Nanoorthogonal Surface Modifications of Gold Nanoparticles and Nanoclusters through Strain-Promoted Cycloaddition Chemistry

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Abstract

This thesis explores the preparation of thiolated gold nanoparticles (AuNPs) and thiolated gold nanoclusters (AuNCs) capable of undergoing post-assembly surface modifications using two common “bioorthogonal” click reactions: the strain-promoted alkyne-azide cycloaddition (SPAAC) reaction (which occurs between a strained-alkyne and an azide) and the strain-promoted alkyne-nitrone cycloaddition (SPANC) reaction (which occurs between a strained-alkyne and a nitrone). Due to their rapid and modifiable reaction kinetics, high chemoselectivity, and stability of the reactive partners, these reactions were originally designed to tether functional substrates to biologically sensitive biomolecules, without altering their structure or perturb the biologically sensitive environments in which they operate in. The research presented herein explores using the SPAAC and SPANC reactions as “nanoorthogonal” click reactions, translating their advantageous characteristics towards surface modifications of thiolated AuNPs and AuNCs in an efficient and straightforward manner without perturbing their chemically sensitive structures.

Chapter 2 describes the development of a reactive AuNP platform with an aliphatic strained-alkyne (specifically, bicyclo[6.1.0]nonyne (BCN)) tethered to its surface. This platform could undergo both interfacial SPAAC (I-SPAAC) and interfacial SPANC (I-SPANC), whose reaction kinetics could be tuned through structural alterations to the complementary azide/nitrone dipolar species, respectively. When highly electron-deficient dipolar species were used, rapid surface modifications could be accomplished. Such predictable alterations to the kinetic profiles of I-SPAAC and I-SPANC allows exclusive reactivity with one highly reactive dipolar species in the presence of a less reactive dipolar species, which altogether provides an efficient and versatile route towards derivatizing AuNP surfaces. To further expand the scope of such rapid modifications of AuNP surfaces, Chapter 3 explores the development of a nitrone-terminated AuNP platform, in which the surface nitrone dipolar species are delocalized into highly electron deficient pyridinium groups. In a prototype kinetic study, nitrones with pyridinium groups on the Nα of the nitrones exhibited rapid reaction kinetics with BCN, whose reaction kinetics could be altered through modifications of the Cα substituents of the nitrone. Unfortunately, due to the high reactivity of the pyridinium-functionalized nitrone group, attempts to incorporate this rapidly reactive moiety to the AuNP surface was not successful due to the synthetic incompatibilities.
between pyridinium-functionalized nitrones and thiols. However, the development of such rapid SPANC chemistry serves as a promising tool for modifications of other nanomaterial systems in which thiols are not present.

Chapter 4 describes the first example of an azide-modified AuNC system (specifically, the \([\text{Au}_{25}(\text{SR})_{18}]^{-1}\) system) that could undergo post-assembly cluster-surface SPAAC (CS-SPAAC) chemistry with complementary strained-alkynes. The molecular structure of this azide-modified platform (specifically \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-N_3)_{18}]^{-1}\) with \(p\)-azidophenylethanolthiolate as the surface ligand) is reported. Whereas larger AuNP systems tend to be more rigid, the structures and integrity of smaller AuNC systems are more chemically sensitive, and the ability to conduct CS-SPAAC in a nanoorthogonal manner without altering the internal structure represents an exciting new paradigm towards AuNC surface modifications. Chapter 5 explores how the reactivity, structure and physical properties of azide-modified \([\text{Au}_{25}(\text{SR})_{18}]^{-1}\) platforms are affected by changing the regioisomeric form of the azide-modified surface ligands. Two isomeric forms of \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-N_3)_{18}]^{-1}\) were developed: \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-m-\text{C}_6\text{H}_4-N_3)_{18}]^{-1}\) and \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4-N_3)_{18}]^{-1}\). The molecular structures of the neutrally charged forms of these three isomers are reported. It was found that although the physical properties appeared to be largely unaffected, the structure and reactivity of these azide-modified platforms appear to be dependent on the regioisomeric form of the azide-modified surface ligand. Chapter 6 describes the first example of a ferrocene-modified \([\text{Au}_{25}(\text{SR})_{18}]^{-1}\) system, which could be accomplished through a CS-SPAAC reaction between the azide-modified \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-N_3)_{18}]^{-1}\) platform and BCN-terminated ferrocene, which highlights the true power of conducting CS-SPAAC chemistry on the surface of \([\text{Au}_{25}(\text{SR})_{18}]^{-1}\) frameworks to incorporate large, functional substrates.

In total, this work describes and explores innovative methodologies that can be used to conduct chemical modifications of AuNP and AuNC surfaces using SPAAC and SPANC, in an efficient and nanoorthogonal manner without altering the parent structures. Using such versatile and effective strategies, it will be possible to develop functional variants of these popular nanomaterial systems more easily for application-based research.
Keywords

Gold Nanoparticles, Gold Nanoclusters, Nanomaterials, Bioorthogonal Chemistry, Nanoorthogonal Chemistry, Click Chemistry, Interfacial Reactions, Surface-Functionalization, Strained-Alkyne, Azide, Nitrone, Strain-Promoted Alkyne-Azide Cycloaddition, Stain-Promoted Alkyne-Nitrone Cycloaddition, Cluster-Surface Strain-Promoted Alkyne-Azide Cycloaddition
Lay Summary

Gold nanoparticles (AuNPs) and gold nanoclusters (AuNCs) are popular nanomaterial frameworks that are promising candidates for application-based research in nanomedicine, bioimaging and catalysis. Both material frameworks have internal gold-containing cores in the nanometer size regime, which are stabilized by an external monolayer of surface ligands. The key distinction between them is that AuNPs are larger (> 2 nm) and typically polydisperse (i.e. broad size range), while AuNCs are smaller (< 2 nm) and typically monodisperse (i.e. narrow size range). To optimize the practicality of these nanomaterial systems, it is important to modify the chemical composition of their external surfaces with functional substrates that can tailor them for desired applications. Common methodologies that are currently employed to achieve such surface modifications share many mutual drawbacks that limit the ability to effectively modify their surfaces. This is caused by the chemical sensitivity of these nanomaterials and the synthetic challenges in developing functional substrates that can be incorporated onto the AuNP and AuNC surfaces. For these reasons, there is a need to explore alternate strategies to incorporate functionality to the surfaces of AuNPs and AuNCs.

This thesis explores the preparation of AuNPs and AuNCs with surface ligands that can undergo post-assembly surface modifications using the strain-promoted alkyne-azide cycloaddition reaction (SPAAC) and strain-promoted alkyne-nitrone cycloaddition reaction (SPANC). These “bioorthogonal” reactions are largely reserved for tethering functional substrates to biologically sensitive biomolecules in an efficient and selective manner without perturbing the biologically sensitive environments in which they reside and operate in. The goal of this thesis is to demonstrate that the SPAAC and SPANC reactions can also be used as “nanoorthogonal” reactions, which can be used to efficiently modify the surfaces of chemically sensitive AuNPs and AuNCs in a straightforward and robust manner without the limitations of other common surface modification strategies.
Co-Authorship Statement

The work described in this thesis contains contributions from the author, as well as co-authors: Max Weisman, Dr. Wilson Luo, Alex M. Polgar, Julia Martin, Jonathan M. Wong, Prof. Pierangelo Gobbo, Prof. Zhifeng Ding, Prof. John F. Corrigan and Prof. Mark S. Workentin. The contributions of each are described below.

Chapter 1 was written by the author and edited by Prof. Mark S. Workentin.

Chapter 2 describes a series of compounds and AuNPs that were synthesized and characterized, for the most part, by the author. Conceptual designs and experimental strategies were developed by author, Prof. Pierangelo Gobbo and Prof. Mark S. Workentin. Prof. Pierangelo Gobbo (at the time a graduate student), supervised by Prof. Mark S. Workentin, helped to develop synthetic strategies for AuNPs, and conducted TEM measurements of AuNP samples. Max Weisman, supervised by Prof. Mark S. Workentin, helped to synthesize AuNPs. All other experiments were conducted by the author. The chapter was written by author in manuscript format for submission, with input and final edits from Prof. Mark S. Workentin.

Gunawardene, P.N.; Gobbo, P.; Weismann, M.; Workentin, M.S. Towards the Development of Self-Sorting Nanomaterials Through Kinetically Directed Orthogonal Control over Interfacial Surface Chemistry. Manuscript prepared for submission.

Chapter 3 describes a series of nitrone compounds that were synthesized and characterized, for the most part, by the author. Conceptual designs and experimental strategies were developed by author and Prof. Mark S. Workentin. Dr. Wilson Luo (at the time a graduate student), supervised by Prof. Mark S. Workentin, helped to synthesize and characterize some of the nitrone and cycloadduct compounds. Alex M. Polgar, supervised by Prof. John F. Corrigan, carried out DFT analysis. All other experiments were performed by the author. The manuscript was written by the author, with input and final edits from Prof. John F. Corrigan and Prof. Mark S. Workentin.

**Chapter 4** describes an AuNC project in which all synthesis and characterization were completed by the author. Conceptual designs and experimental strategies were developed by author. The manuscript was written by the author, with input and final edits from Prof. John F. Corrigan and Prof. Mark S. Workentin.


**Chapter 5** describes a series of AuNCs and compounds that were synthesized and characterized, for the most part, by the author. Conceptual designs and experimental strategies were developed by author. Julia Martin, supervised by Prof. Mark S. Workentin, was a thesis student who helped to synthesize AuNCs and compounds under author’s supervision. Jonathan M. Wong, supervised by Prof. Zhifeng Ding, conducted electrochemical measurements. All other experiments were performed by the author. The chapter was written by the author in manuscript format for submission, with input and final edits from Prof. John F. Corrigan and Prof. Mark S. Workentin.

Gunawardene, P.N.; Martin, J.; Wong, J.M.; Ding, Z.; Corrigan, J.F.; Workentin, M.S. Controlling the Structure, Properties and Surface Reactivity of Clickable Azide-Functionalized \([\text{Au}_{25}\text{(SR)}_{18}]^{-}\) Nanocluster Platforms Through Regioisomeric Ligand Modifications. *Manuscript prepared for submission.*

**Chapter 6** describes an AuNC project in which the synthesis and characterization were completed, for the most part, by the author. Conceptual designs and experimental strategies were developed by author. Jonathan M. Wong, supervised by Prof. Zhifeng Ding, conducted electrochemical measurements. All other experiments were performed by the author. The chapter was written by the author in manuscript format for submission, with input and final edits from Prof. John F. Corrigan and Prof. Mark S. Workentin.

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Chapter 7 was written by the author and edited by Prof. Mark S. Workentin.
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# Table of Contents

Abstract ....................................................................................................................................................... i

Lay Summary ............................................................................................................................................... iv

Co-Authorship Statement .......................................................................................................................... v

Acknowledgements ....................................................................................................................................... viii

Table of Contents ......................................................................................................................................... x

List of Figures ............................................................................................................................................... xxiv

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>.......................................................................................................................... xxiv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2</td>
<td>.......................................................................................................................... xxvii</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>.......................................................................................................................... xxxii</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>.......................................................................................................................... xxxvii</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>.......................................................................................................................... xli</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>........................................................................................................................... xlv</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>.......................................................................................................................... xlvii</td>
</tr>
</tbody>
</table>

List of Schemes ......................................................................................................................................... xlviii

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>.......................................................................................................................... xlviii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2</td>
<td>.......................................................................................................................... xlix</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>.......................................................................................................................... xlix</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>........................................................................................................................... l</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>........................................................................................................................... l</td>
</tr>
</tbody>
</table>
Chapter 6 ................................................................................................................................. 1

Chapter 7 .................................................................................................................................. 1

List of Tables ............................................................................................................................. lii

Chapter 3 ................................................................................................................................... lii

List of Appendices ...................................................................................................................... liv

List of Abbreviations .................................................................................................................. lv

Chapter 1 ..................................................................................................................................... 1

1 Introduction – Strain-Promoted Click Chemistry for Nanoorthogonal Surface Modifications of Gold Nanoparticles and Gold Nanoclusters ........................................ 1

1.1 “Golden Nanochemistry” ..................................................................................................... 1

1.2 Gold Nanoparticles .............................................................................................................. 2

1.2.1 Structure of Gold Nanoparticles ....................................................................................... 2

1.2.2 Synthesis of Gold Nanoparticles ...................................................................................... 3

1.2.3 Properties of Gold Nanoparticles ..................................................................................... 6

1.2.4 Applications of Gold Nanoparticles ............................................................................... 8

1.3 Gold Nanoclusters ............................................................................................................... 12

1.3.1 Structure of Gold Nanoclusters ....................................................................................... 12

1.3.2 Synthesis of Gold Nanoclusters ....................................................................................... 12

1.3.3 The Au$_{25}$(SR)$_{18}$ Nanocluster Framework ..................................................................... 16

1.3.4 Properties of the Au$_{25}$(SR)$_{18}$ Framework ............................................................... 18
1.3.5 Applications of the Au$_{25}$(SR)$_{18}$ Framework ................................................................. 21

1.4 Surface Modifications of AuNPs and AuNCs ................................................................. 24

1.5 Bioorthogonal Click Chemistry ..................................................................................... 27

1.5.1 General Characteristics of Bioorthogonal Click Reactions ............................... 27

1.5.2 Common Bioorthogonal Click Reactions .............................................................. 30

1.5.3 Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) Reaction ................. 32

1.5.4 Strain-Promoted Alkyne-Nitrone Cycloaddition (SPANC) Reaction ............. 34

1.5.5 Kinetically-Variable SPAAC/SPANC ................................................................. 35

1.6 Scope of Thesis ................................................................................................................. 36

1.7 References ....................................................................................................................... 42

Chapter 2 ................................................................................................................................. 61

2 Towards the Design of Self-Sorting Nanomaterials Through Kinetically Directed
Orthogonal Control over Interfacial Surface Chemistry ..................................................... 61

2.1 Introduction ...................................................................................................................... 61

2.2 Results and Discussion ................................................................................................. 63

2.3 Conclusions ...................................................................................................................... 68

2.4 Acknowledgments ............................................................................................................ 68

2.5 References ....................................................................................................................... 68

2.6 Supporting Information ................................................................................................. 71

2.6.1 General Methods and Methods ............................................................................ 71
2.6.2 Experimental Procedures ................................................................. 72
  2.6.2.1 Synthesis of HS-EG$_3$-Me ......................................................... 72
  2.6.2.2 Synthesis of AuNP-OMe ......................................................... 73
  2.6.2.3 Synthesis of STrityl-EG$_4$-NH$_2$ ............................................ 73
  2.6.2.4 Synthesis of HS-EG$_4$-NH$_2$ ............................................... 73
  2.6.2.5 Synthesis of AuNP-NH$_2$ ...................................................... 74
  2.6.2.6 Synthesis of BCN$_{exo}$-OH .................................................. 74
  2.6.2.7 Synthesis of BCN$_{exo}$-O-pNP .............................................. 75
  2.6.2.8 Synthesis of AuNP-BCN ...................................................... 75
  2.6.2.9 Synthesis of 1-azido-3-propanol (azide 1) ............................. 75
  2.6.2.10 Synthesis of 1-azido-4-nitrobenzene (azide 4) ..................... 76
  2.6.2.11 Synthesis of 4-azidopyridine (azide 5) ............................... 76
  2.6.2.12 Synthesis of 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6) ... 77
  2.6.2.13 Synthesis of N-methyl-C-nitrophenyl-nitrone (nitrone 1) ....... 77
  2.6.2.14 Synthesis of N-phenylhydroxylamine .................................. 78
  2.6.2.15 Synthesis of N-phenyl-C-methoxyphenyl-nitrone (nitrone 2) ... 78
  2.6.2.16 Synthesis of N-phenyl-C-phenyl-nitrone (nitrone 3) .............. 79
  2.6.2.17 Synthesis of N-phenyl-C-nitrophenyl-nitrone (nitrone 4) ....... 79
  2.6.2.18 Synthesis of N-phenyl-C-pyridine-nitrone (nitrone 5) .......... 79
  2.6.2.19 Synthesis of N-cyanophenyl-C-pyridine-nitrone (nitrone 6) ... 80

2.6.3 Experimental Spectra and Diagrams ................................................ 81
  2.6.3.1 Experimental Spectra for HS-EG$_3$-Me .................................. 81
  2.6.3.2 Experimental Spectra for AuNP-OMe ...................................... 82
  2.6.3.3 Experimental Spectra for HS-EG$_4$-NH$_2$ .............................. 83
  2.6.3.4 Experimental Spectra for AuNP-NH$_2$ .................................... 84
  2.6.3.5 Experimental Spectra for BCN$_{exo}$-OH .................................. 86
  2.6.3.6 Experimental Spectra for BCN$_{exo}$-O-pNP .............................. 86
  2.6.3.7 Experimental Spectra for AuNP-BCN ...................................... 87
  2.6.3.8 Experimental Spectra for 1-azido-3-propanol (azide 1) ............ 89
  2.6.3.9 Experimental Spectra for 1-azido-4-nitrobenzene (azide 4) ....... 90
  2.6.3.10 Experimental Spectra for 4-azidopyridine (azide 5) ............... 91
  2.6.3.11 Experimental Spectra for 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6) .................. 92
  2.6.3.12 Experimental Spectra for N-methyl-C-nitrophenyl-nitrone (nitrone 1) ........ 93
2.6.3.13 Experimental Spectra for N-phenyl-C-methoxyphenyl-nitrone (nitrone 2).............. 94
2.6.3.14 Experimental Spectra for N-phenyl-C-phenyl-nitrone (nitrone 3)....................... 95
2.6.3.15 Experimental Spectra for N-phenyl-C-nitrophenyl-nitrone (nitrone 4) .............. 96
2.6.3.16 Experimental Spectra for N-phenyl-C-pyridine-nitrone (nitrone 5) ................. 97
2.6.3.17 Experimental Spectra for N-cyanophenyl-C-pyridine-nitrone (nitrone 6) ...... 98

2.6.4 Thermogravimetric Analysis of AuNP-BCN .................................................................. 99
2.6.4.1 General Experimental Details .................................................................................. 99
2.6.4.2 Experimental Spectra for TGA Analysis ................................................................. 99

2.6.5 Kinetic Measurements ................................................................................................. 101
2.6.5.1 General Experimental Details ................................................................................ 101
2.6.5.2 Kinetic Measurements for 1-azido-3-propanol (azide 1) ........................................ 102
2.6.5.3 Kinetic Measurements for 4-azidoanisole (azide 2) ................................................. 103
2.6.5.4 Kinetic Measurements for azidobenzene (azide 3) ................................................ 104
2.6.5.5 Kinetic Measurements for 1-azido-4-nitrobenzene (azide 4) ............................... 105
2.6.5.6 Kinetic Measurements for 4-azidopyridine (azide 5) ............................................. 106
2.6.5.7 Kinetic Measurements for 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6) .... 107
2.6.5.8 Kinetic Measurements for N-methyl-C-nitrophenyl-nitrone (nitrone 1) ........... 108
2.6.5.9 Kinetic Measurements for N-phenyl-C-methoxyphenyl-nitrone (nitrone 2) .... 109
2.6.5.10 Kinetic Measurements for N-phenyl-C-phenyl-nitrone (nitrone 3) ................. 110
2.6.5.11 Kinetic Measurements for N-phenyl-C-nitrophenyl-nitrone (nitrone 4) ............ 111
2.6.5.12 Kinetic Measurements for N-phenyl-C-pyridine-nitrone (nitrone 5) ............... 112
2.6.5.13 Kinetic Measurements for N-cyanophenyl-C-pyridine-nitrone (nitrone 6) ..... 113

2.6.6 Competition Experiments for AuNP-BCN ................................................................ 114
2.6.6.1 General Experimental Details ............................................................................... 114
2.6.6.2 1H NMR Spectra for Competition Experiment between azide 1 and nitrone 6 .... 115
2.6.6.3 1H NMR Spectra for Competition Experiment between azide 3 and nitrone 4 .... 116
2.6.6.4 1H NMR Spectra for Competition Experiment between azide 4 and nitrone 4 .... 117
2.6.6.5 1H NMR Spectra for Competition Experiment between azide 5 and nitrone 2 .... 118
2.6.6.6 1H NMR Spectra for Competition Experiment between azide 6 and nitrone 1 .... 119

2.6.7. References for Supporting Information ..................................................................... 120

Chapter 3 ............................................................................................................................ 121
3 Highly Electron-Deficient Pyridinium-Nitrones for Rapid and Tunable Inverse-Electron-Demand Strain-Promoted Alkyne-Nitrone Cycloaddition to Bicyclo[6.1.0]nonyne ............ 121

3.1 Introduction ................................................................................................................................................................................................................................. 121

3.2 Results and Discussion .............................................................................................................................................................................................................. 123

3.2.1 Towards the Development of Pyridinium-Functionalized Gold Nanoparticles ...... 128

3.3 Conclusion ........................................................................................................................................................................................................................................... 131

3.4 Acknowledgements ....................................................................................................................................................................................................... 131

3.5 References ................................................................................................................................................................................................................................. 132

3.6 Supporting Information ........................................................................................................................................................................................................... 134

3.6.1 General Materials and Methods .................................................................................................................................................................................. 134

3.6.2 Synthesis of Nitrones .............................................................................................................................................................................................................. 136

3.6.2.1 Synthesis of N-(4-methoxyphenyl)-C-(3-pyridine) Nitrone (2a) .......................................................... 136
3.6.2.2 Synthesis of N-(4-methoxyphenyl)-C-(3-methylpyridinium) Nitrone (3a) .................................................. 136
3.6.2.3 Synthesis of N-phenyl-C-(3-pyridine) Nitrone (2b) .............................................................................. 137
3.6.2.4 Synthesis of N-phenyl-C-(3-methylpyridinium) Nitrone (3b) .......................................................... 138
3.6.2.5 Synthesis of N-(4-cyanophenyl)-C-(3-pyridine) Nitrone (2c) .......................................................... 138
3.6.2.6 Synthesis of N-(4-cyanophenyl)-C-(3-methylpyridinium) Nitrone (3c) ............................................. 139
3.6.2.7 Synthesis of N-phenyl-C-phenyl Nitrone (4) ....................................................................................... 139

3.6.3 Synthesis of Cyclooctynes .................................................................................................................................................................................................... 140

3.6.3.1 Synthesis of BCN-OHexo (5) ......................................................................................................................... 140
3.6.3.2 Synthesis of (Z)-5,6-dibromocyclooct-1-ene (6a) ............................................................................... 140
3.6.3.3 Synthesis of (Z)-cyclooct-1-ene-5-yne (6b) ......................................................................................... 141

3.6.4 Synthesis of TEG Ligands ................................................................................................................................................................................................... 141

3.6.4.1 Synthesis of Nitrobenzyl-TEG-OH (A) ................................................................................................. 141
3.6.4.2 Synthesis of Nitrobenzyl-TEG-OTs (B) ............................................................................................ 142
3.6.4.3 Synthesis of Nitrobenzyl-TEG-Thioacetate (C) ................................................................................. 142
3.6.4.4 Synthesis of Synthesis of (C-Pyridinium, N-Phenyl-TEG-Thioacetate)-Nitrone (E)...... 143

3.6.5 Molecular Structures Of Nitrones ............................................................................. 144

3.6.6 General Synthesis of Cycloadducts ......................................................................... 147

3.6.6.1 Synthesis of Cycloadduct between BCN\textsubscript{exo}-OH (5) with Nitrones 3a, 3b, 3c.............. 147
3.6.6.2 Synthesis of Cycloadduct between BCN\textsubscript{exo}-OH with Nitrone 4 and 2b....................... 148
3.6.6.3 Synthesis of Cycloadduct between DBCO-amine with Nitrone 3c ......................... 148
3.6.6.4 Synthesis of Cycloadduct between (Z)-cyclooct-1-ene-5-yne (6b) with Nitrone 3c....... 148

3.6.7 NMR Spectra of Nitrones ....................................................................................... 149

3.6.7.1 Experimental Spectra for N-(4-methoxyphenyl)-C-(3-pyridine) nitrone (Nitrone 2a) .... 149
3.6.7.2 Experimental Spectra for N-(4-methoxyphenyl)-C-(3-methylpyridinium) nitrone (Nitrone 3a) ........................................................................................................ 150
3.6.7.3 Experimental Spectra for N-phenyl-C-(3-pyridine) nitrone (Nitrone 2b)...................... 151
3.6.7.4 Experimental Spectra for N-phenyl-C-(3-methylpyridinium) nitrone (Nitrone 3b) ......... 151
3.6.7.5 Experimental Spectra for N-(4-cyanophenyl)-C-(3-pyridine) nitrone (Nitrone 2c)......... 152
3.6.7.6 Experimental Spectra for N-(4-cyanophenyl)-C-(3-methylpyridinium) nitrone (Nitrone 3c) ........................................................................................................ 152
3.6.7.7 Experimental Spectra for N-phenyl-C-phenyl nitrone (Nitrone 4) ......................... 153

3.6.8 NMR Spectra of TEG Ligands ................................................................................. 154

3.6.8.1 Experimental Spectra for Nitrobenzyl-TEG-OH (A) .................................................. 154
3.6.8.2 Experimental Spectra for Nitrobenzyl-TEG-OTs (B) .................................................. 155
3.6.8.3 Experimental Spectra for Nitrobenzyl-TEG-Thioacetate (C) ................................. 156
3.6.8.4 Experimental Spectra for (C-Pyridinium, N-Phenyl-TEG-Thioacetate)-Nitrone (E) ...... 157

3.6.9 NMR Spectra of Cyclooctynes ............................................................................... 158

3.6.9.1 Experimental Spectra for BCN\textsubscript{exo}-OH (5) .......................................................... 158
3.6.9.2 Experimental Spectra for (Z)-5,6-dibromocyclooct-1-ene (6a) ............................ 159
3.6.9.3 Experimental Spectra for (Z)-cyclooct-1-ene-5-yne (6b) ........................................... 160

3.6.10 NMR Spectra of Cycloadducts between BCN\textsubscript{exo}-OH (5) and Nitrones 4, 2b, 3a, 3b and 3c ........................................................................................................ 160

3.6.11 $^1$H NMR Spectra of Cycloadducts between BCN\textsubscript{exo}-OH (5) and Nitrone 3c in Other Solvents ........................................................................................................ 166
3.6.12 NMR Spectra of Cycloadduct between DBCO-amine and Nitrone 3c ......................... 168
3.6.13 NMR Spectra of Cycloadduct between (Z)-cyclooct-1-ene-5-yne (6b) and Nitrone 3c .................................................................................................................. 169
3.6.14 Stability of Pyridinium-Nitrones 3a, 3b and 3c ......................................................... 170
3.6.15 Kinetic Measurements .............................................................................................. 175
  3.6.15.1 Kinetic Measurements of Cycloaddition Reaction between (4) and BCNexo-OH (5) ..... 176
  3.6.15.2 Kinetic Measurements of Cycloaddition Reaction between (2b) and BCNexo-OH (5) ... 177
  3.6.15.3 Kinetic Measurements of Cycloaddition Reaction between (3a) and BCNexo-OH (5).... 178
  3.6.15.4 Kinetic Measurements of Cycloaddition Reaction between (3b) and BCNexo-OH (5) ... 179
  3.6.15.5 Kinetic Measurements of Cycloaddition Reaction between (3c) and BCNexo-OH (5).... 180
3.6.16 Crystallographic Information ................................................................................. 181
  3.6.16.1 Data Collection and Processing ............................................................................ 181
  3.6.16.2 Structure Solution and Refinement ....................................................................... 182
  3.6.14.3 Summary of Crystallographic Data ..................................................................... 184
3.6.17 Computational Information .................................................................................... 187
  3.6.17.1 Computational Methods ...................................................................................... 187
  3.6.17.2 Optimized Structural Coordinates ...................................................................... 187
  3.6.17.3 Comparison of DFT versus XRD ....................................................................... 192
3.6.18 References – Supporting Information .................................................................... 193

Chapter 4 .......................................................................................................................... 194

4 A Clickable Azide-Functionalized [Au25(SR)18]+ Nanocluster platform for Interfacial Surface Modifications .............................................................................................................. 194

  4.1 Introduction ................................................................................................................ 194
  4.2 Results and Discussion .............................................................................................. 196
  4.3 Conclusions ................................................................................................................. 201
4.4 Acknowledgements ........................................................................................................ 202

4.5 References .................................................................................................................... 202

4.6 Supporting Information ................................................................................................. 205

4.6.1 General Materials and Methods ................................................................................ 205

4.6.2 Experimental Procedures ........................................................................................... 207

4.6.2.1 Synthesis of p-nitro-phenylethanethioacetate ....................................................... 207
4.6.2.2 Synthesis of p-ammonium-phenylethanethioacetate chloride ......................... 207
4.6.2.3 Synthesis of p-azido-phenylethanethioacetate .................................................. 208
4.6.2.4 Synthesis of p-azido-phenylethanethiol .............................................................. 209
4.6.2.5 Synthesis of (Z)-5,6-dibromocyclooct-1-ene ..................................................... 209
4.6.2.6 Synthesis of (Z)-cyclooct-1-ene-5-yne ............................................................... 209
4.6.2.7 Synthesis of bicyclo[6.1.0]nonyne (BCNexo-OH) ............................................. 210
4.6.2.8 Synthesis of [(CH3-(CH2)r)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido) ......... 210
4.6.2.9 Synthesis of [(CH3-(CH2)r)4N][Au25(SCH2CH2-p-C6H4-C10H10N3)18] (4.1-triazole) 211

4.6.3 Experimental Spectra and Diagrams ........................................................................... 213

4.6.3.1 Experimental Spectra and Diagrams for [Au25(SCH2CH2-p-C6H4-N3)18][(CH3-(CH2)r)4N] (4.1-azido) ............................................................... 213
4.6.3.2 Experimental Spectra for [(CH3-(CH2)r)4N][Au25(SCH2CH2-p-C6H4-C10H10N3)18] (4.1-triazole) ................................................................. 217
4.6.3.3 Experimental Spectra for [(CH3-(CH2)r)4N][Au25(SCH2CH2-p-C6H4-C10H14N3O)18] .

Reaction of 4.1-azido with BCNexo-OH ........................................................................ 220
4.6.3.4 Experimental Spectra for p-nitro-phenylethanethioacetate ................................ 223
4.6.3.5 Experimental Spectra for p-ammonium-phenylethanethioacetate chloride ........ 225
4.6.3.6 Experimental Spectra for p-azido-phenylethanethioacetate ................................ 227
4.6.3.7 Experimental Spectra for p-azido-phenylethanethiol ........................................... 229
4.6.3.8 Experimental Spectra for (Z)-5,6-dibromocyclooct-1-ene .................................. 231
4.6.3.9 Experimental Spectra for (Z)-cyclooct-1-ene-5-yne ........................................... 232

4.6.4 Crystallographic Information ..................................................................................... 233

4.6.4.1 Data Collection and Processing ............................................................................ 233
4.6.4.2 Structure Solution and Refinement ....................................................................... 233
4.6.4.3 Summary of Crystal Data ................................................................................. 234

4.6.5 References – Supporting Information ............................................................... 236

Chapter 5 ...................................................................................................................... 237

5 Expanding the Library of Clickable Azide-Functionalized Au$_{25}$SR$_{18}$ Nanocluster Platforms: A study of Ligand Modifications on Structure, Properties and Surface Reactivity .................................................................................................................. 237

5.1 Introduction ............................................................................................................. 237

5.2 Results and Discussion .......................................................................................... 241

5.3 Conclusions ............................................................................................................ 250

5.4 Acknowledgements ............................................................................................... 251

5.5 References ............................................................................................................ 251

5.6 Supporting Information ......................................................................................... 256

5.6.1 General Materials and Methods ....................................................................... 256

5.6.2 Experimental Procedures .................................................................................. 258

5.6.2.1 Synthesis of $p$-nitro-phenylethanethioacetate .............................................. 258

5.6.2.2 Synthesis of $p$-azido-phenylethanethioacetate ............................................. 259

5.6.2.3 Synthesis of $p$-azido-phenylethanethiol ....................................................... 259

5.6.2.4 Synthesis of $m$-nitro-phenylethanethioacetate ........................................... 260

5.6.2.5 Synthesis of $m$-azido-phenylethanethioacetate .......................................... 260

5.6.2.6 Synthesis of $m$-azido-phenylethanethiol ...................................................... 261

5.6.2.7 Synthesis of $o$-nitro-phenylethanethioacetate ............................................ 261

5.6.2.8 Synthesis of $o$-azido-phenylethanethioacetate ............................................ 262

5.6.2.9 Synthesis of $o$-azido-phenylethanethiol ...................................................... 262

5.6.2.10 Synthesis of $(Z)$-cyclooct-1-ene-5-yne ....................................................... 263

5.6.2.11 Synthesis of BCN$_{ex}$-$\text{OH}$ ................................................................... 263

5.6.2.12 Synthesis of [(CH$_3$-(CH$_2$)$_3$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$N_3$)$_{18}$] ($p$-azido$^+$)............ 264
5.6.3 Experimental Spectra and Diagrams .......................................................... 269

5.6.3.1 Experimental Spectra and Diagrams for [(CH$_3$-CH$_2$)$_3$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^1$) ........................................ 269

5.6.3.2 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-N$_3$)$_{18}$] (m-azido$^1$) ........................................ 269

5.6.3.3 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-o-C$_6$H$_4$-N$_3$)$_{18}$] (o-azido$^1$) ........................................ 271

5.6.3.4 Experimental Diagrams for [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^0$) ................................................................. 273

5.6.3.5 Experimental Diagrams for [Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-N$_3$)$_{18}$] (m-azido$^0$) ................................................................. 273

5.6.3.6 Experimental Diagrams for [Au$_{25}$(SCH$_2$CH$_2$-o-C$_6$H$_4$-N$_3$)$_{18}$] (o-azido$^0$) ................................................................. 274

5.6.3.7 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_6$H$_{10}$N$_3$)$_{18}$] (p-triazole$^1$) ................................................................. 275

5.6.3.8 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-C$_6$H$_{10}$N$_3$)$_{18}$] (m-triazole$^1$) ................................................................. 275

5.6.3.9 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-o-C$_6$H$_4$-C$_6$H$_{10}$N$_3$)$_{18}$] (o-triazole$^1$) ................................................................. 277

5.6.3.10 Experimental Spectra for p-nitro-phenylethanethioacetate ................................................................. 279

5.6.3.11 Experimental Spectra for p-azido-phenylethanethioacetate ................................................................. 279

5.6.3.12 Experimental Spectra for p-azido-phenylethanethiol ................................................................. 279

5.6.3.13 Experimental Spectra for m-nitro-phenylethanethioacetate ................................................................. 280

5.6.3.14 Experimental Spectra for m-azido-phenylethanethioacetate ................................................................. 282

5.6.3.15 Experimental Spectra for m-azido-phenylethanethiol ................................................................. 284

5.6.3.16 Experimental Spectra for o-nitro-phenylethanethioacetate ................................................................. 286

5.6.3.17 Experimental Spectra for o-azido-phenylethanethioacetate ................................................................. 288

5.6.3.18 Experimental Spectra for o-azido-phenylethanethiol ................................................................. 290

5.6.4 Electrochemical Graphs .................................................................................. 292

5.6.4.1 Cyclic Voltammetry (CV) Graph of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^1$) ................................................................. 292

5.6.4.2 Cyclic Voltammetry (CV) Graph of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-N$_3$)$_{18}$] (m-azido$^1$) ................................................................. 293
Cluster 6 Expanding the Frontiers of Ultrasmall Gold Nanocluster Surface Composition through
Chapter 6
6.1 Introduction .................................................................................................................. 311
6.2 Results and Discussion ............................................................................................... 313
6.3 Conclusions .................................................................................................................. 318
6.4 Acknowledgements ...................................................................................................... 319
6.5 References .................................................................................................................... 319
6.6 Supporting Information.................................................................................................................. 322

6.6.1 General Methods and Reagents .................................................................................................. 322

6.6.2 Experimental Procedures ........................................................................................................... 325

6.6.2.1 Synthesis of p-nitro-phenylethanethioacetate ........................................................................... 325
6.6.2.2 Synthesis of p-azido-phenylethanethioacetate .......................................................................... 325
6.6.2.3 Synthesis of p-azido-phenylethanol ................................................................. 325
6.6.2.4 Synthesis of [(CH3-(CH2)2)4N][Au25(SCH2CH2-p-C6H4-C8H10N3)18] (6.1-azido) .................. 326
6.6.2.5 Synthesis of BCNexo-OH ...................................................................................................... 326
6.6.2.6 Synthesis of Ferrocene-BCNexo ............................................................................................ 326
6.6.2.7 Synthesis of Ferrocene-Triazole-Thioacetate ............................................................................ 327
6.6.2.8 Synthesis of [(CH3-(CH2)2)4N][Au25(SCH2CH2-p-C6H4-C21H22FeN5O2)18] (6.1-ferrocenyl) ........................................................................................................................................... 327

6.6.3 Experimental Spectra and Diagrams ......................................................................................... 329

6.6.3.1 Experimental Spectra for [(CH3-(CH2)2)4N][Au25(SCH2CH2-p-C6H4-C8H10N3)18] (6.1-azido) .................................................................................................................. 329
6.6.3.2 Experimental Spectra for [(CH3-(CH2)2)4N][Au25(SCH2CH2-p-C6H4-C21H22FeN5O2)18] (6.1-ferrocenyl) .................................................................................................................. 329
6.6.3.3 Experimental Spectra for Ferrocene-BCNexo .......................................................................... 331
6.6.3.4 Experimental Spectra of Ferrocene-Triazole-Thioacetate ...................................................... 333

6.6.4 Electrochemical Graphs .............................................................................................................. 334

6.6.4.1 Cyclic Voltammogram (CV) of [(CH3-(CH2)2)4N][Au25(SCH2CH2-p-C6H4-C8H10N3)18] (1-azido) .................................................................................................................. 334
6.6.4.2 Cyclic Voltammogram (CV) of [(CH3-(CH2)2)4N][Au25(SCH2CH2-p-C6H4-C21H22FeN5O2)18] (6.1-ferrocenyl) .................................................................................................................. 334
6.6.4.3 Cyclic Voltammogram (CV) of p-azido-phenylethanethioacetate .............................................. 335
6.6.4.4 Differential Pulse Voltammogram (DPV) of p-azido-phenylethanethioacetate ............................................ 335
6.6.4.5 Cyclic Voltammogram (CV) of Ferrocene-Triazole-Thioacetate .............................................. 336
6.6.4.6 Differential Pulse Voltammogram (DPV) of Ferrocene-Triazole-Thioacetate ............................................ 336

6.6.5 References – Supporting Information....................................................................................... 337

Chapter 7 .............................................................................................................................................. 338

7 Contributions and Future Perspectives ............................................................................................ 338
7.1 Contributions ........................................................................................................................................ 338

7.2 Future Perspectives ................................................................................................................................ 345

7.2.1 General Comments ................................................................................................................................. 345

7.2.2 Future Perspectives for AuNPs ............................................................................................................... 347

7.2.3 Future Perspectives for AuNCs .............................................................................................................. 348

7.3 References .................................................................................................................................................. 350

Appendices ....................................................................................................................................................... 352

A.1 Permission to Reproduce Copyrighted Material ...................................................................................... 352

A.2 Curriculum Vitae ...................................................................................................................................... 370

xxiii
List of Figures

Chapter 1

Figure 1.1. (a) TEM/SEM images of gold nanoparticles (AuNPs) with different morphologies. Top left. Spherical AuNPs (figure reproduced with permission from Ref [12].) Top Right. Rod-shaped AuNPs (Figure reproduced with permission from Ref [13]). Bottom Left. Prism-shaped AuNPs (Figure reproduced with permission from Ref [14]). Bottom Right. Octahedral AuNPs (Figure reproduced with permission from Ref [15]). (b) Solutions of rod-shaped AuNPs having different sizes. Figure reproduced with permission of Ref [16]……………………………………………...3

Figure 1.2. (a) Optical absorption spectra of 9, 22, 48 and 99 nm spherical AuNPs. The broad peaks correspond to the SPR of each system. Figure reproduced with permission from Ref. [33]. (b) Aggregation-induced shift in SPR wavelength as a function of particle center-center spacing for 72 and 84 nm spherical AuNPs. Figure reproduced with permission from Ref. [37]…………..6

Figure 1.3. (a) Uptake of AuNPs into mammalian cells (A) Number of AuNPs per vesicle diameter versus nanoparticle size (B-F) TEM images of AuNPs with sizes 14, 30, 50, 74 and 100 nm trapped inside vesicles of a Hela cell, respectively. Figure reproduced with permission from Ref. [43] (b) Confocal cell images showing fluorescein-labelled transferrin AuNPs (AuNP-TF) internalized by NPC cells (A) NPC cells without AuNP-TF (B) NPC cells treated with AuNPs without surface transferrin (C) NPC cells treated with AuNP-TF (D) NPC cells treated with albumin-coated AuNPs (E and F) NPC cells co-treated with different proportions of AuNP-TF and albumin-coated AuNPs (1:2 and 1:5, respectively). Figure reproduced with permission from Ref. [45]…………………..8

Figure 1.4. (a) Left. Colorimetric sensing of lead ions, whereby lead adsorption causes dismantling of ([15]-crown-5)-functionalized AuNP aggregates (A) into an AuNP dispersion (B). Right. UV/Vis spectra of A and B (spectra figure reproduced with permission from Ref. [48]). (b) Left. Colorimetric sensing of mercury ions, whereby mercury adsorption caused dispersion of carboxyl-terminated AuNPs (A) to from AuNP aggregates (B). Right. UV/Vis spectra of A and B (spectra figure reproduced with permission from Ref. [49])……………………………………………………………9
Figure 1.5. (a) Schematic representation of doxorubicin (DOX) adsorption onto AuNP surface and intracellular delivery. (b,c) Fluorescence microscopy images of MCF-7 tumor tissues after injection of DOX alone (b) and AuNP-DOX (c). Red = DOX; green = stained vasculature (lectin-FITC). Scale bar, 50 μm. (d) Normalized tumor growth curves after injection with AuNP-DOX (red), DOX alone (black) and phosphate buffered-saline (PBS) (blue). Figures in (b), (c) and (d) reproduced with permission from Ref. [53]………………………………………………………10

Figure 1.6. (a) Schematic representation of development of ‘nano-flare AuNP’ and in vivo release of fluorophore-conjugated reporter sequence after survivin mRNA binding. (b) Differential contrast and fluorescence imaging of survivin-expressing SKBR3 cells treated with survivin nano-flares (left) and non-complementary nano-flares (right). (c) Analogously treated non-survivin expressing C166 cells. Scale bar is 20 μm. Figure in (b) and (c) reproduced with permission from Ref. [56]………………………………………………………………………………………….11

Figure 1.7. Some thiolated AuNC frameworks.

Top (left to right). The Au20(SR)16 framework (where R = C₆H₄-C(CH₃)₃) (figure produced from data with permission from Ref. [59]. The Au23(SR)16 framework (where R = C₆C₁₁) (figure produced from data with permission from Ref. [60]). The Au24(SR)20 framework (where R = CH₂-C₆H₄-C(CH₃)₃) (figure produced from data with permission from Ref. [61]).

Middle (left to right). The Au25(SR)₁₈ framework (where R = CH₂CH₂-C₆H₅) (figure produced from data with permission from Ref. [62]). The Au2₈(SR)₂₀ framework (where R = C₆H₄-C(CH₃)₃) (figure produced from data with permission from Ref. [63]).

