesis of OEG-TUBTS.

Figure 31. Illustration of the formation of an OEG-TUBTSSAM on a cleaned quartz crystal (step I) and the subsequent site-specific covalent immobilization of biotinthiol (step II).

Figure 32. Contact angle measurements obtained with cleaned quartz crystal, OEG-TUBTS SAM and biotinthiol functionalized OEG-TUBTS SAM.

Figure 33. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, sulfur and nitrogen (20° angle relative to the surface) obtained
with cleaned quartz crystal, OEG-TUBTS SAM and biotin-thiol functionalized OEG-TUBTS SAM

Figure 34. EMPAS non-specific and specific adsorption frequency shifts respectively measured with OEG-TUBTS and biotinylated OEG-TUBTS SAMs, using 0.1 mg mL⁻¹ BSA, IgG, avidin solutions in PBS.

Figure 35. EMPAS response profiles for (a) OEG-TUBTS SAM and (b) biotinylated OEG-TUBTS SAM using the avidin solution as sample.

Figure 36. Synthesis of 9-OEG.

Figure 37. Synthesis of 10-OEG-TFA.

Figure 38. Illustration of the formation of 9-OEG and 10-OEG SAM onto a cleaned quartz crystal.

Figure 39. Contact angle measurements obtained with cleaned quartz crystal and 9-OEG SAM on quartz crystal.

Figure 40. Contact angle measurements obtained with cleaned quartz crystal, 10-OEG-TFA SAM and 10-OEG SAM on quartz crystal.

Figure 41. XPS narrow scans and relative atomic percentages for silicon, oxygen, and carbon (20° angle relative to the surface) obtained with 9-OEG SAM and cleaned quartz crystal.

Figure 42. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, and fluorine (20° angle relative to the surface) obtained with 10-OEG-TFA SAM, 10-OEG SAM and cleaned quartz crystal.

Figure 43. EMPAS non-specific adsorption frequency shifts measured with undiluted goat serum using cleaned quartz crystal, 9-OEG and 10-OEG SAMs. Measurements were recorded at 0.86 GHz.

Figure 44. Synthesis of 7-OEG-TFA.

Figure 45. Synthesis of 13-OEG-TFA.

Figure 46. Illustration of the formation of 7-OEG and 13-OEG SAM onto a cleaned quartz crystal.

Figure 47. Contact angle measurements obtained with cleaned quartz crystal and 7-OEG-TFA SAM and 7-OEG SAM on quartz crystal.

Figure 48. Contact angle measurements obtained with cleaned quartz crystal, 13-OEG-TFA SAM and 13-OEG SAM on quartz crystal.
Figure 49. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, and fluorine (20° angle relative to the surface) obtained with 7-OEG-TFA SAM, 7-OEG SAM and cleaned quartz crystal.

Figure 50. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, and fluorine (20° angle relative to the surface) obtained with 13-OEG-TFA SAM, 13-OEG SAM and cleaned quartz crystal.

Figure 51. Relative atomic percentage ratio of carbon to silicon (%C/%Si) and relative atomic percentage ratio of fluorine to carbon (%F/%C) of 7-OEG-TFA, 10-OEG-TFA and 13-OEG-TFA SAMs.

Figure 52. EMPAS non-specific adsorption frequency shifts measured with undiluted goat serum using cleaned quartz crystal, 7-OEG, 10-OEG, 13-OEG SAMs. Measurements were recorded at 0.86 GHz.

Figure 53. Illustration of the formation of an OEG-TUBTS/7-OEG-TFA SAM on a cleaned quartz crystal (step I) and the subsequent site-specific covalent immobilization of biotin thiol (step II).

Figure 54. Contact angle measurements obtained with cleaned quartz crystal, OEG-TUBTS/7-OEG-TFA SAM and biotin thiol functionalized OEG-TUBTS/7-OEG SAM.

Figure 55. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, sulfur, nitrogen and fluorine (20° angle relative to the surface) obtained with cleaned quartz crystal, OEG-TUBTS/7-OEG-TFA SAM and biotin thiol functionalized OEG-TUBTS/7-OEG SAM.

Figure 56. EMPAS non-specific and specific adsorption frequency shifts respectively measured with OEG-TUBTS/7-OEG and biotinylated OEG-TUBTS/7-OEG SAMs, using 0.1 mg mL⁻¹ BSA, IgG and avidin solutions in PBS.

Figure 57. EMPAS calibration curves for avidin and BSA in PBS.

Figure 58. Linear region of EMPAS calibration curves for avidin and BSA in PBS.

Figure 59. EMPAS non-specific and specific adsorption frequency shifts respectively measured with 45.1 mg mL⁻¹ BSA and 45 mg mL⁻¹ BSA with 0.1 mg mL⁻¹ avidin solutions in PBS, using biotinylated TUBTS, OEG-TUBTS and OEG-TUBTS/7-OEG SAMs.

Figure 60. EMPAS non-specific adsorption frequency shifts measured with undiluted goat serum using biotinylated TUBTS, OEG-TUBTS and OEG-TUBTS/7-OEG SAMs.
Figure 61. Illustration of the formation of a TUBTS SAM on a cleaned Si₃N₄ and AlN (step I) and the subsequent site-specific covalent immobilization of biotin-thiol (step II). 106

Figure 62. Contact angle measurements obtained with cleaned Si₃N₄, TUBTS SAM on Si₃N₄ and biotin-thiol functionalized TUBTS SAM on Si₃N₄. 107

Figure 63. Contact angle measurements obtained with cleaned AlN, TUBTS SAM on AlN and biotin-thiol functionalized TUBTS SAM on AlN. 107

Figure 64. XPS narrow scans and relative atomic percentages for silicon, nitrogen, oxygen, carbon, and sulfur (20° angle relative to the surface) obtained with cleaned Si₃N₄, TUBTS SAM on Si₃N₄ and biotin-thiol functionalized TUBTS SAM on Si₃N₄. 109

Figure 65. XPS narrow scans and relative atomic percentages for aluminium, nitrogen, oxygen, carbon, silicon and sulfur (20° angle relative to the surface) obtained with cleaned AlN, TUBTS SAM on AlN and biotin-thiol functionalized TUBTS SAM on AlN. 110

Figure 66. Normalized %S for TUBTS SAM on quartz, Si₃N₄, AlN. 112

Figure 67. Synthesis of DNBTS. 114

Figure 68. Illustration of the formation of an DNBTS SAM on a cleaned TSM crystal (step I and the subsequent site-specific covalent immobilization of biotin-thiol (step II). 115

Figure 69. Contact angle measurements obtained with cleaned TSM crystal, DNBTS SAM and biotin-thiol functionalized DNBTS SAM on TSM crystal. 116

Figure 70. XPS narrow scans and relative atomic percentages for gold, carbon, sulfur and nitrogen (20° angle relative to the surface) obtained with cleaned TSM crystal, DNBTS SAM on TSM crystal and biotin-thiol functionalized DNBTS SAM on TSM crystal. 117

Figure 71. Resonant envelopes acquired with and without the EMPAS FET buffer amplifier on EMPAS 119

Figure 72. Schematic representation of EMPAS coil circuit: a) parallel LC coil circuit; b) FET transistor with tunable resonant circuit; c) band-pass filter; d) clamper circuit; e) impedance matching circuit; f) coupling capacitor circuit. 121

Figure A. 1. ¹H NMR of TUBTS precursor. 137

Figure A. 2. ¹H NMR of TUBTS. 137

Figure B. 1. XPS survey spectra for a cleaned quartz crystal. 138

Figure B. 2. XPS survey spectra for a TUBTS SAM on quartz crystal. 138
Figure B. 3. XPS survey spectra for a biotin-thiol functionalized TUBTS SAM on quartz.

Figure C. 1. An example of an EMPAS measurement profile for TUBTS SAM surface with avidin in PBS as sample.

Figure C. 2. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with avidin in PBS as sample.

Figure C. 3. An example of an EMPAS measurement profile for TUBTS SAM surface with BSA in PBS as sample.

Figure C. 4. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with BSA in PBS as sample.

Figure C. 5. An example of an EMPAS measurement profile for TUBTS SAM surface with IgG in PBS as sample.

Figure C. 6. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with IgG in PBS as sample.

Figure D. 1. $^1$H NMR of OEG-TUBTS precursor.

Figure D. 1. $^1$H NMR of OEG-TUBTS.

Figure E. 1. XPS survey spectra for an OEG-TUBTS SAM on quartz.

Figure E. 2. XPS survey spectra for a biotin-thiol functionalized OEG-TUBTS SAM on quartz.

Figure F. 1. An example of an EMPAS measurement profile for OEG-TUBTS SAM surface with avidin in PBS as sample.

Figure F. 2. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with avidin in PBS as sample.

Figure F. 3. An example of an EMPAS measurement profile for OEG-TUBTS SAM surface with BSA in PBS as sample.

Figure F. 4. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with BSA in PBS as sample.

Figure F. 5. An example of an EMPAS measurement profile for OEG-TUBTS SAM surface with IgG in PBS as sample.

Figure F. 6. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with IgG in PBS as sample.

Figure G. 1. $^1$H NMR of 9-OEG.

Figure G. 2. $^1$H NMR of 10-OEG-TFA precursor.
Figure G. 3. $^1$H NMR of 10-OEG-TFA. 148
Figure G. 4. $^1$H NMR of 7-OEG-TFA. 149
Figure G. 5. $^1$H NMR of 13-OEG-TFA precursor. 149
Figure G. 6. $^1$H NMR of 13-OEG-TFA. 150
Figure H. 1. XPS survey spectra for a 9-OEG SAM on quartz. 150
Figure H. 2. XPS survey spectra for a 10-OEG-TFA SAM on quartz. 151
Figure H. 3. XPS survey spectra for a 10-OEG SAM on quartz. 151
Figure H. 4. XPS survey spectra for a 7-OEG-TFA SAM on quartz. 152
Figure H. 5. XPS survey spectra for a 7-OEG SAM on quartz. 152
Figure H. 6. XPS survey spectra for a 13-OEG-TFA SAM on quartz. 153
Figure H. 7. XPS survey spectra for a 13-OEG SAM on quartz. 153
Figure I. 1. An example of an EMPAS measurement profile for 9-OEG SAM surface with goat serum as sample. 154
Figure I. 2. An example of an EMPAS measurement profile for 10-OEG SAM surface with goat serum as sample. 154
Figure I. 3. An example of an EMPAS measurement profile for 7-OEG SAM surface with goat serum as sample. 155
Figure I. 4. An example of an EMPAS measurement profile for 13-OEG SAM surface with goat serum as sample. 155
Figure J. 1. XPS survey spectra for an OEG-TUBTS/7-OEG SAM on quartz. 156
Figure J. 2. XPS survey spectra for a biotin-thiol functionalized OEG-TUBTS/7-OEG SAM on quartz. 156
Figure K. 1. An example of an EMPAS measurement profile for OEG-TUBTS/7-OEG SAM surface with avidin in PBS as sample. 157
Figure K. 2. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with avidin in PBS as sample. 157
Figure K. 3. An example of an EMPAS measurement profile for OEG-TUBTS/7-OEG SAM surface with BSA in PBS as sample. 158
Figure K. 4. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with BSA in PBS as sample. 158
Figure K. 5. An example of an EMPAS measurement profile for OEG-TUBTS/7-OEG SAM surface with IgG in PBS as sample. 159

Figure K. 6. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with IgG in PBS as sample. 159

Figure L. 1. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with high concentration of BSA in PBS as sample. 160

Figure L. 2. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with high concentration of BSA spiked with avidin in PBS as sample. 160

Figure L. 3. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with goat serum as sample. 161

Figure L. 4. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with high concentration of BSA spiked in PBS as sample. 161

Figure L. 5. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with high concentration of BSA spiked with avidin in PBS as sample. 162

Figure L. 6. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with goat serum as sample. 162

Figure L. 7. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with high concentration of BSA spiked in PBS as sample. 163

Figure L. 8. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with high concentration of BSA spiked with avidin in PBS as sample. 163

Figure L. 9. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with goat serum as sample. 164

Figure M. 1. XPS survey spectra for a cleaned Si$_3$N$_4$. 164

Figure M. 2. XPS survey spectra for a TUBTS SAM on Si$_3$N$_4$. 165

Figure M. 3. XPS survey spectra for a biotin-thiol functionalized TUBTS SAM on Si$_3$N$_4$. 165

Figure N. 1. XPS survey spectra for a cleaned AlN. 166

Figure N. 2. XPS survey spectra for a TUBTS SAM on AlN. 166
Figure N. 3. XPS survey spectra for a biotin-thiol functionalized TUBTS SAM on AlN.

Figure O. $^1$H NMR of DNBTS.

Figure P. 1. XPS survey spectra for a cleaned gold-plated TSM crystals.

Figure P. 2. XPS survey spectra for a DNBTS SAM on gold-plated TSM crystals.

Figure P. 3. XPS survey spectra for a biotin-thiol functionalized DNBTS SAM on gold-plated TSM crystals.

Figure Q. $^1$H NMR of biotin-thiol.
I. Introduction

I. 1. Biosensors

With the development of biosensor technology, biosensors have become more and more attractive as tools in diagnostic and analytical applications. Biosensors are now receiving increasing attention in a variety of fields such as environmental analysis,\textsuperscript{1} food\textsuperscript{2} and drug analysis,\textsuperscript{3} and clinical diagnostics.\textsuperscript{4} In essence, biosensors are analytical devices that use biomolecules imposed on a transducer surface to detect complex biochemical interactions.\textsuperscript{5} This type of detection technology offers a particularly attractive possibility for various areas of applications. This is due to its capability for non-destructive, real-time and label-free detection. In principle, biosensors can be designed to detect and measure any targeted interaction occurring within a biological system. All biosensors consist of three fundamental parts: a biosensing element, a transducer and an output system\textsuperscript{6} (\textit{Figure I}).

\textbf{Figure I.} Schematic representation of a biosensor.

In practice, the biosensing element is engineered to interact specifically with the target analyte. It is linked to a transducer to form a biosensing interface. These biosensing elements can be a variety of biological species, ranging from tissue to small biomolecules. The biochemical signal produced upon interaction with the target analyte is then converted into a measurable electrical response through the transducer. Depending on the nature of the biochemical signal produced during this interaction, different types of transducers can be used (electrodes, piezoelectric devices, and optical devices). In the end,
the output system, typically comprised of a signal amplifier, a processor, and a display, will present the electrical response in a readable format.

In the various fields mentioned above, rapid and reliable analysis is needed. Presently, conventional analytical techniques are expensive, time-consuming and labour-intensive. Occasionally, these techniques even produce unreliable results. Thus, there is a high interest for the development of alternative analytical techniques with improved performance (i.e. operating time, reproducibility, sensitivity, and selectivity of analyte) while reducing cost and including user friendly characteristics. Biosensors, unlike conventional tools, have the unique potential to offer these significant advantages.

Although biosensors have many advantages, they commonly possess several issues that need to be addressed before the technology can be implemented practically. The immobilization of the biosensing element onto the transducer in a controlled manner is, most certainly, one of them. This process is an essential and demanding aspect for the development of a biosensor. The immobilization process must be easily reproducible and the resulting biosensing interface must be stable. Additionally, the biological activity of the biosensing element must be preserved. This can be achieved by controlling the distribution and orientation of the immobilized biosensing element. Lastly, the main reason that biosensors are rarely used in the mentioned fields is due to their often impracticability for real samples. To overcome this, the biosensing interface must feature high selectivity and sensitivity towards the target analyte. Ideally, this will allow the biosensor to provide reliable and reproducible results, even in the presence of interfering species. Addressing these issues is one of the focuses of this manuscript.
I. 2. Interactions between the Biosensing Interface and the Sample

When conducting analyses with biosensors, many complex interactions occur on the surface of the biosensing interface with the sample. Ideally, only interactions between the analyte and the biosensing element will occur. However, depending on the nature of the sample, many undesired interactions will occur between interfering species in the sample and the biosensing interface. These undesired interactions negatively affect biosensor performance since interfering species can absorb and saturate the surface of the biosensing interface.\(^9\)

Generally, undesired adsorption is referred to as “non-specific adsorption” as opposed to desired adsorption, which is referred as “specific adsorption”. The adsorption process starts with the most abundant species in the sample that has affinity for the surface. Eventually these are displaced by other species with higher affinities for the surface (such as the analyte of interest).\(^{10}\) However, some interfering biomolecules can aggregate on the surface and never get displaced by the analyte of interest. This phenomenon can distort the result and inaccurately depict the levels of analyte present. This is a prominent issue when working with biological samples, such as blood, serum and urine, which contain large amounts of interfering species (e. g. cells, proteins, electrolytes, lipid, etc.). Thus, there is a great deal of interest for the development of biosensing interfaces that are resistant to non-specific adsorption. There are numerous studies focused on reducing/preventing these undesirable interactions.\(^{11-14}\) Most involve the use of self-assembling monolayer (SAM) chemistry to prepare and modify surfaces.
I. 3. Self-Assembling Monolayer Chemistry

I. 3. 1. Background

In the past decade, self-assembling monolayer (SAM) chemistry has been the method of choice for the preparation of biosensing interfaces in numerous studies.\textsuperscript{15-20} SAM chemistry utilizes molecules that are designed to spontaneously form ordered molecular assemblies on solid inorganic substrates.

The use of SAMs offers several benefits in the preparation of biosensing interfaces. First, the preparation of SAMs is achieved with ease and minimal resources. Second, the resulting SAMs are highly ordered, chemically stable, and robust.\textsuperscript{21} Lastly, SAMs are of a particular interest due to their customizable nature. Many properties of SAMs can be altered, and adapted as needed by molecule design and modification. In the application of biosensors, SAM molecules are typically engineered as linkers. The linker is first anchored onto the transducer to form the SAM. Subsequently, the biosensing element of interest can be immobilized onto the SAM directly or through a series of chemical transformations\textsuperscript{22} (Figure 2). With proper design of the linker, the optimal biosensing interface can be prepared for biosensors in various applications.

![Figure 2](image.png)

**Figure 2.** Schematic of a SAM and subsequent immobilization of the biosensing element.
When designing SAM linkers, there are three essential and distinct parts to consider: the tail function, the head function and the backbone (Figure 2). The tail function is used to anchor the linker itself onto the surface of the transducer. The head function is there to subsequently immobilize the biosensing element onto the SAM. The long backbone provides space between the tail function and the head function. It also provides the linker with the necessary intermolecular forces to form a stable assembly. In addition, the surface properties of the resulting SAM are influenced by the head function and the backbone. Thus, when designing a SAM linker, all three parts can be tailored for different applications.

I. 3. 2. Tail Function of SAMs

The two most common tail functions of SAMs are trichlorosilyl (-SiCl₃) and thiols (-SH). In practice, trichlorosilyl allows linkers to form SAMs on hydroxylated surfaces. This reaction relies on the formation of strong silicon-oxygen bonds. With the thiol tail function, SAMs can be formed on gold surfaces through the strong interaction between sulfur and gold. Although both classes of SAMs yield highly ordered and well defined monolayers, those based on trichlorosilane offer superior stability since bonding of the linker molecules to both each other and the underlying surface is covalent in nature.

I. 3. 2. 1. Trichlorosilyl Tail Function

Trichlorosilyl is a widely used tail function in SAM chemistry due to its ability to form robust and stable SAMs on hydroxylated surfaces. The formation of trichlorosilane-based SAMs on these hydroxylated surfaces is termed silanization. Despite years of research, there are still many conflicting theories on the mechanism of SAM formation through silanization. Generally, silanization is understood with the following multi-step mechanism. First, the trichlorosilyl groups form trisilanol (-Si(OH)₃) by hydrolysis with water. The water is either adsorbed on the hydroxylated surface or in solution. Second, the resulting trisilanol species undergo a reversible physisorption process onto
the hydroxylated surface via hydrogen bonding.\textsuperscript{29,32} During this process, the adsorbed species can randomly condense with surface hydroxyl groups to form surface-bound silanols (\textit{Figure 3}).\textsuperscript{32} In addition, unreacted silanol groups can form siloxane linkages (-Si-O-Si) by forming hydrogen bonds with neighbouring molecules, and then undergoing another condensation reaction.\textsuperscript{24,29,32} This cross-linking further strengthens the attachment of the monolayer onto the underlying substrate surface\textsuperscript{29} (\textit{Figure 4}). It is believed that the overall silanization process is initiated through island formation and then slowly nucleated over the entire surface, finally aggregating to form the SAM.\textsuperscript{30}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Schematic representation of the silanization process: hydrolysis of the trichlorosilyl group, hydrogen bonding formation and condensation with surface hydroxyl groups.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Schematic representation of the cross-linkage of silanols forming siloxanes.}
\end{figure}

The quality of these monolayers and their reproducibility is dependent on a number of conditions: choice of solvent, temperature, time, substrate properties (\textit{i.e.} surface hydroxyl density, morphology, contamination, etc.) and amount of water available during silanization.\textsuperscript{23,28,33} In particular, the amount of water is crucial for silanization and needs to be carefully controlled. It has been shown that if there is too little water, incomplete
SAM formation occurs. However, too much water will result in the polymerization of the linkers in solution, forming ineffective macromolecules for proper SAM formation. Thus, it is important to completely control the conditions for preparing SAMs in order to achieve high reproducibility.

I. 3. 2. 2. Thiol Tail Function

SAMs with thiol tail functions are key elements in various biosensor technologies. The thiol allows the SAM to be formed on planar gold (Au) surfaces or gold nanoparticles (AuNPs). Despite recent efforts, the mechanism behind the reaction between gold and thiol is still not completely understood. However, it is generally believed to be an oxidative addition of the S-H bond to the gold surface followed by the reductive elimination of the S-H hydrogen (Eq. 1).

\[
R - S - H + Au_n^0 \rightarrow R - S^+Au_n^0 + \frac{1}{2} H_2
\] (1)

SAM formation with thiol-based linkers consists of two distinct adsorption kinetics: a fast step (millisecond to minutes) followed by a slow step (several hours). In the fast step, the linkers are readily chemiosorbed on the gold surface and the high sulfur-gold affinity is the driving force. In the slow step, the linkers undergo constant rearrangement with themselves through lateral diffusion to form an ordered assembly with maximum density and minimum defects. In addition to using the single thiol tail function, SAMs on gold can also be prepared by dithiols such as disulfides, cyclic disulfides and aromatic dithiols (Figure 5).


**Figure 5.** General chemical structure of dithiols tail function for forming SAM on gold.

All these tail functions contain two thiol groups relatively close to each other. This allows the formation of SAMs with both sulfur atoms bonding to the substrate surface, leading to a more rigid anchoring to the substrate. However, SAMs formed from these tail functions are less ordered than their monothiol counterparts. This is mainly due to their increased binding energy, which consequently increases the activation energy for lateral diffusion.\(^{45}\)

### I. 3. 3. Head Function of a Linker

#### I. 3. 3. 1. Immobilization of Biosensing Element onto SAMs

When immobilizing biosensing elements onto SAMs, several basic requirements must be met in order for the biosensing interface to exhibit analytical performance. First, the process of immobilization must be relatively mild so that it would not damage the SAM. Second, the resulting biosensing interface must be stable and durable.\(^{5}\) Lastly, and most importantly, the biosensing element must retain its biological activity after immobilization. It is common for the biological activity of the biosensing element to be reduced or even lost once immobilized to the surface.\(^{46}\) This can be attributed to the properties of the SAM or the solvent used in immobilization.\(^{47,48}\) Additionally, the biosensing element’s conformation can be altered and/or its active site can be sterically hindered due to the drastic change in its surrounding environment.
The immobilization of biosensing elements onto SAMs can be classified into two categories: non-covalent or covalent. Non-covalent immobilization relies on the adsorption of the biosensing element via intermolecular forces (i.e. electrostatic, hydrophobic, and polar interactions). These immobilization processes usually require very mild protocols, however, the resulting attachment is not stable and gradually diminishes with repeated use and washing. In addition, the biosensing element will most likely have random orientations on the SAM. This will decrease the number of available active sites to which the target analyte can bind, leading to poor biosensing performance. There are some non-covalent immobilization methods that exploit receptor-protein and biological recognition pair interactions (e.g. avidin-biotin, DNA hybridization, NTA-Ni and His-tag affinity). Although these methods fix many previously mentioned issues in non-covalent immobilization, the extra preparatory steps to modify the biosensing interface with a specific recognition pair can be costly and time-consuming.