Bottom (left to right). The Au₃₈(SR)₂₄ framework (where R = CH₂CH₂-C₆H₅) (figure produced from data with permission from Ref. [64]). The Au₄₄(SR)₂₈ framework (where R = CH₂CH₂-C₆H₅) (figure produced from data with permission from Ref. [65])……………………………………………………………13

Figure 1.8. (a) Molecular structure of [TOA][Au₂₅(CH₂CH₂Ph)₁₈], where TOA = tetraoctylammonium (not shown). (b) Staple motifs, Au₁₃ kernel, and inner/outer ligands in molecular structure of [Au₂₅(CH₂CH₂Ph)₁₈]⁻¹. Figure produced from data with permission from Ref. [62]………………………………………………………………………………………….18
Figure 1.9. (a) Optical absorption spectra of [TOA][Au$_{25}$(CH$_2$CH$_2$Ph)$_{18}$], where TOA = Tetraoctylammonium (black), Au$_{25}$(CH$_2$CH$_2$Ph)$_{18}$ (red) and [Au$_{25}$(CH$_2$CH$_2$Ph)$_{18}$][C$_6$F$_5$CO$_2$] (blue). Figure reproduced with permission from Ref. [88]. (b) Optical absorption spectrum of [TOA][Au$_{25}$(CH$_2$CH$_2$Ph)$_{18}$] showing principal transitions. Figure reproduced with permission from Ref. [62].

Figure 1.10. (a) Photoluminescence (PL) spectra of [Au$_{25}$(SR)$_{18}$]$^{-1}$ functionalized with phenylethanethiolate (black), dodecanethiolate (red) and hexanethiolate (blue). (b) PL spectra of [Au$_{25}$(SR)$_{18}$]$^{-1}$ (black), [Au$_{25}$(SR)$_{18}$]$_0$ (red), [Au$_{25}$(SR)$_{18}$]$^{+1}$ (blue) and [Au$_{25}$(SR)$_{18}$]$^{+2}$ (blue-green). Figures reproduced with permission from Ref. [81].

Figure 1.11. Differential pulse voltammogram (DPV) of [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{-1}$ at 0.02 V/s in 0.1 M Bu$_4$NPF$_6$ in degassed CH$_2$Cl$_2$ at 0.4 mm diameter Pt working electrode, with Ag wire quasi-reference (AgQRE) and Pt wire counter electrode. * indicates wave for incompletely removed O$_2$. DPV reproduced with permission from Ref. [94].

Figure 1.12. Left. Schematic representation of Hg$^{2+}$ sensing based on fluorescence quenching upon Hg$^{2+}$ binding to Au$_{25}$-BSA. Right. Photoemission spectra ($\lambda_{ex} = 470$ nm) of Au$_{25}$-BSA before Hg$^{2+}$ binding (1) and after Hg$^{2+}$ binding (2). Spectra reproduced with permission from Ref. [97].

Figure 1.13. (a) Catalytic activity of [Au$_{25}$(SR)$_{18}$]$^{-1}$ thermally deposited onto CeO$_2$ support, using different capping ligands, for Ullman heterocoupling between 4-methyl-iodobenzene and 4-nitroiodobenzene. Synthetic data taken from Ref. [102]. (b) Catalytic activity of [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{-1}$ thermally deposited onto TiO$_2$ support, for semi-hydrogenation of terminal alkynes. Synthetic data taken from Ref. [103].

Figure 1.14. Left. Schematic representation of DOX delivery by Au$_{25}$(SR)$_{18}$]$^{-1}$-DOX nanoconjugate. Right. Confocal images of A549 cells after incubation with Au$_{25}$(SR)$_{18}$]$^{-1}$-DOX nanoconjugate after 1 hour (top right) and 3 hours (bottom right). Red = doxorubicin. Confocal images reproduced with permission of Ref. [104].

Figure 1.15. Some commonly used cyclooctynes, with associated second-order rate constants for reaction with benzyl azide. Second-order rate constants taken from references [149] and [150].
Figure 1.1. Top. Kinetically variable SPAAC and SPANC through structural modifications to the azide and nitrone moieties, respectively. Bottom. Structures of azides and nitrones and their associated second-order rate constants.........................................................37

Chapter 2

Figure 2.1. (a) Schematic representation of host/guest directed- and kinetically-directed self-assembly strategies. (b) The strain-promoted alkyne-azide cycloaddition reaction (SPAAC) to form a triazole cycloadduct (right), and the strain-promoted alkyne-nitrone cycloaddition reaction (SPANC) to form an isoxazoline cycloadduct (left). (c) Schematic representation of kinetically-directed self-assembly strategy using SPAAC and SPANC.........................................................63

Figure 2.2. Estimated SPAAC/SPANC and I-SPAAC/I-SPANC reaction rates of azides and nitrones with free BCN and AuNP-BCN, respectively. All rate constants were determined under second order conditions in CD₂Cl₂ at 25°C in duplicate trials using ¹H NMR spectroscopy, and monitored over pre-determined time intervals to determine a second order rate constant. The k₂(rel) value indicates the rate of each k₂ relative to the slowest azide (azide 1).................................66

Figure 2.3. Kinetically-directed competitive reactivity of AuNP-BCN, where one equivalent of AuNP-BCN was reacted with one equivalent of nitrone and one equivalent of azide, and the amount of cycloadduct that was formed was determined using ¹H NMR spectroscopy.............67

Figure S2.1. ¹H NMR spectrum of HS-EG₃-Me in CDCl₃ at 25°C. * denotes residual protio solvent..............................................................................................................................81

Figure S2.2. ¹³C{¹H} NMR spectrum of HS-EG₃-Me in CDCl₃ at 25°C. * indicates CDCl₃ solvent..............................................................................................................................................................81

Figure S2.3. ¹H NMR spectrum of AuNP-OMe in CDCl₃ at 25°C. * denotes residual protio solvent..............................................................................................................................................................82

Figure S2.4. ¹H NMR spectrum of HS-EG₄-NH₂ in CDCl₃ at 25°C. * denotes residual protio solvent..............................................................................................................................................................83
Figure S2.5. $^{13}$C{$^1$H} NMR spectrum of HS-EG$_4$-NH$_2$ in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent

Figure S2.6. $^1$H NMR spectrum of AuNP-NH$_2$ in D$_2$O at 25°C. * denotes residual protio solvent

Figure S2.7. TEM image for AuNP-NH$_2$

Figure S2.8. (a) High-resolution carbon 1s XPS spectrum of AuNP-NH$_2$ (b) High-resolution oxygen 1s XPS spectrum of AuNP-NH$_2$

Figure S2.9. (a) XPS survey scan of AuNP-NH$_2$

Figure S2.10. $^1$H NMR spectrum of BCN$_{exo}$-OH in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S2.11. $^1$H NMR spectrum of BCN$_{exo}$-O-pNP in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S2.12. $^1$H NMR spectrum of AuNP-BCN in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S2.13. TEM image for AuNP-BCN

Figure S2.14. (a) High-resolution carbon 1s XPS spectrum of AuNP-BCN (b) High-resolution oxygen 1s XPS spectrum of AuNP-BCN

Figure S2.15. (a) XPS survey scan of AuNP-BCN

Figure S2.16. $^1$H NMR spectrum of azide 1 in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S2.17. $^{13}$C{$^1$H} NMR spectrum of azide 1 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent

Figure S2.18. $^1$H NMR spectrum of azide 4 in CDCl$_3$ at 25°C. * denotes residual protio solvent
Figure S2.19. $^{13}\text{C}\{1\text{H}\}$ NMR spectrum of azide 4 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent..........................................................................................................................90

Figure S2.20. $^1\text{H}$ NMR spectrum of azide 5 in CDCl$_3$ at 25°C. * denotes residual protio solvent........................................................................................................................................91

Figure S2.21. $^{13}\text{C}\{1\text{H}\}$ NMR spectrum of azide 5 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent........................................................................................................................................91

Figure S2.22. $^{19}\text{F}$ NMR spectrum of azide 6 in CDCl$_3$ at 25°C..................................................92

Figure S2.23. $^{13}\text{C}\{1\text{H}\}$ NMR spectrum of azide 6 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent........................................................................................................................................92

Figure S2.24. $^1\text{H}$ NMR spectrum of nitrone 1 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent........................................................................................................................................93

Figure S2.25. $^{13}\text{C}\{1\text{H}\}$ NMR spectrum of nitrone 1 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent........................................................................................................................................93

Figure S2.26. $^1\text{H}$ NMR spectrum of nitrone 2 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent........................................................................................................................................94

Figure S2.27. $^{13}\text{C}\{1\text{H}\}$ NMR spectrum of nitrone 2 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent........................................................................................................................................94

Figure S2.28. $^1\text{H}$ NMR spectrum of nitrone 3 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent........................................................................................................................................95

Figure S2.29. $^{13}\text{C}\{1\text{H}\}$ NMR spectrum of nitrone 3 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent........................................................................................................................................95

Figure S2.30. $^1\text{H}$ NMR spectrum of nitrone 4 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent........................................................................................................................................96
Figure S2.31. $^{13}\text{C}(1\text{H})$ NMR spectrum of nitrone 4 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.

Figure S2.32. $^1\text{H}$ NMR spectrum of nitrone 5 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

Figure S2.33. $^{13}\text{C}(1\text{H})$ NMR spectrum of nitrone 5 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.

Figure S2.34. $^1\text{H}$ NMR spectrum of nitrone 6 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

Figure S2.35. $^{13}\text{C}(1\text{H})$ NMR spectrum of nitrone 6 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.

Figure S2.36. TGA spectrum for AuNP-BCN.

Figure S2.37. First-derivative of TGA spectrum for AuNP-BCN.

Figure S2.38. Second order kinetics graph for azide 1 with BCN$_{exo}$-OH.

Figure S2.39. Second order kinetics graph for azide 1 with AuNP-BCN.

Figure S2.40. Second order kinetics graph for azide 2 with BCN$_{exo}$-OH.

Figure S2.41. Second order kinetics graph for azide 2 with AuNP-BCN.

Figure S2.42. Second order kinetics graph for azide 3 with BCN$_{exo}$-OH.

Figure S2.43. Second order kinetics graph for azide 3 with AuNP-BCN.

Figure S2.44. Second order kinetics graph for azide 4 with BCN$_{exo}$-OH.

Figure S2.45. Second order kinetics graph for azide 4 with AuNP-BCN.

Figure S2.46. Second order kinetics graph for azide 5 with BCN$_{exo}$-OH.

Figure S2.47. Second order kinetics graph for azide 5 with AuNP-BCN.
Figure S2.48. Second order kinetics graph for azide 6 with BCN_{exo}-OH ...........................................107

Figure S2.49. Second order kinetics graph for azide 6 with AuNP-BCN ...........................................107

Figure S2.50. Second order kinetics graph for nitrone 1 with BCN_{exo}-OH ...........................................108

Figure S2.51. Second order kinetics graph for nitrone 1 with AuNP-BCN ...........................................108

Figure S2.52. Second order kinetics graph for nitrone 2 with BCN_{exo}-OH ...........................................109

Figure S2.53. Second order kinetics graph for nitrone 2 with AuNP-BCN ...........................................109

Figure S2.54. Second order kinetics graph for nitrone 3 with BCN_{exo}-OH ...........................................110

Figure S2.55. Second order kinetics graph for nitrone 3 with AuNP-BCN ...........................................110

Figure S2.56. Second order kinetics graph for nitrone 4 with BCN_{exo}-OH ...........................................111

Figure S2.57. Second order kinetics graph for nitrone 4 with AuNP-BCN ...........................................111

Figure S2.58. Second order kinetics graph for nitrone 5 with BCN_{exo}-OH ...........................................112

Figure S2.59. Second order kinetics graph for nitrone 5 with AuNP-BCN ...........................................112

Figure S2.60. Second order kinetics graph for nitrone 6 with BCN_{exo}-OH ...........................................113

Figure S2.61. Second order kinetics graph for nitrone 6 with AuNP-BCN ...........................................113

Figure S2.62. $^1$H NMR spectrum of equimolar mixture of azide 1 and nitrone 6 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent .................................................................115

Figure S2.63. $^1$H NMR spectrum of equimolar mixture of azide 1 and nitrone 6 and interfacial BCN (in AuNP-BCN) in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent ........................................115

Figure S2.64. $^1$H NMR spectrum of equimolar mixture of azide 3 and nitrone 4 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent .................................................................116
Chapter 3

**Figure 3.1.** Isosurface plots (isoval = 0.03 e au$^{-3}$) for the HOMO of BCN–OH$_{exo}$ (5) and the LUMO of 3a–c, 4, 2b, and energy diagram of the frontier orbitals involved in SPANC between 3a–c, 4, 2b and BCN–OH$_{exo}$ (5)…………………………………………………………………………………….127

**Figure S3.1.** Thermal ellipsoid plot of molecular structure of nitrone 4 at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen)………………………………………………………………………………144

**Figure S3.2.** Space-filling diagrams of X-ray structure of nitrone 4 (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine)………………………………………………………………………………144
Figure S3.3. Thermal ellipsoid plot of molecular structure of nitrone 2b at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen).................................................................................................................................145

Figure S3.4. Space-filling diagrams of X-ray structure of nitrone 2b (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................145

Figure S3.5. Thermal ellipsoid plot of molecular structure of nitrone 3a at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................145

Figure S3.6. Space-filling diagrams of X-ray structure of nitrone 3a (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................146

Figure S3.7. Thermal ellipsoid plot of molecular structure of nitrone 3b at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................146

Figure S3.8. Space-filling diagrams of X-ray structure of nitrone 3b (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................146

Figure S3.9. Thermal ellipsoid plot of molecular structure of nitrone 3c at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................147

Figure S3.10. Space-filling diagrams of X-ray structure of nitrone 3c (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).................................................................................................................................147

Figure S3.11. $^1$H NMR spectrum of nitrone 2a in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent..................................................................................................................................................................................149

Figure S3.12. $^{13}$C{${^1}$H} NMR spectrum of nitrone 2a in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent..................................................................................................................................................................................149
Figure S3.13. $^1$H NMR spectrum of nitrone 3a in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent

Figure S3.14. $^{13}$C{$_1^1$H} NMR spectrum of nitrone 3a in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent

Figure S3.15. $^1$H NMR spectrum of nitrone 3b in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent

Figure S3.16. $^{13}$C{$_1^1$H} NMR spectrum of nitrone 3b in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent

Figure S3.17. $^1$H NMR spectrum of nitrone 3c in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent

Figure S3.18. $^{13}$C{$_1^1$H} NMR spectrum of nitrone 3c in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent

Figure S3.19. $^1$H NMR spectrum of nitrobenzyl-TEG-OH (A) in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S3.20. $^{13}$C{$_1^1$H} NMR spectrum of nitrobenzyl-TEG-OH (A) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent

Figure S3.21. $^1$H NMR spectrum of nitrobenzyl-TEG-OTs (B) in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S3.22. $^{13}$C{$_1^1$H} NMR spectrum of nitrobenzyl-TEG-OTs (B) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent

Figure S3.23. $^1$H NMR spectrum of nitrobenzyl-TEG-Thioacetate (C) in CDCl$_3$ at 25°C. * denotes residual protio solvent

Figure S3.24. $^{13}$C{$_1^1$H} NMR spectrum of nitrobenzyl-TEG-Thioacetate (C) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent
Figure S3.25. $^1$H NMR spectrum of (C-pyridinium, N-phenyl-TEG-thioacetate)-nitrone (E) in CDCl$_3$ at 25°C. * denotes residual protio solvent.

Figure S3.26. $^{13}$C{$^1$H} NMR spectrum of (C-pyridinium-N-phenyl-TEG-thioacetate)-nitrone (E) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.

Figure S3.27. $^1$H NMR spectrum of BCN$_{exo}$-OH (5) in (CD$_3$)$_2$SO at 25°C. Made according to reference 2. * denotes residual protio solvent.

Figure S3.28. $^1$H NMR spectrum of 6a in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

Figure S3.29. $^{13}$C{$^1$H} NMR spectrum of 6a in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.

Figure S3.30. $^1$H NMR spectrum of 6b in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

Figure S3.31. $^{13}$C{$^1$H} NMR spectrum of 6b in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.

Figure S3.32. $^1$H NMR spectrum of 4-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent.

Figure S3.33. $^{13}$C{$^1$H} NMR spectrum of 4-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.

Figure S3.34. $^1$H NMR spectrum of 2b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent.

Figure S3.35. $^{13}$C{$^1$H} NMR spectrum of 2b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.

Figure S3.36. $^1$H NMR spectrum of 3a-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent.
Figure S3.37. $^{13}$C{$^1$H} NMR spectrum of 3a-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent………………………………………………………………………………163

Figure S3.38. $^1$H NMR spectrum of 3b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent………………………………………………………………………………164

Figure S3.39. $^{13}$C{$^1$H} NMR spectrum of 3b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent………………………………………………………………………………164

Figure S3.40. $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent………………………………………………………………………………165

Figure S3.41. $^{13}$C{$^1$H} NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent………………………………………………………………………………165

Figure S3.42. $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C. The solvents were suppressed by software. * denotes residual protio solvent………………………………………………………………………………166

Figure S3.43. $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in 1:1 D$_2$O:(CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent………………………………………………………………………………166

Figure S3.44. $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in CD$_3$OD at 25°C. * denotes residual protio solvent………………………………………………………………………………167

Figure S3.45. $^1$H NMR spectrum of 3c-DBCO-amine cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent………………………………………………………………………………168

Figure S3.46. $^{13}$C{$^1$H} NMR spectrum of 3c-DBCO-amine cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent………………………………………………………………………………168

Figure S3.47. $^1$H NMR spectrum of 3c-6b cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent………………………………………………………………………………169

Figure S3.48. $^{13}$C{$^1$H} NMR spectrum of 3c-6b cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent………………………………………………………………………………169
**Figure S3.49.** $^1$H NMR spectrum of nitrone 3a in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 5 minutes. * denotes residual protio solvent………………………………………………………………………………172

**Figure S3.50.** $^1$H NMR spectrum of nitrone 3a in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 12 hours. * denotes residual protio solvent………………………………………………………………………………172

**Figure S3.51.** $^1$H NMR spectrum of nitrone 3b in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 5 minutes. * denotes residual protio solvent………………………………………………………………………………173

**Figure S3.52.** $^1$H NMR spectrum of nitrone 3b in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 12 hours. * denotes residual protio solvent………………………………………………………………………………173

**Figure S3.53.** $^1$H NMR spectrum of nitrone 3c in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 5 minutes. * denotes residual protio solvent………………………………………………………………………………174

**Figure S3.54.** $^1$H NMR spectrum of nitrone 3c in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 12 hours. * denotes residual protio solvent………………………………………………………………………………174

**Figure S3.55.** Pseudo-first order kinetics graph for nitrone 4 with BCN$_{exo}$-OH (5)……………………176

**Figure S3.56.** Pseudo-first order kinetics graph for nitrone 2b with BCN$_{exo}$-OH (5)……………….177

**Figure S3.57.** Pseudo-first order kinetics graph for nitrone 3a with BCN$_{exo}$-OH (5)……………….178

**Figure S3.58.** Pseudo-first order kinetics graph for nitrone 3b with BCN$_{exo}$-OH (5)……………….179

**Figure S3.59.** Pseudo-first order kinetics graph for nitrone 3c with BCN$_{exo}$-OH (5)……………….180

**Chapter 4**

**Figure 4.1.** Synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{24}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (1-azido)………………196

**Figure 4.2 (a)** Molecular structure of the anion [Au$_{24}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$]$^-$ of 4.1-azido (crystallized in R-3 space group). Tetraoctylammonium counterion is not shown. Au = yellow, S
= red, C = grey, N = green (b) Au25 core configuration (c) Staple motif with three μ2-thiolate ligands (d) p-azido-phenylethanethiolate

Figure 4.3 (a) UV-Vis absorption spectrum of 0.2 mM solution of [(CH3- (CH2)7)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido) (black) and surface modified [(CH3- (CH2)7)4N][Au25(SCH2CH2-p-C6H4-C8H10N3)18] (4.1-triazole) (red) in dichloromethane at 23°C (b) ATR-IR spectrum of 4.1-azido (black) and 4.1-triazole (red) (c) 1H NMR spectrum of 4.1-azido (black) and 4.1-triazole (red), taken in CD2Cl2 at 25°C. Chemical shifts of relevant protons are shown for inner ligands (blue) and outer ligands (green). * denotes residual H2O

Figure S4.1. 600 MHz 1H NMR spectrum of [(CH3-(CH2)7)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido) in CD2Cl2 at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvents

Figure S4.2. COSY NMR spectrum of [(CH3-(CH2)7)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido) in CD2Cl2 at 25°C

Figure S4.3. Negative ion mode ESI mass spectrum of anionic [Au25(SCH2CH2-p-C6H4-N3)18]−

Figure S4.4. Space-filling X-ray structure diagram of [(CH3-(CH2)7)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido). Yellow = gold, red = sulfur, black = carbon, green = nitrogen. Tetraoctylammonium counterion is not shown

Figure S4.5. Molecular structure of [(CH3-(CH2)7)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido) showing disordered tetraoctylammonium counterion. Yellow = gold, red = sulfur, black = carboncluster, light blue = carboncounterion, green = nitrogen

Figure S4.6. Photoluminescence spectrum of a 3 μmol/L solution of [(CH3-(CH2)7)4N][Au25(SCH2CH2-p-C6H4-N3)18] (4.1-azido) in dichloromethane at 22°C, recorded with a 532 nm laser and 0.1 second exposure time
Figure S4.7. 600 MHz $^1$H NMR spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N)$_3$] (4.1-triazole) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvents. .........................................................217

Figure S4.8. COSY spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N)$_3$] (4.1-triazole) in CD$_2$Cl$_2$ at 25°C ........................................................................................................218

Figure S4.9. Linear negative mode MALDI-TOF mass spectrum of anionic [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N)$_3$]$^1^-$ .................................................................218

Figure S4.10. Photoluminescence spectrum of a 3 µmol/L solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N)$_3$] (4.1-triazole) in dichloromethane at 22°C, recorded with a 532 nm laser and 0.1 second exposure time ........................................................................219

Figure S4.11. 600 MHz $^1$H NMR spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{10}$H$_{14}$N)$_3$] in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in region of relevant section of spectrum. * indicates residual protio solvents ..................................................................................220

Figure S4.12. Linear negative mode MALDI-TOF mass spectrum of anionic [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{10}$H$_{14}$N)$_3$]$^1^-$ ..................................................221

Figure S4.13. UV-Vis absorption spectrum of 1x10$^{-4}$ M solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{10}$H$_{14}$N)$_3$] in dichloromethane at 23°C ..................................................221

Figure S4.14. Infrared spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{10}$H$_{14}$N)$_3$] ........................................................................................................222

Figure S4.15. $^1$H NMR spectrum of p-nitro-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates residual protio solvent .................................................................223

Figure S4.16. $^{13}$C{$^1$H} NMR spectrum of p-nitro-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent ........................................................................223

Figure S4.17. Infrared spectrum of p-nitro-phenylethanethioacetate .................................................................................................................224
Figure S4.18. UV-Vis absorption spectrum of 1x10^{-4} M solution of \( p \)-nitrophenylethanethioacetate in dichloromethane at 23°C…………………………………………………224

Figure S4.19. \(^1\)H NMR spectrum of \( p \)-ammonium-phenylethanethioacetate chloride in (CD\(_3\))\( _2\)SO at 25°C. * indicates residual protio solvent……………………………………………………………225

Figure S4.20. \(^{13}\)C\{\(^1\)H\} NMR spectrum of \( p \)-ammonium-phenylethanethioacetate chloride in (CD\(_3\))\( _2\)SO at 25°C. * indicates (CD\(_3\))\( _2\)SO solvent………………………………………………………………………225

Figure S4.21. Infrared spectrum of \( p \)-ammonium-phenylethanethioacetate chloride ………226

Figure S4.22. UV-Vis absorption spectrum of 1x10^{-4} M solution of \( p \)-ammonium-phenylethanethioacetate chloride in dichloromethane at 23°C………………………………………………226

Figure S4.23. \(^1\)H NMR spectrum of \( p \)-azido-phenylethanethioacetate in CDCl\( _3\) at 25°C. * indicates residual protio solvent……………………………………………………………………………227

Figure S4.24. \(^{13}\)C\{\(^1\)H\} NMR spectrum of \( p \)-azido-phenylethanethioacetate in CDCl\( _3\) at 25°C. * indicates CDCl\( _3\) solvent……………………………………………………………………………227

Figure S4.25. Infrared spectrum of \( p \)-azido-phenylethanethioacetate…………………………228

Figure S4.26. UV-Vis absorption spectrum of 1x10^{-4} M solution of \( p \)-azido-phenylethanethioacetate in dichloromethane at 23°C………………………………………………………………………..228

Figure S4.27. \(^1\)H NMR spectrum of \( p \)-azido-phenylethanethiol in CD\(_2\)Cl\(_2\) at 25°C. * indicates residual protio solvent……………………………………………………………………………229

Figure S4.28. \(^{13}\)C\{\(^1\)H\} NMR spectrum of \( p \)-azido-phenylethanethiol in CD\(_2\)Cl\(_2\) at 25°C. * indicates CD\(_2\)Cl\(_2\) solvent……………………………………………………………………………229

Figure S4.29. Infrared spectrum of \( p \)-azido-phenylethanethiol………………………………230

Figure S4.30. UV-Vis absorption spectrum of 1x10^{-4} M solution of \( p \)-azido-phenylethanethiol in dichloromethane at 23°C……………………………………………………………………………230
Figure S4.31. Infrared spectrum of (Z)-5,6-dibromocyclooct-1-ene…………………………..231

Figure S4.32. UV-Vis absorption spectrum of 1x10^{-4} M solution of (Z)-5,6-dibromocyclooct-1-ene in dichloromethane at 23°C…………………………………………………………………231

Figure S4.33. Infrared spectrum of (Z)-cyclooct-1-ene-5-yne…………………………………232

Figure S4.34. UV-Vis absorption spectrum of 1x10^{-4} M solution of (Z)-cyclooct-1-ene-5-yne in dichloromethane at 23°C………………………………………………………………………..232

Chapter 5

Figure 5.1. (a) Left to right. Molecular structure of the neutral form [Au_{25}(SCH_{2}CH_{2}-p-C_{6}H_{4}-N_{3})_{18}]^{0} of p-azido (p-azido^{0}), the neutral form [Au_{25}(SCH_{2}CH_{2}-m-C_{6}H_{4}-N_{3})_{18}]^{0} of m-azido (m-azido^{0}) and the neutral form [Au_{25}(SCH_{2}CH_{2}-o-C_{6}H_{4}-N_{3})_{18}]^{0} of o-azido (o-azido^{0}). Au = yellow, S = red, C = black, N = green. (b) Left to right. Comparison of core structures of p-azido, p-azido^{0}, m-azido^{0} and o-azido^{0}………………………………………………………………………….243

Figure 5.2. Left to right. Differential pulse voltammogram (DPV) spectrum of 0.1 mM solution of p-azido^{1}, m-azido^{1} and o-azido^{1}; in 1:1 acetonitrile:benzene. Supporting electrolyte for DPV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP). Arrows indicate scanning potential direction, and asterisk indicates starting position of measurements. Formal potentials of first oxidation reactions are indicated………………………………………………………………………………………………………245

Figure 5.3. (a) ATR-IR spectra of p-azido^{1-} (black), m-azido^{1-} (blue) and o-azido^{1-} (red). (b) ATR-IR spectra of p-triazole^{1-} (dotted black), m-triazole^{1-} (dotted blue) and o-triazole^{1-} (dotted red). (c) UV-Vis absorption spectra of 0.2 mM solutions of p-azido^{1-} (black), m-azido^{1-} (blue) and o-azido^{1-} (red) in dichloromethane at 23°C. (d) UV-Vis absorption spectra of 0.2 mM solutions of p-triazole^{1-} (dotted black), m-triazole^{1-} (dotted blue) and o-triazole^{1-} (dotted red) in dichloromethane at 23°C……………………………………………………………………………………………248
Figure S5.1. 600 MHz $^1$H NMR spectrum of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}] }$ ($m$-azido$^{1-}$) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvents........................................................................................................................................................................269

Figure S5.2. Negative ion mode ESI mass spectrum of anionic $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}]^1-$..................................................................................................................................................................................................................................................270

Figure S5.3. 600 MHz $^1$H NMR spectrum of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-\text{o-C}_6\text{H}_4\text{-N}_3)_{18}] }$ ($o$-azido$^{1-}$) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvents........................................................................................................................................................................271

Figure S5.4. Negative ion mode ESI mass spectrum of anionic $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{o-C}_6\text{H}_4\text{-N}_3)_{18}]^1-$..................................................................................................................................................................................................................................................272

Figure S5.5. Space-filling X-ray structure diagram of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{p-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($p$-azido$^0$). Au = yellow, S = red, C = black, N = green........................................................................................................................................................................................................................................273

Figure S5.6. Space-filling X-ray structure diagram of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($m$-azido$^0$). Au = yellow, S = red, C = black, N = green........................................................................................................................................................................................................................................273

Figure S5.7. Space-filling X-ray structure diagram of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{o-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($o$-azido$^0$). Au = yellow, S = red, C = black, N = green........................................................................................................................................................................................................................................274

Figure S5.8. 600 MHz $^1$H NMR spectrum of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-C}_8\text{H}_{10}\text{N}_3)_{18}] }$ in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvents........................................................................................................................................................................275

Figure S5.9. Linear negative mode MALDI-TOF mass spectrum of anionic $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-C}_8\text{H}_{10}\text{N}_3)_{18}]^1-$........................................................................................................................................................................................................................................276

Figure S5.10. 600 MHz $^1$H NMR spectrum of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-\text{o-C}_6\text{H}_4\text{-C}_8\text{H}_{10}\text{N}_3)_{18}] }$ in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvents........................................................................................................................................................................................................................................277

Figure S5.11. Linear negative mode MALDI-TOF mass spectrum of anionic $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{o-C}_6\text{H}_4\text{-C}_8\text{H}_{10}\text{N}_3)_{18}]^1-$........................................................................................................................................................................................................................................278
Figure S5.12. $^1$H NMR spectrum of $m$-nitro-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates residual protio solvent…………………………………………………………………………………280

Figure S5.13. $^{13}$C{$^1$H} NMR spectrum of $m$-nitro-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent…………………………………………………………………………………280

Figure S5.14. Infrared spectrum of $m$-nitro-phenylethanethioacetate………………………………………281

Figure S5.15. $^1$H NMR spectrum of $m$-azido-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates residual protio solvent…………………………………………………………………………………282

Figure S5.16. $^{13}$C{$^1$H} NMR spectrum of $m$-azido-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent…………………………………………………………………………………282

Figure S5.17. Infrared spectrum of $m$-azido-phenylethanethioacetate………………………………………283

Figure S5.18. $^1$H NMR spectrum of $m$-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent…………………………………………………………………………………284

Figure S5.19. $^{13}$C{$^1$H} NMR spectrum of $m$-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent…………………………………………………………………………………284

Figure S5.20. Infrared spectrum of $m$-azido-phenylethanethiol……………………………………………………………285

Figure S5.21. $^1$H NMR spectrum of $o$-nitro-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates residual protio solvent…………………………………………………………………………………286

Figure S5.22. $^{13}$C{$^1$H} NMR spectrum of $o$-nitro-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent…………………………………………………………………………………286

Figure S5.23. Infrared spectrum of $o$-nitro-phenylethanethioacetate……………………………………………………………287

Figure S5.24. $^1$H NMR spectrum of $o$-azido-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates residual protio solvent…………………………………………………………………………………288
Figure S5.25. $^{13}$C{$_{1}$}H NMR spectrum of $o$-azido-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent……………………………………………………………………………………………………………………..288

Figure S5.26. Infrared spectrum of $o$-azido-phenylethanethioacetate……………………………………………………………………………………………………………………………………………289

Figure S5.27. $^1$H NMR spectrum of $o$-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent……………………………………………………………………………………………………………………………………………290

Figure S5.28. $^{13}$C{$_{1}$}H NMR spectrum of $o$-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent……………………………………………………………………………………………………………………………………………290

Figure S5.29. Infrared spectrum of $o$-azido-phenylethanethiol…………………………………………………………………………………………………………………………………………………………………291

Figure S5.30. Cyclic voltammetry (CV) graph of 0.1 mM solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$SCH$_2$CH$_2$-$p$-C$_6$H$_4$N$_3$)$_{18}$] ($p$-azido$^{1}$) in 1:1 acetonitrile:benzene. Supporting electrolyte for CV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP)……292

Figure S5.31. Cyclic voltammetry (CV) graph of 0.1 mM solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$SCH$_2$CH$_2$-$m$-C$_6$H$_4$N$_3$)$_{18}$] ($m$-azido$^{1}$) in 1:1 acetonitrile:benzene. Supporting electrolyte for CV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP)……293

Figure S5.32. Cyclic voltammetry (CV) graph of 0.1 mM solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$SCH$_2$CH$_2$-$o$-C$_6$H$_4$N$_3$)$_{18}$] ($o$-azido$^{1}$) in 1:1 acetonitrile:benzene. Supporting electrolyte for CV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP)……294

Figure S5.33. Second order kinetics graph for $p$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 1)…………………………………………………………………………………………………………………………………………………………………………………………………………………………………..296

Figure S5.34. Second order kinetics graph for $p$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 2)…………………………………………………………………………………………………………………………………………………………………………………………………………………………………..296

Figure S5.35. Second order kinetics graph for $m$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 1)…………………………………………………………………………………………………………………………………………………………………………………………………………………………………..297

Figure S5.36. Second order kinetics graph for $m$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 2)…………………………………………………………………………………………………………………………………………………………………………………………………………………………………..297
Figure S5.37. Second order kinetics graph for $o$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 1)…………………………………………………………………………………………298

Figure S5.38. Second order kinetics graph for $o$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 2)…………………………………………………………………………………………298

Figure S5.39. Second order kinetics graph for $p$-azido$^1$ with BCN$_{exo}$-OH (Trial 1)…………299

Figure S5.40. Second order kinetics graph for $p$-azido$^1$ with BCN$_{exo}$-OH (Trial 2)…………299

Figure S5.41. Second order kinetics graph for $m$-azido$^1$ with BCN$_{exo}$-OH (Trial 1)…………300

Figure S5.42. Second order kinetics graph for $m$-azido$^1$ with BCN$_{exo}$-OH (Trial 2)…………300

Chapter 6

Figure 6.1. (a) ATR-IR spectrum of [((CH$_3$)-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (6.1-azido) (black), [((CH$_3$)-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_21$H$_{22}$FeN$_3$O$_2$)$_{18}$] (6.1-ferrocenyl) (red) and ferrocene-BCN$_{exo}$ (blue). (b) UV-Vis absorption spectrum of (6.1-azido) (black, 0.2 mM), 6.1-ferrocenyl (red, 0.2 mM) and ferrocene-BCN$_{exo}$ (blue, 5 μM) in dichloromethane at 23°C. (c) $^1$H NMR spectrum of 6.1-ferrocenyl taken in CD$_2$Cl$_2$ at 25°C. Red insets shows expanded sections of spectrum of 6.1-ferrocenyl, and black inset shows relevant expanded section of $^1$H spectrum of 6.1-azido, which was also taken in CD$_2$Cl$_2$ at 25°C……………………………………………………………..…316

Figure 6.2. (a) Differential pulse voltammogram (DPV) of 0.1 mM solution of 6.1-azido in 1:1 acetonitrile with 0.1M tetra-n-butylammonium perchlorate (TBAP) as a supporting electrolyte. (b) Differential pulse voltammogram (DPV) of 0.1 mM solution of 6.1-ferrocenyl in 1:1 acetonitrile with 0.1M tetra-n-butylammonium perchlorate (TBAP) as a supporting electrolyte. Arrows indicate scanning potential direction. Note the different vertical scales for the two nanoclusters…………………………………………………………………………………..…318
Figure S6.1. 600 MHz $^1$H NMR spectrum of [(CH$_3$-$($CH$_2$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$C_21$H$_{22}$FeN$_3$O$_2$)$_{18}$] (6.1-ferrocenyl) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvents.

Figure S6.2. Linear negative mode MALDI-TOF mass spectrum of anionic [Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$C_21$H$_{22}$FeN$_3$O$_2$)$_{18}$]$^-$.

Figure S6.3. $^1$H NMR spectrum of ferrocene-BCN$_{exo}$ in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent.

Figure S6.4. $^{13}$C{$^1$H} NMR spectrum of ferrocene-BCN$_{exo}$ in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent.

Figure S6.5. Infrared spectrum of ferrocene-BCN$_{exo}$.

Figure S6.6. $^1$H NMR spectrum of ferrocene-triazole-thioacetate in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent.

Figure S6.7. Cyclic voltammogram of a 0.1 mM solution of [(CH$_3$-$($CH$_2$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$C_21$H$_{22}$FeN$_3$O$_2$)$_{18}$] (6.1 ferrocenyl) in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.

Figure S6.8. Cyclic voltammogram of a 1 mM solution of $p$-azido-phenylethanethioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.

Figure S6.9. Differential pulse voltammogram of a 1 mM solution of $p$-azido-phenylethanethioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.

Figure S6.10. Cyclic voltammogram of a 3 mM solution of ferrocene-triazole-thioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.
Figure S6.11. Differential pulse voltammogram of a 3 mM solution of ferrocene-triazole-thioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.336

Chapter 7

Figure 7.1. Chapter 5. Investigation of isomeric effects of the azide position on the surface ligands in azide-functionalized [Au25(SR)18]1 platforms, and related properties and surface reactivity.344

Figure 7.2. Development of functional [Au25(SR)18]1 systems using nanoorthogonal CS-SPAAC chemistry for applications in drug delivery, bioimaging and catalysis.349
List of Schemes

Chapter 1

Scheme 1.1. Modern methods for synthesizing AuNPs. (Method 1) The Brust-Schiffrin method to generate 1.5 to 5 nm AuNPs. (Method 2) The Turkevich method to generate 10 to 100 nm AuNPs. (Method 3) The seed growth method to generate 50 to 200 nm AuNPs.


Scheme 1.3. Synthesis of functional AuNP or AuNC systems for application-based research, using the direct synthetic method (Method 1) or the ligand exchange method (Method 2).

Scheme 1.4. A “bioorthogonal click reaction”. The first reactive group is exogenously added and intracellularly incorporated into the target biomolecule using the cellular machinery. A chemical reporter possessing the second (complementary) reactive group is then exogenously added. The reaction between the first and second reactive groups need to proceed selectively in the presence of all the functionalities found within cellular systems, some of which are shown, and the biomolecule is then labelled with the chemical reporter.

Scheme 1.5. Three common “bioorthogonal click reactions”. (a) The Staudinger-Bertozzi ligation between a terminal azide and phenyl ester-functionalized phosphine. (b) The trans-cyclooctene-tetrazine ligation. (c) The azide-oxanorbornadiene cycloaddition.

Scheme 1.6. Common reactions between terminal azides and alkyne moieties. (a) The alkyne-azide Huisgen cycloaddition reaction between a terminal alkyne and terminal azide under high temperature (and/or pressure). (b) The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction between a terminal alkyne and terminal azide in the presence of a copper (I) catalyst. (c) The strain-promoted alkyne-azide cycloaddition (SPAAC) reaction between a cyclooctyne and terminal azide.
Scheme 1.7. The strain-promoted alkyne-nitrone cycloaddition (SPANC) reaction between a nitrone and cyclooctyne..........................................................34

Scheme 1.8. (a) Synthesis of functional AuNP/AuNC through ligand exchange reaction. Addition of chemical reporter possessing thiol group undergoes exchange with native thiols at the metallic core. Reaction at core can result in altered core framework. Quantitative exchange is difficult to accomplish, leading to mixed monolayer. (b) Synthesis of functional AuNP/AuNC through “nanoorthogonal click reaction”. Addition of chemical reporter possessing second reactive group (azide or cyclooctyne) undergoes chemoselective reaction with nanomaterial platform possessing first (complementary) reactive group on the exterior interface. Reaction at interface less likely to result in altered core framework. Quantitative interfacial reaction can be achieved reliably……39

Chapter 2

Scheme 2.1. Synthesis of AuNP-BCN platform. Right inset. TEM image of AuNP-BCN……65

Chapter 3

Scheme 3.1. Synthesis of pyridinium–nitrone possessing anisole (3a), phenyl (3b) and benzonitrile (3c) substituents. Top inset shows general scheme for strain–promoted alkyne–nitrone cycloaddition (SPANC) reaction between nitrone (blue) and cyclooctyne (red). Bottom: molecular structures of 3a, 3b, 3c in the crystal. Thermal ellipsoids are drawn at the 50% probability level with hydrogen atoms drawn with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, purple = iodine).................................................................124

Scheme 3.2. (a) Proposed synthetic strategy for incorporating the pyridinium-nitrone moiety to the surface of AuNPs, using a pyridinium-functionalized thiol ligand. (b) Synthetic strategy for pyridinium-functionalized thiol ligand.................................................................130
Chapter 4

Scheme 4.1. CS-SPAAC reaction between [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (1-azido) and (Z)-cyclooct-1-ene-5-yne, giving surface modified [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}$] (1-triazole)..................................................................................................................198

Chapter 5

Scheme 5.1. Synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^1$; black), [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-N$_3$)$_{18}$] (m-azido$^1$; blue) and [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-o-C$_6$H$_4$-N$_3$)$_{18}$] (o-azido$^1$; red). Rate of reaction ($k_2$) between AuNCs and BCN$_{exo}$-OH were determined under second order conditions using $^1$H NMR spectroscopy, and are indicated for p-azidophenylethanethiol, m-azidophenylethanethiol, o-azidophenylethanethiol, p-azido$^1$ and m-azido$^1$........................................................................................................................................241

Chapter 6

Scheme 6.1. Synthetic approach to the preparation of fully-ferrocenated [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{21}$H$_{22}$FeN$_3$O$_2$)$_{18}$] (6.1-ferrocenyl) through CS-SPAAC between [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (6.1-azido) and ferrocene-BCN$_{exo}$..............................................................................................................................314

Chapter 7

Scheme 7.1. Scheme illustrating ligand exchange chemistry and nanoorthogonal surface chemistry for surface modifications of polydisperse AuNPs and atomically precise AuNCs. Green = first reactive partner. Blue = second, complementary reactive partner. Brown = functional substrate........................................................................................................................................339
Scheme 7.2. *Chapter 2*. Development of BCN-functionalized AuNP platform (AuNP) for kinetically variable SPAAC and SPANC, and kinetically directed competitive reactions........341

Scheme 7.3. *Chapter 3*. (a) Development of highly reactive pyridinium-nitrones for rapid and tunable SPANC chemistry. (b) Proposed synthesis for the development of pyridinium-nitrone-functionalized AuNP platform. However, the necessary thiolated-ligand possessing the terminal pyridinium-nitrone moiety could not be isolated.................................................................342

Scheme 7.4. *Chapter 4*. Development of azide-functionalized [Au$_{25}$(SR)$_{18}$]$^+$ platform for nanoorthogonal CS-SPAAC chemistry for efficient and reliable surface modifications........343

Scheme 7.5. *Chapter 6*. Development of ferrocene-modified [Au$_{25}$(SR)$_{18}$]$^+$ framework through nanoorthogonal CS-SPAAC chemistry.................................................................345
List of Tables

Chapter 3

Table 3.1. Key parameters of pyridinium–nitrones that vary as electron deficiency (and corresponding reactivity) is increased. $^1$H NMR spectra were taken in deuterated dimethylsulfoxide at 25°C. Bond lengths were determined from crystallographic data. Mulliken charges (Q) and the energy gap between the HOMO of BCN and LUMO of the nitrone ($\Delta E_{\text{HOMO-LUMO}}$) were determined from DFT calculations. Bimolecular rate constants ($k_2$) were determined under pseudo–first order conditions in 2:1 acetonitrile:tetrahydrofuran at 22°C by UV-Vis spectroscopy…………………………………………………………………………………………………….126

Table S3.1. Percentage of nitrone 3a remaining in 6:1 D$_2$O:(CD$_3$)$_2$SO over time, measured as a change in the H$_a$ NMR signal intensity relative to the peak from residual H$_2$O solvent………170

Table S3.2. Percentage of nitrone 3b remaining in 6:1 D$_2$O:(CD$_3$)$_2$SO over time, measured as a change in the H$_a$ NMR signal intensity relative to the peak from residual H$_2$O solvent………170

Table S3.3. Percentage of nitrone 3c remaining in 6:1 D$_2$O:(CD$_3$)$_2$SO over time, measured as a change in the H$_a$ NMR signal intensity relative to the peak from residual H$_2$O solvent………171

Table S3.4. Kinetic data for nitrone 4 and BCN$_\text{exo}$-OH (5)……………………………………….176

Table S3.5. Kinetic data for nitrone 2b and BCN$_\text{exo}$-OH (5)……………………………………….177

Table S3.6. Kinetic data for nitrone 3a and BCN$_\text{exo}$-OH (5)……………………………………….178

Table S3.7. Kinetic data for nitrone 3b and BCN$_\text{exo}$-OH (5)……………………………………….179

Table S3.8. Kinetic data for nitrone 3c and BCN$_\text{exo}$-OH (5)……………………………………….180

Table S3.9. Crystallographic information of for molecular structures of nitrones………………..184

Table S3.10. Structural coordinates for BCN$_\text{exo}$-OH (5)………………………………………….187

Table S3.11. Structural coordinates for nitrone 3a………………………………………………….188
Table S3.12. Structural coordinates for nitrone 3b.................................................................188

Table S3.13. Structural coordinates for nitrone 3c.................................................................189

Table S3.14. Structural coordinates for nitrone 2b.................................................................190

Table S3.15. Structural coordinates for nitrone 4.................................................................191

Table S3.16. Comparison of actual bond lengths and bond angles in molecular structures of nitrones to theoretical bond lengths and bond angles from DFT analysis............................192
List of Appendices

A1.1 Permission to Reproduce Copyrighted Material ........................................352

A1.2 Curriculum Vitae ..................................................................................370
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
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CeO$_2$ cerium (IV) oxide
CH$_2$Cl$_2$ dichloromethane
CH$_3$I iodomethane
cm$^{-1}$ wavenumber
CuAAC copper(I)-catalyzed alkyne-azide cycloaddition
CV cyclic voltammetry
d (in NMR data) doublet
Da Dalton
D$_2$O water-d$_2$
DBCO dibenzocyclooctyne
DCM dichloromethane
dd (in NMR data) doublet of doublets
DIBAC dibenzoazyclooctyne
DIFO difluorinated cyclooctyne
DFT density functional theory
DNA deoxyribonucleic acid
DMF dimethylformamide
DMSO dimethylsulfoxide
DOX doxorubicin
DPV differential pulse voltammetry
dt (in NMR data) doublet of triplets
EG ethylene glycol
EG$_3$ triethylene glycol
EG$_4$ tetraethylene glycol
eq. equivalent
ESI-MS electrospray ionization-mass spectrometry
EtOH ethanol
eV electron volts
F(000) structure factor
FRET fluorescence resonance energy transfer
FT Fourier transform
g gram
GFP green fluorescent protein
GSH glutathione
HAuCl₄ tetrachloroauric acid
hv light
HOMO highest occupied molecular orbital
HRMS high-resolution mass spectrometry
Hz hertz
I-SPAAC interfacial strain-promoted alkyne-azide cycloaddition
I-SPANC interfacial strain-promoted alkyne-nitrone cycloaddition
IED inverse electron demand
IR infrared
J coupling constant
LDI-MS laser desorption ionization mass spectrometry
LUMO lowest unoccupied molecular orbital
K degrees Kelvin
k₂ bimolecular rate constant
k_{obs} observed rate constant
KBr potassium bromide
kcal kilocalorie
kcps kilocounts per second
kDa kilodalton
kJ kilojoule
KSCOCH₃ potassium thioacetate
kV kilovolt
m (in NMR data) multiplet
M molar (mol/L)
mA milliamp
MBA 4-mercaptopbenzoic acid
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<td>Mulliken charge</td>
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quin (in NMR data)  quintet
QRE  quasi-reference electrode
r.t.  room temperature
Ref  reference
RSH  thiol molecule
rpm  revolutions per minute
s (in NMR data)  singlet
s  second
SPAAC  strain-promoted alkyne-azide cycloaddition
SPANC  strain-promoted alkyne-nitrone cycloaddition
SPR  surface plasmon resonance
SR  thiolate molecule
t (in NMR data)  triplet
\(t_0\)  time zero
\(t_{24}\)  time twenty-four hours
TCO  \(trans\)-cyclooctene
TEG  tetraethylene glycol
TEM  transmission electron microscope
THF  tetrahydrofuran
TsCl  tosyl chloride
TLC  thin layer chromatography
TGA  thermogravimetric analysis
\(\text{TiO}_2\)  titanium (IV) oxide
UV-Vis  ultraviolet-visible
V  volts
XPS  x-ray photoelectron spectroscopy
XRD  x-ray diffraction
\(Z\)  number of molecules in asymmetric unit cell
Chapter 1

1 Introduction – Strain-Promoted Click Chemistry for Nanoorthogonal Surface Modifications of Gold Nanoparticles and Gold Nanoclusters

1.1 “Golden Nanochemistry”

Colloidal solutions of gold, in which bulk metallic gold is dismantled into distinct metallic particles on the nanoscale, has been a great source of scientific interest that dates back to Michael Faraday’s time, when he reported his synthesis of “fine particles of gold”, by reducing tetrachloroauric acid (HAuCl₄) with phosphorus and stabilizing the particles through addition of carbon disulfide, producing a “beautiful ruby fluid”.¹ This pioneering study triggered intensive systematic research towards developing colloidal solutions of inorganic metals (such as silver and copper)² but developing different gold colloids received particular interest due to their stunning and vibrant array of colors.

Initial efforts in the early 20th century were directed towards developing different methods for reducing HAuCl₄,³ and as the documentation of different gold colloids started to expand, important concepts of colloidal gold formation started to mature, such as nucleation and growth mechanisms,⁴ surface adsorption of ions⁵ and chemical protection of colloidal dispersions.³ In 1918, Scherrer recognized that the size of colloidal gold could be estimated using X-ray diffraction,⁶ a size-determination strategy that is often still used today. With the commercialization of the transmission electron microscope (TEM) in the mid 20th century, Turkevich later reported that the size and shape of colloidal gold species could be more easily determined using TEM microscopy by directly visualizing them,⁷ which is the primary method for size- and shape-determination used today. This progressively evolving understanding of colloidal gold systems seeded a renaissance of nanoscale gold research in the late 1900s, when the term “nanotechnology” started to emerge. Since then, the age of “golden nanochemistry” has blossomed, dedicated to research on gold colloids that has become one of the dominant fields in nanomaterial research.
The field of “golden nanochemistry” can be dissected into two primary research pursuits. The first and initial pursuit is towards developing synthetic control over the size and shape of the colloidal gold particles, and researchers have developed excellent control over these parameters to construct different three-dimensional structures and frameworks (Section 1.2.1 and Section 1.3.1). With the maturation of synthetic strategies for developing colloidal gold particles with different structures and sizes has come a binary distinction that is based on the size (diameter) of the particles. When colloidal gold particles are greater than approximately 2 nm and polydisperse, they are classified as “gold nanoparticles (AuNPs)” (Section 1.2), and when they are less than approximately 2 nm and monodisperse, they are classified as “gold nanoclusters (AuNCs)” (Section 1.3). Although collectively they have properties that are distinct to bulk metallic gold, individually their properties have sub-distinctions that distinguishes them from each other, with the “nanoparticle size regime” having its own properties (Section 1.2.3), and the “nanocluster size regime” having separate properties that are more molecule-like (as the molecular size threshold is approached) (Section 1.3.4).

The second pursuit within “golden nanochemistry” is dedicated to surface functionalization of colloidal gold particles. Early synthetic efforts afforded colloidal gold particles with largely inert surfaces, and their properties were largely dependent on the properties of the metallic arrangements of the core. However, as our understanding of their overall structure began to improve, structure-property relationships started to arise. The realization that the metallic core cannot exist on its own and requires a protecting surface monolayer was an imperative discovery. It is through manipulations of the surface monolayer, and functionalization with different substrates, that has led to extensive application-based research utilizing gold nanoparticles (Section 1.2.4) and gold nanoclusters (Section 1.3.5) for several scientific disciplines.

1.2 Gold Nanoparticles

1.2.1 Structure of Gold Nanoparticles

When colloidal gold particles are greater than approximately 2 nm, they are classified as “gold nanoparticles (AuNPs)” . Most syntheses for obtaining AuNPs follow a similar mechanism as that first reported by Faraday in 1857 whereby a gold (III) precursor is reduced in the presence of a
surface capping ligand to prevent metallic aggregation and stabilize the internal core. As described in Section 1.2.2, using different synthetic strategies, particle size can be adjusted by varying the relative gold ion : reducing agent or gold ion : stabilizer ratios, with larger (and more polydisperse) systems being obtained when using larger ratios. Several different strategies have also been developed to furnish AuNPs with different shapes, where AuNPs having spherical, rod-shaped, prism-shaped, and octahedral morphologies have been reported (Figure 1.1a), among many others. As shown in Figure 1.1b, as the size of the particles are increased, the size-dependent properties (such as optical properties) changes correspondingly. Although there have been different three-dimensional structures reported, spherical AuNPs are arguably the most common as it is easier to functionalize their surfaces and require simple reaction conditions.

![Figure 1.1](image.png)

**Figure 1.1. (a) TEM/SEM images of gold nanoparticles (AuNPs) with different morphologies. Top left. Spherical AuNPs (Figure reproduced with permission from Ref [12].) Top Right. Rod-shaped AuNPs (Figure reproduced with permission from Ref [13]). Bottom Left. Prism-shaped AuNPs (Figure reproduced with permission from Ref [14]). Bottom Right. Octahedral AuNPs (Figure reproduced with permission from Ref [15]). (b) Solutions of rod-shaped AuNPs having different sizes. (Figure reproduced with permission of Ref [16]).**

### 1.2.2 Synthesis of Gold Nanoparticles

One of the major advantages of operating in the nanoparticle size regime for colloidal gold is the versatility and adaptability of the synthetic strategies available to furnish AuNP systems of different size, shape and surface functionalization/complexity. Given that their larger size imparts greater chemical and physical stability, compared to the nanocluster size regime, and that monodispersity is not an expected feature, the desired AuNP structures are less delicate to the chosen synthetic conditions and are typically made in a particle size distribution, which provides
greater freedom over the methodologies that are employed to acquire these rigid structures. The universal paradigm for all AuNP synthetic strategies is a bottom-up reduction of a gold(III) precursor salt (most commonly tetrachloroauric acid, HAuCl₄) by a reducing agent to form an Au(I)/Au(0) core that is stabilized by an external stabilizing ligand on the core surface. Modern procedures can be classified into three major methods: the Brust-Schiffrin method, the Turkevich Method and the seed growth method (Scheme 1.1).

The classical Brust-Schiffrin method is a modification of Faraday’s two-phase pioneering synthesis,¹ in which HAuCl₄ is phase-transferred from water into toluene in the presence of a phase-transfer agent (most commonly tetraoctylammonium bromide). The Au(III) precursor is then reduced by an external thiol (RSH, where R is an alkyl or aryl group) ligand that strongly binds to gold due to the soft character of both gold and sulfur, which leads to the formation of an Au(I) polymeric intermediate [(Au(I)-SR)ₙ].¹⁷ This intermediate is subsequently reduced by excess sodium borohydride (NaBH₄), which results in a Au(I)/Au(0) core that is stabilized by [-SR-Au(I)-SR-] core surface motifs,¹⁸,¹⁹ and furnishes organic-soluble AuNPs with a particle size distribution of approximately 1.5 to 5 nm (Scheme 1.1, Method 1).²⁰ It should be noted that contemporary Brust-Schiffrin syntheses may or may not include an external phase-transfer agent depending on the thiol chosen, and this method is amendable to different one-phase solvent systems that eliminates the necessity of the phase-transfer agent.²¹ The Brust-Schiffrin method is arguably the most versatile of the three methods for synthesizing AuNP structures, because the size and shape of the structures can be controlled through careful choice of: (1) ratio of thiol to gold (III) precursor (2) structure of the thiol ligand and (3) temperature and rate at which the sodium borohydride is added.²² Furthermore, the method is amenable to a wide variety of different alkyl and aromatic thiol ligands,²³ including thiol ligands possessing nucleophilic groups such as OH and NH₂ groups, providing exceptional control over the surface derivatization of the AuNP structures.

In the Turkevich method, an aqueous solution of HAuCl₄ is reduced by trisodium citrate, which also serves as the required stabilizing ligand for the resultant Au(0) core.²⁴ This method furnishes citrate-stabilized water-soluble AuNPs with a particle size distribution between approximately 10 to 100 nm (Scheme 1.1, Method 2), whose size distribution can primarily be controlled through changing the ratio of citrate to gold (III) precursor.²⁵ As surface citrate anions are bound through a weak electrostatic interaction to the core gold atoms, the template citrate-
stabilized AuNPs can be augmented through addition of a supplementary thiol ligand, in the presence of a surfactant to prevent nanoparticle aggregation during the exchange process.\textsuperscript{26, 27}

The largest AuNP systems can be fabricated using the seed growth method, in which smaller AuNP systems serve as seed particles, or nucleation centers, allowing for the hierarchal assembly of AuNP systems that are larger than can be made by the Brust-Schiffrin and Turkevich methods. Seed particles are most commonly citrate-stabilized AuNPs, and upon addition of a growth solution containing H\textsubscript{2}AuCl\textsubscript{4} and a weak reducing agent, such as hydroquinone, the Au(III) salt is reduced and nascent metal ions are affixed to the surface of the pre-existing seed particles.\textsuperscript{28} As with the Turkevich method, surface anions can be exchanged with thiol ligands to establish the desired surface functionalization. This method furnishes AuNP systems with a particle size distribution between approximately 50 to 200 nm (\textbf{Scheme 1.1, Method 3}),\textsuperscript{29} where the size distribution can be controlled through changing the ratio of Au(III) precursor to seed particle.\textsuperscript{30}

\textbf{Scheme 1.1.} \textit{Modern methods for synthesizing AuNPs.} (Method 1) The Brust-Schiffrin method to generate 1.5 to 5 nm AuNPs. (Method 2) The Turkevich method to generate 10 to 100 nm AuNPs. (Method 3) The seed growth method to generate 50 to 200 nm AuNPs.
1.2.3 Properties of Gold Nanoparticles

Colloidal gold particles in the nanoparticle size regime exhibit physical properties that are distinct to both smaller particles in the nanocluster size regime, and bulk metallic gold. One of the most highly exploited intrinsic properties that is unique to AuNPs is the physical nature in which they interact and respond to incident electromagnetic radiation in the ultraviolet-visible (UV-Vis) range, which is a consequence of their electronic surface structure. Spherical AuNPs above 8 nm exhibit a sharp absorption peak between 500 to 600 nm, which is a result from the collective oscillation of free conduction electrons across the AuNP surface due to the resonant excitation by the incident UV-Vis radiation, and is known as “surface plasmon resonance (SPR)”. It is the SPR that produces the intense colors observed with AuNPs in solution and is primarily influenced by the size of the AuNP system, with smaller AuNPs giving blue-shifted (hypochromic) SPR peaks and larger AuNPs giving red-shifted (bathochromic) SPR peaks (Figure 1.2a). The position of the SPR peak is also influenced by the shape, solvent, surface ligand, core charge and temperature. Although the SPR peak on its own serves as an absorption fingerprint for a given AuNP system, one feature of it that is exploitable for application-based research is that AuNP aggregation causes the SPR peak to become broader, and also undergoes a significant red-shift that increases as the interparticle distance decreases (Figure 1.2b).

Figure 1.2. (a) Optical absorption spectra of 9, 22, 48 and 99 nm spherical AuNPs. The broad peaks correspond to the SPR of each system. Figure reproduced with permission from Ref. [33]. (b) Aggregation-induced shift in SPR wavelength as a function of particle center-center spacing for 72 and 84 nm spherical AuNPs. Figure reproduced with permission from Ref. [37].
Another intrinsic property unique to AuNPs is their superior ability to quench fluorescence of adsorbed or covalently linked fluorophore molecules to their surfaces. This quenching phenomenon is a result of nonradiative fluorescence resonance energy transfer (FRET) that is caused by good overlap between the emission profile of excited donor fluorophores in the UV-Vis region, and the SPR profile of the AuNP core, which serves as a ground state electron acceptor, leading to emission deactivation of the proximal fluorophore. The degree of quenching is dependent on two main factors. The first is the extent of spectral overlap, which is determined by both the identity and emission profile of the surface fluorophore, and size and shape of the AuNP, which determines the spectral position of the SPR. The second factor is the distance between the donor fluorophore and AuNP acceptor, with FRET-based quenching decreasing as the distance between the fluorophore and AuNP core increases.

An extrinsic property of AuNPs is their excellent biocompatibility, which has resulted in substantial AuNP-based applications in biological settings (Section 1.2.4). The ability for AuNP systems to be internalized by cells appear to be largely independent of the cell type and surface functionalization of the AuNP, which is a surprising phenomenon given the high density of surface ligands and foreign nature of the metallic core in intracellular settings, and only requires simple incubation of the desired AuNP system with the cell line that is to internalize the functionalized AuNP. However, polymeric coatings are often tethered to the AuNP surface to enhance cellular permeability and prevent premature extracellular degradation. Cellular entry can also be affected by the size and shape of the AuNP system, with spherical ~50 nm AuNPs having the highest internalization rates (Figure 1.3a). The method of internalization is not well understood, but it is thought to occur through a receptor-mediated endocytotic pathway, in which membrane-bound transport proteins recognize and internalize the functionalized AuNP system through vesicle formation. Evidence of this endocytotic pathway was demonstrated in a study in which transferrin-coated AuNPs were highly internalized by human nasopharyngeal carcinoma (NPC) cells under physiologically relevant conditions, whereas AuNPs not coated with the transferrin protein were not (Figure 1.3b), implicating the transferrin receptor-mediated endocytotic pathway for AuNP internalization.
Figure 1.3. (a) Uptake of AuNPs into mammalian cells (A) Number of AuNPs per vesicle diameter versus nanoparticle size (B-F) TEM images of AuNPs with sizes 14, 30, 50, 74 and 100 nm trapped inside vesicles of a Hela cell, respectively. Figure reproduced with permission from Ref. [43] (b) Confocal cell images showing fluorescein-labelled transferrin AuNPs (AuNP-TF) internalized by NPC cells (A) NPC cells without AuNP-TF (B) NPC cells treated with AuNPs without surface transferrin (C) NPC cells treated with AuNP-TF (D) NPC cells treated with albumin-coated AuNPs (E and F) NPC cells co-treated with different proportions of AuNP-TF and albumin-coated AuNPs (1:2 and 1:5, respectively). Figure reproduced with permission from Ref. [45].

1.2.4 Applications of Gold Nanoparticles

Compared to many other nanomaterial systems, AuNP systems can be synthesized using straightforward yet modifiable strategies that can be manipulated to construct AuNP systems of different size and three-dimensional complexity simply through careful choice of reaction stoichiometries and reactive partners. Furthermore, due to the affinity of thiol moieties towards gold surfaces, the composition and arrangement of their surfaces can be modulated with high precision using a variety of different thiol ligands, most often through simple mix-and-stir reactions. This ability to amend their core and surface structures allow for their size- and composition-dependent properties to be reliably modified in a very predictable manner. This has led to the maturation of a rich and dynamic application-based research field over the past several decades, focusing on the use of functional AuNP systems in a variety of different scientific disciplines.

One of the most exploitable properties of AuNPs for application-based research is their plasmonic optical properties. AuNP aggregation induces interparticle surface plasmon coupling,
resulting in a color change from red to blue that can be measured both visually and spectrophotometrically. This makes AuNPs useful platforms for spectral-based colorimetric sensing of target analytes, whereby the target analyte induces either AuNP aggregation or dispersion. Lin et al. reported the development of an AuNP system functionalized with [15]-crown-5 moieties, which have a high binding affinity for lead ions. In the absence of lead ions, the surface [15]-crown-5 moieties undergo interparticle hydrogen bonding in aqueous solutions, causing AuNP aggregation. Addition of lead ions causes competitive adsorption, preventing interparticle hydrogen bonding and dismantling of the AuNP aggregates, affording a measurable color change (Figure 1.4a). Conversely, Huang et al. reported an AuNP system where the surface ligands were 3-mercaptopropionic acid (MPA), which was a highly sensitive colorimetric sensor for mercury ions. Their MPA-functionalized AuNPs were dispersed in aqueous solution, and addition of mercury ions led to an interaction between surface carboxyl groups and the lead ions that caused AuNP aggregation, causing a change in the spectral pattern of the AuNP system that could be measured (Figure 1.4b).

Figure 1.4. (a) Left. Colorimetric sensing of lead ions, whereby lead adsorption causes dismantling of ([15]-crown-5)-functionalized AuNP aggregates (A) into an AuNP dispersion (B). Right. UV/Vis spectra of A and B (Spectra figure reproduced with permission from Ref. [48]). (b) Left. Colorimetric sensing of mercury ions, whereby mercury adsorption caused dispersion of carboxyl-terminated AuNPs (A) to from AuNP aggregates (B). Right. UV/Vis spectra of A and B (Spectra figure reproduced with permission from Ref. [49]).

AuNP systems are also promising candidates for macromolecular delivery platforms, especially in the delivery of drug molecules. Drug inoculation is often accompanied by premature
deterioration by extracellular matrix proteases due to the foreign nature of the drug molecules, preventing effective intracellular entry and lowering the overall efficacy of the drug.\textsuperscript{50} Given the excellent biocompatibility of AuNPs, drug molecules tethered onto AuNP surfaces has proven to be an effective strategy for their delivery. Due to the high surface area-to-volume ratio, drug molecules are packed densely on the AuNP surface.\textsuperscript{51} This both allows for a high loading of drug molecules per AuNP, and also sterically prevents access to extracellular enzymes, improving therapeutic delivery.\textsuperscript{52} Peng \textit{et al.} reported an AuNP system in which doxorubicin (DOX) was adsorbed onto the surface of 4-mercaptobenzoic acid (MBA)-functionalized AuNPs, in which DOX was able to adsorb onto the surface through $\pi$-$\pi$ interactions (Figure 1.5a).\textsuperscript{53} DOX is one of the most effective anti-cancer chemotherapeutics available today and is used for the treatment of a variety of cancer types.\textsuperscript{54} They found that when these DOX-loaded AuNPs were introduced into breast cancer cell line MCF-7, there was higher delivery of DOX (Figure 1.5c) than when DOX was inoculated on its own (Figure 1.5b).\textsuperscript{53} They also found a greater reduction in tumor volume in mice when the DOX was delivered on the AuNP surface (Figure 1.5d).\textsuperscript{53}

**Figure 1.5.** (a) Schematic representation of doxorubicin (DOX) adsorption onto AuNP surface and intracellular delivery. (b,c) Fluorescence microscopy images of MCF-7 tumor tissues after injection of DOX alone (b) and AuNP-DOX (c). Red = DOX; green = stained vasculature (lectin-FITC). Scale bar, 50 µm. (d) Normalized tumor growth curves after injection with AuNP-DOX (red), DOX alone (black) and phosphate buffered-saline (PBS) (blue). Figures in (b), (c) and (d) reproduced with permission from Ref. [53].
Functionalized AuNPs have also found extensive use in bioimaging applications, which typically involves using AuNPs as molecular beacons whereby ‘reporter’ macromolecules are tethered to the AuNP surface that also have an attached fluorophore, where the fluorescence of the fluorophore is quenched due to non-radiative FRET.\textsuperscript{41,55} Upon introduction of the AuNP system to the intracellular environment, competitive binding of target analytes causes a release of the fluorophore-conjugated reporter agents, which reestablishes fluorescence.\textsuperscript{41} Seferos \emph{et al.} developed an AuNP “nano-flare” system (\textbf{Figure 1.6a}),\textsuperscript{56} in which oligonucleotide-functionalized AuNPs were synthesized through a ligand exchange between citrate-functionalized AuNPs and a thiol-terminated oligonucleotide sequence. The attached sequence (‘reporter sequence’ with attached fluorophore) was complementary to an oligonucleotide sequence that is present in the mRNA sequence of survivin, a protein that is commonly over-expressed in cancer cell lines. When introduced to the intracellular environment of cancer cells, ‘survivin mRNA’ displaced the fluorophore-conjugated reporter sequence, which released the reported sequence from the AuNP surface and reestablished fluorescence of the attached fluorophore. They observed high fluorescence in cancer cell type SKBR3 when the survivin recognition sequence was on the AuNP surface, as opposed to when a different sequence was tethered to the AuNP surface (\textbf{Figure 1.6b}).\textsuperscript{56}

\textbf{Figure 1.6.} (a) Schematic representation of development of ‘nano-flare AuNP’ and \textit{in vivo} release of fluorophore-conjugated reporter sequence after survivin mRNA binding. (b) Differential contrast and fluorescence imaging of survivin-expressing SKBR3 cells treated with survivin nano-flares (\textit{left}) and non-complementary nano-flares (\textit{right}). (c) Analogously treated non-survivin expressing C166 cells. Scale bar is 20 μm. Figure in (b) and (c) reproduced with permission from Ref. [56].
Furthermore, they found the highest fluorescence in survivin-expressing SKBR3 cells as opposed to cell lines that did not express survivin, such as mouse endothelial cell type C166 (Figure 1.6c).\textsuperscript{56}

1.3 Gold Nanoclusters

1.3.1 Structure of Gold Nanoclusters

Atomically precise gold particles are known as gold nanoclusters (AuNCs) and have received tremendous research interest in recent years due to their unique and extraordinary properties that are not seen from their larger AuNP counterparts. Unlike AuNPs, whose optical properties are largely plasmonic, when the nanocluster size regime is accessed, non-plasmonic molecular-type properties begin to manifest that is one of the primary distinctions.\textsuperscript{57} Furthermore, whereas AuNP systems exist as polydisperse systems, AuNC systems can be furnished an in atomically precise fashion, generating true molecules with well-defined molecular weights and formulas, which permits analysis techniques largely reserved for small molecules, such as single crystal X-ray diffraction. Although there has been other capping ligands reported to stabilize the central metallic core, such as carbenes,\textsuperscript{58} as with AuNPs the predominant capping ligand used in AuNC systems has been thiols. This due to the structural versatility of thiolated ligands, allowing for different AuNC frameworks to be generated through careful choice of reaction conditions and strategies (Figure 1.7).\textsuperscript{59-65}

1.3.2 Synthesis of Gold Nanoclusters

Synthetic strategies for AuNPs are aimed towards developing colloidal gold systems with a general size and shape, and although these strategies are highly versatile in developing different three-dimensional nanostructures, AuNPs are synthesized as polydisperse systems, with a particle size distribution that is dependent on the conditions of the reaction. Unlike with AuNPs, when entering the nanocluster size regime, synthetic strategies are often aimed towards developing atomically precise, and thus monodisperse AuNC systems. Most AuNC synthetic strategies involve an initial reaction using the Brust-Schiffrin method, whereby a gold (III) precursor (most commonly HAuCl\textsubscript{4}) is reduced by a thiol ligand to form an Au(I) polymeric intermediate [(Au(I)-SR)\textsubscript{n}], which is subsequently reduced by sodium borohydride (NaBH\textsubscript{4}) to form an Au(I)/Au(0) nanostructure.\textsuperscript{66} While the Brust-Schiffrin method can also be used to fashion AuNP systems (Section 1.2.2),
Figure 1.7. Some thiolated AuNC frameworks.

Top (left to right). The Au\(_{20}\)(SR)\(_{16}\) framework (where R = C\(_6\)H\(_4\)-C(CH\(_3\))\(_3\)) (Figure produced from data with permission from Ref. [59]). The Au\(_{23}\)(SR)\(_{16}\) framework (where R = C\(_6\)C\(_{11}\)) (Figure produced from data with permission from Ref. [60]). The Au\(_{24}\)(SR)\(_{20}\) framework (where R = C\(_6\)H\(_4\)-C(CH\(_3\))\(_3\)) (Figure produced from data with permission from Ref. [61]).

Middle (left to right). The Au\(_{25}\)(SR)\(_{18}\) framework (where R = CH\(_2\)-C\(_6\)H\(_5\)-C(CH\(_3\))\(_3\)) (Figure produced from data with permission from Ref. [62]). The Au\(_{28}\)(SR)\(_{20}\) framework (where R = C\(_6\)H\(_4\)-C(CH\(_3\))\(_3\)) (Figure produced from data with permission from Ref. [63]).

Bottom (left to right). The Au\(_{38}\)(SR)\(_{24}\) framework (where R = CH\(_2\)-CH\(_2\)-C\(_6\)H\(_5\)) (Figure produced from data with permission from Ref. [64]). The Au\(_{44}\)(SR)\(_{28}\) framework (where R = CH\(_2\)-CH\(_2\)-C\(_6\)H\(_5\)) (Figure produced from data with permission from Ref. [65]).
careful choice of reaction conditions, stoichiometries and reactive partners also allows access to the AuNC size regime using the same method. Modern AuNC syntheses can generally be classified depending on whether this Au(I)/Au(0) nanostructure is the desired AuNC, or whether it is subsequently treated in some way to generate the desired AuNC.

During AuNP synthesis, the structure of the [(Au(I)-SR)\textsubscript{n}] intermediate not well-defined, which leads to the formation of a polydisperse system after reduction by sodium borohydride. However, since monodispersity is a desired trait for AuNC systems, more careful attention needs to be given to the structure of the [(Au(I)-SR)\textsubscript{n}] intermediate, which needs to have a more unambivalent arrangement.\textsuperscript{67} The structure of the [(Au(I)-SR)\textsubscript{n}] intermediate is very sensitive to the ratio of gold (III) precursor to thiol, the rate of thiol addition, the temperature at which the gold (III) precursor and thiol are combined/stirred together and the time over which they are stirred,\textsuperscript{68} all of which need to be more carefully considered when synthesizing the [(Au(I)-SR)\textsubscript{n}] intermediate. Upon addition of NaBH\textsubscript{4}, it is generally accepted that larger particles initially form, but then begin to ‘size-focus’ into smaller atomically precise core configurations over time, which is dependent on the amount and rate of sodium borohydride addition, the temperature of the reaction, and structure of the [(Au(I)-SR)\textsubscript{n}] intermediate.\textsuperscript{69} If the acquired Au(I)/Au(0) structure is the desired AuNC system, it is said to have been furnished using this ‘kinetic size-focusing’ method (Scheme 1.2, Method 1),\textsuperscript{70} because typically the structure of the [(Au(I)-SR)\textsubscript{n}] is ‘kinetically controlled’ through careful choice of the reaction conditions, followed by size focusing into the desired configuration after NaBH\textsubscript{4} addition. Monodisperse samples of the Au\textsubscript{23}(SR)\textsubscript{16} and Au\textsubscript{25}(SR)\textsubscript{18} frameworks have been synthesized using this kinetic size-focusing strategy,\textsuperscript{60, 62} which have been successfully characterized by single-crystal X-ray diffraction (Figure 1.7, Section 1.3.1).

Another common strategy involves using pre-made AuNC templates furnished using the kinetic size-focusing method and altering their core configurations through exchange chemistry with a second (structurally different) thiol ligand, which leads to a transformation of the core configuration and overall structure of the AuNC framework.\textsuperscript{71} Various AuNC systems have been furnished using this ‘ligand exchange’ structure-transformation method (Scheme 1.2, Method 2). Using the Au\textsubscript{25}(SR)\textsubscript{18} framework as the template, monodisperse samples of the Au\textsubscript{20}(SR)\textsubscript{16} and Au\textsubscript{28}(SR)\textsubscript{20} frameworks have been synthesized,\textsuperscript{59} and using the Au\textsubscript{23}(SR)\textsubscript{16} framework as the
template, monodisperse samples of the Au\textsubscript{24}(SR)\textsubscript{20} framework has been synthesized,\textsuperscript{61} which have been successfully characterized by single-crystal X-ray diffraction (Figure 1.7, Section 1.3.1).

Many monodisperse AuNC systems have been synthesized using a third common strategy, in which the [(Au(I)-SR)\textsubscript{n}] intermediate is less meticulously synthesized, and then rapidly reduced using NaBH\textsubscript{4} to form a polydisperse AuNC system. This polydisperse AuNC sample is then heated to high temperature (typically \(\geq 80\) °C) in the presence of a second (structurally different) thiol ligand, which leads to ligand exchange/thiol etching on the AuNC surface and size-transformation to fabricate a monodisperse AuNC system (Scheme 1.2, Method 3).\textsuperscript{72} Monodisperse samples of the Au\textsubscript{38}(SR)\textsubscript{24} and Au\textsubscript{44}(SR)\textsubscript{28} frameworks have been synthesized using this ‘high temperature thiol etching’ strategy,\textsuperscript{64, 65} which have been successfully characterized by single-crystal X-ray diffraction (Figure 1.7, Section 1.3.1).

**Scheme 1.2.** Modern methods for synthesizing AuNCs. (Method 1) The kinetic size-focusing method. (Method 2) The ligand exchange method. (Method 3) The high temperature thiol etching method.
1.3.3 The $\text{Au}_{25}(\text{SR})_{18}$ Nanocluster Framework

Early AuNC research by the Whetten group reported the synthesis of large polydisperse glutathione (SG)-protected particles (>30,000 Da). In a later study in which they modified the reaction conditions, they analyzed a sample of SG-protected particles by laser desorption ionization mass spectrometry (LDI-MS), in which they identified a species with an approximate mass of ~8000 Da, among the many other larger species that formed, but couldn’t conclusively determine a molecular formula due to excessive fragmentation of this species. This ~8000 Da species triggered interest because it started to display non-plasmonic optical properties not seen in the larger SG-protected gold particles that formed. The Tsukuda group were then able to suppress the fragmentation during their mass spectrometric analysis, using electrospray ionization (ESI) mass spectrometry, allowing them to unambiguously determine the molecular formula of the ~8000 Da species as $[\text{Au}_{25}(\text{SG})_{18}]^{-}$. This assignment was later confirmed in subsequent studies. In 2007, the Jin group synthesized an $[\text{Au}_{25}(\text{SR})_{18}]^{-}$ system which used the more rigid phenylethanethiol (HSCH$_2$CH$_2$Ph) as the capping ligand instead of glutathione and tetraoctylammonium bromide as a phase-transfer agent, using the kinetic size-focusing method that they first described (Section 1.3.2). It should be noted that it is the anionic form of the $\text{Au}_{25}(\text{SR})_{18}$ framework that is directly accessible using the kinetic size-focusing method, having a tetraoctylammonium counterion. They were able to synthesize a highly monodisperse sample of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}]^{-}$ in high yield that was of sufficient purity and monodispersity to confirm its structure by single-crystal X-ray diffraction.

Since 2007, when the synthetic strategy for $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}]^{-}$ was optimized, and its molecular structure was confirmed, the $[\text{Au}_{25}(\text{SR})_{18}]^{-}$ framework has become the dominant thiolate-protected AuNC, especially for structure-property and application-based research. This can largely be attributed to its superior core stability at ambient conditions in a variety of environments and settings, due to its closed-shell superatom electron configuration (an 8 electron system (i.e. 25 – 18 + 1 = 8) that corresponds to a noble-gas like 1S$^2$1P$^6$ configuration). It is because of its superior stability that, unlike many thiolate-protected AuNC systems, the $[\text{Au}_{25}(\text{SR})_{18}]^{-}$ system is very amenable to different capping thiol ligands other than SG or HSCH$_2$CH$_2$Ph, which is one of the most attractive features of the $[\text{Au}_{25}(\text{SR})_{18}]^{-}$ system. Furthermore, the stability of the $[\text{Au}_{25}(\text{SR})_{18}]^{-}$ framework makes it comparatively easily to
synthesize, and under kinetic control, can be synthesized in high yield reproducibly at ambient conditions.

Another important feature of the [Au$_{25}$(SR)$_{18}$]$^{1-}$ framework is the ability to alter the oxidation state of the framework through redox chemistry, with several examples of [Au$_{25}$(SR)$_{18}$]$^{z}$ (where $z = -1, 0$ and $+1$) with different capping ligands having been reported. With respects to the [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{z}$ framework, the Jin group first reported the synthesis of the [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1-}$ framework through air oxidation of the [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1-}$ framework. In a later study, they were able to oxidize the [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1-}$ framework using hydrogen peroxide to form the same neutral framework.

Whereas the anionic form is a closed-shell 8 electron system, [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{0}$ is a 7 electron paramagnetic system (i.e. $25 - 18 = 7$) that corresponds to a 1S$^2$1P$^5$ electron configuration, attributing its lower stability than the anionic form.

The Maran group subsequently reported the synthesis of the cationic [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1+}$ framework by treating the anionic [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1-}$ framework with bis(pentafluorobenzoyl) peroxide. The cationic [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1+}$ framework is now a 6 electron diamagnetic system (i.e. $25 - 18 - 1 = 6$) that corresponds to a 1S$^2$1P$^4$ electron configuration.

The discovery of the molecular structure of the [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1-}$ framework in 2007 by the Jin group (Figure 1.8a) has also provided valuable insight into the general molecular and bonding features that are common to most thiolate-protected AuNCs. One molecular feature of [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^{1-}$ that is common to most thiolate-protected AuNCs are the [-SR-Au(I)-SR-Au(I)-SR-] core surface motifs (also known as staple motifs) shown in Figure 1.8b, which comprise μ$_2$-thiolate ligands that are bonded to two gold atoms, with each gold atom concurrently bonded to two thiolate ligands. In the core structure, there is an internal Au$_{13}$ icosahedral kernel at the center, with twelve [-SR-Au(I)-SR-Au(I)-SR-] staple motifs at the 12 vertices (Figure 1.8b).

A feature that is unique to [Au$_{25}$(SR)$_{18}$]$^{1-}$ frameworks is the existence of two distinguishable surface ligands sites in each of the twelve [-SR-Au(I)-SR-Au(I)-SR-] staple motifs. Ligands on the two edges of each staple motif occupies one distinguishable surface site, and they are known as “inner ligands”, while ligands attached at the center of each staple motif occupies a separate distinguishable surface site, and they are known as “outer ligands” (Figure 1.8b). Pengo et al. demonstrated that the inner surface sites are more reactive towards exchange chemistry than the
outer ligands,\textsuperscript{65} which creates important consequences in the ligand exchange chemistry of \([\text{Au}_{25}(\text{SR})_{18}]\textsuperscript{1-}\) frameworks.

Figure 1.8. (a) Molecular structure of \([\text{TOA}]\text{[Au}_{25}(\text{CH}_2\text{CH}_2\text{Ph})_{18}]\), where TOA = tetraoctylammonium (not shown). (b) Staple motifs, \(\text{Au}_{13}\) kernel, and inner/outer ligands in molecular structure of \([\text{Au}_{25}(\text{CH}_2\text{CH}_2\text{Ph})_{18}]\textsuperscript{1-}\). Figure produced from data with permission from Ref. [62].

1.3.4 Properties of the \(\text{Au}_{25}(\text{SR})_{18}\) Framework

Colloidal gold particles in the nanocluster size regime exhibit molecular-type physical properties that are distinct from the physical properties of larger AuNPs, as the molecular size regime is approached.\textsuperscript{86} Furthermore, unlike with AuNPs, the properties of AuNCs are highly sensitive to small changes in the structure of the internal core configuration and ligand structure. One of the most distinctive features of AuNC systems, that distinguishes them from larger AuNPs, is the way in which they interact with incident light in the UV-Vis range. Whereas the absorption patterns of AuNPs with particle size distributions above 8 nm exhibit an SPR peak, AuNCs response to incident light that resembles that of molecular species due to the molecular-type electronic structure, with the SPR disappearing entirely below \(~2\) nm.\textsuperscript{87} When the AuNC size regime \((< 2 \text{ nm})\) is accessed, the absorption pattern exhibits discrete quantized electronic transitions due to the presence of discrete molecular-type energy levels, which is distinct to the continuous energy band in the metallic state of larger AuNPs.\textsuperscript{87}
With respects to the [Au25(SCH2CH2Ph)18]1− framework, there are characteristic absorption peaks at ~670, ~450 and ~400 nm, which are common to most [Au25(SR)18]1− frameworks (Figure 1.9a,b). The Aitken group determined that the transition at ~670 nm is a HOMO-to-LUMO transition (intraband sp ← sp transition) (Figure 1.9b), where the HOMO and LUMO are composed of atomic orbital contributions from the Au atoms in the internal Au13 icosahedral atoms, which is characteristic of the [Au25(SCH2CH2Ph)18]1− framework. In the absorption pattern for [Au23(SR)16]1−, which has the same charge state and free electron configuration as [Au25(SR)18]1−, but has an internal Au13 cuboctahedron instead of an internal Au13 icosahedron, there is a peak at ~570 nm (with no peak at ~670 nm), indicating the correlation of this transition to the structure of the internal core configuration. In this way, the peak at 670 nm is a characteristic feature in the absorption pattern of [Au25(SR)18]1−. The peaks at ~450 nm and ~400 nm occur from electronic metal-ligand interband transitions (Figure 1.9b), which are also characteristic for [Au25(SR)18]1− frameworks. In the absorption pattern of [Au25(SCH2CH2Ph)18]0 and [Au25(SCH2CH2Ph)18]1+, the qualitative appearance of the peaks does not change, and there is a retention of the peak at ~670 nm, albeit that the intensity of these peaks changes as the atomic orbital contributions changes as the free electron configuration of the system is changed (Figure 1.9a).

Figure 1.9. (a) Optical absorption spectra of [TOA][Au25(CH2CH2Ph)18], where TOA = Tetraoctylammonium (black), Au25(CH2CH2Ph)18 (red) and [Au25(CH2CH2Ph)18][C6F5CO2−] (blue). Figure reproduced with permission from Ref. [88]. (b) Optical absorption spectrum of [TOA][Au25(CH2CH2Ph)18] showing principal transitions. Figure reproduced with permission from Ref. [62].