On the other hand, covalent immobilization generally offers the best stability and durability.\(^4\) Furthermore, proper orientation and preserved activity of biosensing element can be accomplished. In covalent immobilization, a strong covalent bond is formed between accessible functional groups on the biosensing element (e.g. amine, thiol, or carboxyl acid groups) and head functions of the SAM.

### 1. 3. 3. 2. Covalent Immobilization

One of the most common and well-established methods to covalently immobilize biosensing elements onto SAMs is the use of a carboxyl head function (-COOH) with the N-ethyl-N’-(3-dimethylamino-propyl)-carbodiimide/N-hydroxy-succinimide (EDC/NHS) coupling chemistry (Figure 6). In short, EDC reacts with the carboxyl head function to form O-acylisourea intermediates, which then reacts with NHS to form the more reactive NHS ester head function. As a result, the amine-containing biosensing element can react with the NHS ester, immobilizing it through a strong and stable covalent amide bond.\(^5\)
Despite the common use of these transformations, they can be cumbersome. These transformations often result in an incomplete conversion of the head function, which produces irreproducible SAMs. The EDC/NHS chemistry is also chemically incompatible with the trichlorosilane-based linker. It is impossible to synthesize linkers with both COOH and Cl₃Si moieties, since they readily react together. For this to be done, a protecting group is required on the COOH head function, which leads to an additional de-protection step before the coupling reaction. Furthermore, immobilizing biosensing elements through amine is often not desired due to the large amount of free amines available on biomolecules. This makes site-specific immobilization much more difficult.

![Figure 6. Schematic representation EDC/NHS coupling chemistry.](image)

In order to find the solution to these problems, a linker must be designed with a head function that is both chemically compatible with the tail function and does not require a
pre-activation step for the immobilization of the biosensing element. In addition, the head function must react with other less abundant functional groups to immobilize the biosensing element. One apparent candidate is thiol. Free thiols are much less abundant in biological species and they can be added through various methods if not present. Developing a linker that can form a SAM with the ability to subsequently immobilize biosensing element in a single, straightforward, and site-specific step is one of the focuses of this manuscript.

I. 3. 4. Backbone of a Linker

The backbone of a linker plays a crucial role in forming SAMs. The most common type of backbone in SAM linkers is the alkyl chains. In terms of SAM formation, the backbones position themselves to maximize neighbouring intermolecular interactions (e.g. van der Waals interactions) within the assembly, resulting in denser packing and higher order. In general, linkers consisting of long alkyl backbones (8 to 18 carbons in length) are able to form crystalline-like rigid monolayer assemblies, where the alkyl backbones are densely packed. Those with longer alkyl chains consistently form more ordered layers than those formed from shorter chains.

The properties of the SAM are also affected by the backbones of the linkers that assemble them. There are many studies that utilize different types of backbones to modify the properties of the SAM, such as fluorinated alkyl chains, aromatic chains and ethylene glycol chains. Interestingly, SAMs with ethylene glycol chains in both forms —Poly(Ethylene Glycol) (PEG) and Oligo(Ethylene Glycol) (OEG)— have shown great potential in resisting non-specific adsorption.

The entropy repulsion and water barrier hypotheses have often been separately proposed to explain the mechanism of non-specific adsorption resistance on these surfaces. Entropy repulsion is mainly attributed to the flexibility of the ethylene glycol
chain. These chains exhibit great conformational degrees of freedom. Adsorption of biomolecules on the surface causes the glycol chains to compress. The resulting volume restriction and/or osmotic repulsion leads to a loss in configurational entropy. Thus, the entropy repulsion force repels the approach of biomolecules that have low affinity for the surface. This hypothesis is often used to describe how PEG SAMs prevent non-specific adsorption by virtue of their long chain length. However, the presence of water molecules are not included in the explanation of entropy repulsion. In contrast, the water barrier mechanism is attributed to the presence of tightly bound water at the interface. The oxygen atoms within the ethylene glycol chain allows for hydrogen bonding with water molecules in solution. This forms a physical barrier that prevents direct contact between non-specific biomolecules and the surface. Contrary to PEG SAMs, the non-specific adsorption resistance of OEG SAMs is often explained by the water barrier mechanism. This is due to the fact that OEG SAMs have shorter and more densely packed assemblies, which have less freedom for conformational change upon biomolecule adsorption.

I. 3. 5. Linker/Diluent Mixed SAMs

It is known that SAMs form densely packed assemblies on the surface. However, it is believed that such crowded assemblies could have a negative impact on the subsequent biosensing element immobilization due to steric hindrance around the neighbouring head function. To alleviate this problem, SAM molecules without a functional group (known as diluents) are incorporated into the SAMs to space out the linker molecules within the assembly.

Mixed linker/diluent SAMs can be prepared by mixing the linker with the diluent in a solution with the desired ratio. Subsequently, the substrate is soaked in the solution to allow the SAM formation to occur. The resulting mixed linker/diluent SAMs should offer greater spacing between the biosensing elements on the surface upon immobilization. This has the potential to preserve the biological activity of the biosensing element after
immobilization since the biosensing element is less likely to be denatured (if it is a protein) and can freely interact with analyte.68

I. 4. Surface Characterization Techniques

To ensure the validity of the information gained from measurements performed by the biosensors, the physical and chemical properties (i.e. chemical composition, hydrophobicity, order, thickness and roughness) of the biosensing interface must be thoroughly characterized beforehand. There is a collection of surface characterization techniques that can be used. The choice of such techniques depends on the information desired and their compatibility with the surface. However, it is also influenced by other factors, such as sensitivity, effectiveness, availability, time and cost. Described herein are the two techniques employed in this study: Contact Angle Measurements (CAM) and X-ray Photoelectron Spectroscopy (XPS).

I. 4. 1. Contact Angle Measurements (CAMs)

Contact Angle Measurement (CAM) is a simple and effective method to determine surface wettability and polarity. The technique involves depositing a liquid droplet onto a flat surface. The droplet forms a capped sphere with a finite contact angle (θ) over the substrate surface. The shape that the droplet makes with the surface is affected by the interfacial free energies between the solid-vapour (γ_{SV}), solid-liquid (γ_{SL}) and liquid-vapour (γ_{LV}) interface (Figure 7).
Figure 7. Schematic representation of the contact angle (θ) at the solid (s), liquid (l), and vapour (v) interface.

In the case of an ideal surface (i.e. perfectly flat, smooth, levelled and rigid), the relationship between the thermodynamic parameters $\gamma_{SV}$, $\gamma_{SL}$, $\gamma_{LV}$, and $\theta$ is given by Young’s equation:\(^{69}\)

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta \text{ where } 0^\circ \leq \theta \leq 180^\circ \quad (2)$$

However, the above equation is only valid for ideal homogenous surfaces in thermodynamic equilibrium, which is rarely the case for practical measurement. For this reason, there are several mathematical models that take into account the substrate’s roughness, heterogeneity and defects.\(^{70}\) In practice, a contact angle equal to $0^\circ$ corresponds to a completely wetted surface. For a contact angle between $0^\circ$ and $90^\circ$ the surface is partially wetted. Finally, a surface with contact angle larger than $90^\circ$ exhibits no wetting. More specifically, when water is used as the test liquid, a contact angle close to zero indicates that the surface is highly hydrophilic, and as the angle increases this indicates the surface is becoming more hydrophobic. Thus, this technique offers a simple and rapid determination of relative differences in polarity among different surfaces (e.g. cleaned surfaces and those modified with SAMs).\(^{71}\)
I. 4. 2. X-ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) is a powerful surface analysis technique used to qualitatively and quantitatively measure the elemental composition of a substrate surface at depths of 1-10 nm \(^2\) (Figure 8).

![Figure 8. Schematic representation of a XPS instrument.](image)

Fundamentally, this technique is based on the photoelectric effect, where ejection of core electrons from a surface occurs upon photon bombardment. In essence, a sample is exposed to a monochromatic source of X-ray photons, with energy commonly in the range of 1000-2000 eV.\(^3\) The impact of the X-ray photon ejects electrons from the core shells (\(i.e.\) K, L, or M) of the atoms on the surface (Figure 9).
Core electrons are bound with a certain binding energy, $E_b$, which is characteristic for each element and oxidation state. After the electrons are ejected, they are collected by a system of lenses, and then sorted by an electron energy analyzer according to their kinetic energy, $E_K$. The number of electrons at each kinetic energy is counted and recorded at the detector. At the end, the measured data is processed and displayed by a computer. The electrons that were excited, and escaped without any energy loss contribute to the main photoelectron peaks in the XPS spectrum. On the other hand, electrons that have lost energy through inelastic collisions within the sample contribute to the background noise observed in the spectrum. The binding energy of the electron is calculated from the difference between the detected kinetic energy of the ejected electron and the energy of the incident X-ray photon, $h\nu$. The spectrometer also adds a work function, $\phi$, onto the kinetic energy of the ejected electron, which must be taken into account:

$$E_b = h\nu - E_K - \phi \tag{3}$$

By analyzing the core electron binding energy, the identity of different elements on the sample surface can be determined. This is because each element with a certain oxidation
state has a unique set of binding energies. In addition, the electron count gives quantitative information about the elements (Eq. 4). Thus, with proper peak fitting and calculation, the elemental composition of the sample's surface can be quantified.

\[ I = n \Phi S \]  

(4)

where, \( I \) is the number of photoelectrons detected each second; \( n \) is the number of atoms; \( \Phi \) is the flux of the incident X-ray beam; and \( S \) is the sensitivity factor for the element of interest.

In practice, additional information about the chemical environment of the analyzed element can be obtained with high-resolution XPS. Angle-Resolved XPS (ARXPS) offers the possibility to study the sample at different depths. Hence, the elemental composition of the surface coating and the bulk material can be determined. This is achieved by varying the photoelectron take-off angle (\( \theta \)) when collecting the data (Figure 10). Reducing the angle reduces the depth from which the XPS information is obtained, making the analysis more surface sensitive. On the other hand, when the angle is increased, more information is obtained at greater depth which leads to a higher bulk sensitivity in the analysis.
Figure 10. Illustration of ARXPS analysis: a) at lower angles relative to the surface, greater surface (S) sensitivity is observed; b) at higher angles relative to the surface, greater bulk (B) sensitivity is obtained.

I. 5. Detection of Biochemical Interactions

I. 5. 1. Acoustic Wave Devices

There are many types of biosensors, which are defined by the type of transducer present. There are a variety of transducers available for analysis, and the optimal transducer for a given application depends on the nature of the stimulus involved between the analyte and the biosensing element. In acoustic, wave-based devices, a piezoelectric material is used as the transducing element. Piezoelectric materials have the ability to generate voltage in response to applied mechanical stress, or conversely, experience a mechanical strain upon application of an electrical and/or magnetic field. This is known as the piezoelectric effect and it only occurs in crystals that lack a centre of symmetry. Amongst all piezoelectric materials, quartz is most commonly used in biosensor applications due to its unique physical and chemical properties. AT-cut quartz crystal is especially desirable as the transducing element due to its stability at high frequencies over a wide temperature range (Figure 11).
The application of an electric and/or magnetic field forces the displacement of the quartz atoms—electropositive Si and electronegative O—and creates a charge separation. The displaced atoms return to their original position with the aid of forces from charge separation. Acoustic wave sensors exploit this phenomenon by generating periodic particle displacements, termed acoustic waves, within the crystal. Often, application of a periodically varying electric field is used to achieve this acoustic oscillation at a characteristic resonant frequency. Moreover, changes at the transducer surface reflect changes in the resonant frequency, $\Delta f_R$, of the generated shear wave measured in hertz (Hz) in real-time. This includes binding of the analyte, any non-specific adsorption, changes in viscosity and density of the liquid, properties of surface roughness, electrical effects, addition or removal of mass and any structural changes.

There are several different types of acoustic wave devices that are divided by their mode of wave propagation (Figure 12). The direction of particle displacement and acoustic wave within the crystal are different among these devices. Thickness Shear Mode (TSM)
devices, also known as Bulk Acoustic Wave (BAW) devices or Quartz Crystal Microbalances are the most widely used among these acoustic devices in biosensor application. This is due to their practicality for liquid samples, robustness, low-cost and availability. In these devices, the acoustic wave travels perpendicular to the surface and attenuate into the liquid medium. On the other hand, the acoustic wave in Surface Acoustic Wave (SAW) and Flexural Plate Wave (FPW) travels either directly along or near the surface of the crystal. Consequently, these waves experience heavy attenuation when operated in liquids, rendering them impractical for the study of liquid samples.

![TSM, SAW, FPW](image)

**Figure 12.** Different types of acoustic wave devices.

### 1. 5. 2. **Thickness Shear Mode Acoustic Wave Sensor (TSM)**

The Thickness Shear Mode (TSM) device offers a sensitive, label-free, non-destructive, and real-time detection for biochemical interactions. Generally, a thin (0.2 to 0.5 mm) AT-cut quartz disk with gold electrodes deposited on both sides of the piezoelectric substrate is used as the transducer. By applying a high frequency (1 to 20 MHz), sinusoidal voltage across the electrodes, the quartz crystal is driven to mechanical oscillation at its resonant frequency (Figure 13).
The extent of deformation of the crystal and displacement of the surfaces with respect to each other is determined by the applied voltage. The resonant frequency, $f_R$, generated within the quartz is dependent on the thickness of the disk, $d$ (Eq. 5).

$$f_R = \frac{v_{tr}}{\lambda} = \frac{v_{tr}}{2d}$$

where $v_{tr}$ is the speed of the transverse wave in the substrate and $\lambda$ is wavelength of the resonant wave.$^{82}$

In its earliest form, TSM was first employed by G. Z. Sauerbrey for the measurement of mass loading from gas-phase species on quartz crystal in 1959.$^{88}$ It was reported that there is a linear relationship between the deposited mass ($m_f$) and the frequency response ($\Delta f$) when operating in air (Eq. 6).
\[ \Delta f = -2 \frac{f_0^2 m_f}{A \sqrt{\rho_s \mu_s}} \]  

where \( f_0 \) is the fundamental frequency of the crystal, \( A \) is the surface area, \( \rho_s \) is the quartz density, and \( \mu_s \) is the shear modulus of quartz.

However, this model falls apart when the TSM device operates in liquid. This is because a portion of the acoustic waves’ energy dissipates into the liquid medium (Figure 13). This results from the liquid’s lack of support for shear motion. The density (\( \rho_L \)) and viscosity (\( \eta_L \)) of the liquid effectively decreases \( f_R \) and increases the dissipation. Consequently, a number of factors other than mass loading contribute to the overall sensor response when operated in liquid: viscosity, elasticity, coupling phenomena, slip, dielectric properties and conductivity.\(^{89}\) Changes to any of these variables will alter the resonance condition of the transducer, which is reflected as a change in the impedance of the transducer. A network analysis method is used to monitor the impedance, which involves the application of a sine voltage of varying frequency. As a result, two parameters are derived through this analysis, which are \( \Delta f_R \), and change in motional resistance \( \Delta R \). Mass loading primarily correlates to \( \Delta f_R \). On the other hand, changes in motional resistance represent the damping effect on the system, and is related to changes in viscoelasticity. Thus, TSM can be used to monitor biochemical changes happening at the surface of the transducer after it is modified by biosensing elements.

I. 5. 3. ElectroMagnetic Piezoelectric Acoustic Sensor (EMPAS)

The ElectroMagnetic Piezoelectric Acoustic Sensors (EMPAS) was developed in 2003 in an effort to improve TSM sensor technology.\(^{90,91}\) It is a hybrid between TSM technology and the Magnetic Acoustic Resonator Sensor (MARS)\(^{92}\), combining their benefits and overcoming their limitations. The EMPAS technology features a new configuration for the excitation of acoustic waves within a piezoelectric quartz crystal (Figure 14).
As described above, TSM devices excite quartz crystals to their resonant frequency through the direct application of a sinusoidal potential across the gold electrodes. With MARS technology, both static magnetic fields from a permanent magnet and electromagnetic fields from an AC powered electromagnetic coil are used to remotely generate acoustic resonance within a glass substrate. The EMPAS, as a combination of the TSM and MARS technology, involves the use of an oscillating electromagnetic field from an AC powered electromagnetic coil (Figure 15a) to remotely excite an ultra-thin piezoelectric quartz crystal at its acoustic resonance (Figure 15b, 15c). The coil is placed directly below the crystal with a certain distance of separation, both located in a flow-through cell.

The coil generates the electromagnetic field required to drive the crystal into mechanical resonance. The electromagnetic field will induce a secondary electric field within the quartz crystal, which is able to generate a mechanical resonance by coupling with the piezoelectric tensor of the quartz. Once resonance is established, any change in the resonance condition of the crystal will be associated with a change in the impedance of the coil. Thus, similar to TSM, the device can be used as a biosensor by monitoring the impedance change of the coil.
Figure 15. a) Schematic representation of an AT-cut quartz crystal subjected to an oscillating electromagnetic field which induces a secondary electric field within the crystal; b) quartz crystals used for EMPAS (13.5 mm in diameter, and a thickness of 83 µm); c) hand-wound spiral coil (5 mm in diameter).

The EMPAS offers several advantages over conventional acoustic wave sensors. These advantages are mainly attributed to the fact that the EMPAS does not require the use of metal electrode-plated quartz crystal (no hardwire connection required) and that the resonance excitation is achieved remotely by an electromagnetic field. First, the EMPAS opens up the possibility of using trichlorosilane-based SAM chemistry for the fabrication of biosensing interfaces. Second, the EMPAS overcomes the complications associated with lateral electric fields and fringing fields. Lateral electric fields arise between electrode and bare quartz, and fringing fields occur near electrode edges. Third, the EMPAS has the ability to excite the quartz crystal at much higher harmonics, allowing measurements to be performed at ultra-high frequencies (up to and over 1 GHz) for greater sensitivity and lower limit-of-detections. Lastly, the instrument has the potential
to remotely conduct measurements.\textsuperscript{90} However, unlike TSM, EMPAS only measures $\Delta f_R$, which takes into consideration any changes that occur at the near-surface and bulk of the quartz crystal. This includes both mass density changes and damping in the system.

The EMPAS employed in this study is composed of the following components: a flow-through cell holder, a radio frequency generator, a coil circuit, a lock-in amplifier, an AM diode detector, an oscilloscope and a computer (\textit{Figure 14}). The flow-through cell holder houses the piezoelectric AT-cut crystals as well as the coil of the coil circuit. The crystal is suspended on top of the coil with the aid of a Teflon O-ring. The cell holder consists of two Plexiglas blocks clamped together, where the top contains an input tube and an output tube. The input tubing carries the sample and is connected to a low-pressure HPLC valve and a syringe pump. With the carrier wave (MHz) signal provided by the radio frequency generator, the coil circuit generates the electromagnetic field to excite resonance within the crystal. The lock-in amplifier modulates the carrier wave through a low-frequency (Hz) signal and, in combination with an AM diode detector, monitors amplitude changes in the low-frequency signal (\textit{i.e.} measures the voltage drop across the coil terminals). The oscilloscope constantly monitors the varying signal voltages. The computer controls all the settings of the sensor while processing and displaying the data.

\textbf{I. 6. Thesis Project}

\textbf{I. 6. 1. Background}

In the laboratory of Professor M. Thompson, we are developing a new generation of SAM linkers and diluents for the preparation of biosensing interfaces in order to address many of the issues that hinder biosensors as an analytical tool. This research was first initiated in our lab by Mark E. McGovern back in the late 1990’s with the development of 1-(Thiotrifluoroacetato)-11-(Trichlorosilyl)-Undecane (TTU), a thiol-protected trichlorosilane linker\textsuperscript{94} (\textit{Figure 17}).
The goal was to avoid the use of EDC/NHS coupling chemistry due its irreproducibility. It was anticipated that the linker would be able to immobilize the thiol-containing biosensing element through disulfide bonds after removal of the trifluoroacetyl protecting group. However, due to their close proximity in such packed structures, adjacent free thiols on the linkers would rather interact with each other to form disulfide bonds within the SAMs. This hindered the linkers by preventing the formation of external attachments. In addition, the extra deprotection step was not desirable. This linker was quickly abandoned and other types of head functions on the linker were sought after.

Many other linkers with different head functions were eventually designed and studied (Figure 17). Most of these linkers demonstrated the ability to prepare biosensing interfaces, but with varying degree of success. Many linkers shared the same problems with reproducibility, which was likely due to the head function’s lack of reactivity and/or selectivity towards thiol. Diluents were also incorporated into the SAM in an attempt to alleviate this problem. During these studies, it was also realized that the chemistry of the head function needed to be compatible with aqueous conditions and the resulting SAM needed to be resistant to non-specific adsorption in order to be practical. Thus, the ideal SAM linker and diluent would be able to form a non-specific adsorption resistant SAM and would be able to immobilize thiol-containing biomolecules under aqueous conditions in a single, straightforward, reliable and coupling-free manner.
Figure 17. Chemical structures of different SAM linkers with various head functions.

I. 6. 2. Thiosulfonate Head Function

Thiosulfonates and their derivatives are used in a variety of fields of study, such as organic chemistry, biochemistry, and polymer and material chemistry. They have an especially broad use in biochemistry due to their highly selective reactivity towards thiols. Disulfide bonds can be rapidly formed in water through the reaction of thiosulfonate with thiols. Typically, they are used for applications involving site-specific modifications of cysteine residues. These applications include inhibition, fluorescent labeling, glycosylation and biotinylation. The functional group consists of a residue $R^1$ attached to an electrophilic sulfur atom carrying a sulfinyl leaving group with residue $R^2$ (Figure 18).

Figure 18. General mechanism of the reaction between a thiosulfonate and a thiol.
The electrophilic sulfur atom features high selectivity toward soft nucleophiles, such as thiol. Generally, the reaction rates between thiosulfonate and thiol in aqueous buffer (pH 7) are to the order of $10^3$ to $10^4$ mol$^{-1}$s$^{-1}$, depending on the chemical nature of the R groups.$^{104}$ Even at low concentrations (below 1 mM), the reaction has been shown to proceed to completion within seconds.$^{105}$ Reactions with hard nucleophiles such as alcohols (-OH) and amines (-NH$_2$) are possible but occur at a much slower rate and produce a product is not stable in aqueous conditions. Additionally, thiosulfonate is stable towards hydrolysis. At neutral pH, the reaction half-life of hydrolysis ranges from 10 min to 2 h, and decreases with increasing pH.$^{106}$ Due to these remarkable characteristics and properties, thiosulfonate is an excellent candidate for the head function of a SAM linker and the immobilization of thiol-containing biosensing elements.

I. 6. 3. Thesis Objective and Methods

Having assessed the issues with the existing linkers and diluents, we therefore wanted to develop novel SAM linkers and diluents for the preparation of biosensing interfaces. These novel linkers used the thiosulfonate head function, which allowed for the chemoselective attachment of thiol-containing biosensing elements under mild aqueous conditions in a single, straightforward, reliable, and coupling-free manner. In addition, the OEG backbone was incorporated into the linkers and diluents in an attempt to eliminate non-specific adsorption. Two linkers (TUBTS and OEG-TUBTS) and four diluents (7-OEG-TFA, 9-OEG, 10-OEG-TFA and 13-OEG-TFA) were synthesized and investigated (Figure 19).
The main focus of this study is to evaluate the performance of the biosensing interfaces prepared by these novel linkers and diluents. The main analytical parameters studied were sensitivity, selectivity and resistance to non-specific adsorption. Specifically, we examined which aspects of these linkers and diluents prevented non-specific adsorption. Biotin/avidin was chosen as the test system to evaluate the performance of these interfaces.