Another important optical property of thiolate-protected AuNCs, that is not exhibited by AuNP systems, is their photoluminescence (PL). Thiolate-protected AuNCs typically have PL in the near infrared (NIR) region, that originates from the structure of the ligands in the [-SR-Au(I)-SR-Au(I)-SR-] staple motifs, and in the visible region, which originates from the electronic
composition and structure of the metallic core, with stronger PL being observed in the NIR region than in the visible region.\textsuperscript{90} Regardless of its weak contribution, the PL in the visible region can be enhanced through structural changes to the internal metallic framework,\textsuperscript{91} or especially through doping the core with metals such as silver.\textsuperscript{92} As the NIR PL is stronger, the role of the capping ligand structure on the PL of thiolate-protected AuNCs, especially from Au\textsubscript{25}(SR)\textsubscript{18} frameworks, has received significant attention. Water soluble Au\textsubscript{25}(SR)\textsubscript{18} frameworks (having water soluble surface ligands such as glutathione (SG)) generally have higher NIR PL than their organic soluble counterparts (having organic soluble surface ligands such as phenylethanethiol (SCH\textsubscript{2}CH\textsubscript{2}Ph)).\textsuperscript{81} Wu and Jin reported the strong correlation between the PL of organic-soluble [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{1−} frameworks and the electronic composition of the capping thiol ligands, with stronger PL being observed when more electron-donating capping ligands were used (Figure 1.10a).\textsuperscript{81} They also found that the charge of the Au\textsubscript{25}(SR)\textsubscript{18} framework has important consequences on the PL, with the PL of [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{18}]\textsuperscript{1+} being more than 5 times higher than the PL of [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{18}]\textsuperscript{1−} (Figure 1.10b).\textsuperscript{81}

![Figure 1.10](image_url)

**Figure 1.10.** (a) Photoluminescence (PL) spectra of [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{1−} functionalized with phenylethanethiolate (black), dodecanethiolate (red) and hexanethiolate (blue). (b) PL spectra of [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{1−} (black), [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{0} (red), [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{1+} (blue) and [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{2+} (blue-green). Figures reproduced with permission from Ref. [81].

Another important feature of thiolate-protected AuNC is their electrochemical behaviour. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are the most frequently used methods for evaluating their electrochemical behaviour. Electrochemistry reveals the energies of the HOMO and LUMO of the thiolate-protected AuNC, and the corresponding HOMO-LUMO energy gap, which is defined as the difference between the first oxidation (O1) and first reduction (R1) potentials.\textsuperscript{93} Furthermore, the electrochemical pattern exhibited by a given AuNC system provides an electrochemical fingerprint for the AuNC system. In this way, electrochemistry is a
useful characterization technique to evaluate the core configuration of the AuNC system. Lee et al. reported the CV and DPV for the \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}]^{1-}\) framework at 25 °C,\(^{94}\) which contains peaks at potentials of -1.5 (R1), 0.1 (O1), 0.4 (O2) and 1.1 (O3) V versus an Ag wire quasi-reference electrode (AgQRE) that correspond to the \(\text{Au}_{25}^{2+}, \text{Au}_{25}^{1+}, \text{Au}_{25}^{0}\) and \(\text{Au}_{25}^{-}\) charge states, respectively (Figure 1.11). Using this data, the HOMO-LUMO gap for \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}]^{1-}\) (i.e. the R1-O1 difference) was 1.5 V, which is typical for other \([\text{Au}_{25}(\text{SR})_{18}]^{1-}\) frameworks.\(^{80,95}\)

![Figure 1.11. Differential pulse voltammogram (DPV) of \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}]^{1-}\) at 0.02 V/s in 0.1 M Bu_4NPF_6 in degassed CH_2Cl_2 at 0.4 mm diameter Pt working electrode, with Ag wire quasi-reference (AgQRE) and Pt wire counter electrode. * indicates wave for incompletely removed O_2. DPV reproduced with permission from Ref. [94].](image)

1.3.5 Applications of the \(\text{Au}_{25}(\text{SR})_{18}\) Framework

Due to the comparatively newer discovery of AuNCs and the more recent optimization of their syntheses, application-based research for AuNCs is less developed, and is receiving significant attention in contemporary literature as the relationship between the physical properties and framework structure is becoming more well-defined. The maturation of the synthetic strategies available to acquire different AuNC core configuration and surface structures, and the concurrent ability to fine-tune the associated structure-dependent properties, has made thiolate-protected AuNCs a promising candidate for a variety of applications. Given the synthetic accessibility and chemical robustness of the \(\text{Au}_{25}\) framework and its amenability to the capping thiol ligand, the \(\text{Au}_{25}\) framework has received the most attention in terms of application-based research.
One exploitable property of Au$_{25}$ systems is the way in which they interact with light, which has been used towards colorimetric sensing applications. Whereas colorimetric sensing applications using AuNP systems relies on aggregation-induced variations in the SPR absorption patterns, colorimetric sensing using Au$_{25}$ systems relies on changes in the PL before and after adsorption of the target analyte. Xie et al. developed a “green synthesis” of an Au$_{25}$ system in which treatment with HAuCl$_4$ with bovine serum album (BSA) in alkaline solution resulted in the formation of a BSA-encapsulated Au$_{25}$ core (Au$_{25}$-BSA), with the internal core being stabilized through [-SR-Au(I)-SR] motifs where the surface thiolates are thiol-containing amino acids in BSA. This Au$_{25}$-BSA nanoconjugate exhibited high PL, which was even stronger than the PL from Au$_{25}$(SG)$_{18}$. In a later study, they found that their Au$_{25}$-BSA nanoconjugate was an excellent colorimetric sensor for mercury ions. Treatment of Au$_{25}$-BSA with lead ions led to the adsorption of the lead ions to the internal Au$_{25}$ core (due to the strong interaction between mercury and gold ions), which led to a subsequent quenching of the PL, which could be monitored visually and using fluorescence spectroscopy (Figure 1.12).

![Figure 1.12. Left. Schematic representation of Hg$^{2+}$ sensing based on fluorescence quenching upon Hg$^{2+}$ binding to Au$_{25}$-BSA. Right. Photoemission spectra ($\lambda_{ex} = 470$ nm) of Au$_{25}$-BSA before Hg$^{2+}$ binding (1) and after Hg$^{2+}$ binding (2). Spectra figure reproduced with permission from Ref. [97].](image)

Catalysis is one of the most significant applications of [Au$_{25}$(SR)$_{18}$]$^{1-}$ nanoclusters that is currently being investigated. Catalytic applications of [Au$_{25}$(SR)$_{18}$]$^{1-}$ typically involve thermal deposition of the [Au$_{25}$(SR)$_{18}$]$^{1-}$ system onto a metallic oxide support, which is a necessary component to achieve optimal catalytic activity, with free [Au$_{25}$(SR)$_{18}$]$^{1-}$ systems typically undergoing deterioration under catalytic conditions. Density functional theory (DFT) has provided valuable insight in the molecular and structural features of [Au$_{25}$(SR)$_{18}$]$^{1-}$ contributes to its catalytic activity. The Zeng group used DFT to propose that the post-deposition catalytic sites...
are the Au(I) atoms in the staple motifs, instead of the internal Au(0) atoms in the Au$_{13}$ core.\footnote{100} There is also a strong dependence on the structure and electronic composition of the capping thiol ligand on the surface, which essentially provides an ‘active site’ (similar to the active sites in enzymes) to orient the substrate on the [Au$_{25}$(SR)$_{18}$]$^+$ surface and catalyze the desired transformation. Smaller capping ligands gives reactants better access to the internal catalytic sites,\footnote{101} and ligands containing phenyl moieties are especially effective in increasing catalytic performance that is likely through facilitation of π-π stacking between substrates and ligand \cite{Figure 1.13a}.\footnote{102} To highlight a notable catalytic application of oxide-supported [Au$_{25}$(SR)$_{18}$]$^+$, the Jin group thermally deposited [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^+$ onto a titanium dioxide support, which was an excellent catalytic system for semi-hydrogenation of terminal alkynes to alkenes (Figure 1.13b),\footnote{103} that was more effective than most analogous catalytic systems.

![Figure 1.13. (a) Catalytic activity of [Au$_{25}$(SR)$_{18}$]$^+$ thermally deposited onto CeO$_2$ support, using different capping ligands, for Ullmann heterocoupling between 4-methyl-iodobenzene and 4-nitro-iodobenzene, in presence of K$_2$CO$_3$ in DMF at 130°C for 24 hours. Synthetic data taken from Ref. [101]. (b) Catalytic activity of [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]$^+$ thermally deposited onto TiO$_2$ support, for semi-hydrogenation of terminal alkynes, in presence of pyridine and H$_2$ at 100°C for 20 hours. Synthetic data taken from Ref. [103].](image)

The [Au$_{25}$(SR)$_{18}$]$^{+}$ framework has also been used towards drug delivery applications. As described in Section 1.2.4, when therapeutic drugs such as doxorubicin (DOX) is inoculated as a free molecule, there oftentimes is excessive extracellular degradation by enzymes, preventing optimal therapeutic effects.\footnote{50} However, when these same agents are conjugated onto the surface of nanomaterial carriers, the dense packing of the agent on the nanomaterial surface prevents premature degradation, enhancing their therapeutic effects. Although the mechanism of internalization is not yet known, as with AuNP systems, [Au$_{25}$(SR)$_{18}$]$^+$ frameworks are internalized by a variety of cellular lines. Zhang \textit{et al.} synthesized a carboxyl-terminated
[Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−}] framework, and then conjugated DOX to the terminal carboxyl groups via electrostatic interactions to create a [Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−}–DOX nanoconjugate (Figure 1.14).\textsuperscript{104} This conjugate was readily internalized by A549 lung cancer cells, and due to the fluorescence of the DOX molecules, the efficacy of internalization could be evaluated by fluorescence microscopy. As with AuNP delivery of DOX, they also found that the [Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−}–DOX nanoconjugate was more effective and decreasing A549 cell viability, compared to DOX on its own.\textsuperscript{104}

\textbf{Figure 1.14.} Left. Schematic representation of DOX delivery by Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−}–DOX nanoconjugate. Right. Confocal images of A549 cells after incubation with Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−}–DOX nanoconjugate after 1 hour (top right) and 3 hours (bottom right). Red = doxorubicin. Figures of confocal images reproduced with permission of Ref. [104].

1.4 Surface Modifications of AuNPs and AuNCs

As outlined in Section 1.2.4 and Section 1.3.5, the ability to modulate the surface compositions of AuNPs and AuNCs (particularly the [Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−} framework) is of paramount importance for application-based research, in order to either fine-tune their structure-dependent properties (which is in large part dependent on the surface configurations) or passivate relevant substrates on their surface to induce the desired chemical or biological effect. To this end, in addition to application-based research itself, there has been an extensive pursuit towards developing methodologies capable of modifying the surface structure of AuNPs and AuNCs (particularly the [Au\textsubscript{25}(SR\textsubscript{18})\textsuperscript{1}\textsuperscript{−} framework).
The most obvious strategy would be a direct synthetic approach using the Brust-Schiffrin method, in which the thiolated metallic frameworks are fabricated using a gold (III) precursor and a thiolated ligand possessing the desired structure and/or surface functionality (Scheme 1.3, Method 1). Although simple frameworks such as the \([\text{Au}^{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}]^{-}\) framework can be synthesized using direct methods, more complex systems such as oligonucleotide-functionalized AuNPs or \([\text{Au}^{25}(\text{SR})_{18}]^{-}\) frameworks with therapeutic agents passivated on their surfaces, typically cannot be fabricated using direct synthetic methods because of:

1. The chemical sensitivity of the substrates (such as carbonyl groups) with sodium borohydride.

2. The inability to acquire the desired framework with large, complex thiolated ligands, with this mismatched relationship between ligand structure and framework structure being especially present in \([\text{Au}^{25}(\text{SR})_{18}]^{-}\) syntheses.

As direct synthetic approaches are generally inaccessible to generate functionally complex systems, approaches using post-assembly modifications are generally employed. One strategy is to incorporate reactive nucleophilic handles (such as -OH or -NH\(_2\)) to the exterior of the surface monolayers using direct synthetic methods, and conduct coupling reactions to electrophically-activated substrates.\(^{105,106}\) Another strategy is to manufacture carboxyl-terminated systems using direct synthetic methods and perform post-assembly acyl coupling reactions in the presence of coupling agents.\(^{107,108}\) Although such strategies have found some utility, the main problems associated with them are:

1. The nucleophilicity and reactivity of the reactive handles makes such systems difficult to synthesize and purify.

2. Such post-assembly coupling strategies are often limited by their less than quantitative yields, preventing efficient surface derivatization and can lead to AuNP degradation.

3. The presence of competing reactive groups (such as -NH\(_2\) moieties in oligonucleotides) can initiate undesired reactivity.
Due to these synthetic complexities, the most common method for engineering functional AuNP and $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ systems is through ligand exchange chemistry, which was employed in all the studies described in Section 1.2.4 and Section 1.3.5 in which functional systems were fabricated. In this method, simple and chemically inert thiolated ligands are used to construct AuNP or $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ templates using direct synthetic methods, after which native ligands are exchanged with thiolated-ligands possessing the desired structure or functional substrate, typically through simple mix-and-stir reactions (Scheme 1.3, Method 2). The degree of ligand exchange is dependent on several factors, such as the structure of the native and incoming ligands,\(^{109}\) the electronic charge of the framework\(^ {110}\) and the molar ratio of the metallic system and incoming thiol,\(^ {111}\) with typically large concentrations of thiol being necessary for efficient ligand exchange. Although the ligand exchange strategy has found great utility in developing functional AuNP and $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ systems, there are three common problems associated with it:

1. The functional substrate and thiol moiety are oftentimes not chemically compatible. Furthermore, due to the reactivity of thiols, protection/deprotection strategies are often required to successfully tether the thiol moiety. These conditions may not be compatible with the desired functional substrate and leads to low yields for the desired thiolated-substrate (such as the in the synthesis of thiolated-oligonucleotides).

2. Exchange of native thiols with functional thiolated-substrates that are too largely different in structure can lead to an alteration of the parent system. This especially presents a problem in ligand exchange strategies using $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ systems, in which ligand exchange can alter the internal framework.

3. Finally, the excessive concentrations of the incoming thiol can be limiting if they are synthesized in low yield and/or expensive to make.

It is because of the complications inherent to direct synthetic methods, post-assembly coupling strategies and ligand exchange strategies that it is of paramount importance to develop alternative strategies to engineer functional AuNP and $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ systems to minimize or entirely eliminate such limitations and enhance their usage for application-based research.
Scheme 1.3. Synthesis of functional AuNP or AuNC systems for application-based research, using the direct synthetic method (Method 1) or the ligand exchange method (Method 2).

1.5 Bioorthogonal Click Chemistry

1.5.1 General Characteristics of Bioorthogonal Click Reactions

The inception of “bioorthogonal chemistry” is rooted in the early investigations from the late 20th century when scientists began to develop an intense interest in how biomolecules behave, organize and interact with one another in their natural settings. Although the general shape of cellular systems could be observed with the development of the TEM, the visualization and understanding of internal biochemical processes required scientists to develop in vivo strategies to manipulate biomolecules on the atomic level to produce measurable and visualizable responses. Such manipulations need to be done (1) without altering the structure and spatiotemporal responses of the biomolecule and (2) not perturb the native settings in which they operate in; these criteria encompass the term “bioorthogonal”.

One of the earliest and foremost discoveries towards understanding cellular processes through bioorthogonal strategies was “the discovery and development of green fluorescent protein
(GFP)”, which earned the shared 2008 Nobel Prize in Chemistry to Osamu Shimomura (for first isolating GFP from the jellyfish *Aequorea victoria*)\(^{112}\) Martin Chalfie (for demonstrating the usefulness of GFP as a luminescent genetic tag)\(^ {113}\) and Roger Tsien (for understanding the structural features that makes it fluorescent).\(^ {114}\) Once the nucleic acid sequence of GFP was determined,\(^ {115}\) its sequence could be fused to the ends of recombinant pre-translational sequences as a genetic “reporter”, and cellular translation of the entire sequence furnished fluorescent analogues of the native protein that could be visualized and monitored.\(^ {116-118}\) Although the discovery of GFP marked a landmark breakthrough in understanding native cellular activities, the post-translational size of the GFP protein (238 amino acids) renders it incompatible with some protein structures.\(^ {118}\) Furthermore, GFP can only serve as a genetically encoded tag for proteins, and other important biomolecules such as nucleic acids, lipids and glycans cannot be monitored using genetically encoded reporters.

A major breakthrough in circumventing this limitation came from Roger Tsien’s group in 1998, when he reported the first example of *in vivo* protein labelling, in which recombinant proteins possessing a tetracysteine domain were labelled with small, reactively complementary biarsenical dyes inside the cell.\(^ {119}\) The small size of the organic dye, compared to post-translational size of GFP, presented an important advantage over GFP labelling strategies. However, the most important difference is that the exogenous agent in GFP labelling strategies is recombinant sequences containing the GFP gene, which relies on intracellular translation. This new strategy proposed a revolutionary new labelling paradigm, in which the exogenous agent was a small chemical dye, which was integrated into the cell, bound to the target recombinant protein, and then imaged using florescence microscopy. This early research triggered an intense interest in developing strategies to modify and observe biomolecules in their natural environments with “bioorthogonal chemical reporters” (instead of genetically encoded reporters).

Since the report by the Tsien group, there have been extensive studies describing different strategies for bioorthogonal labeling. Of these, no class of strategies has embodied the concept of “bioorthogonal chemical reporters” better than that first proposed by Carolyn Bertozzi in 2009, who not only first coined the term “bioorthogonal”, but also proposed that biomolecules and reporters be linked together intracellularly using “click chemistry”, and iconized the term “bioorthogonal click chemistry”.\(^ {120}\) The concept of “click chemistry” pre-dates Bertozzi’s
exemplar study, and was a term first coined in 2001 by chemists Barry Sharpless, Hartmuth Kolb and M.G. Finn to describe a series of highly efficient reactions in synthetic chemistry that simply ‘clicked’ together, such as the Diel-Alder reaction and additions to carbon-carbon multiple bonds.\textsuperscript{121} They constrained the term “click reactions” to those that:

1. Have high thermodynamic driving forces.

2. Proceeds rapidly to completion (i.e. rapid reaction kinetics).

3. Be highly selective for one another in the presence of other reactive functional groups (i.e. chemoselective).

4. Proceed in the absence of catalysts.

5. Ideally produce no by-products, that if present, can easily be removed and is non-perturbing.

**Scheme 1.4.** A “*bioorthogonal click reaction*”. The first reactive group is exogenously added and intracellularly incorporated into the target biomolecule using the cellular machinery. A chemical reporter possessing the second (complementary) reactive group is then exogenously added. The reaction between the first and second reactive groups need to proceed selectively in the presence of all the functionalities found within cellular systems, some of which are shown, and the biomolecule is then labelled with the chemical reporter.

The term “click chemistry” was initially confined to highly efficient organic transformations in synthetic settings, and in 2009, Bertozzi recognized the potential for using such highly efficient click reactions for *in vivo* labelling studies. Unlike in synthetic settings, these need
to be done in more delicate settings, and thus she coined the term “bioorthogonal click chemistry” to describe sets of reactions that can be used to ‘click’ reporters and chemically sensitive biomolecules within biologically sensitive environments (Scheme 1.4).

1.5.2 Common Bioorthogonal Click Reactions

To date, there have been many chemical reactions that meet many of the criteria of both “click reactions” and “bioorthogonality”, albeit to different extents. One of the most widely utilized bioorthogonal click reactions is the Staudinger-Bertozzi ligation, which is a modification of the classical Staudinger reaction first reported by Hermann Staudinger in 1919.122 The classical Staudinger reaction is between a terminal azide (N$_3$) dipole and triarylphosphine to form an aza-ylide (and nitrogen gas), which is subsequently hydrolyzed to form a phosphine oxide and primary amine.123 In 2000, Bertozzi reported a modification of the classical Staudinger reaction whereby an ester group was tethered ortho to the phosphorus atom in one of the three phenyl rings in the triarylphosphine. Upon reaction of the terminal azide and the phosphine, the aza-ylide reacts with the ester group to form an amide linkage (instead of a primary amine), ligating the group attached to the azide to the triarylphosphine (Scheme 1.5a).124 Although it is one of the predominant bioorthogonal click reactions utilized to conjugate two chemically sensitive substrates,125-127 the major limitation of the Staudinger-Bertozzi ligation is the slow reaction kinetics ($\sim$10$^{-3}$ to 10$^{-1}$ M$^{-1}$s$^{-1}$),128 which necessitates larger concentrations of the reactive partners in order to drive successful amide formation, limiting their usage in vivo. Furthermore, although the azide moiety is exceptionally stable, the sensitivity of the phosphine moiety to air oxidation, and the lability of the phosphine to enzymatic cellular degradation is another common problem associated with using phosphines, limiting the “bioorthogonal” aspect of the reaction.129

Addressing the slow reaction kinetics of the Staudinger-Bertozzi ligation, in 2008 the Fox group developed another bioorthogonal click reaction between a trans-cyclooctene (TCO) and s-tetrazines, which is known as the “TCO-tetrazine ligation” (Scheme 1.5b), which forms nitrogen gas as the only by-product and proceeds with reaction kinetics reaching as high as 2000 M$^{-1}$s$^{-1}$ (in 9:1 methanol:water).130 The reaction has shown to be tolerable to a variety of solvent systems, although the reaction kinetics are affected by the solvent system used. Although the TCO-tetrazine ligation has found usage for in vivo applications,131-133 the major limitation of the TCO-tetrazine
ligation is the conformationally strained TCO moiety, which in many conditions can spontaneously cycloisomerize into the more stable, less strained cis-isomer. The TCO moiety is also very reactive towards thiols, which can be problematic when used in vivo due to thiol moieties present in protein backbones.

Another noteworthy bioorthogonal click reaction that was developed to mitigate the slow reaction kinetics of the Staudinger-Bertozzi ligation is the reaction between a terminal azide and an oxanorbornadiene, which forms a triazole moiety in a 1,3-dipolar cycloaddition reaction, generating a molecule of furan as the only by-product (Scheme 1.5c). This bioorthogonal click reaction has found usage in biomedical applications. However, as with the Staudinger-Bertozzi ligation, whereas the azide moiety is chemically stable, the major limitation of the azide-

(a) Staudinger-Bertozzi Ligation

\[
R_1\text{-N}=\text{N}^\text{+}^2\text{-N}^\text{2-} + R_2\text{-O}\text{Me} \rightarrow H_2O \xrightarrow{N_2} \xrightarrow{-MeOH} R_1\text{-N}\text{Ph}_2 \text{R}_2
\]

(b) Trans-Cyclooctene-Tetrazine Ligation

(c) Azide-Oxanorbornadiene Cycloaddition

Scheme 1.5. Three common “bioorthogonal click reactions”. (a) The Staudinger-Bertozzi ligation between a terminal azide and phenyl ester-functionalized phosphine. (b) The trans-cyclooctene-tetrazine ligation. (c) The azide-oxanorbornadiene cycloaddition.
oxanorbornadiene cycloaddition reaction is the oxanorbornadiene, which requires electron-deficient moieties to be tethered on to the oxanorbornadiene in order to activate it for the reaction to proceed successfully, a structural restriction that dually presents a risk in biological settings.¹³⁸

1.5.3 Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) Reaction

Most of the limitations commonly associated with other bioorthogonal click reactions, primarily the chemical instability of the reactive groups, can be largely mitigated by the reaction between an azide and terminal alkyne moieties. The prototype reaction was presented in 1963 by Rolf Huisgen, who reported a [3+2] cycloaddition between an azide dipole and terminal alkyne dipolarophile to afford an equimolar mixture of 1,4- and 1,5-disubstituted 1,2,3-triazole cycloadducts (Scheme 1.6a), without the formation of any by-products.¹³⁹ As these two π-systems rarely exist in nature, are largely inert within biological and natural settings, and also feature excellent functional group compatibility, this made the “Huisgen cycloaddition reaction” a promising candidate as a bioorthogonal click reaction. However, in the absence of activation of the alkyne by delocalizing it into electron withdrawing substituents (such as ester groups), the reaction does not proceed efficiently without elevated temperatures and pressure,¹³⁹ which diminishes its bioorthogonality.

In 2002, the Sharpless and Meldal groups independently discovered that the reaction can be made to proceed in the presence of a catalytic amounts of copper (I) salts, which activates the alkyne towards the [3+2] cycloaddition reaction in the absence of functional group activation (Scheme 1.6b), again in the absence of by-product formation.¹⁴⁰,¹⁴¹ This “copper (I)-catalyzed alkyne-azide cycloaddition (CuAAC)” reaction can be performed at physiological temperatures with reaction kinetics ~10⁶ fold faster than the Huisgen cycloaddition reaction.¹⁴¹ It also generates a single 1,4-disubstituted triazole regioisomer, though the regioisomeric nature of the cycloaddition reaction is not generally problematic in biological labelling studies. The ability to conduct the CuAAC reaction under milder reaction conditions made it a more bioorthogonal variant of the Huisgen cycloaddition reaction, but the necessity of the copper(I) catalyst, which is typically cytotoxic,¹⁴² still limited the bioorthogonality of this alkyne-azide reaction.

The first reported [3+2] cycloaddition reaction between cyclooctyne, the smallest stable non-linear cycloalkyne, and phenyl azide was reported by Krebs and Wittig in 1961, who reported that two reacted “like an explosion”.¹⁴³ The utility of this reaction as a potential bioorthogonal
click reaction wasn’t realized until 2004, when the Bertozzi group reported that the reaction can be used to label biomolecules bioorthogonally. By constraining the sp-hybridized alkyne moiety within an eight-membered ring, this creates a large amount of strain energy (~18 kcal/mol), which ‘activates’ the alkyne, and permits the reaction to proceed in the absence of high temperatures, functional group activation or catalysts. Remarkably, although the ring strain associated with the non-linear alkyne activates it towards [3+2] cycloaddition chemistry, it remains largely inert in biological and other chemically sensitive settings. This reaction between a strained-cyclooctyne and azide moiety is termed the “strain-promoted alkyne-azide cycloaddition (SPAAC)” reaction (Scheme 1.6c). As with the Huisgen cycloaddition reaction, it produces two regioisomers that are not typically problematic in biological labelling studies.

**Scheme 1.6. Common reactions between terminal azides and alkyne moieties.** (a) The alkyne-azide Huisgen cycloaddition reaction between a terminal alkyne and terminal azide under high temperature (and/or pressure). (b) The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction between a terminal alkyne and terminal azide in the presence of a copper (I) catalyst. (c) The strain-promoted alkyne-azide cycloaddition (SPAAC) reaction between a cyclooctyne and terminal azide.
The discovery of SPAAC as a bioorthogonal click reaction represented a landmark breakthrough in chemical biology that can be used combine two chemically sensitive substrates in biological settings, that would otherwise be difficult or impossible to achieve. It features the same functional group tolerance and biocompatibility of the two reactive partners used in the Huisgen cycloaddition and CuAAC reactions, but also comes without the limitations of not just these two alkyne-azide cycloaddition variants, but also without the limitations associated with other bioorthogonal click reactions, making it a truly bioorthogonal reaction.

1.5.4 Strain-Promoted Alkyne-Nitrone Cycloaddition (SPANC) Reaction

An analogous variant of the SPAAC reaction was developed by the Pezacki group, where the azide dipole was replaced with a nitrone dipole, and is termed the “strain-promoted alkyne-nitrone cycloaddition (SPANC)” reaction (Scheme 1.7). Although the nitrone dipole is prone to hydrolysis in the presence of strong acids, whereas the azide is not, the SPANC reaction has found great utility in biological labelling studies. One of the key advantages of the SPANC reaction, compared to the SPAAC reaction, is that while the terminal azide moiety has one modifiable site, the nitrone moiety has two modifiable sites, namely the nitrogen (Nα) and carbon (Cα) atoms of the nitrone moiety. As shall be discussed in Section 1.5.5, the ability to more fine-tune the composition of the nitrone dipolar moiety through substituent modifications has important implications on their reactive profile, that cannot be achieved to the same extent using azides.

Scheme 1.7. The strain-promoted alkyne-nitrone cycloaddition (SPANC) reaction between a nitrone and cyclooctyne.
1.5.5 Kinetically-Variable SPAAC/SPANC

In addition to the chemical stability, biocompatibility, functional group tolerance and ability to perform the reactions under ambient physiological conditions, one of the key features of the SPAAC and SPANC reactions that renders it an important tool is the ability to tune their reaction kinetics. Compared to the Staudinger ligation, which is the other predominant bioorthogonal click reaction used in contemporary literature, the SPAAC and SPANC reactions feature more rapid reaction kinetics. Although the TCO-tetrazine ligation is more rapid than the SPAAC and SPANC reactions, trans-cyclooctenes are susceptible to isomerization that inactivates them towards the reaction, which limits its utility. Rapid reaction kinetics is a key feature towards developing efficient bioorthogonal click reactions, because it reduces the effective concentrations of the two reactive partners necessary to obtain optimal coupling yields. If the reaction were too slow, then larger excess of the two reactive partners would be required to acquire the same yield than if the reaction were faster.

Figure 1.15. Some commonly used cyclooctynes, with associated second-order rate constants for reaction with benzyl azide. Second-order rate constants taken from references [149] and [150].
Much of the rapid kinetic profiles of the SPAAC and SPANC reactions can be attributed to the ring strain in the cyclooctyne moieties. In recent years, there has been an interest in further improving the kinetic profiles of the SPAAC and SPANC reactions through structural changes to the cyclooctyne (Figure 1.15). Although addition of electron-withdrawing substituents provides a minor change to their kinetic profile, the greatest increase in reaction kinetics can be achieved by fusing the cyclooctyne ring to aryl rings, rigidifying the cyclooctyne ring and vastly increasing the ring strain of the alkyne moiety. Although such ‘benzoannulated’ cyclooctynes have shown to react much more rapidly than their ‘aliphatic’ counterparts, increasing the ring strain of the cyclooctyne moiety is associated with a problematic tradeoff, in that it concurrently makes them less stable. Benzoannulated strained-alkynes also tend to have shorter shelf lives, and are synthetically more inaccessible than aliphatic cyclooctynes. This presents a restrictive constraint on improving the reactive profiles of the cyclooctyne reactive partner in the SPAAC and SPANC reactions.

It is for this reason that contemporary efforts have converged on improving the reaction profiles of the SPAAC and SPANC reactions through structural modifications to the azide or nitrone dipoles. The Pezacki group first reported that delocalizing the nitrone moieties into electron withdrawing substituents (such as cyano groups) greatly increases the reaction kinetics of the SPANC reactions, and conversely when the nitrone moieties are delocalized in electron donating substituents (such as methoxy groups), the SPANC reaction is decelerated (Figure 1.16). In a later study, the Dommerholt group reported the same effect in the SPAAC reaction with aromatic azides. Delocalizing the azide moiety into electron withdrawing substituents such as pyridinium and pentafluorophenyl groups greatly improved the reaction kinetics of the SPAAC reaction. Conversely, delocalization into electron donating substituents such as anisole groups greatly decelerated the reaction rates (Figure 1.16).

1.6 Scope of Thesis

As outlined in Section 1.2.4 and Section 1.3.5, the ability to adjust and modulate the surface structure of AuNPs and AuNCs (specifically [Au$_{25}$(SR)$_{18}$]$^+$) and develop functional varieties of these nanomaterial frameworks is of paramount importance for application-based research. As direct synthetic methods are typically inaccessible, ligand exchange strategies are often employed.
Figure 1.1. Top. Kinetically variable SPAAC and SPANC through structural modifications to the azide and nitrone moieties, respectively. Bottom. Structures of azides and nitrones and their associated second-order rate constants.

This can have limited utility that can be attributed to the reactivity of the thiol moiety, difficulty in fabricating thiolated functional substrates and an inability to establish efficient surface ligand exchanges (Scheme 1.8a). In particular, whereas polydisperse AuNP structures tend to be quite chemically rigid and less affected by ligand exchange processes, the sensitive relationship between surface ligand structure and core configuration of monodisperse \([\text{Au}_{25}(\text{SR})_{18}]^{1-}\) frameworks presents a risk when attempting similar exchange processes, which can lead to undesired alterations to the parent metallic core and loss of monodispersity (Scheme 1.8a). Such drawbacks of ligand exchange strategies necessitate investigations into alternative strategies to modify the surface structure of these nanomaterial frameworks more reliably, without altering their chemically sensitive core structures.
The conceptual development of “bioorthogonal click chemistry” was a major breakthrough in chemical biology, defining a set of reactions that can be conducted to tether ‘chemical reporters’ to chemically-sensitive biomacromolecule frameworks (e.g. proteins etc.) within biologically sensitive environments. The ideal bioorthogonal click reaction binds the reporter to the biological framework without altering the parent structure (and related properties and functions), and at the same time produce measurable changes to the framework that is inherent to the choice of reporter (e.g. fluorophore-tagged proteins). Of the repertoire of bioorthogonal click reactions currently available, the strain-promoted dipolar cycloaddition reactions, SPAAC and SPANC, have proved to be the most versatile because of they have following the following advantages not shared among other common bioorthogonal click reactions:

1. The terminal azide and cyclooctyne moieties are very stable under most conditions, but at the same time have a high thermodynamic driving force to react chemoselectively with each other.

2. They are functional group and solvent tolerant.

3. Can be performed under mild reactions conditions at room temperature, typically through simple mix-and-stir reactions.

4. Have fast reaction kinetics, which can be modulated through structural considerations to the reactive partners, and so lowers the effective concentrations required to achieve efficient coupling compared to slower reactions.

The aim of this thesis is to translate these advantageous features of the SPAAC and SPANC reactions towards more efficient surface modifications of AuNPs and the \([\text{Au}_{25}(\text{SR})_{18}]^{1-}\) framework, as a more reliable and efficient alternative to ligand exchange strategies that can lead to undesired structural alterations. The conception of these versatile “bioorthogonal” reactions as a tool in chemical biology is rooted in the ability to conduct them without perturbing sensitive biological structures and the sensitive environments in which they operate. Not only does the SPAAC and SPANC reactions meet these criteria, but unlike many other bioorthogonal reactions, the two reactive partners are very stable in biological settings, making the SPAAC and SPANC reactions truly “bioorthogonal click reactions”. Given the ability to conduct them in biologically
sensitive environments, the research herein presented demonstrates that SPAAC and SPANC reactions can likewise be conducted in the chemically sensitive AuNP and (in particular) \([\text{Au}_{25}(\text{SR})_{18}]^{+}\) surface environments. In this way, the aim of this thesis is to present the SPAAC and SPANC reactions as “nanoorthogonal click reactions” (Scheme 1.8b), capable of linking function-altering ‘reporters’ nanoorthogonally to the surfaces of chemically sensitive nanomaterial platforms in an efficient manner without altering their parent structure (Scheme 1.8).

Scheme 1.8. (a) Synthesis of functional AuNP/AuNC through ligand exchange reaction. Addition of chemical reporter possessing thiol group undergoes exchange with native thiols at the metallic core. Reaction at core can result in altered core framework. Quantitative exchange is difficult to accomplish, leading to mixed monolayer. (b) Synthesis of functional AuNP/AuNC through “nanoorthogonal click reaction”. Addition of chemical reporter possessing second reactive group (azide or cyclooctyne) undergoes chemoselective reaction with nanomaterial platform possessing first (complementary) reactive group on the exterior interface. Reaction at interface less likely to result in altered core framework. Quantitative interfacial reaction can be achieved reliably.

Our group has recently explored the use of SPAAC for nanoorthogonal surface modifications of AuNPs. Due to the superior stability and chemical inertness of the azide moiety (compared to the cyclooctyne moiety), our seminal study reported the development of an azide-functionalized AuNP platform (giving “AuNP-azide”) that could undergo post-assembly interfacial SPAAC (I-SPAAC) chemistry with complementary strained-alkynes.\(^{154}\) Realizing that the azide and strained-alkyne groups were compatible with the AuNP surface environments, and
that the I-SPAAC reaction was nanoorthogonal to AuNPs, laid the foundation for many studies to follow.

We subsequently reported a complementary variant of the AuNP-azide platform by incorporating the strained-alkyne moiety, dibenzocyclooctyne (DBCO), to the AuNP surface (giving “AuNP-DBCO”). The advantage of this approach is that the superior stability of the azide moiety makes it easier to tether to complementary functional substrate reporters. However, as described in Section 1.5.5, benzoannulated cyclooctynes are inherently less stable than their aliphatic counterparts, and have shorter self-lives, which can create compatibility issues when trying to conduct surface chemistry in sensitive environments. To make a more stable variant of AuNP-DBCO, Chapter 2 focuses on the incorporation the more stable, aliphatic strained-alkyne bicyclo[6.1.0]nonyne (BCN), to give “AuNP-BCN”. The characterization of AuNP-BCN and quantification of interfacial BCN is described. The kinetically variable reactivity of AuNP-BCN towards a suite of different azides (through I-SPAAC) and nitrones (through I-SPANC) was also explored. Using this kinetic study, competition experiments between equimolar amounts of AuNP-BCN, an azide and a nitrone was investigated.

After the development of AuNP-azide, we also reported a synonymous variant by incorporating the nitrone moiety to the AuNP surface (giving “AuNP-nitrone”), which could undergo interfacial SPANC (I-SPANC) with cyclooctynes. The main drawback of this platform was the slow reactivity of the nitrone chosen for this study, which had kinetically-decelerating electron-donating groups on both the Nα and Cα positions (see Section 1.5.5). To make a more reactive variant of AuNP-nitrone, Chapter 3 reports the development of highly electron-deficient nitrones possessing pyridinium functionalities on the Cα position. Alterations to the kinetic profiles of these “pyridinium-nitrones” could be accomplished through modifications to the Nα position, with either electron-donating or electron-withdrawing substituents. Attempts were made to synthesize a thiolated ligand with a terminal pyridinium-nitrone group to incorporate it to the AuNP surface through either a direct or ligand exchange strategy. However, the pyridinium-nitrone group appears to undergo hydrolysis in the presence of the thiol group, preventing the development of such a ligand.
Having thoroughly explored nanoorthogonal click chemistry for surface modifications of AuNPs, our group became interested in conducting such strategies with AuNCs. This presents a particularly advantageous paradigm for AuNC surface modifications, which relies on coronal surface modifications, instead of risky ligand exchanges at the metallic core that would likely alter the internal configuration and diminish the monodispersity of the parent AuNC system (see Section 1.4). As with our seminal studies exploring SPAAC chemistry on AuNPs, the incorporation of the surface azide moieties was explored due to its superior chemical stability. Chapter 4 describes the first example of an azide-functionalized [Au$_{25}$(SR)$_{18}$]$^{1-}$ platform (giving “$p$-azido”), which was confirmed by single-crystal X-ray diffraction. This $p$-azido platform is capable of undergoing cluster-surface SPAAC (CS-SPAAC) quantitatively, without altering the chemically sensitive core structure. This study demonstrates that the SPAAC reaction can be conducted as a nanoorthogonal reaction for surface modifications of the most popular AuNC framework that is currently being used towards application-based research.

Having developed the $p$-azido platform, Chapter 5 describes the development of two isomeric forms of $p$-azido (namely, “$m$-azido” and “$o$-azido”) to explore how the optical properties, electrochemical properties, and reactivity of these azide-functionalized [Au$_{25}$(SR)$_{18}$]$^{1-}$ platforms correlated with the isomeric forms of the azide-functionalized surface ligands. The molecular structures of the neutral forms of the isomers, [Au$_{25}$(SR)$_{18}$]$^{0}$, are reported. Although the optical properties of the three isomeric forms are nearly identical, the electrochemical responses appear to correlate with the position of the azide groups. Kinetic studies indicate that the reactivity of $p$-azido is higher than that of $m$-azido, while the $o$-azido platform undergoes deterioration when reacted with a strained-alkyne and so is not nanoorthogonal to CS-SPAAC chemistry.

Having established that the $p$-azido platform is the most reactive and the most reliable of the three isomeric variants, Chapter 6 highlights the development of the first example of a ferrocene-functionalized [Au$_{25}$(SR)$_{18}$]$^{1-}$ framework, through CS-SPAAC between $p$-azido and a strained-alkyne-modified ferrocene derivative (to give “$p$-ferrocenyl”). The optical properties of this modified [Au$_{25}$(SR)$_{18}$]$^{1-}$ framework is reported. The electrochemical pattern of $p$-azido and $p$-ferrocenyl is presented, with the spectra of $p$-ferrocenyl containing a large peak that indicates that all surface ferrocene groups are electrochemically accessible.
Chapter 7 summarizes the overall contributions of this work and provides a commentary on how the work presented can be used towards surface modifications of AuNPs and the \([\text{Au}_{25}\text{(SR)}_{18}]^\text{1}\) framework for application-based research.

1.7 References


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Chapter 2

2 Towards the Design of Self-Sorting Nanomaterials Through Kinetically Directed Orthogonal Control over Interfacial Surface Chemistry

This chapter is being submitted as a short paper and is in manuscript format. Max Weismann and Prof. Pierangelo Gobbo are co-authors, along with Prof. Mark S. Workentin.

Max Weismann was an undergraduate student under co-supervision of myself and Prof. Pierangelo Gobbo (who was a graduate student at the time), along with our supervisor Prof. Mark S. Workentin, who assisted in the synthesis and characterization of the AuNP-BCN platform. The draft of the chapter was written by the author and edited by Prof. Mark S. Workentin.

2.1 Introduction

Recent progresses in self-assembly research have explored the development of self-sorting materials, which are materials capable of exclusively reacting orthogonally with complementary analytes in a complex media containing non-competing analytes to form “asocial” or “social” hierarchical structures. Asocial self-sorting encompasses orthogonal assembly of materials systems that are compositionally identical, whereas socially self-sorting materials are capable of orthogonally hetero-assembling in multicomponent structures possessing compositionally different material systems. Such pre-defined organization is achieved by designing material building blocks that store defined chemical information, which determines their role in the self-assembly process and their final location in the self-assembled material. Unlike indiscriminate self-assembly, which involves structural organization that is independent of material type and chemical composition, directed control over assembly of simpler material building blocks to construct complex structures makes it possible to develop material systems whose structure and function can be programmed depending on the chemically pre-determined assembly instructions. Such an approach has led to the generation of hierarchical structures that would not be possible to generate otherwise, paving the way towards the design of new classes of materials that presents new collective properties and extends their potential applications.
Self-assembly is primarily governed by selective orthogonal interactions between material building blocks to trigger co-assembly.\textsuperscript{10, 11} Such orthogonality can be pre-programmed in the chemical information on the surface of the constituent building blocks, which allows for a great deal of control over which higher-order structures can be attained in a complex media containing multiple building blocks.\textsuperscript{12} The most common method to accomplish asocial/social self-sorting is to install molecular units on the surface of individual building blocks, each of which is capable of orthogonally interacting with other complementary units on the surface of other building blocks. Such programmable control is typically achieved through lock/key (specifically host/guest) molecular interactions between hetero-complementary groups, in which a multi-component mixture can be made to assembly into supramolecular structures based on the “lock” and “key” group that is installed on individual components (Figure 2.1a).\textsuperscript{13} Han et al. demonstrated the social self-sorting of different co-assembled families of colloid structures using a mixture of four building blocks, which was programed using high-interaction host/guest recognition chemistry between cyclodextrins (host/lock) and ferrocene/azobenzene (guest/key).\textsuperscript{14} Although such strategies are notable due to the highly predictable manner in which building blocks can assemble, it is limited by the requirement of designing different host material frameworks that is capable of interacting with complementary guest groups on different material building blocks.

The development of self-sorting nanomaterials has not received much attention, because of the difficulty in programming the nanomaterials to display well-defined orthogonal interactions so that self-assembly can be triggered.\textsuperscript{15, 16} Furthermore, host/guest chemistry is difficult to implement on nanomaterial surfaces because of the sensitive relationship between nanomaterial integrity and surface composition. In this work, we not only address the challenge of designing nanomaterial systems in which orthogonal control over surface modifications can be achieved, but also provide an alternative approach to accomplish orthogonal control. As opposed to implementing a host/guest-directed approach to accomplish self-sorting (Figure 2.1a), we herein present a kinetics study where we propose that self-sorting can be accomplished using a kinetically-directed approach (Figure 2.1b). The major drawback to the most common lock/key self-sorting methodologies is the necessity of developing a library of host material frameworks capable of orthogonally interacting with their individual complementary guest partners. Instead of relying on the development of a library of nanomaterial frameworks possessing unique lock groups, we report a singular nanomaterial platform possessing one reactive lock group, which can
orthogonally react with a wide variety of complementary key groups in a kinetically predictable manner, depending on the chemical composition of the key groups.

**Figure 2.1.** (a) Schematic representation of host/guest directed- and kinetically-directed self-assembly strategies. (b) The strain-promoted alkyne-azide cycloaddition reaction (SPAAC) to form a triazole cycloadduct (right), and the strain-promoted alkyne-nitron cycloaddition reaction (SPANC) to form an isooxazoline cycloadduct (left). (c) Schematic representation of kinetically-directed self-assembly strategy using SPAAC and SPANC.

### 2.2 Results and Discussion

For our proof-of-concept study to demonstrate our kinetically-directed approach for modifying nanomaterial surfaces, we used small (~3 nm) gold nanoparticles as a model nanomaterial, which was chosen because they are comparatively stable, easy to characterize, but most importantly, because changes in surface composition can be easily monitored by \(^1\)H NMR spectroscopy. To develop a prototype gold nanoparticle platform capable of kinetically-directed surface modifications, a reactive strained-alkyne moiety (the lock group) was tethered to the nanomaterial surface. The surface strained-alkynes are capable of orthogonally reacting with both azide and nitrone moieties (the key groups) through the strain-promoted alkyne-azide cycloaddition (SPAAC) or the strain-promoted alkyne-nitron cycloaddition (SPANC), respectively.\(^{17}\) In addition to the high chemoselectivity and atom efficiency,\(^{18}\) these cycloaddition reactions were chosen because the reaction kinetics of both SPAAC and SPANC can be regulated through structural modifications to the dipolar species. Directly delocalizing the azide/nitron moieties into
electron-donating groups decelerates reaction kinetics, while delocalization into electron-withdrawing groups accelerates reaction kinetics (Figure 2.1c). The ability to tailor the reaction kinetics of the SPAAC and SPANC reactions presents an opportunity to develop nanomaterial systems that is capable of kinetically-directed orthogonal surface modifications that can be used to trigger reactivity with individual complementary partners in a kinetically pre-determined way. The ability to isolate the reactivity between one lock group and one key group in a mixture of key groups based on pre-defined kinetic information, as opposed to the requirement of changing the chemical information inherent to common host/guest strategies, provides an exciting kinetically-directed alternative to self-sorting methodologies.

The strained-alkyne that was chosen for this study was an aliphatic strained-alkyne known as bicyclononyne (BCN). It was chosen because, compared to other popular benzoannulated strained-alkynes (such as dibenzocyclooctyne (DBCO)), it can be synthesized on large scale using a relatively straightforward protocol and demonstrates exceptional stability. In order to append the BCN moiety to the gold nanoparticle surface, a direct synthesis using a BCN terminated thiol via the typical Brust-Shiffrin method that is most commonly employed to synthesize gold nanoparticles could not be used due to the incompatibility of the strained-alkyne moiety towards the reducing conditions of the reaction. Furthermore, a place exchange reaction with a BCN-terminated thiol onto a template AuNP system could not be performed due to the ability of thiols to undergo a Michael addition onto strained-alkynes.

To mitigate these synthetic complications, an AuNP system with a nucleophilic moiety appended to the surface was first developed, which could then undergo an interfacial substitution reaction with a BCN derivative that had been electrophilically activated (Scheme 2.1, see Supporting Information Sections 2.6.2.2, 2.6.2.5 and 2.6.2.8 for experimental details). First, an inert AuNP system was synthesized by reaction of HAuCl₄ and MeO-EG₃-SH (EG = ethylene glycol) as the protecting ligand in a Brust-Shiffrin synthesis, to give AuNP-OMe. Next, a place exchange reaction was performed between AuNP-OMe and NH₂-EG₄-SH, where the native methoxy-terminated ligands were exchanged with the incoming amine-terminated ligands to incorporate the nucleophilic amine moiety. The surface amino groups in AuNP-NH₂ were then reacted with bicyclo[6.1.0]non-4-yn-9-ylmethyl-(4-nitrophenyl)-carbonate (exo-BCN-O-pNP) to create a new carbamate linkage through an interfacial acyl substitution reaction, which
successfully incorporated the BCN moiety to make AuNP-BCN. X-ray photoelectron (XPS) spectroscopy confirmed the new carbamate linkage after the interfacial acyl substitution reaction (Figure S2.8 and Figure S2.14), and transmission electron microscopy (TEM) indicated that the AuNP-BCN particles are approximately 3 nm in diameter (Figure S2.13), which was similar to the diameter of the AuNP-NH$_2$ particles (Figure S2.7). The successful incorporation of the strained-alkyne moiety was also confirmed by $^1$H NMR spectroscopy. The $^1$H NMR spectrum of AuNP-NH$_2$ has two signals at 3.34 ppm and 3.15 ppm (Figure S2.6), which are produced by surface methoxy moieties in the MeO-EG$_3$-S$^-$ ligands and surface methylene protons alpha to the amino moiety in the NH$_2$-EG$_4$-S$^-$ ligands, respectively. After the interfacial reaction, the signal at 3.15 ppm disappears due to the surface methylene protons now being alpha to a carbamate linkage, and new signals occur at 3.98, 2.41, 2.32, 2.22, 1.38 and 0.80 ppm that correspond to the newly incorporated exo-bicyclononyne moiety (Figure S2.12). The thermogravimetric analysis (TGA) spectrum of AuNP-BCN showed two primary organic components (Figure S2.36), namely the surface MeO-EG$_3$-S$^-$ and BCN-O-(CO-NH)-EG$_4$-S$^-$ ligands, which constituted 28% and 72% of the organic mass, respectively (Figure S2.37). It should be noted that the quantity of surface BCN can be tuned by changing the reaction time and concentration of the place exchange reaction to adjust the amount of surface amine groups, which will amend the number of strained-alkyne groups being introduced to the AuNP surface.

Scheme 2.1. Synthesis of AuNP-BCN platform. Right inset. TEM image of AuNP-BCN.

Subsequently, we designed and synthesized a library of azides and nitrones that were delocalized into either electron donating or electron-withdrawing substituents, giving each their own kinetic profile. Each azide and nitrone were reacted with both exo-bicyclo[6.1.0]non-4-yn-9-ol (BCN$_{exo}$-OH) and AuNP-BCN, and the second order kinetic rate constants were determined by
\(^1\)H NMR spectroscopy by following the decrease in signals from free azide or nitrone. Figure 2.2 summarizes the bimolecular rate constants for the SPAAC and SPANC reactions with free BCN\(_{exo}\)-OH and the *interfacial* SPAAC (*I*-SPAAC) and *interfacial* SPANC (*I*-SPANC) reactions with AuNP-BCN. In most cases, the *I*-SPAAC kinetics of AuNP-BCN were similar to the SPAAC kinetics of free BCN. However, nitro-functionalized azide 4 exhibited a two-fold faster *I*-SPAAC reaction with AuNP-BCN versus free BCN\(_{exo}\)-OH. A similar two-fold enhancement in *I*-SPANC kinetics was observed between nitro-functionalized nitrone 1 and nitrone 4 with AuNP-BCN, providing further evidence that nitro-functionalized dipoles interestingly display faster interfacial cycloaddition kinetics compared to their non-interfacial counterparts.

![Increasing SPAAC Kinetics](Image)

**Figure 2.2.** Estimated SPAAC/SPANC and *I*-SPAAC/*I*-SPANC reaction rates of azides and nitrones with free BCN and AuNP-BCN, respectively. All rate constants were determined under second order conditions in CD\(_2\)Cl\(_2\) at 25°C in duplicate trials using \(^1\)H NMR spectroscopy, and monitored over pre-determined time intervals to determine a second order rate constant. The \(k_2\)(rel) value indicates the rate of each \(k_2\) relative to the slowest azide (azide 1).

Having established that surface reactivity of AuNP-BCN is kinetically modifiable depending on the chemical composition of the complementary azide/nitrone species, we sought to demonstrate that the nanomaterial platform can react exclusively with one reactive partner over another in a mixture of two reactive partners (Figure 2.3). AuNP-BCN was challenged to equimolar quantities of an azide and nitrone and the reaction was monitored by \(^1\)H NMR
spectroscopy. The percentage of each resulting cycloadduct was determined by measuring the change in non-coinciding signals from the constituent azide and nitrones before and after the introduction of AuNP-BCN. As shown in Figure 2.3, when AuNP-BCN was challenged to an azide and nitrone with similar rate constants, similar amounts of each cycloadduct system was observed. However, as the difference between rate constants were increased, exclusive reactivity to one dipolar species over the other was observed. When a equimolar mixture of nitrone 4 \((k_2(\text{I-SPANC}) = 0.61 \text{ M}^{-1}\text{s}^{-1})\) and azide 4 \((k_2(\text{I-SPAAC}) = 0.92 \text{ M}^{-1}\text{s}^{-1})\) was added to AuNP-BCN, similar amounts of each of the two triazole and isooxazoline cycloadduct systems was observed. However, when a mixture of nitrone 4 \((k_2(\text{I-SPANC}) = 0.61 \text{ M}^{-1}\text{s}^{-1})\) and azide 3 \((k_2(\text{I-SPAAC}) = 0.17 \text{ M}^{-1}\text{s}^{-1})\), a significantly greater amount of the isooxazoline cycloadduct was observed. When the difference between the rate constants was increased even further, as exists between azide 1 \((k_2(\text{I-SPAAC}) = 0.0.034 \text{ M}^{-1}\text{s}^{-1})\) and nitrone 6 \((k_2(\text{I-SPANC}) = 2.0 \text{ M}^{-1}\text{s}^{-1})\), there was no observable change in the intensity of the \(^1\text{H}\) NMR signal from the azide protons, indicating exclusive formation of the isooxazoline cycloadduct. Similarly, when AuNP-BCN was challenged to a mixture of azide 6 \((k_2(\text{I-SPAAC}) = 1.6 \text{ M}^{-1}\text{s}^{-1})\) and nitrone 1 \((k_2(\text{I-SPANC}) = 0.015 \text{ M}^{-1}\text{s}^{-1})\), there was no observable change in the \(^1\text{H}\) NMR signal from the nitrone protons, indicating exclusive formation of the triazole cycloadduct. This data is exciting because it demonstrates that AuNP-BCN can not only undergo orthogonal surface chemistry, but it can be done to isolate reactivity to one complementary partner over another in a kinetically predictable way, that is dependent on the electronic composition of the complementary dipolar species.

<table>
<thead>
<tr>
<th>Azide 1 (k_2 = 0.034)</th>
<th>Azide 3 (k_2 = 0.17)</th>
<th>Azide 4 (k_2 = 0.92)</th>
<th>Azide 5 (k_2 = 0.59)</th>
<th>Azide 6 (k_2 = 2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate 6 (k_2 = 2.0)</td>
<td>Nitrate 4 (k_2 = 0.61)</td>
<td>Nitrate 4 (k_2 = 0.61)</td>
<td>Nitrate 2 (k_2 = 0.033)</td>
<td>Nitrate 1 (k_2 = 0.015)</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>55</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>89</td>
<td>45</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 2.3.** Kinetically-directed competitive reactivity of AuNP-BCN, where one equivalent of AuNP-BCN was reacted with one equivalent of nitrone and one equivalent of azide, and the amount of cycloadduct that was formed was determined using \(^1\text{H}\) NMR spectroscopy.

67
2.3 Conclusions

We herein report a versatile nanomaterial platform in which kinetically variable surface modifications can be performed in a multi-component mixture, depending on the electronic and chemical composition of the complementary reactive species in the mixture. By delocalizing the azide and nitrone species into different electronic groups, unique kinetic profiles can be bestowed onto these reactive partners, permitting exclusive reactivity with one species over another in a kinetically predictable way. In this way, complex systems can be constructed from simpler ones simultaneously in a self-sorting manner in a way that depends on the kinetic profile of the desired complementary building block, rather than the chemical identity of large moieties that are tethered to the building blocks that are more difficult to install than azides and nitrones. This presents an exciting new fabrication process for predictable hierarchal construction of chemically distinct materials from a complex mixture of building blocks through a kinetically-directed approach, that doesn’t rely on the common lock-and-key approaches that is most commonly employed to date in self-sorting technology.

2.4 Acknowledgments

This work was funded by NSERC-DG and the University of Western Ontario. We would like to thank D. Hairsine (ESI-MS Facility) and M. Biesinger (Surface Science Western).

2.5 References


2.6 Supporting Information

2.6.1 General Methods and Methods

Reagents and Solvents. The following materials were used as received. Tetraethylene glycol (99%), p-toluenesulfonyl chloride (≥99%), sodium azide (≥99.5%), triethylamine (≥99%), triphenylmethanethiol (97%), triphenylphosphine (99%), trifluoroacetic acid (99%), triisopropylsilane (98%), gold (III) chloride trihydrate (≥99.9% trace metal basis), sodium borohydride (≥98%), rhodium (II) acetate dimer (99.99% trace metals basis), ethyl diazoacetate (contains ≥13 wt% dichloromethane), bromine (reagent grade), lithium aluminum hydride (95%), potassium tert-butoxide (1.0 M in THF), 4-nitrophenyl chloroformate (96%), 3-bromo-1-propanol (97%), 4-azidoanisole solution (0.5 M in tert-butyl methyl ether, ≥90%), azidobenzene solution (0.5 M in tert-butyl methyl ether), 4-nitroaniline (≥99%), 4-bromopyridine hydrochloride (99%), 2,3,4,5,6-pentafluoroaniline, N-methylhydroxylamine hydrochloride (98%), nitrobenzene (≥99%), zinc dust (<10μm, ≥98%), p-anisaldehyde (98%), benzaldehyde (≥99%), 4-nitrobenzaldehyde (98%), 3-pyridinecarboxaldehyde (98%), 4-nitrobenzonitrile (97%) and dichloromethane-D₂ (CD₂Cl₂, 99.5 atom %D) were purchased from Sigma-Aldrich (Millipore Sigma). Chloroform-D₁ (CDCl₃, 99.8 atom %D) was purchased from Cambridge Isotope Laboratories. Sodium chloride, sodium hydroxide pellets, tetrahydrofuran and toluene were purchased from Fischer Scientific. Technical grade ammonium chloride, magnesium sulphate, sodium nitrite, hexanes, dichloromethane, di-ethyl ether, methanol, acetic acid, acetonitrile, pentane and 12 M hydrochloric acid were purchased from Caledon. Ethanol (anhydrous) was purchased from Commercial Alcohols.

Unless otherwise stated, all reactions were performed at ambient conditions.

NMR Spectroscopy. ¹H and ¹³C{¹H} spectra were recorded on either a Bruker AvIII HD 400 spectrometer or Varian INOVA 600 spectrometer, as indicated. ¹H NMR spectra are reported as δ in units of parts per million (ppm), and referenced against residual protio chloroform (7.27 ppm, s) or dichloromethane (5.32 ppm, t), as indicated. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintuplet), m (multiplet) and br (broad signal). Coupling constants are reported as a J value in Hertz (Hz) according to the spectrometer frequency. The
number of protons \((n)\) for a given resonance is indicated as \(n\)H, and is based on spectral integration values. \(^{13}\text{C}\{}^{1}\text{H}\) NMR spectra are reported as \(\delta\) in units of parts per million (ppm) and referenced against the indicated deuterated solvent: chloroform-D\(_1\) (77.0 ppm, t) or dichloromethane-D\(_2\) (54.0 ppm, quin).

**Thermogravimetric Analysis (TGA).** TGA was performed using a Mettler Toledo TGA/SDTA 851 instrument from 25°C to 750°C at a heating rate of 10°C/min under a nitrogen flow of 70 mL/min. The sample was prepared by adding a small sample of AuNP-BCN dissolved in dichloromethane onto a pre-weighed alumina crucible, removing the solvent under argon gas flow and drying the sample overnight under high vacuum, which resulted in the formation of a thin AuNP film on the crucible service.

**X-Ray Photoelectron Spectroscopy (XPS).** The XPS analyses were carried out with a Kratos Axis Ultra spectrometer using a monochromatic Al K(alpha) source (15mA, 14kV). Specimens were mounted on a double side adhesive and the Kratos charge neutralizer system was used on all specimens. Survey scan analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 160 eV. High resolution analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 20 eV. Spectra have been charge corrected to the main line of the carbon 1s spectrum set to 284.5 eV for graphitic/nanotube type species. Spectra were analyzed using CasaXPS software (version 2.3.14)

### 2.6.2 Experimental Procedures

4-Azidoanisole (azide 2) and azidobenzene (azide 3) were purchased from Sigma Aldrich as a 0.5 M solution in tert-butyl methyl ether, which was partitioned between water and ether. The resulting ether phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure.

#### 2.6.2.1 Synthesis of HS-EG\(_3\)-Me

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{O} & \quad \text{SH} \\
\end{align*}
\]

\(^1\text{H}\) NMR (CDCl\(_3\), 400 MHz) \(\delta\)(ppm): 3.66 (m, 8H), 3.57 (m, 2H), 3.39 (s, 3H), 2.71 (q, \(J = 8\) Hz, 2H), 1.60 (t, \(J = 8\) Hz, 1H). \(^{13}\text{C}\) NMR (CDCl\(_3\), 400 MHz) \(\delta\)(ppm):

* Synthesized to our previously reported procedure\(^{176}\).
72.8, 17.8, 70.5, 70.3, 70.1, 58.9, 24.2. HRMS (ESI) m/z calc. for C_{7}H_{16}O_{3}S (M)^{+}: 180.0820, found: 180.0825.

2.6.2.2 Synthesis of AuNP-OMe

*Synthesized to our previously reported procedure.¹

2.6.2.3 Synthesis of STrityl-EG₄-NH₂

*synthesized according to our previously reported procedure.²

H₂N

¹H NMR (400 MHz, CDCl₃): δ 7.39 – 7.43 (m, 6H), 7.18 – 7.30 (m, 9H), 3.55 – 3.63 (m, 6H), 3.49 (t, J = 5.2 Hz, 2H), 3.45 (dd, J₁ = 5.7 Hz, J₂ = 3.9 Hz, 2H), 3.30 (t, J = 6.9 Hz, 2H), 2.85 (t, J = 5.1 Hz, 2H), 2.43 (t, J = 6.9 Hz, 2H), 1.97 (s, 2H). ¹³C NMR (400 MHz, CDCl₃): δ 144.8, 129.6, 127.8, 126.6, 73.0, 70.5, 70.4, 70.2, 70.1, 69.6, 41.6, 31.6. ESI-MS calc. for C_{27}H_{34}NO_{3}S^{+} [M+H^{+}] 452.2259, found 452.2240. ¹³C NMR (400 MHz, CDCl₃): δ 72.7, 70.2, 70.1, 69.9, 69.7, 66.8, 39.6, 24.0.

2.6.2.4 Synthesis of HS-EG₄-NH₂

H₂N

To 4.0 g (8.9 mmol) STrityl-EG₄-NH₂: in 100 mL dry dichloromethane was added 2.2 mL (10.6 mmol) triisopropylsilane and then 34 mL (45 mmol) trifluoroacetic acid. The reaction mixture was stirred for 1 hour while monitoring carefully by TLC, after which the solvent was removed via rotary evaporation. The crude residue was purified by flash column chromatography (3:1
dichloromethane:methanol) to give HS-TEG-NH$_2$ as a thick white oil in 83% yield (1.55 g). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.73 (m, 2H), 3.62 (m, 6H), 3.46 (m, 6H), 3.15 (t, $J$ = 8 Hz, 2H), 2.68 (t, $J$ = 8 Hz, 2H), 1.58 (t, $J$ = 4 Hz, 1H). $^{13}$C NMR (400 MHz, CDCl$_3$): 72.7, 70.2, 70.1, 69.9, 69.7, 66.8, 39.6, 24.0. HRMS (ESI) $m/z$ calc. for C$_8$H$_{19}$NO$_3$S (M)$^+$: 209.1086, found: 209.1083.

2.6.2.5 Synthesis of AuNP-NH$_2$

To 0.3 g AuNP-OMe in 25 mL methanol was added 0.3 g HS-EG$_4$-NH$_2$ in 5 mL methanol in a round bottom flask. The resulting solution was stirred for 2 hours at room temperature, after which the solvent was removed thoroughly via rotary evaporation to form a black AuNP film in the round bottom flask. The film was washed vigorously by added 100 mL portions of dichloromethane and swirling the sample for 15 minutes. The dichloromethane was then poured out, and the film was re-dissolved in methanol and removed under reduced pressure to re-form the AuNP film, which was subsequently washed with two more 100 mL portions of dichloromethane in the same way. After the final wash, the sample was dried thoroughly under high vacuum to give AuNP-NH$_2$ in quantitative yield (0.3 g).

2.6.2.6 Synthesis of BCN$_{exo}$-OH

*Synthesized according to Dommerholt et al.$^3$

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.56 (d, $J$ = 8 Hz, 2H), 2.42 (d, $J$ = 16 Hz, 2H), 2.30 (t, $J$ = 16 Hz, 2H), 2.17 (d, $J$ = 16 Hz, 2H), 1.42 (m, 3H), 0.69 (m, 3H). $^{13}$C NMR (400 MHz, CDCl$_3$): 98.8, 67.1, 33.4, 27.3, 22.6, 21.5. HRMS (ESI) $m/z$ calc. for C$_{10}$H$_{14}$O (M)$^+$: 150.1045, found: 150.1052.
2.6.2.7 Synthesis of BCN_{exo}-O-pNP

*Synthesized according to Dommerholt et al.\textsuperscript{3}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{BCN_{exo}-O-pNP}
\end{figure}

\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400MHz): 8.29 (d, J = 8 Hz, 2H), 7.40 (d, J = 8 Hz, 2H), 4.23 (d, J = 8 Hz, 2H), 2.46 (m, 2H), 2.32 (m, 2H), 2.20 (m, 2H), 1.44 (m, 2H), 0.86 (m, 3H). \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 150MHz): 155.6, 152.4, 145.2, 125.3, 121.7, 98.7, 68.0, 29.1, 21.3, 20.5, 17.2. HRMS (ESI) m/z calc. for C\textsubscript{17}H\textsubscript{17}NO\textsubscript{5} (M\textsuperscript{+}): 315.1107, found: 315.1223.

2.6.2.8 Synthesis of AuNP-BCN

To 350 mg AuNP-NH\textsubscript{2} in 2 mL methanol, 14 mL dichloromethane and 3.5 mL trimethylamine was added BCN_{exo}-O-pNP in 2 mL dichloromethane. The solution was stirred overnight, after which the solvent was removed under reduced pressure and the resulting black film was dried thoroughly under high vacuum. The dried film was washed thoroughly with several 100 mL portions of di ethyl ether to give AuNP-BCN as a shiny black solid (370 mg).

2.6.2.9 Synthesis of 1-azido-3-propanol (azide 1)

To 0.65 mL (1.0 g, 7.2 mmol) 3-bromo-1-propanol in 5 mL 1:1 water:ethanol was added 1.4 g (21 mmol) sodium azide. After refluxing the resulting solution at 55°C for 4 hours, the solution was cooled to room temperature and 50 mL water was added. The aqueous phase was extracted with dichloromethane (3 x 10 mL) and the collected organic phases were dried over magnesium sulphate, and concentrated under streaming Ar\textsubscript{(g)} to give azido-propanol (azide 1) as a light yellow oil in 67\% yield (0.49 g). \textit{Note:} Due to volatility of azido-propanol, solvent should not be evaporated under reduced pressure. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz): δ 3.74 (t, J = 8 Hz, 2H), 3.44 (t, J = 8 Hz, 2H), 2.03 (s, 1H), 1.82 (quin, J = 4 Hz, 2H). \textsuperscript{13}C NMR
(CDCl₃, 400 MHz): δ 59.8, 48.4, 31.4. HRMS (Cl) m/z calc. for C₆H₆N₃O (M+1): 102.0667, found: 102.0672. IR (KBr, cm⁻¹): 3336, 2930, 2882, 2091, 1455, 1344, 1259, 1047.

2.6.2.10 Synthesis of 1-azido-4-nitrobenzene (azide 4)

Synthesized according to Kwok et al., with minor modifications.⁴

To 40 mL 12 M HCl and 40 mL water was slowly added 2.1 g (15 mmol) 4-nitroaniline, after which 15 mL ethanol was slowly added until the solution became transparent yellow. After cooling the solution to 0°C, 1.6 g (23 mmol) sodium nitrite was added slowly and the solution was stirred for 45 minutes at 0°C. Next, sodium azide (1.5 g, 23 mmol) was added very slowly at room temperature and the resulting solution was stirred for 2 hours, after which 50 mL water and 100 mL ether was added. The organic phase was removed and the aqueous phase was extracted with ether (2 x 50 mL). The collected organic phases were extracted with saturated NaHCO₃ (3 x 50 mL) and brine (3 x 50 mL), dried over magnesium sulphate and concentrated via rotary evaporation to give 1-azido-4-nitrobenzene (azide 4) as a yellow solid in 98% yield (2.4 g). ¹H NMR (CDCl₃, 400 MHz): δ 8.25 (d, J = 8 Hz, 2H), 7.15 (d, J = 8 Hz, 2H). ¹³C NMR (CDCl₃, 400 MHz): δ 147.2, 145.0, 125.9, 119.7. HRMS (ESI) m/z calc. for C₆H₄N₄O₂ (M): 164.0334, found: 164.0335. IR (KBr, cm⁻¹): 3113, 3069, 2922, 2403, 2122, 1605, 1590, 1512, 1489, 1369, 1328, 1287, 1177, 1130, 1118, 1105.

2.6.2.11 Synthesis of 4-azidopyridine (azide 5)

Synthesized according to Zhao and Qing, with minor modifications.⁵

To 0.60 g (3.1 mmol) 4-bromopyridine hydrochloride in 6 mL 1:1 water:ethanol (95%) was added 0.060 g (1.5 mmol) sodium hydroxide and 0.50 g (7.7 mmol) sodium azide. After refluxing the resulting solution at 110°C for 4 hours, the solution was cooled to room temperature and 50 mL water and 10 mL dichloromethane was added. The organic phase was removed, and the aqueous phase was extracted with dichloromethane (2 x 10 mL). The collected organic phases were extracted with brine (3 x 30 mL), dried over magnesium sulphate and concentrated under streaming Ar(g) to give 4-azidopyridine (azide 5) as a light yellow oil in 86% yield (0.42 g). Note: Due to volatility of 4-azidopyridine, solvent should not be removed under
reduced pressure. $^1$H NMR (CDCl$_3$, 400 MHz): 8.54 (d, $J = 4$ Hz, 2H), 6.96 (d, $J = 4$ Hz, 2H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ 151.3, 148.9, 114.3. HRMS (ESI) $m/z$ calc. for C$_5$H$_4$N$_4$ (M)$^+$: 120.0436, found: 120.0439. IR (KBr, cm$^{-1}$): 3037, 2919, 2850, 2430, 2136, 1614, 1580, 1565, 1493, 1416, 1343, 1298, 1281, 1215, 1137.

2.6.2.12 Synthesis of 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6)

![Synthesis of 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6)](image)

Synthesized according to Li-Mei et al., with minor modifications.$^6$

To 2.1 g (10 mmol) 2,3,4,5,6-pentafluoroaniline in 25 mL trifluoroacetic acid was slowly added 0.83 g (12 mmol) sodium nitrite at 0°C. The resulting solution was stirred for 1 hour at 0°C, after which 0.98 g (15 mmol) sodium azide was added portion-wise at 0°C. After stirring the solution for 1 hour at 0°C, 50 mL diethyl-ether was added and the aqueous phase was removed. The organic phase was extracted with water (3 x 50 mL) and NaHCO$_3$ (3 x 50 mL), dried over magnesium sulphate and concentrated via rotary evaporation. The resulting crude residue was purified via flash column chromatography (hexanes) to give 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6) as a light yellow oil in 89% yield (2.1 g). $^{19}$F NMR (CDCl$_3$, 400 MHz): -151.6 (d, 2F), -159.8 (t, 1F), -161.6 (t, 2F). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ 142.2, 139.5, 136.9, 110.0. HRMS (ESI) $m/z$ calc. for C$_6$F$_5$N$_3$ (M)$^+$: 209.0012, found: 209.0014. IR (KBr, cm$^{-1}$): 2407, 2120, 1638, 1507, 1462, 1326, 1243, 1103, 1013.

2.6.2.13 Synthesis of N-methyl-C-nitrophenyl-nitrone (nitrone 1)

![Synthesis of N-methyl-C-nitrophenyl-nitrone (nitrone 1)](image)

To 0.25 g (3.0 mmol) N-methylhydroxylamine and 0.36 g (9.0 mmol) sodium hydroxide in 10 mL methanol was added 0.70 g (4.5 mmol) nitrobenzaldehyde. After stirring the mixture overnight at room temperature, the solvent was removed via rotary evaporation, the crude solid was suspended in ether, and the resulting insoluble yellow solid was collected by filtration to give N-methyl-C-nitrophenyl-nitrone (nitrone 1) in 72% yield (0.39 g). $^1$H NMR (CD$_2$Cl$_2$, 400 MHz): 8.38 (d, $J = 8$ Hz, 2H), 8.23 (d, $J = 8$ Hz, 2H), 7.53 (s, 1H), 3.90 (s, 3H). $^{13}$C NMR (CD$_2$Cl$_2$, 400 MHz): $\delta$ 148.2, 137.0, 133.2, 128.9, 124.2, 55.7. HRMS (ESI) $m/z$ calc. for C$_8$H$_8$N$_2$O$_3$ (M)$^+$: 180.0535, found: 180.0531. IR (KBr, cm$^{-1}$): 3108, 3082, 3024, 2958.3, 1597, 1956, 1506, 1333, 1184, 1164, 1110.
2.6.2.14 Synthesis of N-phenylhydroxylamine

To 2.0 g (16 mmol, 1.7 mL) nitrobenzene and 0.95 g (18 mmol) ammonium chloride in 25 mL 1:1 water:ethanol was added 2.1g (32 mmol) zinc dust portion-wise over 5 minutes. Note: rate of zinc dust addition was adjusted so as to maintain the elevated temperature at ~60°C. After stirring the resulting suspension for 20 minutes, the grey solid was removed by vacuum filtration, and washed with 50 mL water and 10 mL ether. The organic phase in the filtrate was removed, the aqueous phase was extracted with ether (2 x 10 mL) until the aqueous phase was nearly colorless, and the collected organic phases were dried over magnesium sulphate and concentrated via rotary evaporation to give N-phenylhydroxylamine in 81% crude yield (1.4 g), which was used without further purification towards the syntheses of Nitrone 2, Nitrone 3, Nitrone 4 and Nitrone 5. Note: Due to the instability of the hydroxylamine, it should be used immediately.

2.6.2.15 Synthesis of N-phenyl-C-methoxyphenyl-nitrone (nitrone 2)

To 0.23 g (2.1 mmol) crude N-phenylhydroxylamine in 5 mL dichloromethane was added 0.34 g (2.5 mmol, 0.30 mL) 4-anisaldehyde and a small amount of magnesium sulphate. The resulting solution was stirred overnight at room temperature, after which the solvent was removed via rotary evaporation. The crude residue was suspended in ether, and the resulting off-white insoluble solid was collected by vacuum filtration to give N-phenyl-C-methoxyphenyl-nitrone (nitrone 2) in 70% overall yield (0.33g). $^1$H NMR (CD$_2$Cl$_2$, 400 MHz): 8.41 (d, $J = 8$ Hz, 2H), 7.88 (s, 1H), 7.76 (d, $J = 8$ Hz, 2H), 7.48 (m, 3H), 7.00 (d, $J = 8$ Hz, 2H), 3.87 (s, 3H). $^{13}$C NMR (CD$_2$Cl$_2$, 400 MHz): δ 162.0, 149.6, 134.0, 131.4, 130.0, 129.6, 124.5, 122.1, 114.4, 55.9. HRMS (ESI) $m/z$ calc. for C$_{14}$H$_{13}$NO$_2$ (M)$^+$: 227.0946, found: 227.0940. IR (KBr, cm$^{-1}$): 3050, 3013, 2962, 2931, 2839, 1603, 1554, 1506, 1484, 1461, 1399, 1305, 1259, 1193, 1176, 1108, 1064, 1023.
2.6.2.16 Synthesis of \(N\)-phenyl-C-phenyl-nitrone (nitron 3)

See Section 3.6.2.7 for detailed synthesis of \(N\)-phenyl-C-phenyl-nitrone (nitron 3)

\[
\begin{array}{c}
\text{O}^\ominus \\
\text{N}^\ominus \\
\text{C}^\ominus \\
\text{O}
\end{array}
\]

\(^1\text{H NMR (CD}_2\text{Cl}_2, 400 \text{ MHz):} \ 8.41 \ (m, \ 2\text{H}), \ 7.95 \ (s, \ 1\text{H}), \ 7.78 \ (m, \ 2\text{H}), \ 7.49 \ (m, \ 6\text{H}). \ \ ^{13}\text{C NMR (CD}_2\text{Cl}_2, 400 \text{ MHz):} \ \delta \ 149.8, \ 134.5, \ 131.6, \ 131.2, \ 130.4, \ 129.6, \ 129.3, \ 129.1, \ 122.2. \ \text{HRMS (ESI) } m/z \ \text{calc. for } \text{C}_{13}\text{H}_{11}\text{NO} \ (M)^+ : \ 197.0841, \ \text{found:} \ 197.0837. \ \text{IR (KBr, cm}^{-1}): \ 3060, \ 1593, \ 1547, \ 1510, \ 1484, \ 1461, \ 1445, \ 1396, \ 1340, \ 1324, \ 1298, \ 1191, \ 1163, \ 1067, \ 1025.
\]

2.6.2.17 Synthesis of \(N\)-phenyl-C-nitrophenyl-nitrone (nitron 4)

To 0.21 g (1.9 mmol) crude \textit{N-phenylhydroxylamine} in 5 mL dichloromethane was added 0.26 g (2.5 mmol, 0.25 mL) benzaldehyde and a small amount of magnesium sulphate. The resulting solution was stirred overnight at room temperature, after which the solvent was removed via rotary evaporation. The crude residue was suspended in ether, and the resulting white insoluble solid was collected by vacuum filtration to give \textit{N-phenyl-C-nitrophenyl-nitrone (nitron 4)} in 64\% overall yield (0.29 g). \(^1\text{H NMR (CD}_2\text{Cl}_2, 400 \text{ MHz):} \ 8.56 \ (d, \ J = 8 \text{ Hz, } 2\text{H}), \ 8.30 \ (d, \ J = 8 \text{ Hz, } 2\text{H}), \ 8.10 \ (s, \ 1\text{H}), \ 7.80 \ (m, \ 2\text{H}), \ 7.54 \ (m, \ 3\text{H}). \ \ ^{13}\text{C NMR (CD}_2\text{Cl}_2, 400 \text{ MHz):} \ \delta \ 149.6, \ 148.4, \ 137.0, \ 132.6, \ 131.1, \ 129.9, \ 129.6, \ 124.4, \ 122.2. \ \text{HRMS (ESI) } m/z \ \text{calc. for } \text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_3 \ (M)^+ : \ 242.0691, \ \text{found:} \ 242.0693. \ \text{IR (KBr, cm}^{-1}): \ 3104, \ 3065, \ 1596, \ 1544, \ 1509, \ 1483, \ 1459, \ 1403, \ 1340, \ 1319, \ 1193, \ 1176, \ 1157, \ 1101, \ 1072.

2.6.2.18 Synthesis of \(N\)-phenyl-C-pyridine-nitrone (nitron 5)

* See Section 3.6.2.3 for detailed synthesis of \textit{N-phenyl-C-pyridine-nitrone (nitron 5)}

\[
\begin{array}{c}
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\text{N}^\ominus \\
\text{C}^\ominus \\
\text{N}
\end{array}
\]

\(^1\text{H NMR (CD}_2\text{Cl}_2, 400 \text{ MHz):} \ \delta \ 9.19 \ (s, \ 1\text{H}), \ 9.07 \ (d, \ J = 8 \text{ Hz, } 1\text{H}), \ 8.65 \ (d, \ J = 4 \text{ Hz, } 1\text{H}), \ 8.05 \ (s, \ 1\text{H}), \ 7.95 \ (d, \ J = 8 \text{ Hz, } 2\text{H}), \ 7.83 \ (d, \ J = 8 \text{ Hz, } 2\text{H}), \ 7.43 \ (m, \ 1\text{H}). \ \ ^{13}\text{NMR (CD}_2\text{Cl}_2, 400 \text{ MHz):} \ \delta \ 151.9, \ 150.9, \ 135.4, \ 133.9, \ 132.8, \ 127.5, \ 124.1, \ 123.1, \ 118.1, \ 114.6. \ \text{HRMS (ESI) } m/z \ \text{calc. for } \text{C}_{12}\text{H}_{10}\text{N}_2\text{O} \ (M)^+ : \ 198.0793, \ \text{found:} \ 198.0796. \ \text{IR (ATR, cm}^{-1}): \ 3130, \ 3065, \ 3062, \ 1582, \ 1555, \ 1484, \ 1466, \ 1403, \ 1335, \ 1272, \ 1203, \ 1175, \ 1164, \ 1072, \ 1022.
2.6.2.19 Synthesis of \( N \)-cyanophenyl-\( C \)-pyridine-nitrone (nitrone 6)

See Section 3.6.2.5 for detailed synthesis of \( N \)-phenyl-\( C \)-phenyl-nitrone (nitrone 6)

\[ \text{\( \text{H NMR (CD}_2\text{Cl}_2, 400 MHz): } \delta 9.17 \text{ (s, 1H), } 9.09 \text{ (d, } J = 8.0 \text{ Hz, 1H), } 8.62 \text{ (d, } J = 8.0 \text{ Hz, 1H), } 8.00 \text{ (s, 1H), } 7.79 \text{ (m, 2H), } 7.50 \text{ (m, 3H), } 7.41 \text{ (m, 1H). } \text{\( 1^3\text{NMR (CD}_2\text{Cl}_2, 400 MHz): } \delta 151.3, 150.7, 149.4, 135.1, 131.6, 130.8, 129.7, 128.0, 124.0, 122.11, 114.4. } \text{HRMS (ESI) } m/z \text{ calc. for } \text{C}_{13}\text{H}_9\text{N}_3\text{O (M)}^+: 223.0746, \text{ found: } 223.0735. \text{ IR (ATR, cm}^{-1})\text{: } 3104, 3082, 3065, 3054, 2242, 1584, 1498, 1425, 1413, 1338, 1297, 1270, 1209, 1172, 1070, 1026. \]
2.6.3 Experimental Spectra and Diagrams

2.6.3.1 Experimental Spectra for HS-EG₃-Me

**Figure S2.1.** $^1$H NMR spectrum of HS-EG₃-Me in CDCl₃ at 25°C. * denotes residual protio solvent and impurities.

**Figure S2.2.** $^{13}$C($^1$H) NMR spectrum of HS-EG₃-Me in CDCl₃ at 25°C.
2.6.3.2 Experimental Spectra for AuNP-OMe

**Figure S2.3.** $^1$H NMR spectrum of AuNP-OMe in CDCl$_3$ at 25°C. * denotes residual protio solvent.
2.6.3.3 Experimental Spectra for HS-EG₄-NH₂

**Figure S2.4.** $^1$H NMR spectrum of HS-EG₄-NH₂ in CDCl₃ at 25°C. * denotes residual protio solvent.

**Figure S2.5.** $^{13}$C{$^1$H} NMR spectrum of HS-EG₄-NH₂ in CDCl₃ at 25°C. * indicates CDCl₃ solvent.
2.6.3.4 Experimental Spectra for AuNP-NH₂

**Figure S2.6.** ¹H NMR spectrum of AuNP-NH₂ in D₂O at 25°C. * denotes residual protio solvent.

**Figure S2.7.** TEM image for AuNP-NH₂.
Figure S2.8. (a) High-resolution carbon 1s XPS spectrum of AuNP-NH₂ (b) High-resolution oxygen 1s XPS spectrum of AuNP-NH₂.

Figure S2.9. (a) XPS survey scan of AuNP-NH₂.
2.6.3.5 Experimental Spectra for BCN\textsubscript{exo}-OH

![Figure S2.10](BCN-OH-Proton.esp)

**Figure S2.10.** $^1$H NMR spectrum of BCN\textsubscript{exo}-OH in CDCl\textsubscript{3} at 25°C. * denotes residual protio solvent.

2.6.3.6 Experimental Spectra for BCN\textsubscript{exo}-O-pNP

![Figure S2.11](PNGPHD908-BCN-oPNP_001000fid)

**Figure S2.11.** $^1$H NMR spectrum of BCN\textsubscript{exo}-O-pNP in CDCl\textsubscript{3} at 25°C. * denotes residual protio solvent and impurities.
2.6.3.7 Experimental Spectra for AuNP-BCN

Figure S2.12. $^1$H NMR spectrum of AuNP-BCN in CDCl$_3$ at 25°C. * denotes residual protio solvent and impurities.

Figure S2.13. TEM image for AuNP-BCN.
Figure S2.14. (a) High-resolution carbon 1s XPS spectrum of AuNP-BCN (b) High-resolution oxygen 1s XPS spectrum of AuNP-BCN.

Figure S2.15. (a) XPS survey scan of AuNP-BCN.
2.6.3.8 Experimental Spectra for 1-azido-3-propanol (azide 1)

**Figure S2.16.** $^1$H NMR spectrum of azide 1 in CDCl$_3$ at 25°C. * denotes residual protio solvent.

**Figure S2.17.** $^{13}$C{$^1$H} NMR spectrum of azide 1 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
2.6.3.9 Experimental Spectra for 1-azido-4-nitrobenzene (azide 4)

**Figure S2.18.** $^1$H NMR spectrum of *azide 4* in CDCl$_3$ at 25°C. * denotes residual protio solvent and impurities.

**Figure S2.19.** $^{13}$C{$^1$H} NMR spectrum of *azide 4* in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
2.6.3.10 Experimental Spectra for 4-azidopyridine (azide 5)

Figure S2.20. $^1$H NMR spectrum of azide 5 in CDCl$_3$ at 25°C. * denotes residual protio solvent.

Figure S2.21. $^{13}$C{$^1$H} NMR spectrum of azide 5 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
2.6.3.11 Experimental Spectra for 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6)

**Figure S2.22.** $^{19}$F NMR spectrum of azide 6 in CDCl$_3$ at 25°C.

**Figure S2.23.** $^{13}$C{${}^{1}$H} NMR spectrum of azide 6 in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
2.6.3.12 Experimental Spectra for \( N \)-methyl-\( C \)-nitrophenyl-nitrone (nitrone 1)

**Figure S2.24.** \( ^1 \)H NMR spectrum of nitrone 1 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * denotes residual protio solvent and impurities.