First, the preparation of the biosensing interface with the TUBTS SAM and the test biosensing element, biotinthiol (Figure 19) will be described. Then, their characterization by CAM and XPS analysis will be described. Then, the performance of the resulting
biosensing interface will be evaluated with the EMPAS. The same analyses will be conducted with the OEG-analog of TUBTS (OEG-TUBTS) in an attempt to prevent non-specific adsorption. Our attention will then turn towards the design of a diluent to further improve the biosensing performance of our surfaces. A detailed study of the diluent with the best potential will be presented. Furthermore, we will examine the possibility of using our chemistry with other materials to further expand its applications. TUBTS will be used on Si$_3$N$_4$ and AlN, and the dithiolane-analog of TUBTS (DNBTS) will be used on gold. Lastly, a previous undocumented EMPAS coil circuit will be reported and discussed.
II. Experimental

II. 1. General Remarks

Anhydrous solvents (THF, MeCN, CH₂Cl₂, CHCl₃, PhMe, Et₂O) were systematically used. Freshly distilled anhydrous MeOH, EtOH and Et₃N (from KOH) were systematically used for biotinthiol immobilization. Avidin (from egg white, lyophilized powder), IgG from Rabbit, bovine serum albumin (BSA), goat serum and Dulbecco’s phosphate buffered saline (PBS) were purchased from Sigma-Aldrich®. Unless otherwise noted, other chemicals were also purchased from Sigma-Aldrich® and used as received. Quartz crystals (AT-cut, 13.5 mm in diameter, 20 MHz fundamental frequency) and TSM crystals (AT-cut, 13.5 mm in diameter, 9 MHz fundamental frequency) were purchased from Lap-Tech Inc®. EMPAS measurements were performed at 864 MHz (43rd harmonic). SAM formation and biotinthiol immobilization were performed in a glovebox maintained under an inert (N₂) and anhydrous (P₂O₅) atmosphere. The crystals were systematically handled with thoroughly pre-cleaned stainless steel tweezers in order to minimize any external contamination. ¹H and ¹³C NMR spectra were recorded at room temperature on a Varian Mercury 300 MHz or a Mercury 400 MHz spectrometers using CDCl₃ and CD₃OD as the NMR solvent. ¹H and ¹³C NMR spectra are referenced to the residual solvent peak (CDCl₃: 7.27 and 77.23 ppm; CD₃OD: 3.31 ppm). Chemical shifts are given in ppm.

II. 2. Chemical Synthesis

II. 2. 1. Synthesis of TUBTS

S-undec-10-enyl benzenethiosulfonate 1. To a stirred solution of 11-bromo-undec-1-ene (95%, 4.6 mL, 20.1 mmol, 1.0 equiv.) in MeCN (100 mL) were added benzenethionosulfonic acid sodium salt (85%, 9.2 g, 39.9 mmol, 2.0 equiv.) at rt. After overnight refluxing, the resulting solution was submitted to a EtOAc/H₂O extraction. The combined organic phases were then dried over anhydrous Na₂SO₄, filtered and finally evaporated in
vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 95/5) to afford 6.15 g (93%) of a pale cloudy yellow oil (> 95% purity). An additional careful column chromatography afforded pure 1 as a pale yellow oil. 1: \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ 7.95 (m, 2H), 7.64 (m, 1H), 7.56 (m, 2H), 5.82 (m, 1H), 5.14-4.91 (m, 2H), 3.00 (t, \(J = 7.2\) Hz, 2H), 2.04 (q, \(J = 7.0\) Hz, 2H), 1.58 (qt, \(J = 7.2\) Hz, 2H), 1.42-1.16 (m, 12H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) δ 145.2, 139.4, 133.7, 129.4, 127.2, 114.4, 36.3, 34.0, 29.5, 29.4, 29.2, 29.1, 29.0, 28.8, 28.7; IR (neat) 3070 cm\(^{-1}\), 1640 cm\(^{-1}\), 1328 cm\(^{-1}\), 1147 cm\(^{-1}\); HRMS (ESI, \(m/z\)) calcd. for C\(_{17}\)H\(_{27}\)S\(_2\)O\(_2\) (MH\(^+\)) 327.1446, found 327.1447.

S-(11-trichlorosilyl-undecenyl) benzenethiosulfonate 2 (TUBTS). In a heavy-walled tube equipped with a magnetic stirring bar, S-undec-10-enyl benzenethiosulfonate 1 (700 mg, 2.13 mmol, 1.0 equiv.) and H\(_2\)PtCl\(_6\).6H\(_2\)O (1 mg, 0.013 mmol, 0.1 mol. %) was placed. The tube was transferred into a glove-box and HSiCl\(_3\) (0.30 mL, 2.94 mmol, 2.1 equiv.) was added to the solution. The tube was tightly fastened and then removed from the glove-box. The resulting solution was stirred at room temperature for 43 h behind a protecting shield. Excess HSiCl\(_3\) was then removed under high vacuum to afford 2 as a viscous orange oil (633 mg, 95%). 2: \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ 7.93 (m, 2H), 7.63 (m, 1H), 7.55 (m, 2H), 2.99 (t, \(J = 7.3\) Hz, 2H), 1.65-1.51 (m, 4H), 1.44-1.16 (m, 16H).

II. 2. 2. Synthesis of OEG-TUBTS

(2-allyloxy-ethoxy)-acetic acid methyl ester 3. To a stirred solution of 2-allyloxy-ethanol (5.5 mL, 50.0 mmol, 1.0 equiv.) in THF (50 mL) was carefully added NaH (60%, 2.4 g, 60.0 mmol, 1.2 equiv.) in small portions at rt. The solution was then refluxed for 1h (until H\(_2\) release ceases). After cooling to 0ºC, the resulting solution is added dropwise to
a stirred solution of Methyl bromoacetate (5.7 mL, 60.0 mmol, 1.2 equiv.) in THF (50 mL). After completion (30 min at 0ºC), the resulting solution was submitted to an EtOAc/H₂O extraction. The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄, then filtered and evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 95/5 to 60/40). 3: yellow oil (6.66 g, 70%); ¹H NMR (400 MHz, CDCl₃) δ 5.96-5.85 (m, 1H), 5.31-5.24 (m, 1H), 5.20-5.15 (m, 1H), 4.17 (s, 2H), 4.04-4.01 (m, 2H), 3.75 (s, 3H), 3.75-3.72 (m, 2H), 3.65-3.62 (m, 2H); ¹³C NMR (100MHz, CDCl₃) δ 171.1, 134.8, 117.3, 72.4, 71.2, 69.7, 68.9, 51.9; IR (neat) 1755 cm⁻¹; HRMS (ESI, m/z) calcd. For C₈H₁₅O₄ (MH⁺) 175.0964, found 175.0960.

2-(2-allyloxy-ethoxy)-ethanol 4. To a stirred solution of ester 3 (9.77 g, 55.1 mmol, 1.0 equiv.) in THF (100 mL) was carefully added one portion of LAH (1.10 g, 27.5 mmol, 0.5 equiv.) at 0ºC. After 30 min, another portion of LAH was carefully added and then the reaction was stirred for an extra 30 min. The reaction was then carefully quenched with a dropwise addition of a Na₂SO₄ saturated aqueous solution. White aluminum salts were then filtered off over a short plug of Celite (EtOAc washings) and the resulting filtrate was finally evaporated in vacuo. Purification was achieved by distillation under reduced pressure using a Kugelrohr distillation apparatus to afforded 4 as a colourless oil (7.99 g, 99%). Spectroscopic data was consistent with those reported in the literature.¹¹ ¹H NMR (400 MHz, CDCl₃) δ 5.92 (ddt, J = 17.3, 10.4, 5.6 Hz, 1H), 5.29 (dq, J = 17.3, 1.6 Hz, 1H), 5.20 (dq, J = 10.4, 1.6 Hz, 1H), 4.04 (dt, J = 5.6, 1.6 Hz, 2H), 3.76-3.73 (m, 2H), 3.71-3.68 (m, 2H), 3.64-3.60 (m, 4H), 2.36 (brs, 1H).

(2-(2-allyloxy-ethoxy)-ethoxy)-acetic acid methyl ester 5. To a stirred solution of alcohol 4 (4.86 g, 33.2 mmol, 1.0 equiv.) in THF (35 mL) was carefully added NaH (60%, 1.60 g, 40.0 mmol, 1.2 equiv.) in small portions at rt. The solution was then refluxed for 1h (until H₂ release ceases). After cooling to 0ºC, the
resulting solution is added dropwise to a stirred solution of Methyl bromoacetate (5.7 mL, 60.0 mmol, 1.2 equiv.) in THF (35 mL). After completion (30 min at 0°C), the resulting solution was submitted to an EtOAc/H₂O extraction. The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄, and then filtered and evaporated in vacuo. Purification was achieved by distillation under high vacuum. 5: colourless oil (5.49 g, 75%); bp = 130-140 0°C (0.09 Torr); ℎ NMR (300 MHz, CDCl₃) δ 5.98-5.84 (m, 1H), 5.32-5.24 (m, 1H), 5.22-5.14 (m, 1H), 4.17 (s, 2H), 4.02 (m, 2H), 3.75 (s, 3H), 3.75-3.58 (m, 8H); ℎ NMR (75 MHz, CDCl₃) δ 171.0, 134.8, 117.1, 72.3, 71.0, 70.8, 70.7, 69.5, 68.7, 51.8; IR (neat) 1754 cm⁻¹; HRMS (ESI, m/z) calcd. for C₁₀H₁₉O₅ (MH⁺) 219.1236, found 219.1227.

2-(2-(2-allyloxy-ethoxy)-ethoxy)-ethanol 6. To a stirred solution of ester 5 (4.60 g, 21.1 mmol, 1.0 equiv.) in THF (60 mL) was carefully added one portion of LAH (0.50 g, 12.5 mmol, 0.5 equiv.) at 0°C. After 30 min, another portion of LAH was carefully added. The reaction was then stirred for an extra 30 min. The reaction was carefully quenched with a dropwise addition of a Na₂SO₄ saturated aqueous solution. White aluminum salts were then filtered off over a short plug of Celite (EtOAc washings) and the resulting filtrate was finally evaporated in vacuo to afford pure 6 (no purification required) as a pale yellow oil (3.89 g, 97%). Spectroscopic data were consistent with those reported in the literature: ℎ NMR (300 MHz, CDCl₃) δ 5.93 (ddt, J = 17.2, 10.3, 5.7 Hz, 1H), 5.28 (dq, J = 17.2, 1.5 Hz, 1H), 5.19 (dq, J = 10.3, 1.5 Hz, 1H), 4.04 (dt, J = 5.7, 1.5 Hz, 2H), 3.78-3.58 (m, 12H), 2.51 (brs, 1H); ℎ NMR (75 MHz, CDCl₃) δ 134.8, 117.3, 72.7, 72.4, 70.8, 70.7, 70.5, 69.5, 61.8.

3-(2-(2-bromo-ethoxy)-ethoxy)-ethoxy)-prop-1-ene 7. To a stirred solution of alcohol 6 (3.83 g, 20.1 mmol, 1.0 equiv.) and pyridine (0.16 mL, 2.00 mmol, 0.1 equiv.) in Et₂O (20 mL) was added dropwise phosphorus tribromide (0.74 mL, 7.60
mmol, 0.36 equiv.) at 0°C. After 30 min at 0°C, the reaction was allowed to warm to room temperature. As the reaction was apparently not complete after 12h of stirring, pyridine (1.60 mL, 20.0 mmol, 1.0 equiv.) and sodium bromide (4.14 g, 40.2 mmol, 2.0 equiv.) were added successively. After 12h of reflux, the resulting solution was submitted to an EtOAc/NH₄Cl saturated aqueous solution extraction. The combined organic phases were then dried over anhydrous Na₂SO₄, filtered and evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 95/5 to 60/40). 7: yellow oil (0.90 g, 18%); ¹H NMR (300 MHz, CDCl₃) δ 5.93 (ddt, J = 17.3, 10.5, 5.7 Hz, 1H), 5.28 (dq, J = 17.3, 1.5 Hz, 1H), 5.19 (dq, J = 10.5, 1.5 Hz, 1H), 4.03 (dt, J = 5.7, 1.5 Hz, 2H), 3.82 (t, J = 6.3 Hz, 2H), 3.71-3.65 (m, 6H), 3.64-3.59 (m, 2H), 3.48 (t, J = 6.3 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 134.9, 117.4, 72.5, 71.4, 70.9, 70.8, 70.7, 69.6, 30.5.

S-(2-(2-(allyloxy-ethoxy)-ethoxy)-ethyl) benzenethiosulfonate 8. To a stirred solution of bromide 7 (0.90 g, 3.6 mmol, 1.0 equiv.) in MeCN (18 mL) was added benzenethionosulfonic acid sodium salt (85%, 1.64 g, 7.1 mmol, 2.0 equiv.) at rt. After overnight refluxing, the resulting solution was submitted to an EtOAc/brine extraction. The combined organic phases were then dried over anhydrous Na₂SO₄, filtered and finally evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 95/5 to 30/70) to afford 1.04 g (84%) of a pale yellow oil (> 95% purity). An additional careful column chromatography afforded pure 8 as a pale yellow oil. 8: ¹H NMR (300 MHz, CDCl₃) δ 7.95 (m, 2H), 7.65 (m, 1H), 7.56 (m, 2H), 5.91 (m, 1H), 5.32-5.14 (m, 2H), 4.01 (m, 2H), 3.72 (m, 10H), 3.20 (t, J = 6.3 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 145.0, 134.9, 133.9, 129.5, 127.2, 117.3, 72.4, 70.8, 70.7, 70.6, 69.6, 69.2, 35.9; IR (neat) 3068, 1647, 1324, 1142 cm⁻¹; HRMS (ESI, m/z) calcd. for C₁₅H₂₃S₂O₅ (MH⁺) 347.0971, found 347.0981.
S-(2-(2-(3-trichlorosilyl-propyloxy)-ethoxy)-ethoxy)-ethyl benzenethiosulfonate 9 (OEG-TUBTS). In a heavy-walled tube equipped with a magnetic stirring bar, benzene-thiosulfonate 8 (347 mg, 1.00 mmol, 1.0 equiv.) and H₂PtCl₆·6H₂O (1 mg, 0.002 mmol, 0.5 mol. %) were placed. The tube was transferred into a glove-box and HSiCl₃ (0.30 mL, 2.94 mmol, 3.0 equiv.) was added to the solution. The tube was tightly fastened and then removed from the glove-box. The resulting solution was stirred at room temperature for 21 h behind a protecting shield. HSiCl₃ excess was then removed under high vacuum to afford 9 as a viscous yellow-orange cloudy oil (444 mg, 92%). 9: ¹H NMR (300 MHz, CDCl₃) δ 7.94 (m, 1H), 7.62 (m, 1H), 7.56 (m, 1H), 7.46 (m, 1H), 7.32 (m, 1H), 3.85-3.52 (m, 16H), 3.20 (t, J = 6.2 Hz, 1H), 2.80 (t, J = 6.2 Hz, 1H).

II. 2. 3. Synthesis of 9-OEG

3-(2-ethoxyethoxy)prop-1-ene 10. To a stirred solution of 2-ethoxyethanol (2.0 mL, 20.6 mmol, 1.0 equiv.) in Et₂O (25 mL) at was carefully added NaH (60%, 0.98 g, 24.7 mmol, 1.2 equiv.) in small portions at rt. The solution was then reflexed for 1h (until H₂ release ceases). After cooling to 0ºC, the resulting solutions is added dropwise to a stirred solution of allyl bromide (2.1 mL, 24.7 mmol, 1.2 equiv.) in Et₂O (25 mL). After completion (1 h at 0ºC), the resulting solution was submitted to an Et₂O/H₂O extraction. The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄ then filtered and evaporated in vacuo. Purification was achieved by column chromatography (Pentanes/Et₂O gradient 100/0 to 85/15). 11: colourless oil (1.24 g, 32%); ¹H NMR (400 MHz, CDCl₃): δ 5.93 (ddt, J = 17.1, 10.6, 5.7 Hz, 1H), 5.28 (m, 1H), 5.19 (m, 1H), 4.04 (m, 2H), 3.60 (s, 4H), 3.54 (q, J = 7.0 Hz, 2H), 1.23 (t, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 134.8, 117.2, 72.3, 69.8, 69.4, 66.7, 15.1.
Trichloro(3-(2-ethoxyethoxy)-propyl silane 13 (9-OEG)

11. In a heavy-walled tube equipped with a magnetic stirring bar, OEG-diluent precursor 10 (1.00 g, 7.7 mmol, 1.0 equiv.) and $\text{H}_2\text{PtCl}_6.6\text{H}_2\text{O}$ (41 mg, 0.08 mmol, 1.0 mol. %) were placed. The tube was then transferred into a glove-box and $\text{HSiCl}_3$ (1.57 mL, 15.4 mmol, 2.0 equiv.) was added to the solution. The tube was tightly fastened and removed from the glove-box. The resulting solution was stirred at room temperature for 20 h behind a protecting shield. Purification was achieved by Kugelrohr distillation under high vacuum. 11: colourless oil (1.63 g, 80%); bp = 105-120 °C (0.09 Torr); $^1\text{H}$ NMR (400 MHz, CDCl$_3$) δ 3.58 (s, 4H), 3.54 (m, 4H), 1.85 (m, 2H), 1.48 (m, 2H), 1.23 (m, 3H); $^{13}\text{C}$ NMR (100 MHz, CDCl$_3$) δ 71.8, 70.2, 69.8, 67.0, 22.7, 21.1, 15.1.

II. 2. 4. Synthesis of 10-OEG-TFA

Trifluoroacetic acid 2-(2-allyloxy-ethoxy)-ethyl ester 12. To a stirred solution of alcohol 4 (2.92 mL, 20.0 mmol, 1.0 equiv.), triethylamine (5.6 mL, 40.0 mmol, 2.0 equiv.) and 4-DMAP (0.25 g, 2.0 mmol, 0.1 equiv.) in CH$_2$Cl$_2$ (40 mL) was added dropwise trifluoroacetic anhydride (3.37 mL, 24.0 mmol, 1.2 equiv.) at 0°C. The solution was then allowed to warm to room temperature and then stirred overnight. The resulting solution was submitted to a CH$_2$Cl$_2$/saturated NH$_4$Cl aqueous solution extraction. The combined organic phases were dried over anhydrous Na$_2$SO$_4$, filtered then evaporated in vacuo. Purification was achieved by distillation under reduced pressure. 12: colourless oil (3.61 g, 70%); bp = 100-110°C (water tap vacuum); $^1\text{H}$ NMR (400 MHz, CDCl$_3$) δ 5.91 (ddt, $J = 17.2$, 11.6, 5.6 Hz, 1H), 5.27 (dq, $J = 17.2$ Hz, 1H), 5.18 (dq, $J = 11.6$, 1H), 4.50 (t, $J = 4.8$ Hz, 2H), 4.02 (dt, $J = 5.6$ Hz, 2H), 3.80 (t, $J = 4.8$ Hz, 2H), 3.64 (m, 4H); $^{13}\text{C}$ NMR (100 MHz, CDCl$_3$) δ 157.8 (q, $J = 42.4$ Hz), 134.2, 117.7, 114.8 (q, $J = 283.9$Hz) 72.2, 71.0, 68.7, 67.8 66.2.
Trifluoroacetic acid 2-(2-(3-trichlorosilyl-propyloxy)-ethoxy)-ethyl ester 15 (10-OEG-TFA). In a heavy-walled tube equipped with a magnetic stirring bar, OEG-diluent precursor 12 (0.50 g, 1.9 mmol, 1.0 equiv.) and H$_2$PtCl$_6$.6H$_2$O (1 mg, 0.002 mmol, 0.1 mol. %) were placed. The tube was transferred into a glove-box and HSiCl$_3$ (0.39 mL, 3.8 mmol, 2.0 equiv.) was added to the solution. The tube was tightly fastened then removed from the glove-box. The resulting solution was stirred at room temperature for 20 h behind a protecting shield. HSiCl$_3$ excess was then removed under high vacuum to afford 13 as an orange-brown oil (0.67 g, 90%). 13: $^1$H NMR (400 MHz, CDCl$_3$) δ 4.50 (m, 2H), 3.79 (m, 2H), 3.65 (m, 4H), 3.51(m, 2H), 1.85 (m, 2H), 1.47 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 157.6 (q, $J = 42.4$ Hz), 114.2 (q, $J = 284.0$ Hz), 71.8, 70.6, 70.2, 68.1, 66.5, 22.2, 20.8

II. 2. 5. Synthesis of 7-OEG-TFA

Trifluoroacetic acid 2-allyloxy-ethyl ester 10. To a stirred solution of 2-allyloxy-ethanol (4.36 mL, 40.0 mmol, 1.0 equiv.), triethylamine (11.2 mL, 80.0 mmol, 2.0 equiv.) and 4-DMAP (0.49 g, 4.0 mmol, 0.1 equiv.) in CH$_2$Cl$_2$ (80 mL) was added dropwise trifluoroacetic anhydride (6.74 mL, 48.0 mmol, 1.2 equiv.) at 0°C. The solution was then allowed to warm to room temperature then stirred overnight. The resulting solution was submitted to a CH$_2$Cl$_2$/saturated NH$_4$Cl aqueous solution extraction. The combined organic phases were dried over anhydrous Na$_2$SO$_4$, filtered then evaporated in vacuo. Purification was achieved by distillation under reduced pressure. 10: colourless oil (5.76 g, 72%); bp = 72-74°C (water tap vacuum); $^1$H NMR (400 MHz, CDCl$_3$) δ 5.88 (m, 1H), 5.29 (brd, $J = 17.2$ Hz, 1H), 5.20 (m, 1H), 4.50 (t, $J = 4.8$ Hz, 2H), 4.03 (m, 2H), 3.73 (t, $J = 4.8$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 157.7 (q, $J = 4.1$ Hz), 134.2, 117.8, 114.7 (q, $J = 283.9$ Hz), 72.4, 67.2, 67.0.
Trifluoroacetic acid 2-(3-trichlorosilyl-propyloxy)-ethyl ester 11 (7-OEG-TFA). In a heavy-walled tube equipped with a magnetic stirring bar, OEG-diluent precursor 10 (3.97 g, 20.0 mmol, 1.0 equiv.) and H₂PtCl₆.6H₂O (104 mg, 0.20 mmol, 1.0 mol. %) were placed. The tube was transferred into a glove-box and HSiCl₃ (4.10 mL, 40.2 mmol, 2.0 equiv.) was added to the solution. The tube was tightly fastened then removed from the glove-box. The resulting solution was stirred at room temperature for 20 h behind a protecting shield. Purification was achieved by Kugelrohr distillation under high vacuum. 11: colourless oil (5.46 g, 82%); bp = 115-120 ºC (0.09 Torr); ¹H NMR (400 MHz, CDCl₃) δ 4.50 (m, 2H), 3.73 (m, 2H), 3.54 (t, J = 6.2 Hz, 2H), 1.85 (m, 2H), 1.48 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 157.7 (q, J = 42.4 Hz), 114.3 (q, J = 284.0 Hz), 71.8, 67.9, 67.0, 22.8, 21.0.

II. 2. 6. Synthesis of 13-OEG-TFA

Trifluoroacetic acid 2-(2-(2-allyloxy-ethoxy)-ethoxy)-ethyl ester 16. To a stirred solution of alcohol 6 (1.92 mL, 10.0 mmol, 1.0 equiv.), triethylamine (2.8 mL, 20.0 mmol, 2.0 equiv.) and 4-DMAP (0.13 g, 1.0 mmol, 0.1 equiv.) in CH₂Cl₂ (20 mL) was added dropwise trifluoroacetic anhydride (1.69 mL, 12.0 mmol, 1.2 equiv.) at 0ºC. The solution was then allowed to warm to room temperature then stirred overnight. The resulting solution was submitted to a CH₂Cl₂/saturated NH₄Cl aqueous solution extraction. The combined organic phases were dried over anhydrous Na₂SO₄, filtered then evaporated in vacuo. Purification was achieved by distillation under high vacuum then followed by a quick column chromatography (CH₂Cl₂/EtOAc gradient 100/0 to 95/5). 16: pale yellow oil (1.72 g, 60%); bp = 140-150ºC (water tap vacuum); ¹H NMR (400 MHz, CDCl₃) δ 5.91 (J = 17.2, 10.4, 5.6 Hz, 1H), 5.27 (dq, J = 17.2, 1.6 Hz, 1H), 5.17 (dq, J = 10.4, 1.6 Hz, 1H) 4.49 (t, J = 4.8 Hz, 2H), 4.02 (dt, J = 5.6 Hz, 2H), 3.79 (t, J = 4.8 Hz, 2H), 3.69-3.58 (m, 8H); ¹³C NMR (100 MHz, CDCl₃) δ 157.8 (q, J = 42.4 Hz), 134.3, 117.5, 114.8 (q, J = 283.8Hz) 72.2, 70.8, 70.7, 70.6, 69.4, 68.2, 67.0.
Trifluoroacetic acid 2-(2-(3-trichlorosilyl-propyloxy)-ethoxy)-ethoxy-ethyl ester 17 (13-OEG-TFA).

In a heavy-walled tube equipped with a magnetic stirring bar, OEG-diluent precursor 16 (0.50 g, 1.7 mmol, 1.0 equiv.) and H₂PtCl₆·6H₂O (1 mg, 0.002 mmol, 0.1 mol. %) were placed. The tube was transferred into a glove-box and HSiCl₃ (0.35 mL, 3.4 mmol, 2.0 equiv.) was added to the solution. The tube was tightly fastened then removed from the glove-box. The resulting solution was stirred at room temperature for 20 h behind a protecting shield. HSiCl₃ excess was then removed under high vacuum to afford 17 as an orange-brown oil (0.64 g, 90%). 17: ¹H NMR (400 MHz, CDCl₃) δ 4.47 (m, 2H), 3.77 (m, 2H), 3.70-3.47 (m, 10H), 1.85 (m, 2H), 1.47 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 157.8 (q, J = 42.6 Hz), 114.4 (q, J = 283.7 Hz) 71.9, 70.8, 70.7, 70.6, 68.2, 67.0, 65.9, 22.4, 21.0.

II. 2. 7. Synthesis of DNBTS

2-(9-bromomononyloxy)-(2H)-tetrahydropyran 18. To a stirred solution of PTSA (0.44 g, 2.3 mmol, 0.1 equiv.) and dihydropyran (2.9 mL, 32 mmol, 1.4 equiv.) in CH₂Cl₂ (40 mL) was added 9-bromomononanol (5.0 g, 23 mmol, 1.0 equiv.) at rt. The solution was stirred overnight at rt. The resulting solution was submitted to a CH₂Cl₂/saturated K₂CO₃ aqueous solution extraction. The combined organic phases were dried over anhydrous Na₂SO₄, filtered then evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 95/5). 18: colourless liquid (7.3 g, 98%); ¹H NMR (400 MHz, CDCl₃) δ 4.56 (t, 1H), 3.87-3.84 (m, 1H), 3.74-3.69 (m, 1H), 3.50-3.47 (m, 1H), 3.40-3.35 (m, 3H), 1.87-1.81 (m, 3H), 1.75-1.64 (m, 1H), 1.60-1.43 (m, 6H), 1.42-1.32 (m, 10H); ¹³C NMR (100 MHz, CDCl₃) δ 98.77, 67.59, 62.26, 33.88, 32.75, 30.73, 29.66, 29.28, 28.63, 28.08, 25.45, 19.64.
2-(9-(tetrahydro-2H-pyran-2-yloxy)nonyl) diethyl malonate 19. To a stirred solution of diethyl malonate (3.88 g, 23.0 mmol, 1.2 equiv.) in THF (30 mL) was carefully added NaH (60%, 0.84 g, 21.1 mmol, 1.1 equiv.) in small portions at rt. The solution was then refluxed for 1h (until H₂ release ceases). After cooling to 0 °C, the resulting solution is added dropwise to a stirred solution of bromide 18 (5.88 g, 19.1 mmol, 1.0 equiv.) in THF (30 mL). After completion (30 min at 0 °C), the resulting solution was submitted to a EtOAc/H₂O extraction. The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄, then filtered and evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 95/5). 19: colourless liquid (5.02 g, 68%); ¹H NMR (400 MHz, CDCl₃) δ 4.58 (t, 1H), 4.22-4.18 (m, 4H). 3.87-3.82 (m, 1H). 3.77-3.74 (m, 1H), 3.52-3.49 (m, 1H), 3.40-3.35 (m, 1H), 3.34-3.32 (m, 1H), 1.92-1.79 (m, 3H), 1.78-1.64 (m, 1H), 1.62-1.47 (m, 6H), 1.40-1.22 (m, 18H).

2-(9-(tetrahydro-2H-pyran-2-yloxy)nonyl)propane-1,3-diol 20. To a stirred solution of diester 19 (4.80 g, 12.4 mmol, 1.0 equiv.) in THF (20 mL) was carefully added one portion of LAH (0.50 g, 12.4 mmol, 1.0 equiv.) at 0°C. After 30 min, another portion of LAH was carefully added then the reaction was stirred for an extra 30 min. The reaction was then carefully quenched with a dropwise addition of a Na₂SO₄ saturated aqueous solution. White aluminum salts were then filtered off over a short plug of Celite (EtOAc washings) and the resulting filtrate was finally evaporated in vacuo to afford pure 20 (no purification required) as a pale yellow oil (3.97 g, 97%); ¹H NMR (400 MHz, CDCl₃) δ 4.58 (t, 1H), 3.90-3.78 (m, 3H), 3.72-3.68 (m, 1H), 3.68-3.60 (m, 2H), 3.50-3.45 (m, 1H), 3.40-3.36 (m, 1H), 2.45-2.35 (b, 2H), 1.81-1.65 (m, 3H), 1.60-1.44 (m, 6H). 1.40-1.18 (m, 14H).
2-(9-(tetrahydro-2H-pyran-2-yloxy)nonyl)propane-1,3-dibromide 21. To a stirred solution of diol 20 (4.20 g, 14.0 mmol, 1.0 equiv.), imidazole (2.60 g, 39.1 mmol, 2.8 equiv.) and Ph₃P (11.0 g, 42.0 mmol, 3.0 equiv.) in CH₂Cl₂ (140 mL) was added dropwise CBr₄ (13.0 g, 39 mmol, 2.8 equiv.) at 0°C. After 30 min at 0°C, the reaction was allowed to warm to room temperature then the reaction was stirred for an extra 1h. The reaction was then carefully quenched with a dropwise addition of a 10% NaSO₃ aqueous solution. The resulting solution was submitted to an EtOAc/H₂O extraction. The combined organic phases were then dried over anhydrous Na₂SO₄, filtered and evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 50/50). 21: yellow oil (5.11 g, 80%); ¹H NMR (400 MHz, CDCl₃) δ 4.58 (t, 1H) 3.90-3.84 (m, 1H), 3.75-3.68 (m, 1H), 3.61-3.58 (m, 2H), 3.53-3.44 (m, 3H), 3.40-3.36 (m, 1H), 1.99-1.91 (m, 1H), 1.86-1.78 (m, 1H), 1.77-1.65 (m, 1H), 1.63-1.22 (m, 20H).

2-(9-(tetrahydro-2H-pyran-2-yloxy)nonyl)propane-1,3-dithioacetate 22. To a stirred solution of dibromide 21 (4.83 g, 11.3 mmol, 1.0 equiv.) and NaI (8.81 g, 58.8 mmol, 5.1 equiv.) in MeCN (150 mL) was added KSAc (6.72 g, 58.8 mmol, 5.1 equiv.) at rt. The reaction was refluxed overnight then submitted to a CH₂Cl₂/H₂O extraction. The combined organic layers were washed with sodium thiosulfate and brine, dried over anhydrous Na₂SO₄, filtered then evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 85/15). 19: brown liquid (4.01 g, 85%); ¹H NMR (400 MHz, CDCl₃) δ 4.57 (t, 1H), 3.90-3.82 (m, 1H), 3.79-3.71 (m, 1H), 3.61-3.58 (m, 2H), 3.53-3.44 (m, 3H), 3.40-3.36 (m, 1H), 1.99-1.91 (m, 1H), 1.86-1.78 (m, 1H), 1.77-1.65 (m, 1H), 1.40-1.18 (m, 14H).
2-(9-(tetrahydro-2H-pyran-2-yloxy)nonyl)propane-1,3-dithiol 23. To a stirred solution of dithioacetate 22 (3.90 g, 9.32 mmol, 1.0 equiv.) in THF (100 mL) was added LAH (95%, 0.74 g, 18.6 mmol, 2.2 equiv.) in small portions at -78°C. After addition, the reaction was allowed to warm to rt then stirred for 1.5h. The reaction was then carefully quenched with a dropwise addition of a Na₂SO₄ saturated aqueous solution. White aluminum salts were then filtered off over a short plug of Celite (EtOAc washings) and the resulting filtrate was finally evaporated in vacuo to afford pure 23 (no purification required) as a pale yellow oil (1.56 g, 50%); ¹H NMR (400 MHz, CDCl₃) δ 4.58 (t, 1H), 3.90-3.82 (m, 1H), 3.75-3.71 (m, 1H), 3.51-3.45 (m, 1H), 3.40-3.36 (m, 1H), 2.77-2.58 (m, 4H), 1.84-1.78 (m, 1H), 1.78-1.64 (m, 2H), 1.60-1.18 (m, 22H).

4-(9-nonanoyl)-1,2-dithiolane 24. To a stirred solution of dithiol 23 (1.56 g, 4.69 mmol, 1.0 equiv.) and sodium acetate (1.81 g, 14.1 mmol, 3 equiv.) in CHCl₃ (100 mL) was added solution of I₂ (0.1 M, 50 mL) at 0 °C. After addition, the reaction was allowed to warm to rt then stirred for overnight. The reaction was decolourized with Na₂S₂O₃ (1 M) and submitted to a CHCl₃/H₂O extraction. The combined organic layers were dried over anhydrous Na₂SO₄, filtered then evaporated under reduced pressure. The residue was immediately dissolved in MeOH (60 mL) then PTSA (88 mg, 0.47 mmol, 0.1 equiv.) was added at rt. The reaction was stirred overnight then submitted to a CHCl₃/saturated K₂CO₃ aqueous solution extraction. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered then evaporated under reduced pressure. Purification was achieved by column chromatography on silica gel (CHCl₃/MeOH gradient 100/0 to 90/10) and provided 1.05 g (90%, 2 steps) of alcohol 24 as a pale yellow oil; ¹H NMR (400 MHz, CDCl₃) δ 3.63 (t, J = 6.4 Hz, 2H), 3.23 (dd, J = 11.2, 6.8 Hz, 2H), 2.78 (dd, J = 11.2, 6.8 Hz), 2.52 (sep, J = 6.8 Hz, 1H), 1.59-1.46 (m, 4H), 1.35-1.23 (m, 12H).
4-(9-bromononyl)-1,2-dithiolane 25. To a stirred solution of alcohol 24 (0.90 g, 3.62 mmol, 1.0 equiv.), imidazole (0.35 g, 5.1 mmol, 1.4 equiv.) and Ph₃P (1.45 g, 5.43 mmol, 1.5 equiv.) in CH₂Cl₂ (60 mL) was added dropwise CBr₄ (1.70 g, 5.1 mmol, 1.4 equiv.) at 0°C. After 30 min at 0°C, the reaction was allowed to warm to room temperature then the reaction was stirred for an extra 1h. The reaction was then carefully quenched with a dropwise addition of a 10% NaSO₃ aqueous solution. The resulting solution was submitted to an EtOAc/H₂O extraction. The combined organic phases were then dried over anhydrous Na₂SO₄, filtered and evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 85/15). 19: yellow liquid (0.98 g, 84%); ¹H NMR (400 MHz, CDCl₃) δ 3.40 (t, J = 7.2 Hz, 2H), 3.25 (dd, J = 11.2, 6.8 Hz, 2H), 2.79 (dd, J = 11.2, 6.8 Hz), 2.51 (sep, J = 6.8 Hz, 1H), 1.87-1.84 (m, 4H), 1.51-1.30 (m, 12H).

S-9-(1,2-dithiolan-4-yl)nonyl benzenesulfonothioate 26 (DNBTS). To a stirred solution of bromide 25 (0.34 g, 1.08 mmol, 1.0 equiv.) in MeCN (10 mL) was added benzenethionosulfonic acid sodium salt (85%, 0.5 g, 2.17 mmol, 2.0 equiv.) at rt. After overnight refluxing, the resulting solution was submitted to an EtOAc/H₂O extraction. The combined organic phases were then dried over anhydrous Na₂SO₄, filtered and finally evaporated in vacuo. Purification was achieved by column chromatography (Hexanes/EtOAc gradient 100/0 to 90/10) to afford 0.41 g (93%) of a pale yellow oil. An additional careful column chromatography afforded pure 26 as a pale yellow oil. 26: ¹H NMR (400 MHz, CDCl₃) δ 7.93 (m, 2H), 7.63 (m, 1H), 7.55 (m, 2H), 3.22 (dd, J = 10.8, 6.8 Hz, 2H), 2.99 (t, J = 7.2 Hz, 2H), 2.79 (dd, J = 10.8, 6.8 Hz, 2H), 2.51 (sep, J = 6.8, 1H), 1.61-1.46 (m, 4H), 1.36-1.21 (m, 12H).
II. 2. 8. Synthesis of Biotinthiol

Biotinthiol was synthesized in five steps from biotin with a 33% overall yield (Figure 18).

**Figure 20.** Synthesis of biotinthiol.

**Biotin methyl ester 27.** To a stirred solution of biotin (900 mg, 3.65 mmol, 1.0 equiv.) in absolute EtOH (30 mL) were added few drops of concentrated H$_2$SO$_4$ at rt. After stirring at rt overnight, the reaction was submitted to a CH$_2$Cl$_2$/Na$_2$CO$_3$-aqueous solution extraction. The combined organic layers were
dried over anhydrous Na$_2$SO$_4$, filtered then evaporated under reduced pressure to provide 961 mg (97%) of ester 27 as a white solid. Spectroscopic data were consistent with those reported in the literature:°°°° ¹H NMR (400 MHz, CDCl$_3$) δ 5.52 (brs, 1H), 5.15 (brs, 1H), 4.52 (m, 1H), 4.32 (m, 1H), 4.13 (q, $J = 7.2$ Hz, 2H), 3.17 (m, 1H), 2.93 (dd, $J = 12.8$, 4.8 Hz, 1H), 2.75 (d, $J = 12.8$ Hz, 1H), 2.33 (t, $J = 7.6$ Hz, 2H), 1.69 (m, 4H), 1.46 (m, 2H), 1.26 (t, $J = 7.2$ Hz, 3H).

Biotinol 28.°°°° To a stirred solution of biotin methyl ester 27 (961 mg, 3.53 mmol, 1.0 equiv.) in CH$_2$Cl$_2$ (10 mL) was added dropwise DIBAL-H (1.0 M in hexanes, 12.4 mL, 12.4 mmol, 3.5 equiv.) at -78°C. After addition, the reaction was allowed to warm to rt then stirred for 2h. The reaction was then carefully quenched, at -78°C, by dropwise addition of MeOH then H$_2$O. After evaporation of the solvents under reduced pressure, the purification was achieved by Soxhlett extraction (EtOH) and provided 796 mg (98%) of biotinol 28 as a white solid;°°°° ¹H NMR (400 MHz, CD$_3$OD) δ 4.49 (dd, $J = 7.8$, 4.8 Hz, 1H), 4.30 (dd, $J = 7.8$, 4.8 Hz, 1H) 3.55 (t, $J = 6.6$ Hz, 2H), 3.21 (m, 1H), 2.93 (dd, $J = 12.6$, 4.8 Hz, 1H), 2.71 (d, $J = 12.6$ Hz, 1H), 2.16 (s, 1H), 1.74 (m, 1H), 1.57 (m, 3H), 1.45 (m, 4H).

Biotin tosylate°°°° 29 and biotin thioacetate 30. To a stirred solution of biotinol 28 (796 mg, 3.46 mmol, 1.0 equiv.) in pyridine (20mL) was added tosyl chloride (1.75 g, 9.09 mmol, 2.6 equiv.) at 0°C. After addition, the reaction was allowed to warm to rt then stirred for 2h. The reaction was then submitted to a CH$_2$Cl$_2$/1 M H$_2$SO$_4$ aqueous solution extraction. The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered then evaporated under reduced pressure. The residue (crude 29) was rapidly purified by column chromatography on
silica gel (EtOAc/MeOH gradient) to provide 697 mg of an off-white solid. The latter was immediately dissolved in anhydrous MeCN (30 mL) then anhydrous NaI (2.65 g, 17.7 mmol, 5.1 equiv. (relative to 28)) and KSAc (2.06 g, 17.7 mmol, 5.1 equiv.) were successively added at rt. The reaction was refluxed overnight then submitted to a CH₂Cl₂/H₂O extraction. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered then evaporated under reduced pressure. Purification was achieved by column chromatography on silica gel (CH₂Cl₂/MeOH gradient) and provided 417 mg (42%, 2 steps) of biotin thioacetate 30 as a beige solid. Spectroscopic data were consistent with those reported in the literature.¹³¹H NMR (400 MHz, CDCl₃) δ 5.09 (brs, 1H), 4.84 (brs, 1H), 4.52 (m, 1H), 4.33 (m, 1H), 3.16 (m, 1H), 2.94 (dd, J = 12.8, 5.2 Hz, 1H), 2.87 (t, J = 7.4 Hz, 2H), 2.75 (d, J = 12.8 Hz, 1H), 2.34 (s, 3H), 1.64 (m, 4H), 1.43 (m, 4H).

Biotinthiol 31.⁸⁴ To a stirred solution of biotin thioacetate 30 (410 mg, 1.42 mmol, 1.0 equiv.) in THF (40 mL) was added LAH (95%, 454 mg, 11.36 mmol, 8.0 equiv.) in small portions at 0°C. After addition, the reaction was allowed to warm to rt then stirred for 1h. The reaction was diluted with EtOAc then carefully quenched with a 1 M HCl aqueous solution. The resulting aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered then evaporated under reduced pressure. Purification was achieved by column chromatography on silica gel (EtOAc/MeOH gradient) and provided 291 mg (83%) of biotinthiol 31 as a white solid. Spectroscopic data were consistent with those reported in the literature.¹³¹H NMR (300 MHz, CDCl₃) δ 5.15 (brs, 1H), 4.98 (brs, 1H), 4.53 (m, 1H), 4.33 (m, 1H), 3.17 (m, 1H), 2.94 (dd, J = 12.8, 5.2 Hz, 1H), 2.75 (d, J = 12.8 Hz, 1H), 2.54 (q, J = 7.3 Hz, 2H), 1.66 (m, 4H), 1.45 (m, 4H), 1.36 (t, J = 7.3 Hz, 1H).
II. 3. Surface Modification

II. 3. 1. Preparation of Cleaned substrates

For quartz crystals, they were first sonicated in 20 mL of concentrated dishwashing soap for 30 min. The crystals were successively rinsed with hot water followed by distilled water, and then gently dried with forced air. The crystals were then individually soaked in 6 mL of piranha solution (70% concentrated sulfuric acid and 30% of a 30% hydrogen peroxide solution) pre-heated to 90°C using a hot water bath. Piranha solutions are extremely energetic and may result in explosion or skin burns if not handled with extreme caution. After 45 min, the crystals were rinsed three times with distilled water, and three times with spectrograde methanol. Next, the crystals were sonicated in methanol for 2 min before being individually stored into vials and placed in an oven maintained at 150°C for drying. After 2 h, the crystals were transferred into a 60%-maintained (MgNO₃.6H₂O) humidity chamber for 24 h.

For TSM, AlN and SiN substrates, they were first sonicated in 20 mL of concentrated dishwashing soap for 30 min. The substrates were successively rinsed with hot water followed by distilled water. Then, they were rinsed three times with deionized water. Finally, they were rinsed three times in acetone and sonicated for 2 min, also in acetone. This process was repeated with ethanol and then methanol. The substrates were then dried with a gentle stream of nitrogen gas and plasma cleared for 5 min.

II. 3. 2. Silanization Procedure

All glassware was pre-treated with an octadecyltrichlorosilane (1/20 (v/v)) solution in anhydrous toluene overnight to prevent any undesired reactions of our trichlorosilyl linker/diluent with the walls of the glassware during the silanization procedure.
For pure SAMs, neat linker (TUBTS or OEG-TUBTS, 10 μL) or neat diluent (7-OEG-TFA, 9-OEG, 10-OEG-TA or 13-OEG-TFA, 10 μL) was first diluted with anhydrous toluene (10 mL). The resulting solution was portioned (1 mL) into test tubes in which cleaned substrate was soaked. The test tubes were then sealed and set aside for 1 h. Afterwards, the substrate was rinsed twice with dry toluene, and then sonicated for 2 min in another portion of toluene. After a final rinsing with toluene, the above procedure was repeated with dry chloroform. Finally, the crystals were gently dried under forced air, and then individually transferred into vials for storage or immediately engaged in the subsequent biosensing element immobilization.

For 1:1 mixed SAMs, a combination of two neat linkers/diluents (OEG-TUBT, 7-OEG-TFA, 9-OEG, 10-OEG-TA or 13-OEG-TFA, 5 μL each) were diluted with anhydrous toluene (10 mL). The resulting solution was portioned (1 mL) into test tubes in which cleaned substrate (quartz crystal, TSM crystal, AlN or SiN substrates) was soaked. The test tubes were then sealed and set aside for 1 h. Afterwards, the substrates were rinsed twice with dry toluene, and then sonicated for 2 min in another portion of toluene. After a final rinsing with toluene, the above rinsing steps were repeated with dry chloroform. Finally, the substrates were gently dried under forced air, and then individually transferred into vials for storage or immediately engaged in the subsequent biosensing element immobilization.

II. 3. 3. Formation of DNBTS SAMs on Gold
A 10 mM solution of DNBTS was prepared by diluting 0.04 g of neat DNBTS with 10 mL of absolute ethanol. The resulting solution was portioned (1 mL) into test tubes in which cleaned TSM crystal were soaked. The test tubes were then sealed and set aside for 24 h. Afterwards, the crystals were rinsed three times with ethanol and sonicated for 5 min in a new portion of ethanol. This step was then repeated with methanol. The crystals were then dried with gentle stream of nitrogen gas.
II. 3. 4. Immobilization Procedure

Biotin thiol (6.0 mg) was dissolved into a solution of 1:1 deionised water and methanol (6.0 mL). Et₃N (6.0 μL) was also added to this solution. The resulting solution (1.0 mg/mL) was portioned (1 mL) into dry test tubes, in which the freshly prepared SAM coated substrate were soaked. The test tubes were then sealed and set aside for 1 h. Afterwards, the substrate was rinsed three times with spectrograde methanol, then sonicated for 2 min in spectrograde methanol, followed by another rinse of spectrograde methanol. Finally, the substrate was gently dried under a stream of nitrogen gas and transferred into individual vials for storage.

II. 3. 5. Cleavage of TFA Head Function Group

A solution of 1:1 deionised water and methanol was portioned (1 mL) into dry test tubes in which the freshly prepared SAM coated substrate were soaked. The test tubes were then sealed and set aside for 1 h. Afterwards, the substrate were rinsed three times with spectrograde methanol, and then sonicated in spectrograde methanol for 2 min followed by another rinse of spectrograde methanol. Finally, the substrate was gently dried under a stream of nitrogen gas. The substrate was transferred into individual vials for storage.

II. 4. Surface Analysis Techniques

II. 4. 1. Contact Angle Measurement (CAM)

CAMs were performed in the Department of Chemistry at the University of Toronto in Toronto, ON, Canada. The surfaces were analyzed with the KSV contact angle measurement system (KSV Instruments Ltd., CAM101) and ultrapure water was used as the test liquid. Once the droplets were gently deposited onto the surfaces, they were allowed to settle for 10s. Five frames were then recorded at 1s intervals.
II. 4. 2. X-ray Photoelectron Spectroscopy (XPS)

XPS analysis was performed with the Thermo Scientific K-Alpha XPS spectrometer (ThermoFisher, East Grinstead, UK), located at Surface Interface Ontario at the University of Toronto in Toronto, ON, Canada. The samples were analyzed with an Al Kα X-ray source, at take-off angles of 20° relative to the surface. The binding energy scale was calibrated to the main C(1s) signal at 285.0 eV. Peak fitting and data analysis was performed using Avantage software provided with the instrument.