**Figure S2.25.** \( ^{13} \)C\( ^1 \)H NMR spectrum of nitrone 1 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * indicates \( \text{CD}_2\text{Cl}_2 \) solvent.
2.6.3.13 Experimental Spectra for \( N\)-phenyl-\( C\)-methoxyphenyl-nitrone (nitrone 2)

Figure S2.26. \(^1\)H NMR spectrum of nitrone 2 in CD\(_2\)Cl\(_2\) at 25°C. * denotes residual protio solvent and impurities.

Figure S2.27. \(^{13}\)C\{}\(^1\)H\} NMR spectrum of nitrone 2 in CD\(_2\)Cl\(_2\) at 25°C. * indicates CD\(_2\)Cl\(_2\) solvent.
2.6.3.14 Experimental Spectra for \( N \)-phenyl-C-phenyl-nitrone (nitrone 3)

**Figure S2.28.** \(^1\)H NMR spectrum of nitrone 3 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * denotes residual protio solvent.

**Figure S2.29.** \(^{13}\)C\{\(^1\)H\} NMR spectrum of nitrone 3 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * indicates \( \text{CD}_2\text{Cl}_2 \) solvent.
2.6.3.15 Experimental Spectra for \( N \)-phenyl-\( C \)-nitrophenyl-nitrone (nitrone 4)

Figure S2.30. \( ^1 \)H NMR spectrum of nitrone 4 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * denotes residual protio solvent and impurities.

Figure S2.31. \( ^{13} \)C\{\( ^1 \)H\} NMR spectrum of nitrone 4 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * indicates \( \text{CD}_2\text{Cl}_2 \) solvent.
2.6.3.16 Experimental Spectra for N-phenyl-C-pyridine-nitron (nitron 5)

**Figure S2.32.** {^1}H NMR spectrum of nitron 5 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent and impurities.

**Figure S2.33.** {^13}C{^1}H NMR spectrum of nitron 5 in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.
2.6.3.17 Experimental Spectra for \( N \)-cyanophenyl-\( C \)-pyridine-nitrone (nitrone 6)

**Figure S2.34.** \(^1\)H NMR spectrum of nitrone 6 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * denotes residual protio solvent.

**Figure S2.35.** \(^{13}\)C\{\(^1\)H\} NMR spectrum of nitrone 6 in \( \text{CD}_2\text{Cl}_2 \) at 25°C. * indicates \( \text{CD}_2\text{Cl}_2 \) solvent.
2.6.4 Thermogravimetric Analysis of AuNP-BCN

2.6.4.1 General Experimental Details

A crucible containing 1.3 mg of AuNP-BCN was heated from 25°C to 750°C under nitrogen for TGA analysis, and the decrease in organic matter was determined with increasing temperature (Figure S2.36).

The derivative of the TGA curve shows two components centered at 233.2°C and 300.0°C resulting from the decomposition of the BCN_{exo}-EG_{4}-S\(^-\) and OMe-EG_{3}-S\(^-\) ligands, respectively, both of which constitutes 64% of the mass of the AuNP sample (Figure S2.37). The area under each normalized curve indicates that BCN_{exo}-EG_{4}-S\(^-\) (MM = 384.492 g/mol) constitutes 28% of the total organic mass (0.23 mg per 1.3 mg AuNP) and OMe-EG_{3}-S\(^-\) constitutes 72% of the total organic mass (0.60 mg per 1.3 mg AuNP). Correcting for the mass of the total mass of the AuNP sample, the TGA analysis indicates that there is 0.46 μmol/mg of AuNP-BCN.

2.6.4.2 Experimental Spectra for TGA Analysis

Figure S2.36. TGA spectrum for AuNP-BCN.
Model: Gauss
Equation: \( y = y_0 + \left( \frac{A}{w \sqrt{Pl/2}} \right) \exp \left( -2 \frac{(x-xc)^2}{w^2} \right) \)

\( \chi^2/\text{DoF} = 1.0896 \times 10^{-9} \)
\( R^2 = 0.98407 \)

- \( y_0 = -1.23408 \times 10^{-5} \pm 5.6559 \times 10^{-7} \)
- \( xc_1 = 233.24937 \pm 0.64402 \)
- \( w_1 = 56.73643 \pm 0.98522 \)
- \( A_1 = -0.0249 \pm 5.2350 \times 10^{-4} \)
- \( xc_2 = 300.03715 \pm 0.19120 \)
- \( w_2 = 49.76629 \pm 0.27563 \)
- \( A_2 = -0.0654 \pm 5.1223 \times 10^{-4} \)

**Figure S2.37.** First-derivative of TGA spectrum for AuNP-BCN.
2.6.5 Kinetic Measurements

2.6.5.1 General Experimental Details

Estimate rate constants for all azides and nitrones were determined under second order conditions ($k_2$) in deuterated dichloromethane at 25°C using $^1$H NMR spectroscopy, by reacting each with both $\text{BCN}_{\text{exo}}$-$\text{OH}$ and $\text{AuNP}$-$\text{BCN}$.

In order to estimate $k_2$ values for each azide/nitrone with $\text{BCN}_{\text{exo}}$-$\text{OH}$, stock solutions of the azides/nitrones and $\text{BCN}_{\text{exo}}$-$\text{OH}$ were first prepared and then equimolar quantities of each were added to an NMR tube. Stock solutions of $\text{BCN}_{\text{exo}}$-$\text{OH}$ and each azide/nitrone were prepared by dissolving 100 μmol in 0.5 mL deuterated dichloromethane to give a 0.2 M stock solution of $\text{BCN}_{\text{exo}}$-$\text{OH}$ and 0.2 M stock solution of each azide/nitrone. Subsequently, 10 μL (2 μmol) of the azide/nitrone stock solution was added to 0.3 mL deuterated dichloromethane in an NMR tube, and a $t_0$ (time zero) $^1$H NMR spectrum was acquired. Next, 10 μL (2 μmol) of the $\text{BCN}_{\text{exo}}$-$\text{OH}$ stock solution was added. This solution was shaken vigorously, and $^1$H NMR spectra were acquired over pre-determined time intervals according to the speed of the reaction.

In order to estimate $k_2$ values for each azide/nitrone with $\text{BCN}_{\text{exo}}$-$\text{OH}$, a solution was prepared by dissolving 4 mg (2 μmol) $\text{AuNP}$-$\text{BCN}$ in 0.3 mL deuterated dichloromethane in an NMR tube. A $t_0$ (time zero) $^1$H NMR spectra was taken of this sample. Subsequently, 10 μL (2 μmol) of the azide/nitrone stock solution was added. This solution was shaken vigorously, and $^1$H NMR spectra were acquired over pre-determined time intervals according to the speed of the reaction.

Upon cycloaddition to $\text{BCN}_{\text{exo}}$-$\text{OH}$ and $\text{AuNP}$-$\text{BCN}$, the methylene protons alpha to the azide in azide 1, and the aromatic protons beta to the azide in azide 2, azide 3, azide 4 and azide 5 produce a $^1$H NMR signal that decreases over time, and produces a new $^1$H NMR signal at a higher chemical shift, and so the decrease in the $^1$H NMR signal from the parent azide was used to obtain a rate of reaction. A rate of reaction for azide 6 was determined by measuring the reduction in the signal at 4.0 ppm (that appears in the $^1$H NMR spectrum of both $\text{BCN}_{\text{exo}}$-$\text{OH}$ and $\text{AuNP}$-$\text{BCN}$), which is not present in the resulting cycloadduct. The hydrogen on the $\alpha$-carbon on each of the nitrones produces a $^1$H NMR signal that decreases over time, and produces a new $^1$H NMR
signal at a higher chemical shift, and so the decrease in the $^1$H NMR signal from the parent azide was used to obtain a rate of reaction.

### 2.6.5.2 Kinetic Measurements for 1-azido-3-propanol (azide 1)

#### Figure S2.38. Second order kinetics graph for azide 1 with $\text{BCN}_{\text{exo}}$-OH.

![Graph](image)

#### Figure S2.39. Second order kinetics graph for azide 1 with $\text{AuNP-BCN}$.

![Graph](image)
2.6.5.3 Kinetic Measurements for 4-azidoanisole (azide 2)

Figure S2.40. Second order kinetics graph for azide 2 with BCN_{exo}-OH.

Figure S2.41. Second order kinetics graph for azide 2 with AuNP-BCN.
2.6.5.4 Kinetic Measurements for azidobenzene (azide 3)

**Figure S2.42.** Second order kinetics graph for azide 3 with BCN$_{exo}$-OH.

**Figure S2.43.** Second order kinetics graph for azide 3 with AuNP-BCN.
2.6.5.5 Kinetic Measurements for 1-azido-4-nitrobenzene (azide 4)

![Kinetics Graph for azide 4 with BCNexo-OH](image1)

**Figure S2.44.** Second order kinetics graph for azide 4 with BCNexo-OH.

![Kinetics Graph for azide 4 with AuNP-BCN](image2)

**Figure S2.45.** Second order kinetics graph for azide 4 with AuNP-BCN.
2.6.5.6 Kinetic Measurements for 4-azidopyridine (azide 5)

Figure S2.46. Second order kinetics graph for azide 5 with BCN$_{exo}$-OH.

Figure S2.47. Second order kinetics graph for azide 5 with AuNP-BCN.
2.6.5.7 Kinetic Measurements for 1-azido-2,3,4,5,6-pentafluorobenzene (azide 6)

Figure S2.48. Second order kinetics graph for azide 6 with BCN<sub>exo</sub>-OH.

Figure S2.49. Second order kinetics graph for azide 6 with AuNP-BCN.
2.6.5.8 Kinetic Measurements for \textit{N}-methyl-\textit{C}-nitrophenyl-nitrone (nitrone 1)

**Figure S2.50.** Second order kinetics graph for \textit{nitron e 1} with BCN\textsubscript{exo}-OH.

**Figure S2.51.** Second order kinetics graph for \textit{nitron e 1} with AuNP-BCN.
2.6.5.9 Kinetic Measurements for $N$-phenyl-$C$-methoxyphenyl-nitrone (nitrone 2)

**Figure S2.52.** Second order kinetics graph for nitrone 2 with BCN$_{exo}$-OH.

**Figure S2.53.** Second order kinetics graph for nitrone 2 with AuNP-BCN.
2.6.5.10 Kinetic Measurements for \textit{N}-phenyl-\textit{C}-phenyl-nitrone (nitrone 3)

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure_s254}
\caption{Second order kinetics graph for \textit{nitrone 3} with BCN_{exo}-OH.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure_s255}
\caption{Second order kinetics graph for \textit{nitrone 3} with AuNP-BCN.}
\end{figure}
2.6.5.11 Kinetic Measurements for $N$-phenyl-$C$-nitrophenyl-nitrone (nitrone 4)

Figure S2.56. Second order kinetics graph for nitrone 4 with BCN$_{exo}$-OH.

Figure S2.57. Second order kinetics graph for nitrone 4 with AuNP-BCN.
2.6.5.12 Kinetic Measurements for $N$-phenyl-$C$-pyridine-nitrone (nitrone 5)

**Figure S2.58.** Second order kinetics graph for nitrone 5 with BCN<sub>exo</sub>-OH.

**Figure S2.59.** Second order kinetics graph for nitrone 5 with AuNP-BCN.
2.6.5.13 Kinetic Measurements for \( N \)-cyanophenyl-\( C \)-pyridine-nitrone (nitrone 6)

Figure S2.60. Second order kinetics graph for nitrone 6 with BCN\textsubscript{exo}-OH.

Figure S2.61. Second order kinetics graph for nitrone 6 with AuNP-BCN.
2.6.6 Competition Experiments for AuNP-BCN

2.6.6.1 General Experimental Details

For the competition experiments between an azide and nitrone, equimolar quantities of one azide, one nitrone and AuNP-BCN were combined in deuterated dichloromethane and monitored by $^1$H NMR spectroscopy.

First, stock solutions for the azide and nitrone used for each experiment were first prepared as described in section 2.6.5.1 to generate 0.2 M solutions of each. Then 0.2 mL of azide stock solution was added to 0.2 mL of nitrone stock solution to obtain 1:1 azide:nitrone stock solutions. Subsequently, 20 μL (2 μmol azide and 2 μmol nitrone) of this 1:1 stock solution was added to 0.3 mL deuterated dichloromethane in an NMR tube, and a $t_0$ (time zero spectrum) $^1$H NMR spectrum was acquired. Non-coinciding $^1$H NMR signals were chosen, one produced from a proton environment in the azide, and one produced from a proton environment in the nitrone, and the integrals were determined for these signals to give the $t_{0(\text{azide})}$ and $t_{0(\text{nitrone})}$ integral values.

Next, a solution was prepared by dissolving 4 mg (2 μmol) AuNP-BCN in 0.3 deuterated dichloromethane in an NMR tube. Subsequently, 20 μL of the 1:1 azide:nitrone solution was added, the solution was shaken vigorously, and a $^1$H NMR spectrum was taken after 24 hours to allow the interfacial reaction to go to completion (24 hour spectrum). The integral of non-coinciding signals from the same proton environments chosen before were determined (which decreased as the interfacial reaction had gone to completion), to give the $t_{24(\text{azide})}$ and $t_{24(\text{nitrone})}$ integral values. To determine the % I-SPAAC (i.e. degree of reaction between AuNP-BCN and the azide species), the $t_{24(\text{azide})}/t_{0(\text{azide})}$ ratio was determined. To determine the % I-SPANC (i.e. degree of reaction between AuNP-BCN and the nitrone species), the $t_{24(\text{nitrone})}/t_{0(\text{nitrone})}$ ratio was determined.

Since azide 6 does not contain any protons, the reacted percentage of nitrone 1 in the competition experiment against azide 6 was determined, and the reacted percentage of azide 6 was calculated by difference.
2.6.6.2 $^1$H NMR Spectra for Competition Experiment between azide 1 and nitrone 6

**Figure S2.62.** $^1$H NMR spectrum of equimolar mixture of azide 1 and nitrone 6 in CD$_2$Cl$_2$ at 25ºC. * denotes residual protio solvent.

**Figure S2.63.** $^1$H NMR spectrum of equimolar mixture of azide 1 and nitrone 6 and interfacial BCN (in AuNP-BCN) in CD$_2$Cl$_2$ at 25ºC. * denotes residual protio solvent.
2.6.6.3 $^1$H NMR Spectra for Competition Experiment between azide 3 and nitrone 4

**Figure S2.64.** $^1$H NMR spectrum of equimolar mixture of azide 3 and nitrone 4 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

**Figure S2.65.** $^1$H NMR spectrum of equimolar mixture of azide 3 and nitrone 4 and interfacial BCN (in AuNP-BCN) in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.
2.6.6.4 $^1$H NMR Spectra for Competition Experiment between azide 4 and nitrone 4

Figure S2.66. $^1$H NMR spectrum of equimolar mixture of azide 4 and nitrone 4 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

Figure S2.67. $^1$H NMR spectrum of equimolar mixture of azide 4 and nitrone 4 and interfacial BCN (in AuNP-BCN) in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.
2.6.6.5 $^1$H NMR Spectra for Competition Experiment between azide 5 and nitrone 2

Figure S2.68. $^1$H NMR spectrum of equimolar mixture of azide 5 and nitrone 2 in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

Figure S2.69. $^1$H NMR spectrum of equimolar mixture of azide 5 and nitrone 2 and interfacial BCN (in AuNP-BCN) in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.
2.6.6.6 \(^1\)H NMR Spectra for Competition Experiment between azide 6 and nitrone 1

**Figure S2.70.** \(^1\)H NMR spectrum of equimolar mixture of **azide 6** and **nitrene 1** in CD\(_2\)Cl\(_2\) at 25°C. * denotes residual protio solvent.

**Figure S2.71.** \(^1\)H NMR spectrum of equimolar mixture of **azide 6** and **nitrene 1** and interfacial BCN (in AuNP-BCN) in CD\(_2\)Cl\(_2\) at 25°C. * denotes residual protio solvent.
2.6.7. References for Supporting Information


4. Kwok, S. W.; Fotsing, J. R.; Fraser, R. J.; Rodionov, V. O.; Fokin, V. V., Transition-Metal-Free Catalytic Synthesis of 1,5-Diaryl-1,2,3-triazoles. *Org. Lett.* **2010**, *12* (19), 4217-4219.


Chapter 3

3 Highly Electron-Deficient Pyridinium-Nitrones for Rapid and Tunable Inverse-Electron-Demand Strain-Promoted Alkyne-Nitrone Cycloaddition to Bicyclo[6.1.0]nonyne

This chapter has been published as a full paper, except for Section 3.2.1 which describes unpublished results and discussion. The corresponding references is: P.N. Gunawardene, W. Luo, A.M. Polgar, John F. Corrigan, M.S. Workentin. *Org. Lett.* **2019**, 21, 14, 5547-5551.

Wilson Luo, under supervision by our supervisor Prof. Mark S. Workentin, assisted in the synthesis and characterization of some of the nitrone and cycloadduct compounds. Alex M. Polgar, under supervision by Prof. John F. Corrigan, conducted DFT analysis on all nitrone compounds and BCN$_{exo}$-OH. The draft of the manuscript was written by the author and edited by Prof. John F. Corrigan and Prof. Mark S. Workentin.

3.1 Introduction

Bimolecular strain–promoted cycloaddition reactions between cycloalkyne dipolarophiles and 1,3 dipoles are a subset of bioorthogonal ‘click’ reactions that have become a powerful tool in chemical biology$^{1-4}$ and nanomaterial science,$^{5-8}$ for the fusion of two complex substrates that is otherwise inaccessible. Their practicality can be largely attributed to the high chemoselectivity and non–perturbing biocompatibility of the complementary reactive partners in the presence of other reactive moieties in both natural and synthetic settings.$^{9,10}$ At the same time, they maintain high reaction kinetics compared to other bioorthogonal reactions such as the Staudinger–Bertozzi ligation and thiol–maleimide reaction.$^{11}$ First developed by Bertozzi and co–workers, the prototype variant of such strain–promoted cycloaddition is the reaction between a cyclooctyne, which is the smallest stable cycloalkyne, and an azide dipole, and is more commonly known as the strain–promoted alkyne–azide cycloaddition (SPAAC).$^{12}$ A less well–known homologous variant of SPAAC is the reaction between a cyclooctyne and nitrone dipole, and is termed the strain–promoted alkyne–nitrone cycloaddition (SPANC).$^{13}$
In recent years, there has been a growing interest toward the acceleration of the reaction kinetics of both SPAAC and SPANC by tailoring the structure and electronic composition of the reactive partners to promote more favorable frontier orbital energy overlap. Such kinetic accelerations allow for tunable and rapid conjugation, reduces the effective concentration of expensively-modified labelling reagents,\textsuperscript{14} and provides a simple strategy for multi–component couplings. Initial efforts toward promoting faster cycloaddition kinetics focused on modifications to the cyclooctyne structure, but such strategies experience a problematic trade–off between cyclooctyne reactivity and stability. Benzoannulation of the cyclooctyne ring has furnished rapidly reactive strained–alkyne moieties, such as dibenzocyclooctyne (DBCO) and biarylazacyclooctynone (BARAC), but such rigidified aryl–fused strained–alkynes suffer from poor stability at ambient conditions. To mitigate these limitations, Dommerholt and co–workers developed an aliphatic cyclooctyne bicyclo[6.1.0]nonyne (BCN),\textsuperscript{15} that demonstrates exceptional stability and synthetic accessibility, but is kinetically less reactive than its benzoannulated counterparts. Regardless, the superior stability of BCN makes it a more convenient strained–alkyne to work with, compared to DBCO and BARAC.

Contemporary efforts toward enhancing the reaction kinetics of SPAAC/SPANC have converged on structural modifications to the complementary dipolar species. Delocalization of the dipolar species into electron–withdrawing substituents accelerates both SPAAC and SPANC reaction kinetics, while electron–donating substituents decelerate the reaction kinetics.\textsuperscript{14, 16, 17} It should be noted that, unlike SPAAC, an advantageous characteristic of SPANC is that there are three modifiable sites on the nitrone functionality, while terminal azides possess only one modifiable site. Much of the kinetic enhancement of SPANC has been pioneered by Pezacki and co–workers,\textsuperscript{14, 17} who demonstrated the kinetic consequences of substituent modifications on acyclic nitrones, with their electron–deficient acyclic nitrones having the most accelerated reaction rates. They proceeded to demonstrate that cyclic nitrones possess even greater reaction kinetics through increasing the strain at the nitrone functionality. To the best of our knowledge, the fastest SPANC reaction between a cyclic nitrone and a stable cyclooctyne (BCN) has a bimolecular rate constant ($k_2$) of 1.5 M$^{-1}$s$^{-1}$ in methanol.\textsuperscript{17}

In order to investigate further acceleration of the SPANC reaction between a nitrone and BCN, we were intrigued by the fastest SPAAC reaction to BCN reported by Dommerholt and co-
which occurs between a pyridinium–functionalized azide and BCN \((k_2 = 2.0 \text{ M}^{-1}\text{s}^{-1} \text{ in} \ 9:1 \text{ THF:} \text{H}_2\text{O})\). We speculated that delocalizing the nitro moiety into a pyridinium functionality would similarly hasten the SPANC reaction. To that end, we report rapid strain–promoted cycloaddition between a stable cyclooctyne (BCN\(_{\text{exo}}\)-OH) and highly electron–deficient nitrones possessing a pyridinium functionality on the nitrone \(\alpha\)–carbon. The rapid reactivity of these “pyridinium–nitrones” can be rationalized in terms of favorable energetic overlap between the frontier orbitals of the two reactive partners, that is only observed through inclusion of the pyridinium functionality on the nitrene moiety. To further evaluate the tunability of the reaction kinetics and emphasize the advantageous opportunity for multi–site modifications to nitrones that cannot be achieved with azides, anisole (3a), phenyl (3b) and benzonitrile (3c) substituents were incorporated to the nitrene nitrogen, which results in substantial alterations to the kinetic profiles.

### 3.2 Results and Discussion

The three pyridinium-nitrones (3a–c) can be conveniently synthesized in gram–scale quantities using inexpensive, commercially available reagents and can be purified without the need of chromatography. They are made through condensation of the corresponding hydroxylamine onto 3–pyridinecarboxyaldehyde to generate the pyridine–nitrones (2a–c) in good yield, which can be subsequently \(N\)–alkylated using methyl iodide to generate the methylated pyridinium–nitrone species in high yield (Scheme 3.1). Due to the hydrophilicity of the pyridinium functionality, 3a–c are most soluble in polar solvents such as dimethyl sulfoxide, acetonitrile, methanol and water. The characteristic \(^1\text{H} \text{NMR signals of 3a–c are produced by the 2’ aromatic proton in the pyridinium ring (3a and 3c, 10.21 ppm and 3b, 10.23 ppm) and the methyl protons of the pyridinium functionality (3a, 4.44 ppm, 3b and 3c, 4.46 ppm) (see Section 3.5.7). The infrared spectra of 3a–c show a strong band between 1616–1629 cm\(^{-1}\) that does not appear in the infrared spectra of 2a–c (see Section 3.6.2) and can be assigned to C=\(\text{N}\) stretching vibrations characteristic of the quaternary nitrogen in the pyridinium ring.\(^{18}\)

Single crystal X–ray crystallography has been used to verify the structures of 3a–c (see Section 3.6.16), which confirms the planarity of the nitrene moiety and aromatic substituents, as
well as the trigonal planar geometry of the nitrone moiety. The bond length between the nitronitrone nitrogen (N₂) and α–carbon (C₇) in 3a–c is consistent with previously reported C=N bond lengths in iminium ions (~1.305 Å),¹⁹ which are longer than C=N bond lengths in imines (~1.279 Å)²⁰ due to the larger single bond character in iminium ions. Interestingly, the crystallographic data indicates that there is a slight increase in this N₂–C₇ bond length in 3a–c as increasingly electron–withdrawing substituents are fused to the nitronitrone moiety (Table 3.1), indicating a weakening of the N₂–C₇ double bond as more electron–withdrawing substituents are fused to the pyridinium–nitrones. Single crystal X-ray crystallography of model compounds 2b and 4 are included for comparison. The molecular structure of 4 has been reported previously for data collected at room temperature,²¹ but the data included in Table 3.1 is measured at 110 K for consistency with the data of 3a-3c.

Scheme 3.1. Synthesis of pyridinium–nitrones possessing anisole (3a), phenyl (3b) and benzonitrile (3c) substituents. Top inset shows general scheme for strain–promoted alkyne–nitronitrone cycloaddition (SPANC) reaction between nitronitrone (blue) and cyclooctyne (red). Bottom: molecular structures of 3a, 3b, 3c in the crystal. Thermal ellipsoids are drawn at the 50% probability level with hydrogen atoms drawn with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, purple = iodine)
The reaction kinetics of 3a–c with BCN$_{exo}$-OH (5) were evaluated under pseudo-first order conditions, according to a previously reported methodology using UV–Vis spectroscopy. A solvent mixture of 2:1 acetonitrile:tetrahydrofuran was used for this study, instead of methanol or water, to minimize any solvent–dependent acceleration in the more polar solvents that would make measuring the kinetics more difficult. This solvent choice was also chosen to minimize possible hydrolysis of the nitrone, which was observed for 3b in 6:1 D$_2$O:(CD$_3$)$_2$SO after 12 hours (although 3a and 3c were largely unaltered in this solvent after 12 hours) (see Section 3.6.14). Rate constants were measured in duplicate at 22°C (Table 3.1) by observing the decrease in absorption at 345 nm produced by the nitrone moiety in 3a–c that is not produced by the resulting isoxazoline product (see Section 3.6.15). It should be noted that the BCN$_{exo}$-OH isomer was used for this study as it produced in higher quantities during the strained–alkyne synthesis, but the endo–isomer can also be used as it would have similar kinetics. Mass spectrometry and NMR spectroscopy (in (CD$_3$)$_2$SO)) was used to confirm that the pyridinium–nitrones undergoes the SPANC reaction with BCN$_{exo}$-OH (see Section 3.6.10). In addition to the three pyridinium–nitrones 3a–c, the rate of reaction for two nitrones delocalized into a phenyl (4) and pyridine (2b) ring instead of a pyridinium ring was determined, by measuring the decrease in absorption at 316 nm and 320 nm, respectively. Nitrone 4 displayed the slowest rate constant ($k_2 = 0.066$ M$^{-1}$s$^{-1}$) in the series, due to the presence of the least electron-withdrawing α–carbon phenyl and N–phenyl substituents. Replacement of the α–carbon phenyl substituent with the more electron–withdrawing α–carbon pyridine substituent in nitrone 2b results in a two-fold acceleration ($k_2 = 0.15$ M$^{-1}$s$^{-1}$). However, replacement of the α–carbon pyridine with an α–carbon pyridinium ring in 3b results in an approximate twenty–fold acceleration in the reaction rate ($k_2 = 3.3$ M$^{-1}$s$^{-1}$), which demonstrates the substantial acceleration of SPANC kinetics through pyridinium–conjugation. To illustrate the tunability of SPANC kinetics through the multi–site modification that only exists with SPANC, replacement of the N–phenyl substituent in 3b with a more electron-donating N–anisole substituent in 3a results in an approximate two-fold deceleration of the reaction rate ($k_2 = 1.6$ M$^{-1}$s$^{-1}$). Conversely, exchange of the N–phenyl substituent in 3b with the more electron-withdrawing N–benzonitrile substituent in 3c triggered a significant three–fold acceleration in the reaction rate ($k_2 = 8.3$ M$^{-1}$s$^{-1}$). This is the fastest click reaction between a dipolar species and BCN reported to date and represents a substantial improvement to the kinetic profile of strain–promoted cycloaddition chemistry.
Table 3.1. Key parameters of pyridinium–nitrone that vary as electron deficiency (and corresponding reactivity) is increased. $^1$H NMR spectra were taken in deuterated dimethylsulfoxide at 25°C. Bond lengths were determined from crystallographic data. Mulliken charges (Q) and the energy gap between the HOMO of BCN and LUMO of the nitrone ($\Delta E_{\text{HOMO-LUMO}}$) were determined from DFT calculations. Bimolecular rate constants ($k_2$) were determined under pseudo–first order conditions in 2:1 acetonitrile:tetrahydrofuran at 22°C by UV-Vis spectroscopy.

<table>
<thead>
<tr>
<th></th>
<th>$^1$H NMR $\delta$</th>
<th>N$_2$–C$_7$ Bond</th>
<th>N$_2$–C$_8$ Bond</th>
<th>Q at C$_7$</th>
<th>$\Delta E_{\text{BCN-HOMO-Nitron LUMO}}$</th>
<th>$k_2$ (M$^{-1}$s$^{-1}$)</th>
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<tr>
<td>4</td>
<td>R$_1$ = phenyl</td>
<td>8.50</td>
<td>1.3126(17)</td>
<td>1.4612(16)</td>
<td>–0.0412</td>
<td>–4.81</td>
</tr>
<tr>
<td></td>
<td>Y = H</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2b</td>
<td>R$_1$ = pyridine</td>
<td>8.63</td>
<td>1.3126(12)</td>
<td>1.4555(12)</td>
<td>–0.0360</td>
<td>–4.58</td>
</tr>
<tr>
<td></td>
<td>Y = H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>R$_1$ = pyridinium–CH$_3$</td>
<td>8.91</td>
<td>1.305(4)</td>
<td>1.459(4)</td>
<td>–0.0371</td>
<td>–0.532</td>
</tr>
<tr>
<td></td>
<td>Y = OCH$_3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>R$_1$ = pyridinium–CH$_3$</td>
<td>8.98</td>
<td>1.3130(15)</td>
<td>1.4607(15)</td>
<td>–0.0302</td>
<td>–0.335</td>
</tr>
<tr>
<td></td>
<td>Y = H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>R$_1$ = pyridinium–CH$_3$</td>
<td>9.07</td>
<td>1.314(4)</td>
<td>1.485(4)</td>
<td>–0.0255</td>
<td>–0.0533</td>
</tr>
<tr>
<td></td>
<td>Y = CN</td>
<td></td>
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</table>

To illustrate the flexibility of the reaction to solvent choice (that may be applicable for future biological applications), the SPANC reaction between 3c and BCN$_{exo}$–OH was successfully carried out and products confirmed by $^1$H NMR spectroscopy in 6:1 D$_2$O:(CD$_3$)$_2$SO, 1:1 D$_2$O:(CD$_3$)$_2$SO and CD$_3$OD (see Section 3.6.11). Furthermore, the generality of the reaction between 3c and different strained alkynes other than BCN$_{exo}$–OH was probed by challenging 3c to commercially available dibenzycyclooctyne-amine (DBCO–amine) (see Section 3.6.12) and a small symmetrical strained–alkyne, (Z)-cyclooct-1-ene-5-yne (6b) (see Section 3.6.13). Both SPANC reactions were confirmed by NMR spectroscopy and mass spectrometry.

$^1$H NMR spectroscopy allows for simple, preliminary estimations of nitrone reactivity by comparing the chemical shift of the H$_a$ in the nitrone moiety. As shown in Table 3.1, the H$_a$ in the electron–rich nitrone 3a, which has the slowest reaction kinetics, has the lowest chemical shift (8.91 ppm). Incorporation of increasingly electron–deficient substituents results in $^1$H NMR signal
of the Hₐ appearing at higher chemical shifts as the kinetic reactivity is increased, increasing to 8.98 ppm in 3b and 9.07 ppm in 3c. Determination of Mulliken atomic charges²² show that better electron withdrawing groups result in a smaller partial negative charge on the α–carbon (C₇) (Table 3.1), which is a consequence of a less electron–rich nitrone moiety and rationalizes the observed deshielding of the Hₐ as more electron–withdrawing substituents are fused to the pyridinium–nitrone.

The reactivity differences between 3a–c were probed by density functional theory (DFT) computations using the GAUSSIAN09²³ suite of software at the level B3LYP/6–31G*.²⁴,²⁵ The geometries of the three pyridinium–nitrone were optimized and agree closely with the crystallographic data (see Section 3.6.17). The DFT data confirms that, as reported by Dommerholt and co–workers for the SPAAC reaction,¹⁷ the SPANC reaction proceeds via an inverse electron–demand mechanism (IED–SPANC). That is, the frontier orbitals involved in the SPANC reaction are the HOMO of 5, which is localized mainly on the C–C triple bond, and the LUMO of the pyridinium–nitrone, which is delocalized primarily over the both the nitrone and pyridinium moieties, but also has contributions from the nitrone N–substituent (Figure 3.1). Because of these contributions, the energy of the LUMO in IED–SPANC varies according to the electronic nature of the N–substituent on the nitrone moiety, with the electron–donating anisole

![Isosurface plots for the HOMO and LUMO](image)

Figure 3.1. Isosurface plots (isoval = 0.03 e au⁻³) for the HOMO of BCNexo-OH (5) and the LUMO of 3a–c, 4, 2b, and energy diagram of the frontier orbitals involved in SPANC between 3a–c, 4, 2b and BCNexo-OH (5).
substituent in \textbf{3a} destabilizing the LUMO relative to \textbf{3b}, and the electron–withdrawing benzonitrile substituent in \textbf{3c} lowering the LUMO energy relative to \textbf{3b}. This stabilizing effect results in the convergence of the pyridinium–nitrone LUMO energy level and the HOMO energy level of \textbf{5} in the IED–SPANC reaction (Table \textbf{3.1}). The expected rate enhancement can hence be rationalized in terms of favorable energetic overlap between the two reactants when more electron–withdrawing nitrone \textit{N}–substituents are present. In fact, our DFT calculations indicate that \textbf{3c} possesses a miniscule \textit{HOMO}_{BCN}–\textit{LUMO}_{nitrone} energy gap (\textit{\textendash}0.0533 eV). This suggests that \textbf{3c} is a highly idealized reactive partner for BCN (a stable cyclooctyne), and that any additional tuning to accelerate the reactivity further may provide only small enhancements to the cycloaddition reaction to BCN.

\textbf{3.2.1 Towards the Development of Pyridinium-Functionalized Gold Nanoparticles}

Having developed a general methodology for synthesizing pyridinium-functionalized nitrones, the ability to execute such rapid and tunable SPANC chemistry on the surface of gold nanoparticles (AuNPs) was investigated. AuNPs was chosen for this prototype study because they are easy to synthesize in high yield compared to other nanomaterial systems, and can be easily characterized by \textsuperscript{1}H NMR spectroscopy, allowing for straightforward analyses of modifications to the organic surface structure. Given the potential use of functionally complex AuNPs in a variety of applications in biomedicine, imaging and nanoscience, the ability to implement rapid SPANC chemistry to tether functional substrates (such as biological macromolecules, targeting agents and other nanomaterial systems) to the surface of AuNPs would be a worthwhile investigation.

AuNPs are typically synthesized in the presence of moderate reducing agents, such as NaBH\textsubscript{4}, in the presence of a thiol ligand (RSH) that serves to encircle and protect the interior metallic core. Given the chemical sensitivity of many functional substrates, direct synthetic strategies with a thiol ligand that has a functional substrate incorporated onto it is often unfeasible because of incompatibilities with reducing conditions of the synthesis. For this reason, the most common strategy to incorporate functional substrates to the surface of AuNPs is through place exchange chemistry, in which the inert AuNP system is mixed with a functional thiol ligand, and native inert thiol ligands are exchanged with the incoming functional thiol. The most common
problem with this strategy is that protection-deprotection strategies are often required to tether functional substrates to thiol ligands, which is often chemically incompatible with the functional substrate. Given this limitation, our group explores methodologies in which post-assembly modifications can be used, in which a AuNP platform is created that has a reactive group tethered to the surface, after which a functional substrate with the complementary reactive group can be added and reacts exclusively with the reactive group on the AuNP surface. To this end, if the reactive pyridinium-nitrone moiety could be incorporated to the gold nanoparticle surface, then functional substrates with the complementary strained-alkyne moiety could be tethered to the surface through rapid interfacial SPANC. Furthermore, if multiple nitrone moieties could be incorporated to the AuNP surface, each having a different kinetic profile, then this would provide temporal and kinetic control over surface modification chemistry, allowing for the development of multi-functional AuNP systems.

As with the bicyclononyne-functionalized gold nanoparticle system describe in Chapter 2, the inert AuNP system that was chosen was methoxy-functionalized AuNPs (AuNP-OMe), because they can be synthesized in high yield, easily characterizable, and are soluble in a variety of solvents. It was proposed that if this AuNP-OMe were mixed with a tetraethylene glycol (TEG)-based thiol ligand possessing a terminal pyridinium-nitrone moiety, then the pyridinium-nitrone moiety could be incorporated to the AuNP surface through place exchange chemistry (Scheme 3.2a).

The synthetic strategy for the proposed TEG-based pyridinium-nitrone-functionalized thiol ligand is shown in Scheme 3.2b (see Section 3.6.4 for experimental details). First, tetraethylene glycol was monosubstituted with nitrobenzyl bromide in the presence of sodium hydride to give A, which was subsequently tosylated at the alcohol moiety (to give B) and then substituted once more with potassium thioacetate to give C, which possessed a protected thiol in the form of a thioacetate moiety. Then, the nitrophenyl-moiety was transformed into the pyridinium-nitrone moiety, using the same general strategy as described in Section 3.2, to give E.

Having successfully made compound E, the final step was the deprotection of the thioacetate moiety by saponification with sodium hydroxide to give the corresponding thiolate moiety, and the subsequent protonation with hydrochloric acid to give the desired thiol ligand F.
However, this step was unsuccessful, due to the sensitivity of the pyridinium-nitrone moiety. It has been reported that nitro moieties are prone to hydrolysis in the presence of acidic species, splitting the nitro moiety apart into the parent aldehyde and amine moieties.\textsuperscript{14,17} Even at the dilute concentrations of hydrochloric acid used during the deprotection strategy, there was significant hydrolysis observed, with only a small amount of compound F being formed. Furthermore, once the small amount of compound F that formed was isolated and purified, it

Scheme 3.2. (a) Proposed synthetic strategy for incorporating the pyridinium-nitrone moiety to the surface of AuNPs, using a pyridinium-functionalized thiol ligand. (b) Synthetic strategy for pyridinium-functionalized thiol ligand.
underwent further hydrolysis on its own quite rapidly, preventing characterization of F entirely. This indicated that, even if it could by synthesized, the pyridinium-nitrone moiety was not compatible with the thiol moiety. Although the pyridinium-nitrone moiety was demonstrated to be quite stable in the presence of water and alcohol solvents towards undesired hydrolysis, as described and shown in Section 3.2, Section 3.6.11 and Section 3.6.14, the higher acidity of SH moieties (compared to OH moieties) makes the pyridinium-nitrone moiety more susceptible to hydrolysis in the presence of SH moieties. This susceptibility prevents the thiol moiety from being tethered to a ligand possessing a pyridinium-nitrone moiety, which unfortunately prevents the pyridinium-nitrone moiety from being incorporated to the surface of AuNPs.

3.3 Conclusion

In conclusion, the incorporation of pyridinium functionalities into nitrone moieties serves as a simple method for substantial acceleration of IED SPANC to BCN. As benzoannulated cyclooctynes are comparatively unstable and expensive to synthesize, it is highly beneficial to utilize more stable aliphatic strained–alkynes like BCN. Furthermore, the acceleration of the cycloaddition reaction serves as an important tool for the efficient conjugation of expensively–modified substrates in chemical biology and nanomaterial sciences to produce reliable and reproducible results at nano– and pico–molar concentrations. It is important to note that our approach not only creates a general method for acceleration of the SPANC reaction, but also incorporates an additional modifiable site: the pyridine functionality. In this prototype study we coupled a methyl group to the pyridine functionality to simplify the analysis. However, given the numerous methodologies for N–alkylation of pyridine rings, it should be possible to incorporate the pyridinium–nitrone moiety with alkyl ligands bearing additional functionality for applications using such methodologies, while retaining the rapid kinetics of the pyridinium–nitrone moiety. We are currently exploring such methodologies.

3.4 Acknowledgements

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3.5 References


### 3.6 Supporting Information

#### 3.6.1 General Materials and Methods

**Reagents and Solvents.** The following materials were used as received. 4-nitroanisole, zinc dust (<10µm, ≥98%), 3-pyridinecarboxaldehyde (98%), iodomethane (99.5%), nitrobenzene (≥99%), 4-nitrobenzonitrile (97%), benzaldehyde (≥99.5%), dibenzocyclooctyne-amine, dimethyl sulfoxide-D₆ ((CD₃)₂SO, 99.96 atom %D), methanol-D₄ (CD₃OD, 99.8% atom %D)
dichlotomethane-D$_2$ (CD$_2$Cl, 99.5 atom %D) and water-D$_2$ (D2O, 99.9% atom %D) were purchased from Sigma-Aldrich (Millipore Sigma). Chloroform-D$_1$ (99.8 atom %D) was purchased from Cambridge Isotope Laboratories. Technical grade ammonium chloride, magnesium sulphate, hexanes, dichloromethane, dimethyl sulfoxide, di-ethyl ether, methanol, acetonitrile were purchased from Caledon. Ethanol (anhydrous) was purchased from Commercial Alcohols.

Unless otherwise state, all reactions were performed at ambient conditions.

**NMR Spectroscopy.** $^1$H and $^{13}$C{$^1$H} spectra were recorded on a Bruker AvIII HD 400 spectrometer. $^1$H NMR spectra are reported as δ in units of parts per million (ppm), and referenced against residual protio dimethylsulfoxide (2.50 ppm, quin), water (4.75 ppm, s) or methanol (3.31 ppm, quin; 4.87 ppm, s) as indicated. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), quin (quintuplet), m (multiplet), dt (doublet of triplets) and dd (doublet of doublets). Coupling constants are reported as a $J$ value in Hertz (Hz) according to the spectrometer frequency. The number of protons ($n$) for a given resonance is indicated as $n$H, and is based on spectral integration values. $^{13}$C{$^1$H} NMR spectra are reported as δ in units of parts per million (ppm) and referenced against the indicated deuterated solvent: dimethylsulfoxide-D$_6$ (39.5 ppm, septet), chloroform-D$_1$ (77.0 ppm, t).

**Mass Spectrometry.** Electrospray ionization (ESI) mass spectra were obtained in positive-ion mode using a Bruker microTOF II spectrometer.

**UV-Visible (UV-Vis) Spectroscopy.** UV-Vis absorption spectra were recorded using a Cary 5000 scan instrument using standard quartz cells (1cm path length) with a scan range of 200-1000nm. The background spectrum of the indicated solvent was subtracted internally by the software.

**Infrared (IR) spectroscopy.** Attenuated total reflectance IR (ATR-IR) spectra were recorded using a PerkinElmer Spectrum Two FT-IR spectrometer.
3.6.2 Synthesis of Nitrones

3.6.2.1 Synthesis of \( N-(4\text{-methoxyphenyl})\text{-C-(3-pyridine)} \) Nitrone (2a)

To 1.0 g (6.5 mmol, 1eq) 4-nitroanisole and 0.37 g (7.0 mmol, 1.1 eq) ammonium chloride in 10 mL distilled water and 10 mL ethanol was added 1.3 g (20 mmol, 3eq) zinc dust portion-wise over 10 minutes at room temperature. The resulting heterogenous mixture was stirred vigorously for 20 minutes, after which the insoluble solid was removed by vacuum filtration. The supernatant was diluted with brine (50 mL) and extracted with diethyl ether (3 x 10 mL), dried over magnesium sulphate and concentrated to give the crude hydroxylamine as an off-white solid, which was used without further purification.