II. 4. 3. ElectroMagnetic Piezoelectric Acoustic Sensor (EMPAS)

AT-cut 20.0 MHz quartz crystals were placed in a flow-through system. One side of the crystal was exposed to liquid, and the other side was exposed to air. Runs were performed with the crystal in the horizontal position and at ambient temperature. The crystal was secured in the holder using an O-ring. A computer equipped with LabView 6.0 controled the radio frequency generator and lock-in amplifier; while acquiring data during the course of the measurement.

Avidin or BSA buffer stock solutions (1 mg/mL) were prepared by dissolving 2.0 mg of solid avidin or BSA into 2.0 mL of PBS buffer. Avidin buffer sample solutions (0.001 mg/mL to 0.2 mg/mL) were prepared by serial dilutions from the stock solution with PBS buffer. BSA buffer sample solution (0.1 mg/mL) was prepared by adding 90 μL of PBS buffer and 10 μL of BSA buffer stock solution. IgG sample solution (0.1 mg/mL) was prepared by diluting an IgG solution as purchased with the correct volume of PBS. Goat serum sample solution was used without modification or dilution.

Each slide was thoroughly flushed with PBS at a rate of 50 μL/min using a syringe pump. After ensuring uniform coverage of the disc with PBS (i.e. no bubbles) the resonant frequency of the disc was manually found using an oscilloscope. This was achieved by
plugging the cable from the lock-in amplifier into the oscilloscope and then manually scanning frequencies to find the 41st harmonic (0.863 GHz). This frequency was then inputted into the LabView program to be used as the standard during the course of the EMPAS measurement. After initializing the run, the frequency signal was allowed to stabilize for 10-15 min. Then, 50 μL of sample was injected into the flow-through system using a low-pressure chromatography valve. This was followed with the uninterrupted flow of PBS at a rate of 50 μL/min to remove any non-specifically or loosely bound species. PBS was flowed until a stable baseline was achieved (10-15 min). Changes to the resonant frequency were noted during the course of the run. Prior to the introduction of a new slide the system was vacuumed dry using a peristaltic pump and carefully dried using a KimWipe®
III. Results and Discussion

III. 1. Thiosulfonate-Based Linker: TUBTS

In an effort to design a thiosulfonate-based SAM linker, benzenethiosulfonate was chosen as the head function. This head function provides better reactivity when compared to other thiosulfonate derivatives, such as methanethiosulfonate, due to stabilization of the leaving group by benzene. Furthermore, the planar nature of this head function, as well as the possibility of π-stacking interactions through the benzene provides extra stability for SAM formation. An alkyl chain with a length of 11 carbons was chosen as the backbone of the linker. This ensured that there was enough neighbouring intermolecular interactions for SAM formation. In addition, the long alkyl chain provided separation between the head function and the tail function. A trichlorosilyl tail function was chosen as the tail function due to interests in applying this linker on hydroxylated surfaces, such as quartz.

III. 1. 1. Synthesis of TUBTS

The synthesis of TUBTS was achieved in two steps from commercially available 11-bromo-undec-1-ene. The synthesis returned an excellent overall yield of 88% (Figure 20).

![Figure 21. Synthesis of TUBTS.](image-url)
The first step consisted of the S-alkylation of benzenethiosulfonic acid sodium salt by 11-bromo-undec-1-ene. The subsequent hydrosilylation of the terminal olefin with HSiCl$_3$ produced TUBTS (*Appendix A*).

The purity of the TUBTS precursor before hydrosilylation was very important. After the S-alkylation, a by-product with an almost identical $R_f$ was produced (*Figure 22*). The presence of this by-product during hydrosilylation drastically hindered the catalytic reaction, resulting in very little yield. As a consequence, purifications through multiple time-consuming chromatography columns were required. Other synthetic routes, such as a reaction through sulfide with benzenesulfonyl chloride, could be explored to avoid this issue. TUBTS was not purified due to its high molecular weight. Without an ultra-high vacuum pump, the molecule underwent thermal degradation during Kugelrohr distillation before it was purified. The minimum amount of catalyst (0.1 mol %) was used to minimize impurity since purification could not be carried out.

*Figure 22.* Sections of $^1$H NMR spectra for the TUBTS precursor: a) with the impurity; b) without the impurity.
III. 1. 2. SAM Formation

The formation of TUBTS SAMs onto quartz crystal was performed with a dilute solution of TUBTS in toluene (Figure 23). The reaction time associated with this process was optimized in a previous study.\textsuperscript{107}

\textbf{Figure 23}. Illustration of the formation of a TUBTS SAM onto a cleaned quartz crystal.
CAMs were taken for each surface before and after silanization, with deionised water as the test liquid (Figure 24).

![Cleaned Quartz Crystal: 12° ± 2° vs TUBTS SAM: 96° ± 3°](image)

**Figure 24.** Contact angle measurements obtained with cleaned quartz crystal and TUBTS SAM on quartz crystal.

As shown in Figure 24, CAM values increased significantly after silanization with TUBTS (from 12° ± 2° to 96° ± 3°). This indicated that the TUBTS was deposited onto the cleaned quartz crystals, making it more hydrophobic. This was due to the hydroxyl groups, originally on the quartz surface, reacting with TUBTS which replaced them with hydrophobic moieties such as benzene rings and long alkyl chains. ARXPS analysis was also conducted on these surfaces at an angle of 20° relative to the surface. The same conclusion was drawn from examining the surface XPS analyses of silicon, oxygen, carbon and sulfur (**Figure 25 & Appendix A**). These elements were of interest because they are the characteristic elements contained in TUBTS (Si, O, C and S) and quartz (Si and O).
According to the XPS results in Figure 25, the relative atomic percentages of silicon ($\%_\text{Si}$) and oxygen ($\%_\text{O}$) significantly decreased after silanization (40.3% to 17.8% and 52.8% to 28.0%, respectively) while the opposite trend was observed with carbon ($\%_\text{C}$) and sulfur ($\%_\text{S}$) (6.9% to 51.3% and 0.0% to 3.0%, respectively). These observations were in agreement with CAMs and showed that TUBTS SAM was formed on the surface of the quartz crystal. It should be noted that a small amount of $\%_\text{C}$ (6.9%) was observed on cleaned quartz crystal. These carbons are due to contamination from hydrocarbons in the atmosphere. A small amount of hydrocarbons can absorb onto the surface once the substrate comes into contact with the atmosphere. This contamination was observed throughout all studies conducted in this thesis. This could be avoided by transporting the sample in a vacuum chamber if necessary. The increase of the $\%_\text{S}$ and $\%_\text{C}$ could be explained by TUBTS molecules being deposited onto the surface, since sulfur and carbon.

**Figure 25.** XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon and sulfur (20° angle relative to the surface) obtained with TUBTS SAM and cleaned quartz crystal.
are both characteristic to TUBTS molecules. Furthermore, there were two peaks for the sulfur signal which were attributed to the two sulfur atoms in different oxidation states (S^0 and S^{IV}) on the head function of TUBTS (S_{2p} in Figure 25).

The decrease of the %Si and %O could be explained by the formation of the SAM over the surface of the quartz crystal. Fewer characteristic atoms of the quartz surface (Si and O) were seen by the XPS analysis. This is because XPS detects only those electrons that have actually escaped from the sample to the detector. When there is a coating over the substrate, it is more likely for the electrons coming from the substrate to undergo inelastic collisions, or other processes which prevent electrons from escaping the sample. Thus, the magnitude of the XPS signals coming from the substrate atoms was reduced. Additionally, %Si and %O were also reduced because of the many other types of atoms which were added to the surface. These atoms pushed down the relative abundances of silicon and oxygen. Altogether, both data from CAMs and ARXPS are consistent with the SAM formation of TUBTS on quartz crystal surface.

It should be noted that no study was done to examine the quality of the SAM (e.g. density, thickness and roughness). We expect the surfaces prepared in this thesis to be lower in quality (i.e. less uniform) than ideal. This is due to the conditions these SAMs were prepared in. Ideally, SAMs need to be prepared in a cleanroom to achieve high quality. However, one of our goals was to be able to rapidly prepare biosensing interfaces with minimum cost and effort. Thus, the quality of the SAM was not sought after.

### III. 1. 3. Biotin Immobilization

After preparing TUBTS SAM-coated quartz crystals, the immobilization of our test molecule, biotin-thiol, was performed. This was achieved by immersion of freshly prepared TUBTS SAM-coated quartz crystals (120 min silanization) into a solution of biotin-thiol (1.0 mg/mL), methanol and deionised water (in a 1:1 ratio), and triethylamine
(1.0 µL/mL) for 1 h at room temperature (Figure 26). The reaction time associated with this process was optimized in a previous study.107

**Figure 26.** Illustration of the immobilization of biotinthiol onto a TUBTS SAM.

CAMs were taken for each surface before and after biotinthiol immobilization, with deionised water as the test liquid (Figure 27).
According to Figure 27, CAM values decreased after biotinthiol immobilization (96° ± 3° to 78° ± 3°). This suggested that the hydrophobic head function of TUBTS was replaced by biotinthiol, which is a more polar moiety. This observation was also confirmed by surface XPS analysis on nitrogen and sulfur (Figure 28 & Appendix B). Nitrogen was of interest because it is unique to biotinthiol. The change in the two sulfur signals (S\textsuperscript{0} and S\textsuperscript{IV}) after immobilization was also a measurement of interest.

As shown in Figure 28, a peak appeared in the nitrogen signal after biotinthiol immobilization (0.0% to 3.3%). This suggested that biotinthiol was present on the SAM.
Furthermore, the peak profiles of the two sulfur signals (S$_{2p}$ in Figure 28) indicated that biotinthiol was indeed immobilized by a reaction with the TUBTS head function. XPS analysis showed two different peaks for sulfur. The peak at $\sim$163 eV corresponds to sulfur in oxidation state 0 ($S^0$). This is found in the TUBTS head function (sulfide -S-SO$_2$-) and in biotinthiol (in the ring and the thiol tail). The other peak at $\sim$168 eV corresponds to sulfur in oxidation stage +4 ($S^{IV}$) and is found in the leaving group of the TUBTS head function (sulfone: -S-SO$_2$-). After biotinthiol immobilization, the peak of $S^{IV}$ disappeared while the area of $S^0$ peak increased significantly. This indicated that the $S^{IV}$ atom, contained in the leaving group of TUBTS head function, was replaced by the $S^0$ atom in biotinthiol upon reaction to form a disulfide bond. This observation was in agreement with the reaction scheme between thiol and thiosulfonate shown previously (Figure 18). Both CAMs and ARXPS are consistent with the immobilization of biotinthiol onto TUBTS SAM through the formation of a disulfide bond.

III. 1. 4. EMPAS Measurements

Performance of the biosensing interface constructed by TUBTS SAMs was studied with the EMPAS system at an ultra-high resonating frequency of 0.86 GHz (43$^{rd}$ harmonic). Three different proteins (avidin, BSA and IgG) were used separately in the EMPAS experiments. Avidin was the target analyte. With each protein, two sets of independent frequency shift measurements (4 replicates per set) were conducted using 0.1 mg mL$^{-1}$ solutions of the appropriate protein in PBS (Appendix C). For the first set, biotinthiol functionalized TUBTS SAMs were used to quantify the specific adsorption of the protein to biotin. For the next set, TUBTS SAMs without biotinthiol modification were used as a control to record non-specific adsorption of the protein on the biosensing interface. The same mass concentration of avidin, BSA and IgG was used instead of same molar concentration due to the fact that the EMPAS is sensitive to mass change. The specific to non-specific adsorption frequency shift ratio ($R_{S/NS}$) and relative standard deviations (RSD) for each protein were then calculated and compared. The ratio of the avidin-specific adsorption frequency shift to the average non-avidin (IgG and BSA) specific adsorption frequency shifts ($R_{A/NA}$) was also calculated. The $R_{S/NS}$ and $R_{A/NA}$ were used to
assess the ability of the biosensing interface to specifically detect avidin. RSD reflected the reproducibility of the measurements (Figure 29).

Ideally, a large positive EMPAS response should only be observed in the specific adsorption measurement, with avidin as the sample. A large positive frequency shift in the EMPAS correlates to more mass being adsorbed onto the biosensing interface. On the other hand, low or no response should be observed with other measurements to indicate the ability of the biosensing interface to resist non-specific adsorption. Thus, high $R_{S/NS}$ and $R_{A/NA}$ are desired. This represents high detection specificity and selectivity for the biosensing interface. Lastly, low RSDs show high reproducibility in the measurements.

![Figure 29](image)

**Figure 29.** EMPAS non-specific and specific adsorption frequency shifts respectively measured with TUBTS and biotinylated TUBTS SAMs, using 0.1 mg mL$^{-1}$ BSA, IgG, avidin solutions in PBS.

The frequency shifts for the specific adsorption of avidin (18.0 kHz) were significantly greater than those recorded for the non-specific adsorption (12.0 kHz). The specific absorption of avidin was also significantly higher than the specific adsorption of BSA.
(6.0 kHz) and IgG (12.5 kHz), which gave an \( R_{A/NA} \) of 2.0. This clearly demonstrated the ability of the system to capture and detect avidin. In addition, all of the measurements were reasonably reproducible (average RSD = 17%) with the exception of the ones with BSA (22% and 35%).

However, the \( R_{S/NS} \) of avidin (1.5/1) was relatively low due to the high non-specific adsorption response. This was likely due to the interactions between the proteins and the hydrophobic surface of densely packed TUBTS SAMs. Proteins tend to denature and aggregate onto hydrophobic surfaces upon contact. Hydrophobic interactions are the dominating attractive force in non-specific protein adsorption onto surfaces.\(^{108}\) Moreover, a positive response was observed when BSA was used as the sample (\( R_{S/NS} \) of BSA = 1.3/1). Although the magnitude of specific BSA responses was much smaller than the responses of avidin, this suggested that there was still a problem with the detection specificity.

In summary, after TUBTS was successfully synthesized, it was used to prepare SAM-coated piezoelectric quartz crystals. These quartz crystals then had the ability to chemoselectively immobilize thiol-containing biomolecules (biotin-thiol in our case) and create a biosensing interface. This biosensing interface was then used to measure interactions between surface-bound biotin and avidin in solution with the EMPAS. However, it was revealed that these biosensing interfaces had problems with detection specificity due to non-specific adsorption. Thus, the attention of this study was turned towards the incorporation of an OEG backbone into the linker in an attempt to address the issue.
III. 2. OEG Backbone Thiosulfonate-Based Linker: OEG-TUBTS

In order to incorporate an OEG backbone into the linker, OEG-TUBTS was designed. A length of 3 OEG units was used as the backbone in order to keep the length of the OEG-TUBTS and TUBTS as close as possible. The head and tail function of OEG-TUBTS remained the same as TUBTS. This allowed the two linkers to be compared directly in order to see the effect of the OEG backbone on the performance of our system.

III. 2.1. Synthesis of OEG-TUBTS

The synthesis of OEG-TUBTS was achieved in seven steps from commercially available 2-allyloxy-ethanol. The overall yield was 6% (*Figure 30*). First was the $\alpha$-alkoxylation of 2-allyloxy-ethanol and methyl bromoacetate. The resulting ester 3 was subsequently reduced with lithium aluminum hydride (LAH) into the alcohol 4. The same sequence was run on the alcohol, eventually producing alcohol 6, which was then transformed into bromide 7 with PBr$_3$. S-alkylation of benzenethionosulfonic acid sodium salt by 7 gave benzenethiosulfonate 8. The subsequent hydrosilylation of its terminal olefin with HSiCl$_3$ finally provided OEG-TUBTS (*Appendix D*).
The low yields observed with both α-alkoxyations of methyl bromoacetate were due to the volatility of the products. A significant amount of product was lost during purification. The usage of solvents with a lower boiling point, such as pentane and diethyl ether, is an option to avoid this issue. The extremely low yield observed for the halogenation of the alcohol was due to the destabilization of the carbocation intermediate by the oxygen atom next to the β-carbon. When tosylation was used instead of halogenation, the yield was improved slightly. However, tosylation made it difficult to determine when the subsequent reaction would be completed. Purification was also more difficult due to the similarities between the chemical structure of the starting material and the product. The synthesis of OEG-TUBTS also shared some of the same issues as the synthesis of TUBTS. First, the precursor before the hydrosilylation needed to be pure and free of by-products from S-alkylation. Second, OEG-TUBTS was too heavy to purify without an
ultra-high vacuum pump, so the minimum amount of catalyst (0.1 mol %) needed to be used.

III. 2. 2. Preparation of a Biosensing Interface

A biosensing interface was prepared with OEG-TUBTS for EMPAS measurements. (Figure 31). The conditions used to prepare these new surfaces were the exact same as the conditions used to prepare TUBTS in the previous section.

Figure 31. Illustration of the formation of an OEG-TUBTS SAM on a cleaned quartz crystal (step I) and the subsequent site-specific covalent immobilization of biotinthiol (step II).
CAMs were taken for each surface before and after each step. Deionised water was used as the test liquid (*Figure 32*).

![Figure 32](image)

*Figure 32.* Contact angle measurements obtained with cleaned quartz crystal, OEG-TUBTS SAM and biotinthiol functionalized OEG-TUBTS SAM.

The same trends as TUBTS were observed with OEG-TUBTS. CAM values significantly increased after silanization while slightly decreasing after biotinthiol immobilization. The CAM value increased after the formation of the OEG-TUBTS SAM (12° ± 1° and 72° ± 4°). The deposition of OEG-TUBTS onto the quartz surface made the surface more hydrophobic. The CAM value decreased after the displacement of the benzenethiosulfonate head function of OEG-TUBTS (72° ± 4° and 64° ± 3°) with the more hydrophilic moiety, biotinthiol. This suggested that the OEG-TUBTS SAM was successfully formed onto the quartz crystal and biotinthiol was subsequently immobilized onto the SAM. In addition, the CAM values of OEG-TUBTS SAM (72° ± 4°) and biotinthiol functionalized OEG-TUBTS SAM (64° ± 3°) were lower than their counterparts in TUBTS SAM formation (96° ± 3° and 78° ± 3°), respectively. This could be explained by the presence of oxygen atoms in the backbone of OEG-TUBTS, which made the SAM less hydrophobic. These surfaces were also characterized by ARXPS analysis by examining the relevant elements (*Figure 33 & Appendix E*).
Figure 33. XPS narrow scans for silicon, oxygen, carbon, sulfur and nitrogen (20° angle relative to the surface) obtained with cleaned quartz crystal, OEG-TUBTS SAM and biotin-thiol functionalized OEG-TUBTS SAM.

As shown in Figure 33, XPS scans of the OEG-TUBTS surfaces showed the same trend as the TUBTS surfaces. After silanization, the relative atomic percentages of sulfur (\%S) and carbon (\%C) increased significantly (0.0% to 2.8% and 6.9% to 56.0%, respectively), while the percentages of silicon (\%Si) and oxygen (\%O) showed significant declines (40.3% to 12.8% and 52.8% to 28.4%, respectively). The increase in \%S and \%C could be explained by the deposition of OEG-TUBTS molecules onto the surface. In addition, two
sulfur peaks were also observed ($S_{2p}$ in **Figure 33**). These were due to the two sulfur atoms in different oxidation states ($S^0$ and $S^{IV}$) on OEG-TUBTS. Also, less characteristic atoms of quartz (Si and O) were seen by surface XPS analysis. This decrease of $\%_{Si}$ and $\%_{O}$ was due to the SAM formation, which covered the surface of the quartz crystal and added more atoms of different types to the surface. It is interesting to note that the oxygen signal of OEG-TUBTS SAM decreased less than that of TUBTS SAM after silanization ($O_{1s}$ in **Figure 28 & 33**). This could be explained by the oxygen atoms on the backbone of OEG-TUBTS contributing to the XPS oxygen signal after SAM formation on the quartz surface. These observations strongly indicated that OEG-TUBTS SAM formed successfully on the quartz surface.

Furthermore, the presence of a nitrogen signal appeared after biotinthiol immobilization (0.0% to 3.1%). This was due to the two nitrogen atoms unique to biotinthiol. Correspondingly, the $S^0$ peak also increased while the $S^{IV}$ peak disappeared. This was due to the displacement of the $S^{IV}$ atom in the leaving group of the OEG-TUBTS head function with the $S^0$ atom and nitrogen atom in biotinthiol. This indicated that biotinthiol was successfully immobilized onto the OEG-TUBTS SAM. All in all, the data from both CAM and ARXPS indicated that the preparation of the biosensing interface with OEG-TUBTS and biotinthiol was successful.

**III. 2. 3. EMPAS Measurements**

The performance of the biosensing interface constructed by OEG-TUBTS SAMs was studied with the EMPAS system at an ultra-high resonating frequency of 0.86 GHz ($43^{rd}$ harmonics). For a direct comparison to TUBTS, the same experiments were carried out with these SAMs. Three different proteins (avidin, BSA and IgG) were used separately in the EMPAS experiments. Avidin was the target analyte. With each protein, two sets of independent frequency shift measurements (4 replicates per set) were conducted using 0.1 mg mL$^{-1}$ solutions of the appropriate protein in PBS (**Appendix F**). For the first set, biotinthiol functionalized OEG-TUBTS SAMs were used to quantify the specific
adsorption of the protein to biotin. For the other set, OEG-TUBTS SAMs without biotin thiol modification was used as the control to record the non-specific adsorption of the protein on the biosensing interface. The same mass concentration of avidin, BSA and IgG was used instead of the same molar concentration due to the fact that the EMPAS is sensitive to mass change. The specific to non-specific adsorption frequency shift ratio ($R_{S/NS}$) and relative standard deviations (RSD) were then calculated and compared for each protein. The ratio of the avidin-specific adsorption frequency shift to the average non-avidin (IgG and BSA) specific adsorption frequency shifts ($R_{A/NA}$) was also calculated. The $R_{S/NS}$ and $R_{A/NA}$ were used to assess the ability of the biosensing interface to specifically detect avidin. RSD reflected the reproducibility of the measurements (Figure 34).

![Figure 34](image)

Figure 34. EMPAS non-specific and specific adsorption frequency shifts respectively measured with OEG-TUBTS and biotinylated OEG-TUBTS SAMs, using 0.1 mg mL$^{-1}$ BSA, IgG and avidin solutions in PBS.

The addition of the OEG backbone only brought minor improvements to the performance of the system (Figure 34). The $R_{S/NS}$ of avidin increased slightly from 1.5/1 to 1.8/1 and the $R_{A/NA}$ also increased slightly from 2.0/1 to 2.3/1. In addition, all frequency shifts
obtained from BSA and IgG decreased about 40% on average. Although these improvements were minor, the results still confirmed the general observation that OEG SAMs exhibited non-fouling properties.

It is interesting to note that the EMPAS response profile for non-specific adsorption always curves up after the frequency drop (Figure 35a). This phenomenon is likely due to the fact that loosely bound protein is ultimately rinsed off the surface by the buffer flow. This phenomenon, however, was not observed for the specific adsorption EMPAS response profile for avidin. This may be because avidin was bound tightly to the surface through biotinyl residues and resistant to being rinsed off by buffer flow (Figure 35b). In addition, all the RSDs were lowered (from an average of 17% to 12%). However, detection specificity remained a concern for these measurements, as the $R_{S/NS}$ obtained from IgG (1.48/1) was quite high and similar to $R_{S/NS}$ of avidin (1.8/1).

Figure 35. EMPAS response profiles for (a) OEG-TUBTS SAM and (b) biotinylated OEG-TUBTS SAM using the avidin solution as sample.

In conclusion, the incorporation of an OEG backbone into the linker did not result in the improvements that were expected. It was hypothesized that a tightly packed SAM could hinder the effectiveness of the OEG backbone in preventing non-specific adsorption. When linkers form a tightly packed assembly, the exposure of their backbone to the SAM-liquid interface is greatly reduced. This can negatively affect their ability to form
the water barrier over the SAM, which prevents non-specific adsorption. This led to the decision to include a diluent molecule within the SAM to space out the tightly packed linker in order to further improve the performance of our system.

III. 3. OEG-Based Diluents

In order to design an OEG-based diluent for the OEG-TUBTS system, several different head functions and lengths of the diluent had to be considered. The goal was to find a diluent that could further improve the performance of our system in terms of non-specific adsorption resistance. First, a study was done to determine the importance of the –OH group as a head function for the resistance of non-specific adsorption. For this purpose, 9-OEG and 10-OEG-TFA were synthesized and compared with the EMPAS study (Figure 22). After cleaving the protecting head group of 10-OEG-TFA, 10-OEG is yielded. 10-OEG has an extra –OH group when compared to 9-OEG. Next, 7-OEG-TFA, 10-OEG-TFA and 13-OEG-TFA were synthesized to study the effect of different diluent lengths on non-specific adsorption resistance using EMPAS. These diluents varied by the length of their OEG units.

III. 3. 1. Head Function of OEG-Based Diluents

III. 3. 1. 1. Synthesis of 9-OEG

The synthesis of 9-OEG was achieved in two steps from commercially available 2-ethoxyethanol. The overall yield was 41% (Figure 36). The first step was the α-alkoxylation of allyl bromide with 2-ethoxyethanol. The subsequent hydrosilylation of the terminal olefin with HSiCl₃ produced 9-OEG (Appendix G).
Figure 36. Synthesis of 9-OEG.

The yield from α-alkoxylation was very low due to the high volatility of the product. A significant amount of product was lost during purification, even when very low boiling point solvents (i.e. pentane and diethyl ether) were used.

III. 3. 1. 2. Synthesis of 10-OEG-TFA

Since it is impossible to synthesize a molecule containing both an –OH and a trichlorosilyl group, a protecting group for the –OH in 10-OEG was necessary. In this case, trifluoroacetate was chosen as the protecting group. Trifluoroacetate is very susceptible to hydrolysis, which made it easy to de-protect the –OH without damaging the quality of the SAM.

The synthesis of 10-OEG-TFA was achieved in four steps from commercially available 2-allyloxy-ethanol. The overall yield was 59% (Figure 37).
The first step consisted of the α-alkoxylation of methyl bromoacetate with 2-allyloxyethanol. The resulting ester 3 was subsequently reduced with lithium aluminum hydride (LAH) into the alcohol 4. Afterward, the alcohol moiety was protected with trifluoroacetic anhydride. The subsequent hydrosilylation of the terminal olefin with HSiCl3 finally produced 10-OEG-TFA (Appendix G). 10-OEG-TFA was not purified due to its high molecular weight. Without an ultra-high vacuum pump, the molecule underwent thermal degradation during Kugelrohr distillation before purified was completed. Since purification could not be carried out, the minimum amount of catalyst (0.1 mol %) was used to minimize impurity.
III. 3. 1. 3. SAM Formation

The formation of 9-OEG and 10-OEG-TFA SAMs onto quartz crystal was performed with their respective dilute solution of 9-OEG and 10-OEG-TFA in toluene (1µL/1 mL) (Figure 38). In order to yield 10-OEG SAMs, the protecting head group of 10-OEG-TFA was cleaved by soaking the SAM-coated quartz crystal in a solution of methanol and deionised water (at a 1:1 ratio) for 24 h at room temperature (Figure 38).

Figure 38. Illustration of the formation of 9-OEG and 10-OEG SAM onto a cleaned quartz crystal.
CAMs were taken for each surface before and after silanization, with deionised water as the test liquid (Figure 39 & 40)

![Cleaned Quartz Crystal: 12° ± 1°](image1) ![9-OEG SAM: 68° ± 3°](image2)

**Figure 39.** Contact angle measurements obtained with cleaned quartz crystal and 9-OEG SAM on quartz crystal.

![Cleaned Quartz Crystal: 12° ± 1°](image3) ![10-OEG-TFA SAM: 70° ± 2°](image4) ![10-OEG SAM: 25° ± 4°](image5)

**Figure 40.** Contact angle measurements obtained with cleaned quartz crystal, 10-OEG-TFA SAM and 10-OEG SAM on quartz crystal.

The usual trend for silanization was observed with both diluents. CAM values increased significantly after silanization due to the deposition of 9-OEG and 10-OEG-TFA diluent onto the surface of the quartz crystal (12° ± 1° to 68° ± 3° and 12° ± 1° to 70° ± 2°, respectively). This was due to the hydroxyl groups, originally on the quartz surface, reacting with the diluents which replaced them. This placed the more hydrophobic moiety, ethylene glycol chains, onto the surface of the quartz crystal. As for the 10-OEG-TFA
SAM, the CAM values decreased after the protecting head group was cleaved (70° ± 2° to 25° ± 4°). This cleavage yielded the 10-OEG SAM. These observations suggested that 9-OEG and 10-OEG SAMs were formed onto their respective quartz crystals. Each surface prepared by each diluent was also characterized by ARXPS analysis (Appendix H). An examination of the relevant elements indicated whether the diluent SAM was formed successfully (Figure 41 & 42).

![XPS narrow scans and relative atomic percentages for silicon, oxygen, and carbon](image)

*Figure 41.* XPS narrow scans and relative atomic percentages for silicon, oxygen, and carbon (20° angle relative to the surface) obtained with 9-OEG SAM and cleaned quartz crystal.

Regrettably, 9-OEG did not possess unique atoms for XPS analysis. Only signal changes in carbon, oxygen and silicon could be used to determine 9-OEG silanization. After silanization with 9-OEG, the relative atomic percentage of carbon (\%C) increased (6.9% to 32.1%), while the percentage of silicon (\%Si) and oxygen (\%O) decreased (40.3% to 29.8% and 52.8% to 37.4%, respectively). The increase of the \%C could be explained by 9-OEG molecule being deposited onto the surface which added more carbon atoms to the
surface. The decreases in the \%_{\text{Si}} and \%_{\text{O}} were due to 9-OEG SAM formation covering the surface of the quartz crystal. This added many more of other types of atoms to the surface. These observations suggested that the SAM formation of 9-OEG onto the surface of the quartz crystal was successful.

![XPS spectra](image)

Figure 42. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, and fluorine (20° angle relative to the surface) obtained with 10-OEG-TFA SAM, 10-OEG SAM and cleaned quartz crystal.

For 10-OEG-TFA, the fluorine signal was also used to indicate the silanization process, since it is unique to the diluent. After silanization with 10-OEG-TFA, the relative atomic percentages of fluorine (\%_{\text{F}}) and carbon (\%_{\text{C}}) increased (0.0% to 3.8%, and 6.9% to 20.2%, respectively), while the percentages of silicon (\%_{\text{Si}}) and oxygen (\%_{\text{O}}) slightly decreased (40.3% to 37.5%, and 52.8% to 38.6%, respectively). The increases in \%_{\text{F}} and \%_{\text{C}} could be explained by the deposition of the 10-OEG-TFA molecule onto the surface which added more fluorine and carbon atoms to the surface. The decreases in
the $\%_{\text{Si}}$ and $\%_{\text{O}}$ were due to 10-OEG-TFA SAM formation over the surface of the quartz crystal. This added more atoms of other types to the surface. These observations corresponded to the deposition of 10-OEG-TFA onto the surface of the quartz crystal. In addition, the fluorine signals completely disappeared after the diluent SAM was treated with a solution of methanol and deionised water (3.8% to 0.0%). This confirmed that the trifluoroacetate protecting head function of 10-OEG-TFA was cleaved and that the 10-OEG SAM was successfully produced. To summarize, both CAMs and ARXPS data are consistent with the SAM formation of 9-OEG and 10-OEG SAM on quartz crystal surfaces.

III. 3. 1. 4. EMPAS Measurements

To investigate the non-specific adsorption resistance of 9-OEG and 10-OEG SAM, EMPAS experiments were run using undiluted goat serum as the sample. Goat serum was used because it contains various types of interfering biomolecules, at high concentrations. EMPAS frequency shift measurements (4 replicates) were conducted with each diluent system. Cleaned quartz crystals were used as the control. Undiluted goat serum was introduced to each system and the non-specific adsorption response was recorded (Appendix I).

In this experiment, low or no frequency shifts in EMPAS were desired because this represented a high degree of resistance towards non-specific adsorption. Frequency shifts and relative standard deviations (RSD) for each system were then calculated and compared. The frequency shifts were used to assess the non-specific adsorption resistances of each system. The RSD was used to assess the reproducibility of the measurements. Ideally, much lower EMPAS responses should be observed with these diluent SAMs when compared to the ones obtained with clean quartz crystal. Lower frequency shifts in EMPAS correlate to less mass being adsorbed onto the biosensing interface. Thus, in this experiment, with goat serum, a lower frequency shift would
represent increasing resistance to protein non-specific adsorption. Lastly, low RSD represented a high reproducibility in the measurements.

Figure 43. EMPAS non-specific adsorption frequency shifts measured with undiluted goat serum using cleaned quartz crystal, 9-OEG and 10-OEG SAMs. Measurements were recorded at 0.86 GHz.

According to Figure 43, both the 9-OEG and 10-OEG systems showed a significant reduction (> 50%) in EMPAS responses when compared to cleaned quartz crystal. This demonstrated the ability of the OEG moiety in preventing non-specific adsorption, as shown in previous section. More importantly, EMPAS responses obtained with the 10-OEG SAM (2700 Hz) were much lower than the ones obtained with the 9-OEG SAM (11600 Hz). This suggested that the –OH head group of 10-OEG played a crucial role in preventing non-specific adsorption against the numerous interfering species in goat serum. This could be explained by the –OH head group on the SAM aiding in the formation of a water barrier over the SAM through an extensive hydrogen bonding network with water molecules. Henceforth, the –OH head group, protected by trifluoroacetate, was added to all future designs of diluent used in this study.
It should be noted that cleaned quartz crystals also have –OH groups over its surface. However, these –OH groups exhibited a high degree of non-specific adsorption. Their EMPAS responses (26300 Hz) were ~10 fold higher than the ones obtained with 10-OEG. This suggested that the OEG moiety within the backbone of 10-OEG and its –OH head group, were both crucial for the SAM to achieve a high resistance of non-specific adsorption. The same conclusion was also drawn in another study with a diluent containing an –OH head function but without the OEG moiety in the backbone. From these observations, it is unlikely that the mechanism of non-specific adsorption resistance on ethylene glycol chain surface can be explained by entropy repulsion or the water barrier hypotheses alone. We believe the mechanism is a concerted effort between both entropy repulsion and the water barrier hypotheses.

In aqueous solutions, ethylene glycol chains are solvated and disordered. Adsorption of biomolecules on the surface causes the glycol chains to compress, accompanied by desolvation. In a concerted effort, the entropic penalty provoked by the compression of the SAM and the energetic penalty of transferring the tightly bound water to the bulk both serve to resist non-specific adsorption. Thus, in order for biomolecules to stay bound to the surface, the interactions between the biomolecules and the surface need to be strong enough to compensate for these two energy barriers.

III. 3. 2. Length of OEG-Based Diluents

After deciding on the head function of our OEG-based diluents, the attention of this study was turned towards the length of the diluent. For this purpose, 7-OEG-TFA and 13-OEG-TFA were synthesized. Along with 10-OEG-TFA, these new diluents were then used to study the effect of diluent length on non-specific adsorption with EMPAS.

III. 3. 2. 1. Synthesis of 7-OEG-TFA
The synthesis of 7-OEG-TFA was achieved in two steps from commercially available 2-allyloxy-ethanol. The overall yield was 59% (Figure 44). First, the alcohol moiety was protected with trifluoroacetic anhydride. The subsequent hydrosilylation of the terminal olefin with HSiCl₃ produced 7-OEG-TFA (Appendix G).

![Chemical diagram](image)

**Figure 44.** Synthesis of 7-OEG-TFA.

### III. 3. 2. 2. Synthesis of 13-OEG-TFA

The synthesis of 13-OEG-TFA was achieved in four steps from commercially available 2-allyloxy-ethanol. The overall yield of 13-OEG-TFA was 25% (Figure 45). The first step consisted of the α-alkoxylation of methyl bromoacetate with 2-allyloxy-ethanol. The resulting ester 3 was subsequently reduced with lithium aluminum hydride (LAH) into alcohol 4. The same sequence was run on alcohol 4, eventually producing alcohol 6. This was then protected with trifluoroacetic anhydride. The subsequent hydrosilylation of the terminal olefin with HSiCl₃ finally produced 13-OEG-TFA (Appendix G).

![Chemical diagram](image)

After the protection of the alcohol with trifluoroacetic anhydride in the last step, an impurity was produced as a by-product of the reaction. The impurity was indicated in the ¹H NMR as a singlet peak at 7.61 ppm. In the presence of this impurity, the hydrosilylation of the terminal olefin was impossible. The reaction spontaneously
solidified upon the addition of the catalyst. This solidification of the catalyst was most likely due to the coordination of the impurity to the catalyst. To resolve this issue, a quick column chromatography (CH$_2$Cl$_2$/EtOAc gradient 100/0 to 95/5) was used to remove the impurity after distillation. In the synthesis of 7-OEG-TFA and 10-OEG-TFA, this impurity was easily removed during distillation. However, with 13-OEG-TFA synthesis, the boiling point of the impurity was very close to the desired product. In addition, the R$_f$ values of the impurity and the desired product were identical in a hexanes/EtOAc solvent system. Lastly, after hydrosilylation in the final step, 13-OEG-TFA could not be purified by Kugelrohr distillation due to its high molecular weight. Without an ultra-high vacuum pump, the molecule underwent thermal degradation during the distillation process before it purification completed. The minimum amount of catalyst (0.1 mol %) was used to minimize the impurity since purification could not be carried out.

**Figure 45.** Synthesis of 13-OEG-TFA.
III. 3. 2. 3. SAM Formation

The formation of 7-OEG-TFA and 13-OEG-TFA SAMs onto quartz crystal was performed individually with a dilute solution of 7-OEG-TFA and 13-OEG-TFA in toluene (Figure 46). Afterwards, the protecting head group of both types of SAMs was cleaved by soaking the SAM-coated quartz crystal in a solution of methanol and deionised water (1:1 ratio) for 24 h at room temperature (Figure 46). As a result, the 7-OEG SAM and the 13-OEG SAM were formed.
Figure 46. Illustration of the formation of 7-OEG and 13-OEG SAM onto a cleaned quartz crystal.

CAMs were taken for each surface before and after silanization, with deionised water as the test liquid (Figure 47 & 48)

Figure 47. Contact angle measurements obtained with cleaned quartz crystal and 7-OEG-TFA SAM and 7-OEG SAM on quartz crystal.

Figure 48. Contact angle measurements obtained with cleaned quartz crystal, 13-OEG-TFA SAM and 13-OEG SAM on quartz crystal.

The same CAM trends of 10-OEG-TFA were observed for both 7-OEG-TFA and 13-OEG-TFA diluents. CAM values increased significantly after silanization (12° ± 1° to
66° ± 2° and 12° ± 1° to 77° ± 3°) due to the deposition of the two diluents onto the surface of the quartz crystal. After cleavage of the protecting head function, the CAM values decreased for both types of SAMs (20 ± 5° for 7 OEG SAM and 24 ± 3° for 13-OEG SAM). These observations suggested that 7-OEG and 13-OEG SAMs were formed onto their respective quartz crystal. Each surface, prepared with a diluent, was also characterized by ARXPS analysis (Appendix H). An examination of the relevant elements indicated whether the diluent SAM was formed successfully (Figure 49 & 50).

**Figure 49.** XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, and fluorine (20° angle relative to the surface) obtained with 7-OEG-TFA SAM, 7-OEG SAM and cleaned quartz crystal.
**Figure 50.** XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, and fluorine (20° angle relative to the surface) obtained with 13-OEG-TFA SAM, 13-OEG SAM and cleaned quartz crystal.

Same as with 10-OEG-TFA, the fluorine signals appeared after silanization with 7-OEG-TFA and 13-OEG-TFA (0.0% to 4.3% and 0.0% to 1.7%, respectively). In addition, the relative atomic percentages of carbon (%C) increased, while the percentages of silicon (%Si) and oxygen (%C) decreased (**Figure 49 & 50**). These observations corresponded to the deposition of 7-OEG-TFA and 13-OEG-TFA onto the surface of the quartz crystal.

Interestingly, a trend could be observed in the relative atomic percentage ratio of fluorine to carbon (%C/%F), as well as the relative atomic percentage ratio of carbon to silicon (%C/%Si) among the diluents with different lengths (7-OEG-TFA, 10-OEG-TFA and 13-OEG-TFA SAMs) (**Figure 51**). The %C/%F increased as the diluent length increased. This corresponded to the increasing number of carbon atoms in the backbone, relative to the fluorine atoms in the protecting head function, as the length of the diluent increased.
The same trend was observed with %C/%Si. This suggested that the increasing diluent length contributed more carbon atoms within the SAM, relative to the quartz crystal substrate. With these observations, the formation of the SAM with 7-OEG-TFA, 10-OEG-TFA and 13-OEG-TFA was validated further.

**Figure 51.** Relative atomic percentage ratio of carbon to silicon (%C/%Si) and relative atomic percentage ratio of fluorine to carbon (%F/%C) of 7-OEG-TFA, 10-OEG-TFA and 13-OEG-TFA SAMs.

When the diluents were deprotected, the fluorine signals completely disappeared after treatment with a solution of methanol and deionised water (*Figure 49 & 50*). This occurred in both 7-OEG-TFA and 13-OEG-TFA SAMs. This confirmed that the trifluoroacetate protecting head function of both SAMs were cleaved and that the 7-OEG SAM and 13-OEG SAM were produced onto their respective surfaces. In conclusion, both CAMs and ARXPS are consistent with the formation of the SAM with 7-OEG and 13-OEG SAM on quartz crystal surfaces.
III. 3. 2. 4. EMPAS Measurements

To investigate the effect of diluent length on non-specific adsorption resistance, EMPAS experiments were individually run with 7-OEG, 10-OEG and 13-OEG SAMs. Undiluted goat serum was used as the sample since it contained various types of interfering biomolecules at high concentrations. EMPAS frequency shift measurements (4 replicates) were conducted with each diluent system. Cleaned quartz crystals were used as the control.

Undiluted goat serum was introduced to each system and the non-specific adsorption response was recorded (Appendix I). Frequency shifts and relative standard deviations (RSD) for each system were then calculated and compared. The frequency shifts were used to assess the non-specific adsorption resistances of each system and the RSD was used to assess the reproducibility of the measurements.

![Graph of EMPAS non-specific adsorption frequency shifts](image)

**Figure 52.** EMPAS non-specific adsorption frequency shifts measured with undiluted goat serum using cleaned quartz crystal, 7-OEG, 10-OEG, 13-OEG SAMs. Measurements were recorded at 0.86 GHz.
According to Figure 52, all three diluent systems showed a significant reduction (> 60%) in EMPAS responses when compared to cleaned quartz crystal. More importantly, EMPAS responses obtained with 7-OEG was 27-fold lower than those obtained with cleaned quartz crystal. This demonstrated the impressive ability of OEG diluents with –OH head functions at preventing non-specific adsorption. Furthermore, EMPAS responses obtained with the 7-OEG SAM (950 Hz) were much lower than the ones obtained with the longer diluent SAMs: 10-OEG SAM and 13-OEG SAM (2600 Hz and 9800 Hz). In fact, a trend was observed amongst all three diluents. This suggested that shorter diluents possessed better resistance to non-specific adsorption.

We believe that this was the case due to the packing of these diluent SAMs. SAMs formed by shorter molecules are more disordered and liquid-like, with lower packing density and coverage. As the chain length increases, the SAM becomes more densely packed, forming a more crystalline-like and rigid assembly.\textsuperscript{110} As a result, the ethylene glycol chain within the shorter diluent SAM is more solvated by water molecules than the longer diluent SAMs. This increases both the entropic and the enthalpic energy barriers in resisting non-specific adsorption. In addition, the chains within shorter diluent SAMs are more flexible since they possess a more liquid-like assembly than the crystalline-like ones. Lastly, the low quality of our SAM (due to the less stringent SAM formation conditions) could also contribute to its ability to resist non-specific adsorption since poor quality SAMs tend to be more disordered, allowing increased water penetration.

This explanation was supported by a neutron reflectometry study done in our lab. Neutron reflectometry is a neutron diffraction technique that measures the structure of thin films. It is very sensitive to lighter elements (hydrogen, carbon, oxygen, etc). In the neutron reflectometry study of 7-OEG SAM, it showed that there was an extensive penetration of water molecules within the SAM.\textsuperscript{111} We believe that this is a key link to 7-OEG-SAM’s impressive ability in preventing non-specific adsorption. Both the water
barrier hypothesis and entropy repulsion mechanisms work in a concerted effort to achieve a high degree of resistance for non-specific adsorption in the surface.

To further understand the proposed mechanism, efforts were made to study these SAMs with regards to their packing using FTIR (Fourier Transform Infrared Spectroscopy) in ATR (Attenuated Total Reflectance) mode at a 30° angle to the sample surface. One can look at the asymmetric and symmetric $\nu_{\text{C–H}}$ stretching to determine the packing of the SAM. Lower wavenumbers of the $\nu_{\text{C–H}}$ stretching correspond to a higher degree of order. However, these SAMs are too thin to give a response that could be used for comparison. ATR at grazing angle is needed to achieve the sensitivity necessary for analyzing these ultra-thin monolayers. Further neutron reflectometry studies can be done on longer diluent SAM, 10-OEG and 13-OEG SAM, to study their degree of water penetration. These studies will give more insight and confirmation on the proposed mechanism.
III. 4. OEG-TUBTS/7-OEG: Towards Elimination of Non-Specific Adsorption

III. 4.1. Preparation of Biosensing Interface

After determining that 7-OEG-TFA offered the best potential in reducing non-specific adsorption, it was chosen as the diluent to further improve the performance of the biosensing interface. Cleaned quartz crystals were silanized with a dilute solution of OEG-TUBTS/7-OEG-TFA in toluene. Biotinthiol was subsequently immobilized onto the resultant SAM (Figure 53).

Figure 53. Illustration of the formation of an OEG-TUBTS/7-OEG-TFA SAM on a cleaned quartz crystal (step I) and the subsequent site-specific covalent immobilization of biotinthiol (step II).
CAMs were taken for each surface before and after each process. Deionised water was used as the test liquid (*Figure 54*).

![Contact angle measurements](image)

**Figure 54.** Contact angle measurements obtained with cleaned quartz crystal, OEG-TUBTS/7-OEG-TFA SAM and biotinthiol functionalized OEG-TUBTS/7-OEG SAM.

The usual trends for silanization and subsequent biotinthiol immobilization were observed for these surfaces. CAM values increased significantly after silanization (11° ± 1° to 64° ± 4°) due to OEG-TUBTS and 7-OEG-TFA depositing onto the quartz surface. In addition, the CAM values of OEG-TUBTS/7-OEG-TFA SAM (64° ± 4°) were lower than that of OEG-TUBTS (72° ± 4°). This could be explained by the diluent spacing out the linker, which effectively exposed more OEG backbone and made the surface less hydrophobic.

After biotinthiol immobilization, CAM values decreased slightly (64° ± 4° to 55° ± 6°) due to the replacement of the benzenethiosulphonate head function on OEG-TUBTS with biotinthiol. In addition, the cleavage of the trifluoroacetate head function on 7-OEG-TFA yielded 7-OEG with an –OH head function, which also contributed to the decreased CAM values. These observations suggested that the OEG-TUBTS/7-OEG-TFA SAM was successfully formed on quartz crystal and that biotinthiol was also successfully
immobilized onto the SAM. Finally, each surface was also characterized by ARXPS analysis by examining the relevant element signal (Figure 55 & Appendix J).

Figure 55. XPS narrow scans and relative atomic percentages for silicon, oxygen, carbon, sulfur, nitrogen and fluorine (20° angle relative to the surface) obtained with cleaned quartz crystal, OEG-TUBTS/7-OEG-TFA SAM and biotin thiol functionalized OEG-TUBTS/7-OEG SAM.