To the crude hydroxylamine in 15 mL dichloromethane was added 1.2 mL (13 mmol, 2 eq) 3-pyridinecarboxaldehyde and a small amount of magnesium sulphate. The resulting heterogenous mixture was stirred overnight, after which the magnesium sulphate was removed by vacuum filtration. The supernatant was concentrated via rotary evaporation and the crude residue was purified by dissolving in minimal dichloromethane and precipitating the product out with excess di-ethyl ether at -20°C overnight to give \( N-(4\text{-methoxyphenyl})\text{-C-(3-pyridine)} \) nitrone (2a) as an off-white crystalline solid in 52% overall yield (0.79 g, 3.4 mmol). \(^1\)H NMR ((CD\(_3\)_2SO, 400 MHz): \( \delta \) 9.38 (d, \( J=1.6 \text{ Hz} \), 1H), 8.97 (dt, \( J_1=8.0 \text{ Hz} \), \( J_2 = 1.6 \text{ Hz} \), 1H), 8.62 (dd, \( J_1 = 4.8 \text{ Hz} \), \( J_2 = 1.6 \text{ Hz} \), 1H), 8.56 (s, 1H), 7.90 (m, 2H), 7.52 (dd, \( J_1 = 8.0 \text{ Hz} \), \( J_2 = 4.8 \text{ Hz} \), 1H), 7.09 (m, 2H), 3.84 (s, 3H). \(^{13}\)NMR ((CD\(_3\)_2SO, 400 MHz): \( \delta \) 159.9, 149.9, 149.4, 141.0, 134.1, 129.6, 127.2, 123.1, 122.3, 113.6, 55.2. HRMS (ESI) \( m/z \) calc. for C\(_{12}\)H\(_{12}\)N\(_2\)O\(_2\) (M): 228.0899, found: 228.0867. IR (ATR, \( \text{cm}^{-1} \)): 3134, 3044, 2947, 2838, 1597, 1550, 1501, 1301, 1249, 1169, 1078, 1029.

3.6.2.2 Synthesis of \( N-(4\text{-methoxyphenyl})\text{-C-(3-methylpyridinium)} \) Nitrone (3a)

To 0.20 g (0.88 mmol, 1eq) \( N-(4\text{-methoxyphenyl})\text{-C-(3-pyridine)} \) nitrone (2a) in 4 mL dry dichloromethane was added 0.54 mL (8.8 mmol, 10 eq) iodomethane. The colorless solution was stirred overnight, resulting in the precipitation of a yellow solid. The yellow
precipitate was collected by vacuum filtration and washed thoroughly with dichloromethane to give \(N\)-(4-methoxyphenyl)-C-(3-methylpyridinium) nitrone (3a) as a yellow powdery solid in 90% yield (0.29 g, 0.79 mmol). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 10.21 (s, 1H), 9.03 (dd, \(J_1 = 21.6\) Hz, \(J_2 = 8.4\) Hz, 2H), 8.91 (s, 1H), 8.24 (dd, \(J_1 = 8.4\) Hz, \(J_2 = 6.4\) Hz, 1H), 7.94 (m, 2H), 7.16 (m, 2H), 4.45 (s, 3H), 3.86 (s, 3H). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 161.1, 145.6, 143.2, 143.0, 140.6, 130.7, 127.6, 127.5, 122.8, 114.3, 55.8, 48.7. HRMS (ESI) \(m/z\) calc. for \(C_{14}H_{15}N_2O_2\) (M)\(^{+}\): 243.1128, found: 243.1138. IR (ATR, cm\(^{-1}\)): 3136, 3073, 2983, 2835, 1628, 1542, 1499, 1472, 1301, 1254, 1162, 1081, 1014.

3.6.2.3 Synthesis of \(N\)-phenyl-C-(3-pyridine) Nitrone (2b)

\[
\begin{array}{c}
\text{To 1.7 mL (16 mmol, 1 eq) nitrobenzene and 0.91 g (17 mmol, 1.1eq) ammonium chloride in 20 mL distilled water and 20 mL ethanol was added 3.2 g (50 mmol, 3 eq) zinc dust portion-wise over 10 minutes at room temperature. The resulting heterogeneous mixture was stirred vigorously for 20 minutes, after which the insoluble white solid was removed by vacuum filtration. The supernatant was diluted with brine (100 mL) and extracted with diethyl ether (3 x 20 mL), dried over magnesium sulphate and concentrated to give the crude hydroxylamine as a light yellow solid, which was used without further purification.}
\end{array}
\]

To the crude hydroxylamine in 20 mL dichloromethane was added 1.9 mL (20 mmol, 1.3 eq) 3-pyridinecarboxaldehyde and a small amount of magnesium sulphate. The resulting heterogeneous mixture was stirred overnight, after which the magnesium sulphate was removed by vacuum filtration. The supernatant was concentrated via rotary evaporation and the crude residue was purified by dissolving in minimal dichloromethane and precipitating the product out with excess di-ethyl ether at -20°C overnight to give \(N\)-phenyl-C-(3-pyridine) nitrone (2b) as an white crystalline solid in 49% overall yield (1.56 g, 7.9 mmol). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 9.40 (d, \(J = 2.0\) Hz, 1H), 8.99 (dt, \(J_1 = 8.4\) Hz, \(J_2 = 1.6\) Hz, 1H), 8.63 (m, 1H), 8.62 (s, 1H), 7.93 (m, 2H), 7.56 (m, 4H). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 150.3, 149.8, 148.0, 134.5, 131.1, 130.0, 129.0, 127.2, 123.4, 121.3. HRMS (ESI) \(m/z\) calc. for \(C_{12}H_{10}N_2O\) (M)\(^{+}\): 198.0793, found: 198.0796. IR (ATR, cm\(^{-1}\)): 3130, 3065, 3062, 1582, 1555, 1484, 1466, 1403, 1335, 1272, 1203, 1175, 1164, 1072, 1022.
*Spectral data is similar to previously reported data in CDCl$_3$.$^1$

3.6.2.4 Synthesis of $N$-phenyl-$C$-(3-methylpyridinium) Nitrone (3b)

To 0.50 g (2.5 mmol, 1 eq) $N$-phenyl-$C$-(3-pyridine) nitrone (2b) in 10 mL dry dichloromethane was added 1.6 mL (25 mmol, 10 eq) iodomethane. The colorless solution was stirred overnight, resulting in the precipitation of a yellow solid. The yellow precipitate was collected by vacuum filtration and washed thoroughly with dichloromethane to give $N$-phenyl-$C$-(3-methylpyridinium) nitrone (3b) as a yellow powdery solid in 96% yield (0.82 g, 2.4 mmol). $^1$H NMR ((CD$_3$)$_2$SO, 400 MHz): $\delta$ 10.23 (s, 1H), 9.06 (dd, $J_1 = 22.4$ Hz, $J_2 = 8.4$ Hz, 2H), 8.98 (s, 1H), 8.26 (dd, $J_1 = 8.4$ Hz, $J_2 = 6.4$ Hz, 1H), 7.97 (m, 2H), 7.64 (m, 3H), 4.46 (s, 3H). $^{13}$NMR ((CD$_3$)$_2$SO, 400 MHz): $\delta$ 147.7, 146.1, 143.5, 143.4, 131.4, 129.7, 129.2, 127.8, 121.6, 48.9. HRMS (ESI) $m/z$ calc. for C$_{13}$H$_{13}$N$_2$O$^+$ (M)$^+$: 213.1022, found: 213.1029. IR (ATR, cm$^{-1}$): 3099, 3049, 2922, 1616, 1549, 1490, 1464, 1404, 1190, 1154, 1082.

3.6.2.5 Synthesis of $N$-(4-cyanophenyl)-$C$-(3-pyridine) Nitrone (2c)

To 2.0 g (14 mmol, 1 eq) 4-nitrobenzonitrile and 0.79 g (15 mmol, 1.1 eq) ammonium chloride in 20 mL distilled water and 20 mL ethanol was added 2.7 g (41 mmol, 3 eq) zinc dust portionwise over 10 minutes at room temperature. The resulting heterogenous mixture was stirred vigorously for 20 minutes, after which the insoluble white solid was removed by vacuum filtration. The supernatant was diluted with brine (100 mL) and extracted with diethyl ether (3 x 20 mL), dried over magnesium sulphate and concentrated to give the crude hydroxylamine as a light yellow oil, which was used without further purification.

To the crude hydroxylamine in 25 mL dichloromethane was added 3.0 mL (32 mmol, 2.2 eq) 3-pyridinecarboxaldehyde and a small amount of magnesium sulphate. The resulting heterogenous mixture was stirred overnight, after which the magnesium sulphate was removed by vacuum filtration. The supernatant was concentrated via rotary evaporation and the crude residue was purified by dissolving in minimal dichloromethane and precipitating the product out with excess di-ethyl ether at -20°C overnight to give $N$-(4-cyanophenyl)-$C$-(3-pyridine) nitrone (2c)
as an light yellow solid in 54% overall yield (1.7 g, 7.6 mmol). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 9.42 (d, \(J = 2.0\) Hz, 1H), 9.00 (dt, \(J_1 = 8.0\) Hz, \(J_2 = 2.0\) Hz, 1H), 8.77 (s, 1H), 8.67 (dd, \(J_1 = 4.8\) Hz, \(J_2 = 2.0\) Hz, 1H), 8.17 (m, 2H), 8.10 (m, 2H), 7.56 (dd, \(J_1 = 8.0\) Hz, \(J_2 = 4.8\) Hz, 1H). \(^{13}\)NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 151.3, 150.9, 150.5, 135.3, 133.8, 133.1, 127.3, 123.9, 122.8, 118.1, 113.0. HRMS (ESI) \(m/z\) calc. for C\(_{13}\)H\(_9\)N\(_3\)O (M): 223.0746, found: 223.0735. IR (ATR, cm\(^{-1}\)): 3104, 3082, 3065, 3054, 2242, 1584, 1498, 1425, 1413, 1338, 1297, 1270, 1172, 1070, 1026.

3.6.2.6 Synthesis of \(N-\)phenyl-\(C\)-(3-methylpyridinium) Nitrone (3c)

To 0.50 g (2.2 mmol, 1 eq) \(N-\)phenyl-\(C\)-(3-pyridine) nitrone (2c) in 10 mL dry dichloromethane was added 1.4 mL (22 mmol, 10 eq) iodomethane. The light yellow solution was stirred overnight, resulting in the precipitation of a yellow solid. The yellow precipitate was collected by vacuum filtration and washed thoroughly with dichloromethane to give \(N-(4\)cyanophenyl)-\(C\)-(3-methylpyridinium) nitrone (3c) as a yellow powdery solid in 86% yield (0.68 g, 1.9 mmol). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 10.21 (s, 1H), 9.07 (m, 3H), 8.27 (dd, \(J_1 = 8.0\) Hz, \(J_2 = 6.0\) Hz, 1H), 8.17 (m, 4H), 4.46 (s, 3H). \(^{13}\)NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) 150.0, 146.4, 143.6, 143.6, 133.9, 130.6, 130.0, 127.7, 122.6, 117.8, 113.7, 48.8. HRMS (ESI) \(m/z\) calc. for C\(_{14}\)H\(_{12}\)N\(_3\)O (M): 238.0975, found: 238.0965. IR (ATR, cm\(^{-1}\)): 3114, 3049, 3024, 2933, 2230, 1629, 1596, 1555, 1499, 1309, 1160, 1097, 1064, 1024.

3.6.2.7 Synthesis of \(N\)---phenyl-\(C\)---phenyl Nitrone (4)

To 2.0 g (16 mmol, 1.7 mL) nitrobenzene and 0.95 g (18 mmol) ammonium chloride in 12 mL ethanol and 12 mL distilled water was added 2.1 g (32 mmol) zinc dust portion-wise over 10 minutes. The resulting heterogeneous mixture was stirred vigorously for 20 minutes, after which the insoluble solid was removed by vacuum filtration. The supernatant was diluted with brine (100 mL) and extracted with diethyl ether (3 x 20 mL), dried over magnesium sulphate and concentrated to give the \textit{crude hydroxylamine} as a light yellow oil, which was used without further purification.
To the crude hydroxylamine in 20 mL dichloromethane was added 2.1 g (20 mmol, 2.0 mL) benzaldehyde and a small amount of magnesium sulphate. The resulting heterogenous mixture was stirred overnight, after which the magnesium sulphate was removed by vacuum filtration. The supernatant was concentrated via rotary evaporation and the crude residue was purified by dissolving in minimal dichloromethane and precipitating the product out with excess di-ethyl ether at -20°C overnight to give N-phenyl-C-phenyl nitrone (4) in 75% overall yield (2.4 g). ¹H NMR ((CD₃)₂SO, 400 MHz): 8.49 (m, 3H), 7.92 (m, 2H), 7.53 (m, 6H). ¹³C NMR ((CD₃)₂SO, 400 MHz): δ 148.5, 133.5, 131.1, 130.6, 129.9, 129.1, 128.8, 128.5, 121.5. HRMS (ESI) m/z calc. for C₁₃H₁₁NO (M⁺): 197.0841, found: 197.0837. IR (ATR, cm⁻¹): 3060, 1593, 1547, 1510, 1484, 1461, 1445, 1396, 1340, 1324, 1298, 1191, 1163, 1067, 1025.

*Spectral data is similar to previously reported data in CDCl₃.¹

3.6.3 Synthesis of Cyclooctynes

3.6.3.1 Synthesis of BCN-OH_{exo} (5)

Synthesized according to Dommerholt et al.² See Section 2.6.2.6 for characterization data.

3.6.3.2 Synthesis of (Z)-5,6-dibromocyclooct-1-ene (6a)

To 20 mL (163 mmol) cyclooctadiene in 400 mL dichloromethane was added 3.5 mL (68 mmol) bromine in 100 mL dichloromethane dropwise over 30 minutes at room temperature. The resulting solution was stirred for 20 minutes, after which the solvent was thoroughly removed by rotary evaporation. The crude residue was purified by flash column chromatography (hexanes) to give (Z)-5,6-dibromocyclooct-1-ene as a colorless oil in 90% yield (16 g). ¹H NMR (CD₂Cl₂, 400 MHz): δ (ppm) 5.65 (m, 2H), 4.67 (m, 2H), 2.67 (m, 4H), 2.24 (m, 4H). ¹³C NMR (CD₂Cl₂, 400 MHz): δ (ppm) 129.2, 60.7, 36.0, 25.6. HRMS (ESI) m/z calc. for C₈H₁₂Br₂⁺ (M⁺): 265.9306, found: 265.9315. IR (ATR-IR, cm⁻¹): 3016, 2924, 1653, 1477, 1429, 1346, 1296, 1204, 1147.
*Analysis matches previously reported data*³

### 3.6.3.3 Synthesis of (Z)-cyclooct-1-ene-5-yne (6b)

To 2.0 g (7.5 mmol) (Z)-5,6-dibromocyclooct-1-ene in 4 mL dry ether was added 11 mL (11 mmol) of 1 M potassium tert-butoxide in tetrahydrofuran dropwise over 10 minutes at 0°C. The solution was stirred for 30 minutes at 0°C and 30 minutes at room temperature, after which an additional 12 mL (12 mmol) of 1M potassium tert-butoxide in tetrahydrofuran was added over 10 minutes at room temperature. The solution was stirred for 2 hours at 40°C, after which the solution was cooled to room temperature and 100 mL pentane was added, resulting in cloudy dark orange solution. The resulting suspension was gravity filtered to remove insoluble solids. To the filtrate was added magnesium sulphate, and the insoluble solids were once again removed by gravity filtration, giving a yellow filtrate. The filtrate was concentrated by rotary evaporation under mild vacuum, and the resulting crude residue was purified by column chromatography (pentanes) to give (Z)-cyclooct-1-ene-5-yne as a yellow oil in 52% yield (0.41 g). NOTE: product is volatile (bp < 100°C), and so care must be taken when removing solvents using rotary evaporation. Also, we found that quenching the reaction with water significantly reduced the yield of product. ¹H NMR (CD₂Cl₂, 400 MHz): δ (ppm) 5.85 (m, 2H), 2.56 (m, 4H), 2.14 (m, 2H). ¹³C NMR (CDCl₃, 400 MHz): δ (ppm) 131.9, 100.8, 31.2, 19.5. HRMS (ESI) m/z calc. for C₈H₁₀(M⁺): 106.0783, found: 106.0782. IR (ATR-IR, cm⁻¹): 3008, 2924, 2850, 1635, 1450, 1315, 1207.

*Compound is previously reported, but no experimental or spectral data was provided*⁴

### 3.6.4 Synthesis of TEG Ligands

#### 3.6.4.1 Synthesis of Nitrobenzyl-TEG-OH (A)

To 15 mL (87 mmol) tetraethylene glyol in 500 mL dimethylformamide was added 0.21 g (8.7 mmol) sodium hydride, portionwise over 10 minutes at 0°C, resulting in some bubbling. After stirring the heterogenous mixture for 60 minutes at room temperature, a solution of 2.2 g (10 mmol) nitrobenzyl bromide in 25 mL dimethylformamide was
The reaction was stirred overnight, after which 100 mL water was added. The solution was extracted with dichloromethane (3 x 50 mL), and the solvent was removed via rotary evaporation. The crude residue was purified via flash column chromatography (1:10 ethanol:dichloromethane) to give nitrobenzyl-TEG-OH (A) as a pale yellow oil in 65% yield (1.9 g). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.17 (d, J = 12.0 Hz, 2H), 7.50 (d, J = 12.0 Hz, 2H), 4.65 (s, 2H), 3.66 (m, 17H), 3.58 (m, 2H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 170.1, 160.92, 147.22, 145.91, 127.7, 123.5, 72.5, 71.9, 70.5, 70.5, 70.2, 70.1, 65.7, 61.6, 60.3, 20.9, 15.2, 14.1. HRMS (ESI) $m/z$ calc. for C$_{15}$H$_{23}$NO$_7$+ ($M^+$): 329.1475, found: 329.1462.

### 3.6.4.2 Synthesis of Nitrobenzyl-TEG-OTs (B)

To 1.5 g (4.6 mmol) nitrobenzyl-TEG-OH (A) in 100 mL dry dichloromethane was added 1.0 g (5.5 mmol) tosyl chloride in 25 mL dry dichloromethane, and then 1.9 mL (13.8 mmol) triethylamine. The reaction was stirred overnight, after which the solvent was removed via rotary evaporation. The crude residue was purified via flash column chromatography (dichloromethane to 1:10 ethanol:dichloromethane) to give nitrobenzyl-TEG-OTs (B) as a pale yellow oil in 92% yield (2.0 g). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.20 (d, J = 8.0 Hz, 2H), 7.80 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.68 (s, 2H), 4.16 (d, J = 4.0 Hz, 2H), 3.65 (m 16H), 2.45 (s, 3H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 147.6, 146.4, 145.1, 133.3, 130.1, 128.3, 128.0, 123.9, 72.3, 71.1, 71.0, 70.9, 70.5, 69.5, 69.0, 21.9. HRMS (ESI) $m/z$ calc. for C$_{22}$H$_{29}$NO$_9$S$^+$ ($M^+$): 483.1563, found: 483.1521.

### 3.6.4.3 Synthesis of Nitrobenzyl-TEG-Thioacetate (C)

To 2.0 g (4.2 mmol) nitrobenzyl-TEG-OTs (B) in 150 mL acetonitrile was added 0.96 g (8.4 mmol) potassium thioacetate. The resulting heterogenous mixture was stirred overnight, after which the solvent was removed via rotary evaporation. The crude residue was purified via flash column chromatography (1:10 ethanol:dichloromethane) to give nitrobenzyl-TEG-Thioacetate (C) as a yellow oil in 95% yield (1.6 g). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.21 (d, J = 8.0 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H),
4.68 (s, 2H), 3.67 (m, 12H), 3.60 (t, J = 8.0 Hz, 2H), 3.09 (t, J = 8.0 Hz, 2H), 2.34 (s, 3H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 195.2, 170.8, 147.0, 145.8, 127.4, 123.4, 71.7, 70.4, 70.4, 70.3, 70.2, 70.1, 69.9, 69.5, 60.1, 30.2, 28.5, 20.7, 13.9. HRMS (ESI) $m/z$ calc. for C$_{17}$H$_{26}$N$_7$O$_7$S$^+$ (M$^+$): 387.1352, found: 387.1332.

3.6.4.4 Synthesis of (C-Pyridinium, N-Phenyl-TEG-Thioacetate)-Nitrone (E)

To 1.6 g (4.1 mmol) nitrobenzyl-TEG-Thioacetate (C) and 0.26 g (4.9 mmol) ammonium chloride in 10 mL ethanol and 10 mL distilled water was added 0.54 g (8.2 mmol) zinc dust portion-wise over 10 minutes. The resulting heterogenous mixture was stirred vigorously for 20 minutes, after which the insoluble solid was removed via vacuum filtration. The supernatant was diluted with brine (50 mL) and extracted with diethyl ether (3 x 25 mL), dried over magnesium sulphate and concentrated to give the crude hydroxylamine as a yellow oil, which was used without further purification.

To the crude hydroxylamine in 30 mL dichloromethane was added 0.77 mL (8.2 mmol) 3-pyridine carboxaldehyde and a small amount of magnesium sulphate. The resulting heterogenous mixture was stirred overnight, after which the magnesium sulphate was removed by vacuum filtration. The supernatant was concentrated via rotary evaporation and the crude residue was purified via flash column chromatography to give (C-Pyridinium, N-Phenyl-TEG-Thioacetate)-Nitrone (D) as a yellow oil.

To 0.92 g (2 mmol) (C-Pyridine, N-Phenyl-TEG-Thioacetate)-Nitrone (D) in 25 mL dry dichloromethane was added 1.2 mL (20 mmol) iodomethane. The resulting solution was stirred overnight, after which the solvent and unreacted iodomethane was removed via rotary evaporation, to give (C-Pyridinium, N-Phenyl-TEG-Thioacetate)-Nitrone (E) as a deep yellow oil in 46 % yield (1.1 g), which was used without further purification. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 10.47 (s, 1H), 9.61 (d, J = 8.0 Hz, 1H), 9.18 (d, J = 8.0 Hz, 1H), 9.09 (s, 1H), 8.12 (t, J = 4.0 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 8.0 Hz, 2H), 4.64 (s, 2H), 4.60 (s, 3H), 3.69 (m, 16H), 3.04
(t, J = 4.0 Hz, 2H), 2.34 (s, 3H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 196.0, 146.3, 144.6, 143.5, 143.1, 141.5, 131.2, 128.1, 127.6, 127.4, 121.4, 71.8, 70.1, 70.0, 70.0, 69.9, 69.7, 69.6, 69.5, 53.1, 49.4, 30.4, 29.3, 28.5, 18.1. HRMS (ESI) $m/z$ calc. for C$_{24}$H$_{33}$IN$_2$O$_6$S$^+$ (M$^+$): 604.1104, found: 604.1139.

### 3.6.5 Molecular Structures Of Nitrones

**Figure S3.1.** Thermal ellipsoid plot of molecular structure of nitrone 4 at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen).

**Figure S3.2.** Space-filling diagrams of X-ray structure of nitrone 4 (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).
Figure S3.3. Thermal ellipsoid plot of molecular structure of nitrone 2b at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen).

Figure S3.4. Space-filling diagrams of X-ray structure of nitrone 2b (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).

Figure S3.5. Thermal ellipsoid plot of molecular structure of nitrone 3a at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).
**Figure S3.6.** Space-filling diagrams of X-ray structure of nitrone 3a (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).

**Figure S3.7.** Thermal ellipsoid plot of molecular structure of nitrone 3b at 50% probability level. Hydrogens are drawn in with arbitrary radii (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).

**Figure S3.8.** Space-filling diagrams of X-ray structure of nitrone 3b (black = carbon, red = oxygen, green = nitrogen, grey = hydrogen, purple = iodine).
3.6.6 General Synthesis of Cycloadducts

3.6.6.1 Synthesis of Cycloadduct between BCN\textsubscript{exo}-OH (5) with Nitrones 3a, 3b, 3c

To 1 equivalent of nitrone 3a, 3b or 3c in dimethylformamide (10 mg/10 mL) was added 1.1 equivalent of BCN\textsubscript{exo}-OH (5). The reaction mixture was stirred for 20 minutes, after which excess cold di-ethyl ether was added to precipitate out the product. The crude semi-solid was washed with numerous portions of cold di-ethyl ether to give each cycloadduct as a yellow oil. See Section 3.6.10 for $^1$H NMR spectra, $^{13}$C NMR spectra and mass spectrometry data of cycloadducts.
To explore the SPANC reactivity of pyridinium-nitrones in different solvents systems (see Section 3.6.11), 1 equivalent of nitrone 3c was added to 1 equivalent of BCN-OH$_{\text{exo}}$ (5) in 6:1 D$_2$O:(CD$_3$)$_2$SO (see Figure S3.42 for $^1$H NMR spectrum), 1:1 D$_2$O:(CD$_3$)$_2$SO (see Figure S3.43 for $^1$H NMR spectrum) and CD$_3$OD (see Figure S3.44 for $^1$H NMR spectrum).

3.6.6.2 Synthesis of Cycloadduct between BCN$_{\text{exo}}$-OH with Nitrone 4 and 2b

To 1 equivalent of nitrone 4 or 2b in dichloromethane (10 mg/mL) was added 1.1 equivalent of BCN$_{\text{exo}}$-OH (5). The reaction mixture was stirred for 6 hours, after which excess cold di-ethyl ether was added to precipitate out the product. The crude semi-solid was washed with numerous portions of cold di-ethyl ether to give each cycloadduct as a yellow oil. See Section 3.6.10 for $^1$H NMR spectra, $^{13}$C NMR spectra and mass spectrometry data of cycloadducts.

3.6.6.3 Synthesis of Cycloadduct between DBCO-amine with Nitrone 3c

To 1 equivalent of nitrone 3c in acetonitrile (10 mg/10 mL) was added 1.1 equivalent of DBCO-amine. The reaction mixture was stirred for 20 minutes, after which excess cold di-ethyl ether was added to precipitate out the product. The crude semi-solid was washed with numerous portions of cold di-ethyl ether to give the cycloadduct as a yellow oil. See Section 3.6.12 for $^1$H NMR spectrum, $^{13}$C NMR spectrum and mass spectrometry data of cycloadduct.

3.6.6.4 Synthesis of Cycloadduct between (Z)-cyclooct-1-ene-5-yne (6b) with Nitrone 3c

To 1 equivalent of nitrone 3c in acetonitrile (10 mg/10 mL) was added 1.1 equivalent of (Z)-cyclooct-1-ene-5-yne (6b). The reaction mixture was stirred for 20 minutes, after which excess cold pentane was added to precipitate out the product. The crude semi-solid was washed with numerous portions of cold pentane to give the cycloadduct as a yellow oil. See Section 3.6.13 for $^1$H NMR spectrum, $^{13}$C NMR spectrum and mass spectrometry data of cycloadduct.
3.6.7 NMR Spectra of Nitrones

3.6.7.1 Experimental Spectra for \( N \)-(4-methoxyphenyl)-\( C \)-(3-pyridine) nitrone (Nitron 2a)

![Chemical Structures](image1)

**Figure S3.11.** \( ^1 \)H NMR spectrum of nitrone 2a in (CD\(_3\))\(_2\)SO at 25°C. * denotes residual protio solvent and impurities.

![Chemical Structures](image2)

**Figure S3.12.** \( ^{13} \)C\{\( ^1 \)H\} NMR spectrum of nitrone 2a in (CD\(_3\))\(_2\)SO at 25°C. * indicates (CD\(_3\))\(_2\)SO solvent.
3.6.7.2 Experimental Spectra for \( N \)-(4-methoxyphenyl)-\( C \)-(3-methylpyridinium) nitrone (Nitrone 3a)

**Figure S3.13.** \(^1\)H NMR spectrum of nitrone 3a in \((\text{CD}_3)_2\text{SO}\) at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.14.** \(^{13}\)C\(^{\text{H}}\) NMR spectrum of nitrone 3a in \((\text{CD}_3)_2\text{SO}\) at 25°C. * indicates \((\text{CD}_3)_2\text{SO}\) solvent.
3.6.7.3 Experimental Spectra for \textit{N}-phenyl-C-(3-pyridine) nitrone (Nitrone 2b)

See Section 2.6.3.16 for $^1$H NMR and $^{13}$C{$^1$H} NMR spectra of nitrone 2b.

3.6.7.4 Experimental Spectra for \textit{N}-phenyl-C-(3-methylpyridinium) nitrone (Nitrone 3b)

**Figure S3.15.** $^1$H NMR spectrum of nitrone 3b in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.16.** $^{13}$C{$^1$H} NMR spectrum of nitrone 3b in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.
3.6.7.5 Experimental Spectra for $N$-(4-cyanophenyl)$-C$-(3-pyridine) nitrone (Nitrone 2c)

See Section 2.6.3.17 for $^1$H NMR and $^{13}$C($^1$H) NMR spectra of nitrone 2c.

3.6.7.6 Experimental Spectra for $N$-(4-cyanophenyl)$-C$-(3-methylpyridinium) nitrone (Nitrone 3c)

![1H NMR spectrum of nitrone 3c in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.](image)

**Figure S3.17.** $^1$H NMR spectrum of nitrone 3c in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.
Figure S3.18. $^{13}$C{$^{1}$H} NMR spectrum of nitrone 3c in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.

3.6.7.7 Experimental Spectra for N-phenyl-C-phenyl nitrone (Nitron 4)

See Section 2.6.3.14 for $^1$H NMR and $^{13}$C{$^{1}$H} NMR spectra of nitrone 4.
3.6.8 NMR Spectra of TEG Ligands

3.6.8.1 Experimental Spectra for Nitrobenzyl-TEG-OH (A)

**Figure S3.19.** $^1$H NMR spectrum of nitrobenzyl-TEG-OH (A) in CDCl$_3$ at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.20.** $^{13}$C($^1$H) NMR spectrum of nitrobenzyl-TEG-OH (A) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
3.6.8.2 Experimental Spectra for Nitrobenzyl-TEG-OTs (B)

**Figure S3.21.** $^1$H NMR spectrum of nitrobenzyl-TEG-OTs (B) in CDCl$_3$ at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.22.** $^{13}$C($^1$H) NMR spectrum of nitrobenzyl-TEG-OTs (B) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
3.6.8.3 Experimental Spectra for Nitrobenzyl-TEG-Thioacetate (C)

Figure S3.23. $^1$H NMR spectrum of nitrobenzyl-TEG-Thioacetate (C) in CDCl$_3$ at 25°C. * denotes residual protio solvent and impurities.

Figure S3.24. $^{13}$C{$^1$H} NMR spectrum of nitrobenzyl-TEG-Thioacetate (C) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
3.6.8.4 Experimental Spectra for (C-Pyridinium, N-Phenyl-TEG-Thioacetate)-Nitrone (E)

Figure S3.25. $^1$H NMR spectrum of (C-pyridinium, N-phenyl-TEG-thioacetate)-nitrone (E) in CDCl$_3$ at 25°C. * denotes residual protio solvent and impurities.

Figure S3.26. $^{13}$C($^1$H) NMR spectrum of (C-pyridinium-N-phenyl-TEG-thioacetate)-nitrone (E) in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
3.6.9 NMR Spectra of Cyclooctynes

3.6.9.1 Experimental Spectra for BCN$_{exo}$-OH (5)

**Figure S3.27.** $^1$H NMR spectrum of BCN$_{exo}$-OH (5) in (CD$_3$)$_2$SO at 25°C. Made according to reference 2. * denotes residual protio solvent and impurities.
3.6.9.2 Experimental Spectra for (Z)-5,6-dibromocyclooct-1-ene (6a)

**Figure S3.28.** $^1$H NMR spectrum of (Z)-5,6-dibromocyclooct-1-ene (6a) in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent.

**Figure S3.29.** $^{13}$C($^1$H) NMR spectrum of (Z)-5,6-dibromocyclooct-1-ene (6a) in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.
3.6.9.3 Experimental Spectra for (Z)-cyclooct-1-ene-5-yne (6b)

**Figure S3.30.** $^1$H NMR spectrum of (Z)-cyclooct-1-ene-5-yne (6b) in CD$_2$Cl$_2$ at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.31.** $^{13}$C{$_^1$H} NMR spectrum of (Z)-cyclooct-1-ene-5-yne (6b) in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.
3.6.10 NMR Spectra of Cycloadducts between BCN\textsubscript{exo}-OH (5) and Nitrones 4, 2b, 3a, 3b and 3c

**Figure S3.32.** \(^1\)H NMR spectrum of 4-BCN\textsubscript{exo}-OH cycloadduct (2 isomers) in (CD\(_3\))\(_2\)SO at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.33.** \(^{13}\)C\textsuperscript{\(^1\)H} NMR spectrum of 4-BCN\textsubscript{exo}-OH cycloadduct (2 isomers) in (CD\(_3\))\(_2\)SO at 25°C. * indicates (CD\(_3\))\(_2\)SO solvent.

HRMS (ES) for C\(_{23}\)H\(_{25}\)NO\(_2\)^+: calc. = 347.1885 Da
found = 347.1867 Da
Figure S3.34. $^1$H NMR spectrum of 2b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.

Figure S3.35. $^{13}$C\{$^1$H\} NMR spectrum of 2b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.
Figure S3.36. $^1$H NMR spectrum of 3a-BCNexo-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.

HRMS (ESI) for C$_{24}$H$_{29}$N$_2$O$_3^+$: calc. = 393.2173 Da
found = 393.2178 Da

Figure S3.37. $^{13}$C($^1$H) NMR spectrum of 3a-BCNexo-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.
**Figure S3.38.** $^1$H NMR spectrum of 3b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.39.** $^{13}$C$[^1]$H NMR spectrum of 3b-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.
**Figure S3.40.** $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.

**Figure S3.41.** $^{13}$C($^1$H) NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.
3.6.11 $^1$H NMR Spectra of Cycloadducts between BCN$_{exo}$-OH (5) and Nitrone 3c in Other Solvents

**Figure S3.42.** $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C. The solvents were suppressed by software. * denotes residual protio solvent.

**Figure S3.43.** $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloadduct (2 isomers) in 1:1 D$_2$O:(CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent.
Figure S3.44. $^1$H NMR spectrum of 3c-BCN$_{exo}$-OH cycloaduct (2 isomers) in CD$_3$OD at 25°C. * denotes residual protio solvent and impurities.
3.6.12 NMR Spectra of Cycloadduct between DBCO-amine and Nitrone 3c

HRMS (ESI) for C_{32}H_{28}N_{5}O_{2}+: calc. = 514.6085 Da
found = 514.6082 Da

Figure S3.45. $^1$H NMR spectrum of 3c-DBCO-amine cycloadduct (2 isomers) in $(\text{CD}_3)_2\text{SO}$ at 25°C. * denotes residual protio solvent and impurities.

Figure S3.46. $^{13}$C\{$^1$H\} NMR spectrum of 3c-DBCO-amine cycloadduct (2 isomers) in $(\text{CD}_3)_2\text{SO}$ at 25°C. * indicates $(\text{CD}_3)_2\text{SO}$ solvent.
3.6.13 NMR Spectra of Cycloadduct between (Z)-cyclooct-1-ene-5-yne (6b) and Nitrone 3c

HRMS (ESI) for C_{22}H_{22}N_{3}O^+: calc. = 344.1757 Da
found = 344.1782 Da

Figure S3.47. $^1$H NMR spectrum of 3c-6b cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * denotes residual protio solvent and impurities.

Figure S3.48. $^{13}$C($^1$H) NMR spectrum of 3c-6b cycloadduct (2 isomers) in (CD$_3$)$_2$SO at 25°C. * indicates (CD$_3$)$_2$SO solvent.
3.6.14 Stability of Pyridinium-Nitrones 3a, 3b and 3c

To probe the stability of nitrone 3a, 3b and 3c towards solvent-induced hydrolysis, a solution of each nitrone (10 mg/mL) was prepared in 6:1 D$_2$O:(CD$_3$)$_2$SO. The decrease in the $^1$H NMR signal from H$_a$ (see Table 3.1 in Section 3.2) was observed after 5 minutes, 1 hour, 4 hours and 12 hours relative to the residual H$_2$O peak. The change in the intensity of this signal was determined as a percentage of the signal after 5 minutes in the solvent system. $^1$H NMR spectra after 5 minutes and 12 hours are shown below.

Table S3.1. Percentage of nitrone 3a remaining in 6:1 D$_2$O:(CD$_3$)$_2$SO over time, measured as a change in the H$_a$ NMR signal intensity relative to the peak from residual H$_2$O solvent.

<table>
<thead>
<tr>
<th>Time</th>
<th>Percentage of nitrone remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes</td>
<td>100</td>
</tr>
<tr>
<td>1 hour</td>
<td>100</td>
</tr>
<tr>
<td>4 hours</td>
<td>100</td>
</tr>
<tr>
<td>12 hours</td>
<td>100</td>
</tr>
</tbody>
</table>

Table S3.2. Percentage of nitrone 3b remaining in 6:1 D$_2$O:(CD$_3$)$_2$SO over time, measured as a change in the H$_a$ NMR signal intensity relative to the peak from residual H$_2$O solvent.

<table>
<thead>
<tr>
<th>Time</th>
<th>Percentage of nitrone remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes</td>
<td>100</td>
</tr>
<tr>
<td>1 hour</td>
<td>99</td>
</tr>
<tr>
<td>4 hours</td>
<td>98</td>
</tr>
<tr>
<td>12 hours</td>
<td>80</td>
</tr>
</tbody>
</table>
Table S3.3. Percentage of nitrone 3c remaining in 6:1 D₂O:(CD₃)₂SO over time, measured as a change in the H₆ NMR signal intensity relative to the peak from residual H₂O solvent.

<table>
<thead>
<tr>
<th>Time</th>
<th>Percentage of nitrone remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes</td>
<td>100</td>
</tr>
<tr>
<td>1 hour</td>
<td>100</td>
</tr>
<tr>
<td>4 hours</td>
<td>99</td>
</tr>
<tr>
<td>12 hours</td>
<td>94</td>
</tr>
</tbody>
</table>
Figure S3.49. $^1$H NMR spectrum of nitronate 3a in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 5 minutes. * denotes residual protio solvent.

Figure S3.50. $^1$H NMR spectrum of nitronate 3a in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 12 hours. * denotes residual protio solvent.
Figure S3.51. $^1$H NMR spectrum of nitrones 3b in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 5 minutes. * denotes residual protio solvent.

Figure S3.52. $^1$H NMR spectrum of nitrones 3b in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 12 hours. * denotes residual protio solvent.
Figure S3.53. $^1$H NMR spectrum of nitrone 3c in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 5 minutes. * denotes residual protio solvent.

Figure S3.54. $^1$H NMR spectrum of nitrone 3c in 6:1 D$_2$O:(CD$_3$)$_2$SO at 25°C after 12 hours. * denotes residual protio solvent.
3.6.15 Kinetic Measurements

*Kinetic measurements were performed according to a previously reported procedure.\textsuperscript{5}

Estimated rate constants for the pyridinium-nitrones were determined under pseudo-first order conditions in 2:1 acetonitrile:methanol at 25°C using UV-Vis absorption spectroscopy. Each nitrone was added to excess $\text{BCN}_{\text{exo}}\text{-OH}$ (5) (50eq, 75eq, 100eq, 125 eq), with each trial being performed in duplicate. As the cycloaddition reaction with $\text{BCN}_{\text{exo}}\text{-OH}$ proceeded, the decrease in intensity of the absorbance at 345 nm (for nitrones 3a-c), 316 nm (for nitrone 4) and 320 nm (for nitrone 2b) was monitored over time.

The natural logarithm of the absorbance was then plotted over time to obtain four averaged observed rate constants ($k_{\text{obs}}$) under pseudo-first order conditions for each nitrone at the four excess equivalencies of $\text{BCN}_{\text{exo}}\text{-OH}$ (5). The observed rate constants were then plotted against the concentration of $\text{BCN}_{\text{exo}}\text{-OH}$ (5), with the slope of the curve representing the second-order rate constant for each cycloaddition reaction of each nitrone.
3.6.15.1 Kinetic Measurements of Cycloaddition Reaction between (4) and BCN\textsubscript{exo}-OH (5)

![Chemical structures](image)

Table S3.4. Kinetic data for nitrone 4 and BCN\textsubscript{exo}-OH (5)

<table>
<thead>
<tr>
<th>Concentration of BCN (mM)</th>
<th>Observed Rate Constant, (k_{\text{obs}}) (s(^{-1})) – Trial 1</th>
<th>Observed Rate Constant, (k_{\text{obs}}) (s(^{-1})) – Trial 2</th>
<th>Average Rate Constant, (k_{\text{obs}}) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>1.1 \times 10(^{-5})</td>
<td>1.3 \times 10(^{-5})</td>
<td>1.2 \times 10(^{-5})</td>
</tr>
<tr>
<td>0.68</td>
<td>2.3 \times 10(^{-5})</td>
<td>2.7 \times 10(^{-5})</td>
<td>2.5 \times 10(^{-5})</td>
</tr>
<tr>
<td>0.91</td>
<td>3.5 \times 10(^{-5})</td>
<td>4.1 \times 10(^{-5})</td>
<td>3.8 \times 10(^{-5})</td>
</tr>
<tr>
<td>1.1</td>
<td>5.3 \times 10(^{-5})</td>
<td>6.0 \times 10(^{-5})</td>
<td>5.7 \times 10(^{-5})</td>
</tr>
</tbody>
</table>

Figure S3.55. Pseudo-first order kinetics graph for nitrone 4 with BCN\textsubscript{exo}-OH (5).
3.6.15.2 Kinetic Measurements of Cycloaddition Reaction between (2b) and BCN$_{exo}$-OH (5)

![Chemical structure of nitrone 2b and BCN$_{exo}$-OH (5)]

Table S3.5. Kinetic data for nitrone 2b and BCN$_{exo}$-OH (5).

<table>
<thead>
<tr>
<th>Concentration of BCN (mM)</th>
<th>$k_{obs}$ (s$^{-1}$) – Trial 1</th>
<th>$k_{obs}$ (s$^{-1}$) – Trial 2</th>
<th>Average Rate Constant, $k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>$5.8 \times 10^{-5}$</td>
<td>$6.3 \times 10^{-5}$</td>
<td>$6.1 \times 10^{-5}$</td>
</tr>
<tr>
<td>0.68</td>
<td>$8.5 \times 10^{-5}$</td>
<td>$9.8 \times 10^{-5}$</td>
<td>$9.2 \times 10^{-5}$</td>
</tr>
<tr>
<td>0.91</td>
<td>$1.2 \times 10^{-4}$</td>
<td>$1.3 \times 10^{-4}$</td>
<td>$1.2 \times 10^{-4}$</td>
</tr>
<tr>
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<td>$1.5 \times 10^{-4}$</td>
<td>$1.7 \times 10^{-4}$</td>
<td>$1.6 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Figure S3.56. Pseudo-first order kinetics graph for nitrone 2b with BCN$_{exo}$-OH (5).
3.6.15.3 Kinetic Measurements of Cycloaddition Reaction between (3a) and BCN_{exo}-OH (5)

![Reaction Scheme]

Table S3.6. Kinetic data for nitrone 3a and BCN_{exo}-OH (5).