According to Figure 55, the relative atomic percentages of sulfur (%S), fluorine (%F) and carbon (%C) were significantly increased after silanization (0.0% to 1.7%, 0.0% to 2.0%, 40.3% to 52.8% respectively).
and 6.9% to 30.3%, respectively). The opposite trends were observed with the percentages of silicon (\(\%_{Si}\)) and oxygen (\(\%_{O}\)) (40.3% to 18.0%, and 52.8% to 48.0%, respectively). The increase in the \(\%_{S}\) was attributed to the OEG-TUBTS. The increase in the \(\%_{F}\) was attributed to the 7-OEG-TFA. Together, these increases signified that both molecules were anchored onto the quartz surface, thus forming a mixed SAM.

The sulfur signal, however, was much lower than expected. This was likely due to the fact that the same mass concentration was used for both molecules to make the preparation solution. Since 7-OEG-TFA (MW = 333.59 g/mol) has a significantly lower molecular weight than OEG-TUBTS (MW = 481.91 g/mol), there were ~45% more 7-OEG-TFA molecules in the solution than OEG-TUBTS. These compete with OEG-TUBTS for anchoring sites on the surface. The decreases in the \(\%_{Si}\) and \(\%_{O}\) were due to OEG-TUBTS/7-OEG-TFA SAM formation over the surface of the quartz crystal and the presence of other types of atoms from the SAM on the surface.

As for biotinthiol immobilization, the expected nitrogen signal appeared after the reaction (0.0% to 2.5%). At the same time, the \(S^0\) peak increased and \(S^{IV}\) peak disappeared (\(S_{2p}\) in Figure 55). This suggested that the biotinthiol was successfully immobilized onto OEG-TUBTS. In addition, the fluorine signal also disappeared after biotinthiol immobilization (2.0% to 0.0%). This indicated that the cleavage of the head function of 7-OEG-TFA to yield 7-OEG on the surface was successful. In summary, the preparation of the biosensing interface with OEG-TUBTS, 7-OEG-TFA and biotinthiol appeared to be successful, as suggested by both CAM and ARXPS.
III. 4. 2. EMPAS Measurements

The performance of the biosensing interface constructed by the OEG-TUBTS/7-OEG-TFA SAM was studied with the EMPAS system at an ultra-high resonating frequency of 0.86GHz (43rd harmonics). In order to directly compare this biosensing interface to the other biosensing interfaces constructed in this study, the same experiments were carried out. Three different proteins (avidin, BSA and IgG) were used separately in the EMPAS experiments, with avidin being the target analyte. For each protein, two sets of independent frequency shift measurements (4 replicates per set) were conducted using 0.1 mg mL\(^{-1}\) solutions of the appropriate protein in PBS (Appendix K).

For the first set, biotin-thiol functionalized OEG-TUBTS/7-OEG SAMs were used to quantify the specific adsorption of the protein to biotin. For the next set, an OEG-TUBTS/7-OEG SAM with the 7-OEG-TFA head function cleaved was used as a control to record the non-specific adsorption of the protein to the biosensing interface. The same mass concentration of avidin, BSA and IgG was used instead of molar concentration due to the fact that EMPAS is sensitive to mass change. The specific to non-specific adsorption frequency shift ratio (R\(_{S/NS}\)) and relative standard deviations (RSD) for each protein were then calculated and compared. The ratio of the avidin-specific adsorption frequency shift to the average non-avidin (IgG and BSA) specific adsorption frequency shifts (R\(_{A/NA}\)) was also calculated. The R\(_{S/NS}\) and R\(_{A/NA}\) were used to assess the ability of the biosensing interface to specifically detect avidin. RSD reflected the reproducibility of the measurements (Figure 56).
Figure 56. EMPAS non-specific and specific adsorption frequency shifts respectively measured with OEG-TUBTS/7-OEG and biotinylated OEG-TUBTS/7-OEG SAMs, using 0.1 mg mL\(^{-1}\) BSA, IgG and avidin solutions in PBS. EMPAS measurements were recorded at 0.86 GHz.

As shown in Figure 56, the incorporation of 7-OEG within the SAM resulted in a massive improvement to the performance, when compared to the system with only OEG-TUBTS. The \(R_{S/NS}\) of avidin was substantially increased by more than 8 fold (from 1.8/1 to 15/1). The surface specificity towards avidin, indicated by \(R_{A/NA}\), was also significantly increased by more than 6 fold (from 2.3/1 to 14/1). In addition, the system exhibited excellent non-specific adsorption resistance. This was demonstrated by the fact that all responses obtained with this new system, excluding the specific adsorption of avidin, were decreased by 75%. Despite the excellent non-specific adsorption resistance, it was exceptional to observe that the specific adsorption response of avidin remained relatively the same compared to the previous system. It is also important to note that the standard deviation of these responses were exceptionally low (± 385 Hz on average). This RSD is very close to the background noise of the EMPAS (± 200 Hz). This was likely due to the reduction of random non-specific adsorption. More importantly, for the first time in this study, the detection specificity was not an issue as positive responses were not observed with IgG or BSA.
In conclusion, the incorporation of the diluent, 7-OEG, within the SAM brought considerable improvements to the system. It is unknown whether the improvement was due to the spacing out of the longer linkers, allowing for entropy repulsion, or the inherent ability of 7-OEG in preventing non-specific adsorption, or both of these effects.

III. 4.3. EMPAS Calibration Study

To further study the biosensing performance of the OEG-TUBTS/7-OEG system, a calibration study was done with different concentrations of avidin in PBS. For each concentration, 3 replicates of independent EMPAS frequency shift measurements were gathered and plotted against their molar concentration (Figure 57 & 58). In addition, different concentrations of BSA in PBS were used as a control against non-specific adsorption.

\[ K_d = 1.7 \times 10^{-6} \pm 4 \times 10^{-7} \text{ M}^{-1} \]

**Figure 57.** EMPAS calibration curves for avidin and BSA in PBS.

EMPAS frequency shifts are mainly dependent on the adsorption of molecules onto the surface of its biosensing interface. As expected, the calibration curve obtained with
avidin resembles a Langmuir adsorption isotherm (Figure 57). Although avidin has 4 identical binding sites, we assumed that only 1 binding site was able to bind the biotin moiety on the surface of the biosensing interface because of steric hindrance. With this assumption, the curve could be fitted with a Langmuir adsorption equation. The apparent K_d value for avidin on the biosensing interface was calculated as $1.7 \times 10^{-6} \pm 4 \times 10^{-7} \text{M}^{-1}$. Comparably, this apparent K_d value was much greater than the literature K_d value for avidin-biotin binding ($K_d = 10^{-15} \text{M}^{-1}$)\textsuperscript{112}. This could be explained by the steric hindrance imposed by the distance between the surface-bound biotin moiety and the large size of avidin (66 kDa). In addition, the binding equilibrium between the immobilized biotin moiety and avidin in solution was likely not reached due to the constant buffer flow during measurements.

![Figure 58](image.png)

**Figure 58.** Linear region of EMPAS calibration curves for avidin and BSA in PBS.

An examination of the linear region of the EMPAS calibration curves showed that there was a good linear correlation between the frequency shift and the concentration of avidin ($R^2 = 0.9947$). The limit of detection (LoD) was calculated as 9 nM (Figure 58). On the other hand, no linear region could be found for BSA (Figure 58). Compared to avidin, the system was not responsive to BSA concentration changes at all. This was shown by the low $R^2$ and slope. The LoD of BSA was calculated as 1.2 µM, which is ~150 times higher than the LoD of avidin. These observations indicated good specificity, sensitivity
and LoD of the biosensor towards its target, avidin. However, the dynamic range was very narrow (0.03 µM to 0.15 µM). This was due to the constant flow of buffer not allowing the system to reach binding equilibrium. This is acceptable since the goal of this type of detection strategy is to use the biosensor as a preliminary, rapid, qualitative analysis. After detection is established, other quantitative analyses may be used afterwards, if necessary.

### III. 4.4 EMPAS Measurements with Complex Samples

As a preliminary study on the performance of these systems in real-world detection scenarios, a high concentration of BSA (45 mg mL⁻¹) was used as the sample matrix in an attempt to mimic a serum matrix. Serum is a type of biological sample that biosensors commonly have problems with. This is due to the presence of many interfering biomolecules, which contributes to non-specific adsorption. Serum was not used as a better representation of a real-world sample due to the fact that serum contains biotin. All avidin molecules would, instead, bind to the biotin in the serum and the concentration of available avidin would be unknown. Samples with a high concentration of BSA were used to mimic serum because serum albumin is the most abundant protein in blood plasma of mammals (roughly 34 – 54 mg mL⁻¹ in human blood).

Two sets of independent frequency shift measurements (4 replicates per set) were conducted, with each of the three systems (TUBTS, OEG-TUBTS, and OEG-TUBTS/7-OEG SAM) functionalized by biotin-thiol (*Appendix L*). First, each system was exposed to a solution of BSA (45 mg mL⁻¹) spiked with avidin (0.1 mg mL⁻¹) in PBS in order to record specific biotin-avidin interactions. For controls, the same surfaces were exposed to a solution of BSA (45.1 mg mL⁻¹) in PBS in order to record non-specific adsorption. The specific to non-specific adsorption frequency shift ratio (Rₛ/NS) and relative standard deviations (RSD) for each protein were then calculated and compared. The Rₛ/NS was used to assess the ability of the biosensing interface to specifically detect avidin in a
complex sample matrix. The RSD was used to represent the reproducibility of the measurements (*Figure 59*).

*Figure 59.* EMPAS non-specific and specific adsorption frequency shifts respectively measured with 45.1 mg mL\(^{-1}\) BSA and 45 mg mL\(^{-1}\) BSA with 0.1 mg mL\(^{-1}\) avidin solutions in PBS, using biotinylated TUBTS, OEG-TUBTS and OEG-TUBTS/7-OEG SAMs. EMPAS measurements were recorded at 0.86 GHz.

Figure 59 summarizes the EMPAS responses obtained when a high concentration of BSA was used as the sample matrix for each system. It is clear that the performance of each system improved with each modification. The R\(_{S/NS}\) went from 1.2/1 to 2.1/1 to 4.1/1 with the addition of the OEG backbone and 7-OEG. A similar trend in reduction was also observed in the RSD of these responses. In addition, the non-specific adsorption responses also significantly decreased (by more than 5 fold) after both modifications. From these two observations it was apparent that both modifications together were necessary to construct a surface with high degrees of non-specific adsorption resistance.
It is important to note that the responses obtained with all these samples were lower than expected (*Figure 59*). The concentration of BSA was increased 450 times but the signal responses did not increase by nearly the same magnitude. This was likely because of the large amounts of BSA molecules in the sample competing amongst themselves to randomly bind to the surface in a reversible fashion. When the sample was spiked with avidin, these random non-specific binding events were most likely replaced by the specific binding of avidin to biotin. Proteins with higher mobility and abundance would first absorb onto the surface before being ultimately displaced by less mobile, higher affinity entities. This was supported by the observation of rinse-off in the EMPAS response profile for biotin-avidin interaction (*Figure 35*). Thus, non-specific binding of undesired proteins will likely be limited when the target analyte is present.

To further investigate the performance of this system in real-world detection scenarios, experiments were run with undiluted goat serum as the sample. Although spiking avidin into goat serum is useless when looking at the specific adsorption detection of this system, it is still worthwhile to use serum to assess the non-specific adsorption resistances of each system. EMPAS frequency shift measurements (4 replicates) were conducted with each system that was functionalized by biotin-thiol. Each system was exposed to undiluted goat serum and the non-specific adsorption response was recorded. Frequency shifts and RSD for each system were then calculated and compared to assess the non-specific adsorption resistances of each system, and the reproducibility of the measurements, respectively (*Figure 60*).
As shown in Figure 60, only the OEG-TUBTS/7-OEG system showed a significant reduction in both EMPAS response and RSD. No significant changes were seen in the TUBTS and OEG-TUBTS systems. Once again, this demonstrated the importance of the incorporation of 7-OEG within the SAM to prevent non-specific adsorption. However, these responses were a lot higher than expected, which suggested that a high degree of non-specific adsorption was still occurring over the surface. All the responses obtained in this experiment were as high as the specific responses obtained with avidin shown in previous section.

This also showed that the previous experiments, done with a high concentration of BSA, were not a good substitution for serum. Non-specific adsorption EMPAS responses obtained with a high concentration of BSA (9800 Hz on average) were much lower than the ones obtained with goat serum (20500 Hz on average). This could be explained by the presence of many more different species, which have the potential to bind to the surface, in goat serum than in the BSA substitute solution. Nevertheless the previous section still demonstrated that the OEG-TUBTS/7-OEG system possessed excellent selectivity.
The responses obtained with the pure 7-OEG system (950 Hz ± 600 Hz) (Figure 52) were much lower than the ones obtained with OEG-TUBTS/7-OEG (14800 Hz ± 3400 Hz). This indicated that having OEG-TUBTS within the SAM actually promoted non-specific adsorption. This also suggested that the vast improvement of the system after 7-OEG incorporation was most likely due to the 7-OEG’s inherent ability to prevent non-specific adsorption, as opposed to 7-OEG providing the ability of entropy repulsion by spacing out the longer linkers. This reduction in non-specific adsorption resistance could be due to several reasons. First, the presence of OEG-TUBTS within the SAM could reduce the number of –OH head groups which can form a water barrier over the surface. Second, OEG-TUBTS is a long molecule with a head function capable of dipole-dipole interactions and π-stacking interaction (benzenethiosulfonate head function). Thus, during SAM formation, OEG-TUBTS could assist in the formation of a denser SAM and reduce the amount of water penetration within the SAM. Lastly, species within the goat serum could be binding to biotin-thiol on the surface specifically or non-specifically. To further understand this phenomenon, more studies will need to be done. This includes studies on the density and linker/diluent distribution of the SAM with labelling and imaging techniques as well as studies on water penetration within the SAM with techniques such as neutron reflectometry.

In conclusion, the performance of our biosensing interface with our complex sample mimic was very promising. As expected, the OEG-TUBTS/7-OEG system offered the best potential for conducting measurements in complex samples. However, more improvements are needed to deal with real world samples such as serum and blood. There are many modifications that could be done to the system to improve its performance. This includes optimization of the SAM formation conditions (i.e. silanization time, linker/diluent ratio, and concentration), varying the length of the linker, and using different head functions on the diluent.
III. 5. Expanding the Chemistry of the Thiosulfonate-Base Linker

With the success of the thiosulfonate-based linker shown in this study, efforts were undertaken to use this chemistry for various other applications. This system has been used to construct an aptamer-based biosensor for the detection of cocaine\textsuperscript{114} and an antibody-coated steel stent for improved biocompatibility\textsuperscript{115}. There were also efforts to use 7-OEG-TFA in the modification of biomedical polymers in order to prevent the fouling and clotting of blood\textsuperscript{116}. This has led to interests in expanding this chemistry in other types of substrates for wider applications. In this preliminary study, we focus on expanding the chemistry to silicon nitride (Si\textsubscript{3}N\textsubscript{4}), Aluminium nitride (AlN) and Gold (Au) substrates. Si\textsubscript{3}N\textsubscript{4} is an insulator that is often used to make Ion-Selective Field Effect Transistors (ISFET) which have great potential in electrical biosensing technology\textsuperscript{117}. For AlN, there is great interest in its use in acoustic wave biosensing technology due to AlN’s excellent piezoelectric properties (high Q value and resonance frequency)\textsuperscript{118}. Lastly, gold is a very commonly used substrate in the bioanalytical field (e. g. TSM, gold nanoparticle-based biosensing technologies and etc). Although gold surfaces already have the capability of immobilizing thiol-containing molecules, our system will allow us to make a surface with high resistance to non-specific adsorption, while maintaining the capability of immobilizing thiol-containing molecules.
III. 5. 1. TUBTS on Si$_3$N$_4$ and AlN

The preparation of a TUBTS SAM and its subsequent biotinthiol immobilization was performed on Si$_3$N$_4$ and AlN in an attempt to expand our chemistry to these surfaces (Figure 61). TUBTS was used in this study instead of OEG-TUBTS because the synthesis of TUBTS was relatively straightforward and less time-consuming. Nitrogen plasma cleaning was used instead of piranha solution (3:1 concentrated sulfuric acid: 30% H$_2$O$_2$) due to compatibility issues. The exact same conditions as the quartz crystal from the previous section were used for silanization and biotinthiol immobilization.

Figure 61. Illustration of the formation of a TUBTS SAM on a cleaned Si$_3$N$_4$ and AlN (step I) and the subsequent site-specific covalent immobilization of biotinthiol (step II).
CAMs were taken for each surface before and after each step. Deionized water was used as the test liquid (*Figure 62 & 63*).

*Figure 62*. Contact angle measurements obtained with cleaned Si₃N₄, TUBTS SAM on Si₃N₄ and biotinthiol functionalized TUBTS SAM on Si₃N₄.

The usual trends for silanization and subsequent biotinthiol immobilization were observed for both types of surface. CAM values significantly increased after silanization.
due to the deposition of TUBTS onto the surface of both Si$_3$N$_4$ and AlN ($5^\circ \pm 4^\circ$ to $73^\circ \pm 4^\circ$ and $4^\circ \pm 2^\circ$ to $81^\circ \pm 4^\circ$). After biotinthiol immobilization, the CAM values decreased slightly ($5^\circ \pm 4^\circ$ to $5^\circ \pm 4^\circ$, and $5^\circ \pm 4^\circ$ to $5^\circ \pm 4^\circ$, respectively). This was due to the displacement of the head function of TUBTS with biotinthiol. These observations suggested that the TUBTS SAM was formed onto both Si$_3$N$_4$ and AlN substrate and that biotinthiol was subsequently immobilized onto the SAM. Each SAM, prepared on either substrate, was also characterized by ARXPS analysis by examining the relevant elements (Figure 64 & 65) (Appendix M & N).

With ARXPS analysis on the Si$_3$N$_4$ substrate, the relative atomic percentage of carbon ($\%_C$) increased significantly (10.5% to 44.6%). Two sulfur peaks also appeared after silanization. These observations corresponded to the deposition of TUBTS onto the surface of the substrate. Moreover, there were significant declines in the relative atomic percentage of silicon ($\%_S$), nitrogen ($\%_N$), and oxygen ($\%_O$) after silanization (41.0% to 27.9%, 21.8% to 6.9%, and 26.7% to 19.0%, respectively). All declines in relative atomic percentage were due to the SAM formation over the surface of Si$_3$N$_4$. The decrease in $\%_O$ was due to the formation of the SAM over the thin layer of oxidation on the surface of cleaned Si$_3$N$_4$. Thus, less characteristic atoms of Si$_3$N$_4$ and its oxidized layer were seen by surface XPS analysis.

Regrettably, the nitrogen signal could not be used to confirm biotinthiol immobilization. This was due to the large nitrogen signal from the substrate. Thus, only sulfur signals could be used to confirm the immobilization. The $S^0$ peak increased and the $S^{IV}$ peak completely disappeared after biotinthiol immobilization ($S_{2p}$ in Figure 64). This was due to the displacement of the $S^{IV}$ atom in the leaving group of the TUBTS head function with the $S^0$ atom in biotinthiol. This indicated that the biotinthiol was successfully immobilized onto the surface of Si$_3$N$_4$ through TUBTS SAM.
Figure 64. XPS narrow scans and relative atomic percentages for silicon, nitrogen, oxygen, carbon, and sulfur (20° angle relative to the surface) obtained with cleaned Si$_3$N$_4$, TUBTS SAM on Si$_3$N$_4$ and biotinthiol functionalized TUBTS SAM on Si$_3$N$_4$. 
Figure 65. XPS narrow scans and relative atomic percentages for aluminium, nitrogen, oxygen, carbon, silicon and sulfur (20° angle relative to the surface) obtained with cleaned AlN, TUBTS SAM on AlN and biotin-thiol functionalized TUBTS SAM on AlN.

With ARXPS analysis on the AlN substrate, the relative atomic percentage of carbon (\%\textsubscript{C}) significantly increased after silanization (19.5% to 23.0%). In addition, two sulfur peaks and a silicon peak also appeared. These observations corresponded to the deposition of TUBTS onto the surface of the substrate. Since AlN has no silicon atom, the appearance of the silicon signal (0.0% to 1.1%) from the TUBTS tail function further confirms SAM formation. In addition, there was a decline in the relative atomic percentage of nitrogen
(\%N) and oxygen (\%O) (12.1% to 10.3% and 37.2% and 28.8%, respectively). These declines were due to SAM formation over the surface of AlN, making the characteristic atoms of AlN and its oxidized layer less likely to be detected by surface XPS analysis. However, it was unknown why the relative atomic percentage of aluminium (\%Al) slightly increased after silanization (31.1% and 34.5%). This may be due to an incomplete SAM formation and/or differences in the composition of each specific AlN sample substrate.

After biotinthiol immobilization, a second nitrogen peak emerged and became more apparent in the narrow range of the nitrogen signal (N$_{1s}$ in **Figure 65**). This was likely due to the nitrogen atom from biotinthiol. The first and main peak before biotinthiol immobilization was contributed by the nitrogen from AlN. The binding energy difference between the two nitrogen atoms could be explained by the difference in the local chemical and physical environments that the two atoms were in. In addition, the S$^0$ peak increased and S$^{IV}$ peak disappeared after biotinthiol immobilization (S$_{2p}$ in **Figure 65**). This was due to the displacement of the S$^{IV}$ atom in the leaving group of the TUBTS head function with the S$^0$ atom in biotinthiol. This indicated that the biotinthiol was successfully immobilized onto the surface of AlN through TUBTS SAM.

Although it was demonstrated that TUBTS could be used on Si$_3$N$_4$ and AlN substrates, it was important to compare the quality of the SAM formed on these new substrates with the one formed on quartz in order to further assess the compatibility of our chemistry on these new substrates. One quick way to do this was to estimate the amount of TUBTS deposited onto the surface with XPS. For this purpose, the relative atomic percentage of sulfur (\%S) in TUBTS SAM on each substrate was normalized and compared (**Figure 66**). The \%S was normalized to the relative atomic percentages of a single unique element in their respective substrate (Si for quartz and Si$_3$N$_4$, Al for AlN). Normalized values of \%S were used in order to minimize discrepancies between measurements done on separate substrates in different days. Since the measurements were done in different
batches, experimental conditions may be different. In addition, the normalized %S values were corrected by the atomic distribution factor of their respective substrates. The atomic distribution factors were determined by the ratio of atoms in each type of cleaned substrate (Quartz: 1/3 Si; Si$_3$N$_4$: 1/2; AlN: 3/4 Al). This was done to account for the fact that different types of substrates were used in the comparison and their atomic distribution is different. Without this correction, the results would be skewed.

![Figure 66](image.png)

**Figure 66.** Normalized %S for TUBTS SAM on quartz, Si$_3$N$_4$, AlN.

According to the data summarized in Figure 66, the TUBTS SAM on quartz had a much higher normalized %S than the TUBTS SAM on Si$_3$N$_4$ and AlN. This suggested that many more TUBTS molecules were deposited onto the quartz surface, forming a denser SAM than the other two substrates. This could be explained by the differences in –OH group density over the surface of these substrates. Quartz crystals were treated with piranha solution (3:1 concentrated sulfuric acid: 30% H$_2$O$_2$) for cleaning and generated –OH groups over the surface. However, piranha solution could not be used with Si$_3$N$_4$ and AlN since it corrodes these substrates. As an alternative, a nitrogen plasma cleaning method was used due to availability. Regrettably, it did not generate as many -OH groups as the piranha solution. Although generating a less dense SAM could be beneficial in preventing non-specific adsorption, generating an incomplete SAM could lead to
negative outcomes. Therefore, to ensure that this was not the case, more studies will need to be done. In addition, an alternative cleaning method is needed to generate higher concentrations of $-\text{OH}$ groups over the surface of $\text{Si}_3\text{N}_4$ and AlN. Some possible alternative cleaning methods are ozonolysis and oxygen plasma cleaning.

**III. 5. 2. DNBTS on Gold**

In order to design a thiosulfonate-based linker to form a SAM on gold substrates, a thiol-based tail function must be used. However, a simple thiol tail function is not compatible with the thiosulfonate head function since these two functional groups react with each other. To avoid this issue, dithiolane, a cyclic disulfide tail function, was used instead. The two thiols in dithiolane would occupy each other in a disulfide bond, making them incapable of reacting with the thiosulfonate head function. The backbone of the linker was extended from the 4-position of dithiolane to avoid chirality in the molecule, which simplified the synthesis.

**III. 5. 2. 1. DNBTS Synthesis**

The synthesis of DNBTS was achieved in nine steps from commercially available 9-bromononanol with an overall yield of 15% (*Figure 67*). The first step consisted of the protection of the alcohol moiety with dihydropyran. The resulting product 18 was subsequently $\alpha$-alkoxylated with diethyl malonate into the diester 19. Through a series of functional group interconversions, the diester was transformed into dithiolane 24. At the same time, the other end of the molecule was deprotected and then transformed into bromide 25 with CBr$_4$. S-alkylation of the benzenethionosulfonic acid sodium salt by 25 gave the final product, DNBTS (*Appendix O*).
Figure 67. Synthesis of DNBTS.
III. 5. 2. 2. DNBTS SAM Preparation

The preparation of the DNBTS SAM and subsequent biotinthiol immobilization was performed on gold in an attempt to expand our chemistry to these surfaces (Figure 68). For this purpose, cleaned gold-plated TSM crystals were treated with a dilute solution of DNBTS in absolute ethanol (10mM). Afterwards, TSM crystals coated with the DNBTS SAM were immersed into a solution of biotinthiol in methanol (1 mg/1 mL).

Figure 68. Illustration of the formation of an DNBTS SAM on a cleaned TSM crystal (step I) and the subsequent site-specific covalent immobilization of biotinthiol (step II).
CAMs were taken for each surface before and after each step, with deionized water as the test liquid (Figure 69).

![Cleaned TSM Crystal: 10° ± 2°](image1) ![TUBTS SAM on TSM Crystal: 69° ± 2°](image2) ![Biotinthiol functionalized DNBTS SAM on TSM: 53° ± 3°](image3)

**Figure 69.** Contact angle measurements obtained with cleaned TSM crystal, DNBTS SAM and biotinthiol functionalized DNBTS SAM on TSM crystal.

As shown in Figure 69, CAM values increased significantly after treatment with DNBTS (from 10° ± 2° to 69° ± 2°). This indicated that DNBTS formed a SAM on the cleaned gold surface of TSM crystals, making it more hydrophobic. After biotinthiol immobilization, the CAM values decreased slightly (from 69° ± 2° to 53° ± 3°). This suggested that the benzenethiosulfonate head function of DNBTS was replaced by biotinthiol. These surfaces were also characterized by ARXPS analysis by examining the relevant elements (Au, C, S and N) (Figure 70 & Appendix P).
Figure 70. XPS narrow scans and relative atomic percentages for gold, carbon, sulfur and nitrogen (20° angle relative to the surface) obtained with cleaned gold-plated TSM crystal, DNBTS SAM and biotinthiol functionalized DNBTS SAM.

ARXPS analysis on the TSM crystals showed that the relative atomic percentages of carbon (%C) and sulfur (%S) increased significantly after treatment with DNBTS (32.1% to 44.0% and 0.1% to 3.5%, respectively). This observation suggested that the deposition of DNBTS onto the gold surface of the TSM crystal was successful. In addition, there were significant declines in the relative atomic percentages of gold (%Au) (41.0% to 27.9%). This decline was due to DNBTS being deposited over the gold surface. Less gold atoms from the surface were seen by the XPS analysis.

As expected, the head function of DNBTS did not react with the dithiolane tail function during the SAM formation and remained intact after the formation. This was indicated by the two peaks in the sulfur signal, which represented the two sulfur atoms in different oxidation states in the head function of DNBTS. After biotinthiol immobilization, the
nitrogen signal appeared (0.1% to 4.9%), while the S⁰ peak increased and the S⁴ peak disappeared (S₂p in Figure 70). When these results were observed for the other substrates used in this study, they confirmed that biotin-thiol had been immobilized onto the SAM by a reaction with the head function of the thiosulfonate-based linker. However, this was not the case with the gold substrate. Gold can naturally chemisorb sulfur onto its surface. As a result, during biotin-thiol immobilization, biotin-thiol could displace the DNBTS on the surface and chemisorb onto the gold surface instead. Although it is unlikely for a monodentate biotin-thiol to displace a bidentate DNBTS, XPS analysis and CAMs alone cannot confirm that such a phenomenon did not take place. Therefore, experiments such as isotopic labeling will be needed to test the feasibility of expanding our chemistry to gold surfaces.

In summary, preliminary results regarding the expansion of the thiosulfonate-based linkers to Si₃N₄ and AlN were very promising. However, the expansion onto gold was uncertain. The TUBTS SAM was formed onto Si₃N₄ and AlN substrates and biotin-thiol was successfully immobilized onto the SAM. Nonetheless, alternate cleaning methods may be needed to generate more –OH groups over the surface to form a better quality SAM. For gold, the DNBTS SAM was successfully formed onto the surface. However, more studies are required to ensure that thiol-containing molecules do not replace DNBTS on the surface during immobilization.

III. 6. EMPAS Coil Circuit

III. 6. 1. Purpose

In EMPAS, an electromagnetic coil is required to generate the necessary electromagnetic field to remotely drive the transducer (quartz crystal) into mechanical resonance. Any changes in the resonance of the crystal needs to feedback to the coil and relay the signal to a detector. To facilitate this, the EMPAS coil circuit used in this study was composed of a parallel LC (Inductor-Capacitor) coil circuit and a FET (Field-Effect Transistor)
buffer amplifier. With a high frequency AC current (close to 1 GHz) provided by a radio frequency generator, the parallel LC coil circuit was able to resonate and output the necessary electromagnetic field to excite the quartz crystal. To receive and detect the feedback, the AC current was modulated by an external low frequency (Hz) signal from a lock-in amplifier. The FET buffer amplifier was placed between the LC coil circuit and the detector to relay the signal. The FET buffer amplifier was there to minimize noise and pickup in the connecting lines while boosting the signal before it reached the detector. The FET buffer amplifier also provided gains close to the coil circuit before the signal-to-noise ratio was permanently degraded. Without the FET buffer amplifier, the signal received at the detector was very weak and deformed (Figure 71). Consequently, it was crucial to include this circuit in EMPAS for it to gather data properly. However, the designs of this EMPAS coil circuit were previously undocumented. Thus, a detailed discussion of the design of the circuit is presented in the next section.

Figure 71. Resonant envelopes acquired with and without the EMPAS FET buffer amplifier on EMPAS

III. 6. 2. Design

The design of the EMPAS coil circuit was composed of a hand-wound flat spiral coil (≈ 5mm diameter) of polyurethane-coated copper wire (total diameter of metal and insulating layer, 105 µm) (Figure 72a). This coil facilitated the actual output of the electromagnetic field and received the feedback signal. A capacitor trimmer (0.3 pF to 3 pF) was placed in parallel with the coil terminals to make the parallel LC coil circuit.
This allowed the electrical resonant frequency of the coil to be tuned and matched with the acoustic resonance of the quartz crystal. This was done so that the generated electromagnetic field would have sufficient energy to drive the crystal into mechanical resonance. Furthermore, tuning the capacitance not only enhanced the resonant envelope but, in theory, would also allowed for the instrument to operate at higher harmonics for greater sensitivity.

A FET buffer amplifier was inserted between the parallel LC coil circuit and the detector in order to buffer the signal to the detectors while also providing a small boost to the signal. The design of the FET buffer amplifier contained a FET transistor with a tunable resonant circuit (parallel LC circuit) powered by an external power source (-7.55V, 10mA) (Figure 72b). The FET transistor provided gains to the signal with the help of the external power source. The tunable resonant circuit further amplified the signal by tuning its resonant frequency to match the incoming signal. A band-pass filter was placed between the external power source and the FET buffer amplifier to filter out noise from the power source (Figure 72c).

A clamper circuit was included at the beginning of the circuit for input protection for the FET transistor (Figure 72d). After the clamper circuit, an impedance matching circuit was included to match the impedance of the radio frequency generator. This allowed maximum power transfer from the radio frequency generator to the EMPAS coil circuit (Figure 72e). A coupling capacitor was used at the end of the buffer amplifier to connect the circuit to the detector such that the DC signal is blocked and only the AC signal passes through to the detector. However, due to the high input impedance of the detector and high operating frequency, the coupling capacitor remained charged when the signal passed through to the detector. This did not allow the coupling capacity enough time to discharge, which led to the signal being lost. As a consequence, a lower value resistor was added in parallel right after the coupling capacitor (Figure 72f). This enabled the coupling capacitor to discharge through the resistor to the ground. This way, the coupling
capacitor could undergo its charge/discharge cycles so that the detector could pick up the voltage drop as signal between the capacitor and the resistor.

**Figure 72.** Schematic representation of the EMPAS coil circuit: a) parallel LC coil circuit; b) FET transistor with tunable resonant circuit; c) band-pass filter; d) clamper circuit; e) impedance matching circuit; f) coupling capacitor circuit.

### III. 6. 3. Possible Improvements

Since the EMPAS coil circuit was a prototype unit, there are several possible improvements that can be done to the circuit for future use. Specifically, the noise of the instrument could be vastly improved with a few changes to the EMPAS coil circuit.

First, the electromagnetic coil could be placed on a copper-clad board and the rest of the circuit could be fitted in a metal casing. This would greatly reduce the noise from the surrounding environment. This would also prevent electrical interference from sources that operate at similar frequencies, since the electromagnetic coil and the resonant circuit
behave like antennas. Second, a resistor could be added in the LC coil circuit to reduce the chance of interference by reducing the resonant frequency bandwidth. However, this would make circuit tuning more difficult and sacrifice some intensity in the signal. Lastly, electronically controlled capacitors could be used instead of mechanically controlled ones. Mechanically controlled capacitors are prone to physical interference. Vibration or movement could change their capacitance slightly and change the electrical condition of the circuit. This is a necessary change for the instrument in order to make it portable and conduct measurement remotely. More importantly, electronically controlled capacitors allow more precise and easy tuning of the resonance frequency.
IV. Conclusions

Within this thesis, I presented a biosensing interface preparation method for hydroxylated surfaces. This method involved the use of thiosulfonate-based trichlorosilane linkers to construct robust and durable SAMs onto a hydroxylated surface. The resulting SAMs had the ability to chemoselectively immobilize thiol-containing molecules under aqueous condition in a single, straightforward, reliable and coupling-free manner. Our preliminary work consisted of the construction of TUBTS SAMs on quartz crystals and the subsequent immobilization of biotinthiol. The resulting SAMs were then characterized by CAMs and ARXPS. The biosensing properties of our interface in terms of specific and non-specific adsorption was successfully evaluated with EMPAS using avidin, BSA, and IgG in PBS solution. However, the resulting biosensing interface had problems with detection specificity due to high non-specific adsorption. This was most likely due to the hydrophobic interactions between the various proteins and the hydrophobic SAM. It was then suggested that the incorporation of an OEG backbone could prevent/reduce non-specific adsorption. With OEG-TUBTS, an OEG-backboned TUBTS analog, the non-specific adsorption was moderately reduced but the problem with detection specificity was still present. This led us to believe that the tightly packed linkers were hindering the effect of the OEG backbone. It was then suggested that the incorporation of a diluent could alleviate this issue.

Different diluents were designed and synthesized. The construction of SAMs on quartz crystals with each diluent was characterized by CAMs and ARXPS. The non-specific adsorption resistance of these SAMs was evaluated with EMPAS using undiluted goat serum. Remarkably, 7-OEG SAM reduced non-specific adsorption by more than 27-fold when compared to bare quartz crystal, thus outperforming all other diluents. It was demonstrated that the –OH head function in conjunction with OEG moiety in the backbone was crucial for preventing non-specific adsorption. In addition, the formation of a less-ordered and liquid-like SAM, promoted by the short length of 7-OEG-TFA, was also important for preventing non-specific adsorption. This led us to believe that both
enthalpy-driven and entropy-driven mechanisms work in a concerted effort to achieve a high resistance to non-specific adsorption for these surfaces.

With the incorporation of 7-OEG-TFA as the diluent in OEG-TUBTS system, the specific/non-specific ratio was increased by 15/1, which is 8-fold better than OEG-TUBTS without the diluent. The surface specificity towards avidin, indicated by \( R_{\text{A/NA}} \), was also significantly increased by more than 6 fold (from 2.3/1 to 14/1). In addition, all the non-specific adsorption responses were reduced by 75% on average. The calibration study was done to showcase the correlation of the EMPAS response and the concentration of avidin. Excellent LoD and sensitivity for avidin was observed. A preliminary study with a complex sample mimic demonstrated the excellent selectivity of the OEG-TUBTS/7-OEG system. It maintained a 4.07/1 specific/non-specific ratio despite the background species being 450 times more concentrated than the avidin analyte. However, with goat serum as the sample, it was shown that the complex sample mimic was not a true representative for real world samples such as serum and blood. Thus, more improvements are needed for dealing with such samples.

With the success of the OEG-TUBTS/7-OEG system, efforts were taken to expand this chemistry to other type of substrates. A preliminary study on the expansion of the thiosulfonate-based linkers to \( \text{Si}_3\text{N}_4 \) and \( \text{AlN} \) with TUBTS was successful. However, the current cleaning method for these surfaces did not generate a sufficient amount of –OH groups on the surface to form a SAM that was as dense as the one on quartz crystal. Regrettably, the expansion of the thiosulfonate-based linkers to gold with DNBTS was inconclusive. It was uncertain to us whether the biotin thiol was immobilized onto the gold surface through DNBTS or whether it replaced DNBTS.

A previous undocumentd EMPAS coil circuit was reported in detail. The FET buffer amplifier within the circuit was crucial in relaying the signal from the coil to the detector.
Without the FET buffer amplifier, the signal was very weak and deformed. Possible improvements to the circuit were suggested and are ready for implementation.
V. Future Work

The results presented in this manuscript demonstrated the potential of using an OEG-based thiosulfonate linker combined with a short OEG-based diluent to construct biosensing interfaces with high non-specific adsorption resistance. Our method, as opposed to some of the already well-established methods (EDC/NHS coupling chemistry), provides potentially chemoselective immobilization of thiol-containing biomolecule under aqueous condition in a single, straightforward, reliable and coupling-free manner. It also showed great potential for dealing with complex samples. However, more improvements are required for the method to be applicable in a real world setting.

First, more studies need to be done on the diluents to further confirm and understand its mechanism for preventing non-specific adsorption. FTIR in ATR mode sampling at a grazing angle can give insight to the packing order of our diluent SAMs. Neutron reflectometry on longer diluents can be done to measure the penetration of water molecules within the SAMs. These results can help us design more strategies to offer better non-specific adsorption resistance in our biosensing interface. Further manipulation of the packing order in our system can be achieved by changing the head function of the linker from benzenethiosulfonate to methanethiosulfonate. This SAM will have lower packing density without the pi-stacking interaction from the benzene ring. Other ways to change the packing order includes changing the linker to diluent ratio and changing the length of the linkers. Alternative ways to reduce non-specific adsorption, such as trying different head function and/or backbone of the diluent, will also be explored.

To continue the effort in expanding our chemistry to different types of surfaces, more experiments need to be done with Si$_3$N$_4$, AlN, and gold. An alternative cleaning method to generate more –OH groups over the surface of Si$_3$N$_4$ and AlN is needed. There are already efforts underway to test new cleaning methods for AlN. More studies, such as isotopic labelling, need to be done on gold to ensure that thiol-containing molecules do
not replace DNBTS on the surface during immobilization. We also want to continue to expand the chemistry to a larger variety of surfaces for more applications. There are already plans to use this chemistry to construct an antibody-coated steel stent for improved bio-compatibility and 7-OEG SAM coated plastic and glass for anti-biofouling.

Various improvements to EMPAS are also underway. Improvements to the coil circuit would reduce the amount of noise generated within the system as well as the noise received from the surrounding environment. This will allow the system to conduct its measurement reliably at an even higher frequency for greater sensitivity. It is hoped that the continuing improvements to the EMPAS will allow it to reach a wider audience and become an active member of current biosensing techniques. In conjunction with our thiosulfonate linker, the system has already been used to construct an aptamer-based biosensor for the detection of cocaine.

Overall, I hope that the work presented in this thesis, as well as its future extensions, will have a positive impact in bioanalytical research and other fields of interest. I hope that thiosulfonate-based linkers, such as OEG-TUBTS, will become a widely applied surface linking chemistry for the immobilization of thiol-containing biomolecules in the foreseeable future. In addition, I hope the ability to prevent non-specific adsorption with 7-OEG SAM will bring us one step closer to solve the issue of non-specific adsorption and biofouling in related fields. Finally, I hope the success of this study will bring EMPAS one step closer to implementation in a real world setting as an inexpensive biosensor platform that offers rapid and remote detection for various analytes.
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Appendix A: $^1$H NMR of TUBTS

Figure A. 1. $^1$H NMR of TUBTS precursor.

Figure A. 2. $^1$H NMR of TUBTS.
Appendix B: XPS survey spectra for TUBTS SAM on quartz crystal

Figure B. 1. XPS survey spectra for a cleaned quartz crystal.

Figure B. 2. XPS survey spectra for a TUBTS SAM on quartz crystal.
Figure B. 3. XPS survey spectra for a biotin-thiol functionalized TUBTS SAM on quartz.

Appendix C: Example of EMPAS measurement profiles for TUBTS system

Figure C. 1. An example of an EMPAS measurement profile for TUBTS SAM surface with avidin in PBS as sample.
Figure C. 2. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with avidin in PBS as sample.

Figure C. 3. An example of an EMPAS measurement profile for TUBTS SAM surface with BSA in PBS as sample.
**Figure C. 4.** An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with BSA in PBS as sample.

**Figure C. 5.** An example of an EMPAS measurement profile for TUBTS SAM surface with IgG in PBS as sample.
**Figure C. 6.** An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with IgG in PBS as sample.

**Appendix D: $^1$H NMR of OEG-TUBTS**

**Figure D. 1.** $^1$H NMR of OEG-TUBTS precursor.
Figure D. 1. $^1$H NMR of OEG-TUBTS.

Appendix E: XPS survey spectra for OEG-TUBTS SAM on quartz crystal

Figure E. 1. XPS survey spectra for an OEG-TUBTS SAM on quartz.
Figure E. 2. XPS survey spectra for a biotin-thiol functionalized OEG-TUBTS SAM on quartz.

Appendix F: Example of EMPAS profiles for OEG-TUBTS system

Figure F. 1. An example of an EMPAS measurement profile for OEG-TUBTS SAM surface with avidin in PBS as sample.
Figure F. 2. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with avidin in PBS as sample.

Figure F. 3. An example of an EMPAS measurement profile for OEG-TUBTS SAM surface with BSA in PBS as sample.
**Figure F. 4.** An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with BSA in PBS as sample.

**Figure F. 5.** An example of an EMPAS measurement profile for OEG-TUBTS SAM surface with IgG in PBS as sample.
Figure F. 6. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with IgG in PBS as sample.

Appendix G: $^1$H NMR of Diluents

Figure G. 1. $^1$H NMR of 9-OEG.
Figure G. 2. $^1$H NMR of 10-OEG-TFA precursor.

Figure G. 3. $^1$H NMR of 10-OEG-TFA.
Figure G. 4. $^1$H NMR of 7-OEG-TFA.

Figure G. 5. $^1$H NMR of 13-OEG-TFA precursor.
Figure G. 6. $^1$H NMR of 13-OEG-TFA.

Appendix H: XPS survey spectra for diluent SAM on quartz crystal

Figure H. 1. XPS survey spectra for a 9-OEG SAM on quartz.
Figure H. 2. XPS survey spectra for a 10-OEG-TFA SAM on quartz.

Figure H. 3. XPS survey spectra for a 10-OEG SAM on quartz.
**Figure H. 4.** XPS survey spectra for a 7-OEG-TFA SAM on quartz.

**Figure H. 5.** XPS survey spectra for a 7-OEG SAM on quartz.
Figure H. 6. XPS survey spectra for a 13-OEG-TFA SAM on quartz.

Figure H. 7. XPS survey spectra for a 13-OEG SAM on quartz.
Appendix I: Example of EMPAS profiles for pure diluent systems

**Figure I. 1.** An example of an EMPAS measurement profile for 9-OEG SAM surface with goat serum as sample.

**Figure I. 2.** An example of an EMPAS measurement profile for 10-OEG SAM surface with goat serum as sample.
**Figure I.3.** An example of an EMPAS measurement profile for 7-OEG SAM surface with goat serum as sample.

**Figure I.4.** An example of an EMPAS measurement profile for 13-OEG SAM surface with goat serum as sample.
Appendix J: XPS survey spectra for OEG-TUBTS/7-OEG SAM on quartz crystal

Figure J. 1. XPS survey spectra for an OEG-TUBTS/7-OEG SAM on quartz.

Figure J. 2. XPS survey spectra for a biotin-thiol functionalized OEG-TUBTS/7-OEG SAM on quartz.
Appendix K: Example of EMPAS profiles for OEG-TUBTS/7-OEG system

Figure K. 1. An example of an EMPAS measurement profile for OEG-TUBTS/7-OEG SAM surface with avidin in PBS as sample.

Figure K. 2. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with avidin in PBS as sample.
Figure K. 3. An example of an EMPAS measurement profile for OEG-TUBTS/7-OEG SAM surface with BSA in PBS as sample.

Figure K. 4. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with BSA in PBS as sample.
Figure K. 5. An example of an EMPAS measurement profile for OEG-TUBTS/7-OEG SAM surface with IgG in PBS as sample.

Figure K. 6. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with IgG in PBS as sample.
Appendix L: Example of EMPAS profiles for complex sample studies

**Figure L. 1.** An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with high concentration of BSA in PBS as sample.

**Figure L. 2.** An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with high concentration of BSA spiked with avidin in PBS as sample.
Figure L. 3. An example of an EMPAS measurement profile for biotinylated TUBTS SAM surface with goat serum as sample.

Figure L. 4. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with high concentration of BSA spiked in PBS as sample.
Figure L. 5. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with high concentration of BSA spiked with avidin in PBS as sample.

Figure L. 6. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with goat serum as sample.
Figure L. 7. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with high concentration of BSA spiked in PBS as sample.

Figure L. 8. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS/7-OEG SAM surface with high concentration of BSA spiked with avidin in PBS as sample.
Figure L. 9. An example of an EMPAS measurement profile for biotinylated OEG-TUBTS SAM surface with goat serum as sample.

Appendix M: XPS survey spectra for TUBTS SAM on Si$_3$N$_4$

Figure M. 1. XPS survey spectra for a cleaned Si$_3$N$_4$. 
Figure M. 2. XPS survey spectra for a TUBTS SAM on Si$_3$N$_4$.

Figure M. 3. XPS survey spectra for a biotin-thiol functionalized TUBTS SAM on Si$_3$N$_4$. 
Appendix N: XPS survey spectra for TUBTS SAM on AlN

Figure N. 1. XPS survey spectra for a cleaned AlN.

Figure N. 2. XPS survey spectra for a TUBTS SAM on AlN.
Figure N. 3. XPS survey spectra for a biotin-thiol functionalized TUBTS SAM on AlN.

Appendix O: $^1$H NMR of DNBTS

Figure O. $^1$H NMR of DNBTS.
Appendix P: XPS survey spectra for DNBTS SAM on gold-plated TSM crystals

Figure P. 1. XPS survey spectra for a cleaned gold-plated TSM crystals.

Figure P. 2. XPS survey spectra for a DNBTS SAM on gold-plated TSM crystals.
Figure N. 3. XPS survey spectra for a biotinthiol functionalized DNBTS SAM on gold-plated TSM crystals.

Appendix Q: $^1$H NMR of Biotinthiol

Figure Q. $^1$H NMR of biotinthiol