<table>
<thead>
<tr>
<th>Concentration of BCN (mM)</th>
<th>Observed Rate Constant, k_{obs} (s^{-1}) – Trial 1</th>
<th>Observed Rate Constant, k_{obs} (s^{-1}) – Trial 2</th>
<th>Average Rate Constant, k_{obs} (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>7.6 x 10^{-4}</td>
<td>8.1 x 10^{-4}</td>
<td>7.9 x 10^{-4}</td>
</tr>
<tr>
<td>0.68</td>
<td>1.2 x 10^{-3}</td>
<td>1.1 x 10^{-3}</td>
<td>1.2 x 10^{-3}</td>
</tr>
<tr>
<td>0.91</td>
<td>1.5 x 10^{-3}</td>
<td>1.4 x 10^{-3}</td>
<td>1.5 x 10^{-3}</td>
</tr>
<tr>
<td>1.1</td>
<td>1.9 x 10^{-3}</td>
<td>1.8 x 10^{-3}</td>
<td>1.9 x 10^{-3}</td>
</tr>
</tbody>
</table>

![Graph]

Figure S3.57. Pseudo-first order kinetics graph for nitrone 3a with BCN_{exo}-OH (5).
3.6.15.4 Kinetic Measurements of Cycloaddition Reaction between (3b) and BCN$_{exo}$-OH (5)

![Chemical structures of nitrones and BCN$_{exo}$-OH](image)

Table S3.7. Kinetic data for nitrone 3b and BCN$_{exo}$-OH (5).

<table>
<thead>
<tr>
<th>Concentration of BCN (mM)</th>
<th>Observed Rate Constant, $k_{obs}$ (s$^{-1}$) – Trial 1</th>
<th>Observed Rate Constant, $k_{obs}$ (s$^{-1}$) – Trial 2</th>
<th>Average Rate Constant, $k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>1.0 x 10$^{-4}$</td>
<td>1.1 x 10$^{-4}$</td>
<td>1.1 x 10$^{-4}$</td>
</tr>
<tr>
<td>0.68</td>
<td>1.4 x 10$^{-3}$</td>
<td>1.0 x 10$^{-3}$</td>
<td>1.2 x 10$^{-3}$</td>
</tr>
<tr>
<td>0.91</td>
<td>1.9 x 10$^{-3}$</td>
<td>1.5 x 10$^{-3}$</td>
<td>1.7 x 10$^{-3}$</td>
</tr>
<tr>
<td>1.1</td>
<td>2.4 x 10$^{-3}$</td>
<td>2.2 x 10$^{-3}$</td>
<td>2.3 x 10$^{-2}$</td>
</tr>
</tbody>
</table>

Figure S3.58. Pseudo-first order kinetics graph for nitrone 3b with BCN$_{exo}$-OH (5).
3.6.15.5 Kinetic Measurements of Cycloaddition Reaction between (3c) and BCN_{exo}-OH (5)

Table S3.8. Kinetic data for nitrone 3c and BCN_{exo}-OH (5).

<table>
<thead>
<tr>
<th>Concentration of BCN (mM)</th>
<th>Observed Rate Constant, $k_{obs}$ (s$^{-1}$) – Trial 1</th>
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Figure S3.59. Pseudo-first order kinetics graph for nitrone 3c with BCN_{exo}-OH (5).
3.6.16 Crystallographic Information

3.6.16.1 Data Collection and Processing

The molecular structure of 4 has been previously reported\(^6\), where the data was collected at room temperature. However, in order to allow for direct crystallographic comparisons with 3a-3c, we include our data for Nitrone 4, which was collected at 110K – the same temperature used to collect structures 3a-3c and 2b.

Nitrone 4 (\textit{N-phenyl-C-phenyl-nitrone}) was crystallized out of a 1:4 mixture of dichloromethane:di-ethyl ether. Crystals of nitrone 4 was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker-Nonius KappaCCD Apex2 diffractometer at a temperature of 110 K. The unit cell dimensions were determined from a symmetry constrained fit of 7598 reflections with 6.82° < 2\(\theta\) < 132.52°. The data collection strategy was a number of \(\omega\) and \(\varphi\) scans which collected data up to 133.05° (2\(\theta\)). The frame integration was performed using SAINT.\(^7\) The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS.\(^8\)

Nitrone 2b (\textit{N-phenyl-C-(3-pyridine)-nitrone}) was crystallized out of a 1:4 mixture of dichloromethane:di-ethyl ether. Crystals of nitrone 2b was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker Kappa Axis Apex2 diffractometer at a temperature of 110 K. The unit cell dimensions were determined from a symmetry constrained fit of 9938 reflections with 4.9° < 2\(\theta\) < 81.22°. The data collection strategy was a number of \(\omega\) and \(\varphi\) scans which collected data up to 86.246° (2\(\theta\)). The frame integration was performed using SAINT.\(^7\) The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS.\(^8\)

Nitrone 3a (\textit{N-(4-methoxyphenyl)-C-(3-methylpyridinium) nitrone}) was crystallized out of a 1:2 mixture of dimethyl sulfoxide:dichloromethane. Crystals of nitrone 3a was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker-Nonius KappaCCD Apex2 diffractometer at a temperature of 110 K. The unit cell dimensions were determined from a symmetry constrained fit of 9872
reflections with $5.26^\circ < 2\theta < 132.74^\circ$. The data collection strategy was a number of $\omega$ and $\varphi$ scans which collected data up to $132.722^\circ (2\theta)$. The frame integration was performed using SAINT. The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS.

**Nitrone 3b** (*N*-phenyl-C-(3-methylpyridinium) nitrone) was crystallized out of a 1:2 mixture of dimethyl sulfoxide:dichloromethane. Crystals of nitrone 3b was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker Kappa Axis Apex2 diffractometer at a temperature of 223 K. The unit cell dimensions were determined from a symmetry constrained fit of 9948 reflections with $5.66^\circ < 2\theta < 62.14^\circ$. The data collection strategy was a number of $\omega$ and $\varphi$ scans which collected data up to $81.114^\circ (2\theta)$. The frame integration was performed using SAINT. The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS.

**Nitrone 3c** (*N*-(4-cyanophenyl)-C-(3-methylpyridinium) nitrone) was crystallized out of a 1:2 mixture of dimethyl sulfoxide:dichloromethane. Crystals of nitrone 3c was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker-Nonius KappaCCD Apex2 diffractometer at a temperature of 110 K. The unit cell dimensions were determined from a symmetry constrained fit of 9994 reflections with $8.92^\circ < 2\theta < 130.88^\circ$. The data collection strategy was a number of $\omega$ and $\varphi$ scans which collected data up to $132.602^\circ (2\theta)$. The frame integration was performed using SAINT. The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS.

**3.6.16.2 Structure Solution and Refinement**

The structure for nitrone 4 was solved by using a dual space methodology using the SHELXT program. All non-hydrogen atoms were obtained from the initial solution. The hydrogen atoms were introduced at idealized positions and were allowed to refine isotropically. The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL program from the SHELX suite of crystallographic
Graphic plots were produced using the NRCVAX program suite. Additional information and other relevant literature references can be found in the reference section of this website (http://xray.chem.uwo.ca).

The structure for nitrone 2b was solved by using a dual space methodology using the SHELXT program. All non-hydrogen atoms were obtained from the initial solution. The hydrogen atom positions were recovered from a difference Fourier map and were allowed to refine isotropically. The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL program from the SHELX suite of crystallographic software. Graphic plots were produced using the NRCVAX program suite.

The structure for nitrone 3a was solved by using a dual space methodology using the SHELXT program. All non-hydrogen atoms were obtained from the initial solution. The iodine atom resided on two half occupancy higher symmetry sites (an inversion centre and along a two-fold axis). The charge is balanced. The hydrogen atoms were introduced at idealized positions and were allowed to ride on the parent atom. The N bound oxygen exhibited a disorder. The normalized occupancy of the major conformer refined to a value of 0.81(9). The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL-2014 program from the SHELX suite of crystallographic software. Graphic plots were produced using the NRCVAX program suite.

The structure for nitrone 3b was solved by using a dual space methodology using the SHELXT program. All non-hydrogen atoms were obtained from the initial solution. The hydrogen atoms were introduced at idealized positions and were allowed to refine isotropically. The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL-2014 program from the SHELX suite of crystallographic software. Graphic plots were produced using the NRCVAX program suite.

The structure for 9 was solved by using a dual space methodology using the SHELXT program. All non-hydrogen atoms were obtained from the initial solution. The hydrogen atoms
were introduced at idealized positions and were allowed to refine isotropically. During the refinement the highly obtuse $\beta$ angle (130.3°) induced high correlations between the parameters in the least-squares calculation. To reduce these correlations, the basis vectors for the lattice were transformed from the C centred to I centred monoclinic ($\beta = 98.1°$) setting. The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL program from the SHELX suite of crystallographic software. Graphic plots were produced using the NRCVAX program suite.

3.6.14.3 Summary of Crystallographic Data

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Where:

\[
R_1 = \sum\left( |Fo| - |Fc| \right) / \sum Fo
\]

\[
wR_2 = \left[ \sum (w(Fo^2 - Fc^2)^2) / \sum (wFo^4) \right]^{1/2}
\]
3.6.17 Computational Information

3.6.17.1 Computational Methods

Calculations were carried out in Gaussian 09 revision A.02 at the B3LYP/6-31G* level of theory. Geometry optimization of the gas-phase singlet ground state was supplemented with frequency analysis to ensure the optimized structure is located at a potential energy minimum. Computed HOMO and LUMO energies and Mulliken atomic charges were from the DFT-optimized structure. Kohn–Sham orbitals were plotted using Visualization for Electronic and Structural Analysis,12 version 3.3.2.

3.6.17.2 Optimized Structural Coordinates

Table S3.10. Structural coordinates for BCN<sub>exo</sub>-OH (5).

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### Table S3.12. Structural coordinates for nitrone 3b.

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**Table S3.14.** Structural coordinates for nitron 2b.
Table S3.15. Structural coordinates for nitrone 4.

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### 3.6.17.3 Comparison of DFT versus XRD

**Table S3.16.** Comparison of actual bond lengths and bond angles in molecular structures of nitrones to theoretical bond lengths and bond angles from DFT analysis.

![Nitro compound structure](image)

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* Defined as the acute angle between the two aryl rings
3.6.18 References – Supporting Information


6. Bruker-AXS, SAINT version 2013.8, **2013**, Bruker-AXS, Madison, WI 53711, USA.

7. Bruker-AXS, SADABS version 2012.1, **2013**, Bruker-AXS, Madison, WI 53711, USA.


Chapter 4

4 A Clickable Azide-Functionalized [Au$_{25}$(SR)$_{18}$]$^-$ Nanocluster platform for Interfacial Surface Modifications


All synthesis and characterization were completed by the author. The manuscript was written by the author, and edited by Prof. John F. Corrigan and Prof. Mark S. Workentin.

4.1 Introduction

The molecular-type properties and high stability of atomically precise monolayer-protected gold nanoclusters in the ultrasmall size regime (<2nm) render them prominent nanomaterials for structure-property studies$^{1,2}$ with applications in chemical sensing,$^{3,4}$ catalysis,$^{5,6}$ nanomedicine,$^{7-9}$ and optical imaging.$^{10,11}$ Such physical and chemical properties of gold nanoclusters are manifested in a manner that is primarily determined by the core configuration and ligand structure.$^1$ However, the notoriously sensitive relationship between ligand structure and nanocluster integrity leads to only specific ligand types and structures being compatible with direct syntheses of gold nanocluster frameworks. There recently have been reports of robust carbene-stabilized gold nanoclusters.$^{12}$ However, the ease-of-synthesis and ability to introduce structural diversity onto thiol (R-SH) ligands make them the most common type of stabilizing ligands and there have been a wide range of exceptionally stable thiolate-protected gold nanocluster frameworks reported.$^{1,2,13-16}$

To date, direct syntheses of thiolate-protected gold nanoclusters utilize relatively simple, non-functional thiols as protecting ligands (phenylethanethiol being the most popular). To introduce functional diversity onto the gold nanocluster, ligand exchange has been demonstrated$^{17}$ but this methodology is limited by an inability to establish complete surface exchange of native thiols$^{18}$ and by synthetic incompatibilities when ligating thiols to functional substrates. This
highlights a deficiency in the gold nanocluster literature - a variant that can undergo interfacial surface chemistry. Given our history of developing methodologies for post-assembly interfacial modifications of nanomaterials,\textsuperscript{19,20} we believed that the strain-promoted alkyne-azide cycloaddition (SPAAC) would be an excellent reaction candidate for this purpose. The SPAAC reaction is a subset of bioorthogonal “click” chemistry that occurs between a largely inert azide (N\textsubscript{3}-) dipole, and a strained cyclooctyne dipolarophile to generate a triazole moiety.\textsuperscript{21} This reaction occurs with high chemoselectivity, reaction kinetics and atom efficiency without the need of a catalyst. The ability to incorporate a compatible reactive azide group to the nanocluster surface as a template provides a route to deliver property-enhancing substrates to the nanocluster framework through interfacial reaction with functional substrates possessing the complementary strained cyclooctyne reactive partner. Such efficient post-assembly interfacial modifications will transform gold nanoclusters from a simple nanomaterial framework into a surface-reactive nanomaterial platform.

Within the rich library of atomically precise thiolate-protected gold nanoclusters, the [Au\textsubscript{25}(SR)\textsubscript{18}] framework is the most prominent because, unlike many other popular thiolated frameworks, it can be synthesized at room temperature in high yield under mild conditions.\textsuperscript{2} Furthermore, there are three charge states (z = -1, 0, +1) that are accessible, manifesting charge-dependent properties in addition to the pre-existent structure-dependent properties, providing an additional level of control over structure-property relationships. Here, we report the first direct synthesis of a surface reactive gold nanocluster platform, which utilizes an azide-functionalized thiol ligand (HSCH\textsubscript{2}CH\textsubscript{2}-p-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3}) to give a surface reactive [Au\textsubscript{25}(SR)\textsubscript{18}] platform. Although two other charge states are theoretically accessible, we focused our attention on the directly accessible anionic (-1) form, [(CH\textsubscript{3}-(CH\textsubscript{2})\textsubscript{7})\textsubscript{4}N][Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-p-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}] (hereafter referred to as 4.1-azido in current chapter (and referred to as p-azido\textsuperscript{1} in Chapter 5 and 6.1-azido in Chapter 6). To establish simple proof-of-concept cluster-surface SPAAC (CS-SPAAC) reactivity of the platform, we reacted it with a symmetrical strained-cyclooctyne, which greatly aided the characterization of the modified platform. Remarkably, the data show that all surface azide moieties are available for CS-SPAAC, while retaining the internal nanocluster core configuration. Such post-assembly CS-SPAAC on our azide-functionalized [Au\textsubscript{25}(SR)\textsubscript{18}] platform provides an exciting new avenue towards the development of structurally and multifunctionally complex
[Au$_{25}$(SR)$_{18}$] nanoclusters whose molecular-type physical and chemical properties can more easily be tuned for potential applications.

### 4.2 Results and Discussion

In order to selectively synthesize a fully clickable [Au$_{25}$(SR)$_{18}$]$^{-1}$ platform in high yield, we developed an azide-modified thiol ligand that structurally mimicked the standard phenylethanethiol that is most commonly used. The simple four-step synthetic strategy ligates an azide moiety directly to the 4-position of the aryl-ring, giving rise to HSCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$, or $p$-azido-phenylethanethiol (see Section 4.6.2.1 to Section 4.6.2.4 for detailed synthesis). The synthesis of the 4.1-azido platform (Figure 4.1) was carried out with modifications to our previously reported procedure$^{22}$ for synthesizing the [(CH$_3$-(CH$_2$)$_7$)N][Au$_{25}$(SCH$_2$CH$_2$-C$_6$H$_5$)$_{18}$] framework$^{23}$ (hereafter referred to as 4.1-phenyl) (see Section 4.6.2.8 for detailed synthesis). The negative mode ESI-MS spectrum of the purified sample is shown in Figure S4.3, which shows a large peak centered at 8132.9 Da that corresponds to the parent [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$]$^{-1}$ anion of the 4.1-azido cluster (expected m/z = 8132.4 Da).

![Figure 4.1. Synthesis of [(CH$_3$-(CH$_2$)$_7$)N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (1-azido).](image)

The core configuration and monolayer composition of 4.1-azido was confirmed by single-crystal X-ray diffraction (Figure 4.2(a)). Crystals were obtained in a saturated solution of 3:1 ethanol:toluene at 0 °C over 2 days. As with 4.1-phenyl,$^{23}$ the internal body-centered icosahedron is comprised of one central Au atom ligated to Au atoms at 12 vertices (Figure 4.2(b)). The centered icosahedral kernel has an external scaffold of six “staple motifs” (-SR-Au-SR-Au-SR-) (Figure 4.2(c)). Each of the six staple motifs possess three $\mu_2$-thiolate ligands in which the central $\mu_2$-thiolate ligands occupy a distinguishable surface site (“outer” ligands, site 2 in Figure 4.2(c)), while the remaining twelve $\mu_2$-thiolates on the staple edges occupy a second distinguishable
surface site (“inner” ligands, site 1 in Figure 4.2(c)). In the cluster-surface thiolates (Figure 4.2(d)), the $N_\alpha$ of the azide moieties are ligated on each aryl ring at an average C-C-$N_\alpha$ angle of $122.7 \pm 1.3^\circ$, indicating the primarily sp$^2$ character of $N_\alpha$, while the $N_\alpha$-$N_\beta$-$N_\gamma$ bond angle is $173.3 \pm 1.5^\circ$, indicating the near-linear nature of the azide moiety. The bond distance ranges for the azide moiety are 1.23(3) to 1.28(4) Å ($N_\alpha$-$N_\beta$) and 1.18(4) to 1.21(5) Å ($N_\beta$-$N_\gamma$), which is similar to a previously reported phenyl azide structure,$^{24}$ although the uncertainties associated with these values preclude any further distinctions. The space-filling diagram (Figure S4.4) shows that the azide moieties are extended outward in a highly symmetrical and stellated pattern that suggests that the azide groups are all available to undergo the CS-SPAAC reaction.

![Click on cluster-surface](image)

**Figure 4.2** (a) Molecular structure of the anion $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{p-C}_6\text{H}_4-\text{N}_3)_1\text{8}]^{-}$ of 4.1-azido (crystallized in R-3 space group). Tetraoctylammonium counterion is not shown. Au = yellow, S = red, C = grey, N = green (b) Au$_{25}$ core configuration (c) Staple motif with three $\mu_2$-thiolate ligands (d) p-azido-phenylethananthiolate.

To investigate a proof-of-concept CS-SPAAC, we reacted one equivalent of 4.1-azido with excess $\text{(Z)}$-cyclooct-1-ene-5-yne, transforming the 4.1-azido platform to the surface-modified, $[(\text{CH}_3-\text{CH}_2)_7\text{t4N}]\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-\text{p-C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)\text{18]}$ framework (hereafter referred to as 4.1-triazole in current chapter (and referred to as p-triazole$^{1}$ in Chapter 5) (Scheme 4.1). Given the
non-regioselective nature of the SPAAC reaction, a symmetric, achiral cyclooctyne creates a single regioisomer after the CS-SPAAC reaction,\textsuperscript{25} facilitating characterization (especially NMR spectroscopy) after the cluster-surface reaction. The synthesis is a simple mix and stir reaction and can be done at room temperature in a variety of solvents such as tetrahydrofuran, dichloromethane and toluene. Due to the high strain of the alkyne moiety, the CS-SPAAC reaction is complete in under 5 minutes at millimolar concentrations regardless of solvent choice. Removal of excess (Z)-cyclooct-1-ene-5-yne was accomplished through simple trituration with acetonitrile, in which 4.1-triazole is insoluble. The linear negative mode MALDI-TOF spectrum (\textbf{Figure S4.9}) of the purified product has a well-resolved peak at 10048.4 Da that can be assigned to the parent [Au$_{25}$(SCH$_2$CH$_2$p-C$_6$H$_4$C$_8$H$_{10}$N$_3$)$_{18}$]$^{-1}$ anion of the 4.1-triazole cluster (expected \textit{m/z} = 10043.4 Da). To demonstrate the generality of the CS-SPAAC reaction, 4.1-azido was also reacted with \textit{exo}-bicyclo[6.1.0]non-4-yn-9-ol (BCN$_{\text{exo}}$-OH), a bicyclo[6.1.0]nonyne (BCN) derivative that contains a functionalizable -OH handle. The linear negative mode MALDI-TOF spectrum (\textbf{Figure S4.12}) of the purified product has a well-resolved peak at 10837.5 Da that matches the parent surface-modified [Au$_{25}$(SCH$_2$CH$_2$p-C$_6$H$_4$C$_{10}$H$_{14}$N$_3$O)$_{18}$]$^{-1}$ anionic product (expected \textit{m/z} = 10836.3 Da).

\textbf{Figure 4.3(a)} (black) shows the UV-Vis absorption spectrum of 4.1-azido, which features prominent optical absorption bands at 682 nm, 443 nm and 404 nm, which correlates well with the established optical fingerprint of conventional 4.1-phenyl nanoclusters\textsuperscript{26}. The UV-Vis absorption spectrum of the CS-SPAAC product, 4.1-triazole is also shown in \textbf{Figure 4.3(a)} (red), and as can be seen, the optical fingerprint of the anionic [Au$_{25}$(SR)$_{18}$]$^{-1}$ framework has been retained after the

\textbf{Scheme 4.1.} CS-SPAAC reaction between [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$p-C$_6$H$_4$N$_3$)$_{18}$] (4.1-azido) and (Z)-cyclooct-1-ene-5-yne, giving surface modified [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$p-C$_6$H$_4$C$_8$H$_{10}$N$_3$)$_{18}$] (4.1-triazole).
CS-SPAAC reaction, showing distinct absorption maxima at 682 nm, 446 nm and 394 nm. As the absorption maximum at 682 nm occurs from an intra-band metallic transition whose energy is characteristic of the Au$_{25}$ core,$^{26}$ the retention of this absorption maximum after the CS-SPAAC reaction further demonstrates that the anionic [Au$_{25}$(SR)$_{18}$]$^{1-}$ core configuration has been preserved.

Because of the diagnostic N$_3$ stretch, IR spectroscopy is a useful tool in both the characterization of 4.1-azido and subsequent surface chemistry. As shown in Figure 4.3(b) (black), the IR spectrum of 4.1-azido contains a well-resolved peak at 2098 cm$^{-1}$ that is also found in the IR spectrum of free $p$-azido-phenylethanethiol, which can be attributed to the N$_3$ stretch of the ligated azide groups. In the IR spectrum of 4.1-triazole (Figure 4.3(b) (red)), the peak at 2098 cm$^{-1}$ has completely disappeared, indicating that all surface azide moieties have been consumed during the CS-SPAAC reaction.

The synthesis of 4.1-azido was also confirmed using $^1$H NMR spectroscopy (Figure 4.3(c) (black)). As with 4.1-phenyl, the tetraoctylammonium signals appear at 0.90 (-CH$_3$), 1.31 (-CH$_2$), 1.37 (-CH$_2$-) and 3.09 ppm (N$^+$-CH$_2$-). As with other [Au$_{25}$(SR)$_{18}$]$^{1-}$ frameworks,$^{27-29}$ appending SCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$ to the Au$_{25}$ core results in a 12:6 inner:outer ligand distinction (inner: those attached at position 2 in Figure 4.2(b) and outer: those attached at position 1 in Figure 4.2(b)) that is distinguishable in the $^1$H NMR spectrum and was confirmed by COSY spectroscopy. The methylene protons alpha to the thiol (H$_\alpha$ protons) in the 12 inner ligands produce a well-resolved triplet at 3.54 ppm, which is downfield to the methylene protons beta to the thiol (H$_\beta$ protons) that produce a peak at 3.09 ppm. In the remaining 6 outer ligands, the downfield H$_\alpha$ protons produce a peak at 3.09 ppm while the H$_\beta$ protons produce a well-resolved triplet at 2.93 ppm. In spite of their remoteness to the sulfur atom, appending SCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$ to the Au$_{25}$ core results in a change in the chemical environment of both binary sets of aromatic protons, which produce doublets at 7.20 and 6.98 ppm in $p$-azido-phenylethanethiol (Figure S4.27). As with H$_\alpha$ and H$_\beta$, the aromatic protons appear in two resolvable sets, with the 12 inner ligands producing well-resolved doublets at 7.17 and 6.80 ppm and the 6 outer ligands producing well-resolved doublets at 6.86 and 7.12 ppm. Hence, the total removal of residual thiol and disulfide can be assessed through the total disappearance of the signals at 7.20 and 6.98 ppm from $p$-azido-phenylethanethiol.
The CS-SPAAC reaction between 4.1-azido and (Z)-cyclooct-1-ene-5-yne generates a triazole ring, which gives a characteristic change in the chemical environment of the binary sets of aromatic protons. As can be seen in the NMR spectrum of 4.1-triazole (Figure 4.3(c) (red)), there are new sets of doublets at 7.37 and 7.20 ppm from the 12 outer ligands, while the 6 outer ligands produce new doublets at 7.28 and 7.18 ppm. The appearance of these new downfield signals is a

**Figure 4.3** (a) UV-Vis spectrum of 0.2 mM solution of [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-N₃)₁₈] (4.1-azido) (black) and surface modified [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-C₆H₁₀N₃)₁₈] (4.1-triazole) (red) in dichloromethane at 23°C (b) ATR-IR spectrum of 4.1-azido (black) and 4.1-triazole (red) (c) ¹H NMR spectrum of 4.1-azido (black) and 4.1-triazole (red), taken in CD₂Cl₂ at 25°C. Chemical shifts of relevant protons are shown for inner ligands (blue) and outer ligands (green). * denotes residual H₂O.
diagnostic NMR fingerprint for successful CS-SPAAC chemistry on the surface of 4.1-azido, regardless of the cyclooctyne structure (this can also be seen in the $^1$H NMR spectrum of $[(\text{CH}_3(\text{CH}_2)_7)_4\text{N}][\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p\text{-C}_6\text{H}_4\text{-C}_10\text{H}_4\text{N}_3\text{O})_{18}]$ that forms from the reaction between 4.1-azido and BCN$_{\text{exo}}$-OH (Figure S4.11)). The protons associated with the eight-membered ring also occur in a 2:1 ratio and appear at a higher chemical shift compared to (Z)-cyclooct-1-ene-5-yne, which can be attributed to the loss of the alkyne moiety. Expectedly, given the more electron-deficient aryl ring, the signals from the H$_\beta$ protons also appear at higher chemical shifts. However, the signals from the H$_\alpha$ protons also appear at higher chemical shifts, with the 12 inner ligands producing a well-resolved triplet at 3.69 ppm, and the 6 outer ligands producing a signal at 3.22 ppm. Given their proximity to the metallic core, which has remained chemically unaltered, we believe that the change in the H$_\alpha$ signals can be attributed to a change in the packing of the surface monolayer after the CS-SPAAC reaction, altering the electronic environment of the H$_\alpha$ protons relative to the gold core.

4.3 Conclusions

Here we have described the direct synthesis and characterization of a stable azide-modified $[(\text{CH}_3(\text{CH}_2)_7)_4\text{N}][\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p\text{-C}_6\text{H}_4\text{-N}_3)_{18}]$ that is structurally similar to the well-studied benchmark $[(\text{CH}_3(\text{CH}_2)_7)_4\text{N}][\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{-C}_6\text{H}_5)_{18}]$. The incorporation of a reactive azide group amenable to cluster-surface strain-promoted alkyne-azide cycloaddition transforms the [Au$_{25}$(SR)$_{18}$]$^{1-}$ framework into a surface-modifiable [Au$_{25}$(SR)$_{18}$]$^{1-}$ platform, capable of being structurally modified through a simple-to-perform interfacial post-assembly click reaction, which will address many of the challenges for developing functional Au$_{25}$ nanoclusters. As there are many established protocols for incorporating strained-alkynes into a variety of substrates (such as fluorophores, biomolecules and redox species), this new Au$_{25}$ platform allows an easy approach to append additional functionality via the CS-SPAAC reaction to tune the properties of Au$_{25}$ nanoclusters. Furthermore, because each azide is chemically accessible from this single platform, it can allow the preparation of multifunctional Au$_{25}$ nanoclusters by challenging the platform to a mixture of functional strained alkyynes. Studies directed at highlighting these new advantages are currently ongoing.
4.4 Acknowledgements

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4.5 References


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### 4.6 Supporting Information

#### 4.6.1 General Materials and Methods

**Reagents and Solvents.** The following materials were used as received. Potassium thioacetate (98%), zinc dust (<10\(\mu\)m, ≥98%), sodium azide (≥99.5%), gold (III) chloride trihydrate (≥99.9% trace metal basis), tetraoctylammonium bromide (98%), sodium borohydride (≥98%), 1,5-cyclooctadiene (≥99%), bromine (reagent grade), potassium tert-butoxide solution (1.0M in THF), dichloromethane-D\(_2\) (CD\(_2\)Cl\(_2\), 99.5 atom %D) and dimethyl sulfoxide-D\(_6\) ((CD\(_3\))\(_2\)SO, 99.96 atom %D) were purchased from Sigma-Aldrich (Millipore Sigma). 4-nitrophenylethyl bromide was purchased from Oakwood Chemicals. Sodium chloride, sodium hydroxide pellets and tetrahydrofuran were purchased from Fischer Scientific. Technical grade ammonium chloride, magnesium sulphate, hexanes, dichloromethane, ethyl acetate, 12M hydrochloric acid, di-ethyl ether, dimethyl sulfoxide, sodium nitrite, methanol, toluene, isopropanol, acetonitrile and pentane
were purchased from Caledon. Chloroform-D$_1$ (CDCl$_3$, 99.8 atom %D) was purchased from Cambridge Isotope Laboratories. Ethanol (anhydrous) was purchased from Commercial Alcohols. Unless otherwise stated, all reactions were performed at ambient conditions.

**NMR Spectroscopy.** $^1$H and $^{13}$C{$^1$H} spectra were recorded on either a Bruker AvIII HD 400 spectrometer or Varian INOVA 600 spectrometer, as indicated. $^1$H NMR spectra are reported as $\delta$ in units of parts per million (ppm), and referenced against residual protio chloroform (7.27 ppm, s), dimethylsulfoxide (2.50 ppm, quin) or dichloromethane (5.32 ppm, t), as indicated. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintuplet), m (multiplet) and br (broad signal). Coupling constants are reported as a $J$ value in Hertz (Hz) according to the spectrometer frequency. The number of protons ($n$) for a given resonance is indicated as $n$H, and is based on spectral integration values. $^{13}$C{$^1$H} NMR spectra are reported as $\delta$ in units of parts per million (ppm) and referenced against the indicated deuterated solvent: chloroform-D$_1$ (77.0 ppm, t), dimethylsulfoxide-D$_6$ (39.5 ppm, septet) or dichloromethane-D$_2$ (54.0 ppm, quin).

**Mass Spectrometry.** Electrospray ionization (ESI) mass spectra were obtained in either positive-ion or negative-ion mode using a Bruker microTOF II spectrometer. Set capillary was 4000 V, set end plate offset was -400 V, set nebulizer was 1.0 Bar and set dry heater was 100°C. To obtain the ESI spectrum of [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] nanoclusters (obtained in negative-ion mode), a sample was dissolved in 1:5 toluene:methanol (10mg/mL). We generally found that in order to obtain mass spectra of [Au$_{25}$SR$_{18}$] clusters, the sample solution must contain some methanol in order to minimize excessive fragmentation. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were obtained using an AB Sciex 5800 TOF/TOF system. To obtain the MALDI-TOF spectrum of [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}$] nanoclusters (obtained in linear negative mode), a 1 g/L sample solution was mixed with a 10 g/L solution of trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in a ratio of 1:400 by mass. Data acquisition and data processing were respectively done using a TOF TOF Series Explore and Data Explorer (both from AB Sciex). The laser pulse rate was set to 400Hz. The mass spectrum was collected as a sum of 1000 shots.
**UV-Visible (UV-Vis) Spectroscopy.** UV-Vis absorption spectra were recorded using a Cary 5000 scan instrument using standard quartz cells (1 cm path length) with a scan range of 200-1000nm. Samples were dissolved in the indicated solvents at the indicated concentrations. The background spectrum of the indicated solvent was subtracted internally by the software.

**Infrared (IR) spectroscopy.** Attenuated total reflectance IR (ATR-IR) spectra were recorded using a PerkinElmer Spectrum Two FT-IR spectrometer.

### 4.6.2 Experimental Procedures

#### 4.6.2.1 Synthesis of \( p \)-nitro-phenylethanethioacetate

![Chemical Structure](image)

To 8.6 g (38 mmol, 1 eq.) 4-nitrophenylethyl bromide in 250 mL acetone was added 6.5 g (57 mmol, 1.5 eq.) potassium thioacetate. The resulting mixture was stirred at room temperature for 6 hours, after which the solid was removed by gravity filtration and the solution was concentrated by rotary evaporation. The resultant crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give \( p \)-nitro-phenylethanethioacetate as a yellow solid in 98% yield (8.4 g). \(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 8.17 (d, 2H, J=8 Hz), 7.39 (d, 2H, J=8 Hz), 3.15 (t, 2H, J=8 Hz), 2.99 (t, 2H, J=8 Hz), 2.35 (s, 3H). \(^{13}\)C NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 195.2, 147.4, 146.8, 129.5, 123.7, 35.6, 30.6, 29.7. HRMS (ESI) \( m/z \) calc. for C\(_{10}\)H\(_{11}\)NO\(_3\)S (M\(^+\)): 225.0460, found: 225.0467. IR (ATR-IR, cm\(^{-1}\)): 3025, 1680, 1597, 1513, 1448, 1411, 1340, 1280, 1129, 1096. UV-Vis (Dichloromethane, 1x10\(^{-4}\) mol.L\(^{-1}\)): \( \lambda_{\text{max}} = 276 \text{ nm, } \varepsilon = 1100 \text{ M}^{-1}\text{cm}^{-1} \).

#### 4.6.2.2 Synthesis of \( p \)-ammonium-phenylethanethioacetate chloride

![Chemical Structure](image)

To 8.4 g (37 mmol, 1eq.) \( p \)-nitro-phenylethanethioacetate in 70 mL ethanol and 70 mL distilled water was added 15.5 g (296 mmol, 8 eq) technical grade ammonium chloride, and then 12.0 g (185 mmol, 4 eq) zinc dust portion-wise under vigorous stirring over 5 minutes. The resulting suspension was stirred for 2 hours at room temperature, after which the solids were filtered off. The solids were washed with 50 mL of ethanol, after which 100 mL brine was added to the supernatant, which was then extracted with ethyl acetate (3 x 50 mL). The collected organic phases were diluted to
approximately 500 mL of ethyl acetate, after which 10 mL of 12 M hydrochloric acid was added slowly to the colorless solution under vigorous stirring, resulting in a pale yellow solution. After stirring the solution for 15 minutes, and then removing the solvent by rotary evaporation, the resulting crude residue was suspended in di-ethyl ether and the precipitate was collected by vacuum filtration to give **p-ammonium-phenylethanethioacetate chloride** as a bright white solid in 82% yield (7.0 g). \(^1\)H NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) (ppm) 7.34 (m, 4H), 4.01 (br, 3H), 3.10 (t, 2H, J=8 Hz), 2.84 (t, 2H, J=8 Hz), 2.31 (s, 3H). \(^13\)C NMR ((CD\(_3\))\(_2\)SO, 400 MHz): \(\delta\) (ppm) 195.1, 139.9, 130.0, 129.8, 123.2, 34.3, 30.6, 29.6. HRMS (ESI) \(m/z\) calcd. for C\(_{10}\)H\(_{14}\)NOS\(^+\) (M\(^+\)): 196.0791, found: 196.0719. IR (ATR-IR, cm\(^{-1}\)): 2828, 2595, 1950, 1680, 1620, 1556, 1507, 1356, 1315, 1206, 1140, 1105. UV-Vis (Dimethyl sulfoxide, 1x10\(^{-4}\) mol.L\(^{-1}\)): \(\lambda_{max}\) = 300 nm, \(\varepsilon\) = 1400 M\(^{-1}\)cm\(^{-1}\).

4.6.2.3 Synthesis of p-azido-phenylethanethioacetate

To 7.0 g (30 mmol, 1 eq.) **p-ammonium-phenylethanethioacetate chloride** in 350 mL 1 M hydrochloric acid was added 3.1 g (45 mmol, 1.5 eq) sodium nitrite in 75 mL distilled water dropwise over 20 minutes at 0°C. After stirring the solution for an additional 20 minutes at 0°C, 3.9 g (60 mmol, 2 eq) sodium azide in 75 mL distilled water was added dropwise over 10 minutes at 0°C. The solution was stirred for 20 minutes at 0°C, and then an additional 30 minutes at room temperature, after which the crude solution was extracted with dichloromethane (3 x 50 mL), dried over magnesium sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give **p-nitro-phenylethanethioacetate** as a pale yellow oil in 57% yield (3.8 g). \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) (ppm) 7.21 (d, 2H, J=8 Hz), 6.97 (d, 2H, J=8 Hz), 3.10 (t, 2H, J=8 Hz), 2.85 (t, 2H, J=8 Hz), 2.34 (s, 3H). \(^13\)C NMR (CDCl\(_3\), 400 MHz): \(\delta\) (ppm) 195.6, 138.3, 136.7, 130.0, 119.1, 35.1, 30.7, 30.4. HRMS (ESI) \(m/z\) calcd. for C\(_{10}\)H\(_{14}\)NOS\(^+\) (M\(^+\)): 221.0623, found: 221.0630. IR (ATR-IR, cm\(^{-1}\)): 3026, 2106, 1690, 1602, 1506, 1418, 1356, 1285, 1128, 1102. UV-Vis (Dichloromethane, 1x10\(^{-4}\) mol.L\(^{-1}\)): \(\lambda_{max}\) = 255 nm, \(\varepsilon\) = 2340 M\(^{-1}\)cm\(^{-1}\); \(\lambda_{max}\) = 283 nm, \(\varepsilon\) = 870 M\(^{-1}\)cm\(^{-1}\).
4.6.2.4 Synthesis of \(p\)-azido-phenylethanethiol

To 3.8 g (17 mmol, 1 eq.) \(p\)-nitro-phenylethanethioacetate in 100 mL deoxygenated methanol was added 25 mL (25 mmol, 1.5 eq.) of deoxygenated 1 M sodium hydroxide in water and the solution was stirred for 20 minutes at room temperature under argon, resulting in a yellow solution. Next, 50 mL (50 mmol, 3 eq.) of deoxygenated 1 M hydrochloric acid in water was added. The solution was stirred for 15 minutes at room temperature under argon, resulting in a pale yellow solution, after which distilled water (100 mL) was added and the product was extracted with dichloromethane (3 x 25 mL), dried over magnesium sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give \(p\)-azido-phenylethanethiol as a pale yellow oil in 94% yield (2.9 g). \(^1\)H NMR (CD\(_2\)Cl\(_2\), 400 MHz): \(\delta\) (ppm) 7.20 (d, 2H, J=8 Hz), 6.98 (d, 2H, J=8 Hz), 2.89 (t, 2H, J=8 Hz), 2.75 (q, 2H, J=8 Hz), 1.40 (t, 2H, J=8 Hz). \(^{13}\)C NMR (CD\(_2\)Cl\(_2\), 400 MHz): \(\delta\) (ppm) 138.8, 137.4, 130.6, 119.5, 40.0, 26.5. HRMS (ESI) \(m/z\) calc. for C\(_{10}\)H\(_{14}\)NOS\(^+\) (M\(^+\)): 179.0517, found: 179.0514. IR (ATR-IR, cm\(^{-1}\)): 3035, 2936, 2559, 2106, 1601, 1504, 1430, 1283, 1131. UV-Vis (Dichloromethane, 1x10\(^{-4}\) mol.L\(^{-1}\)): \(\lambda\)\(_{\text{max}}\) = 256 nm, \(\varepsilon\) = 2350 M\(^{-1}\)cm\(^{-1}\); \(\lambda\)\(_{\text{max}}\) = 283 nm, \(\varepsilon\) = 890 M\(^{-1}\)cm\(^{-1}\).

4.6.2.5 Synthesis of (Z)-5,6-dibromocyclooct-1-ene

\(\text{Br} \quad \text{Br} \) See Section 3.6.3.2 for detailed synthesis of (Z)-5,6-dibromocyclooct-1-ene.

4.6.2.6 Synthesis of (Z)-cyclooct-1-ene-5-yne

See Section 3.6.3.3 for detailed synthesis of (Z)-cyclooct-1-ene-5-yne.
4.6.2.7 Synthesis of bicyclo[6.1.0]nonyne (BCN<sub>exo</sub>-OH)

Synthesized according to Dommerholt <i>et al.</i> See Section 2.6.2.6 for characterization data.

4.6.2.8 Synthesis of [(CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>N]<Au<sub>25</sub>(SCH<sub>2</sub>CH<sub>2</sub>-<i>p</i>-C<sub>6</sub>H<sub>4</sub>-N<sub>3</sub>)<sub>18</sub>] (4.1-azido)

To a yellow solution of 393 mg (1.0 mmol) tetrachloroauric(III) acid trihydrate in 41 mL tetrahydrofuran was added 600 mg (1.1 eq, 1.1 mmol) tetraoctylammonium bromide at a stirring speed of 600 rpm. The solution was stirred for 15 minutes at room temperature and 30 minutes at 0°C, resulting in a dark red solution. The stirring was reducing to 80 rpm, and to the red solution was added an ice-cold solution of 1.07 g (6 eq, 6.0 mmol) of <i>p</i>-azido-phenylethanethiol in 1 mL tetrahydrofuran over 6 minutes at 0°C at 80 rpm. The resulting solution was stirred at 0°C for 1 hour at 80 rpm, then at room temperature for 3 hours at 80 rpm, then at room temperature for 1 hour at 600 rpm. Over the stirring period, the solution turned from dark red to yellow within the first hour, and once the temperature was increased to room temperature and stirred for four hours, it turned a very pale yellow color. After the stirring period, the pale yellow solution was cooled to 0°C for 15 minutes at 600 rpm, after which an ice-cold, freshly-prepared solution of 378 mg (10 mmol) sodium borohydride in 7.7 mL milli-Q water was added over approximately 15-20 seconds at 600 rpm, resulting in bubbling and an immediate color change from pale yellow to black. The neck of the flask was covered with a cap, and a light stream of argon was passed over the top of the flask, and the solution was stirred for 18 hours at room temperature at 600 rpm. After the overnight reaction, there was white precipitate on the side of the flask, and the solution turned from black to a reddish-brown color. The crude solution was filtered through glass wool, and the tetrahydrofuran was removed by rotary evaporation. The crude residue was dissolved in 50mL toluene and extracted
with 200 mL milli-Q water. The organic phase was removed, and the toluene was removed by rotary evaporation. To the crude brownish-red oil was added approximately 20 mL isopropanol, resulting in the formation of small insoluble black solids. The solids were collected by filtering the suspension through glass wool in a funnel. NOTE: the solution may have to be filtered more than once to collect all black solids. The glass wool was washed thoroughly with isopropanol to fully remove residual thiols and disulfides. After the isopropanol washes, 10 mL acetonitrile was added, resulting in dissolution of most of the black solids to give reddish brown filtrate. The acetonitrile was removed by rotary evaporation, resulting in a film on the flask wall. The film was washed again with isopropanol, and then re-dissolved in acetonitrile and filtered once more through glass wool. Removal of the acetonitrile by rotary evaporation gave a reddish-black oily film of purified [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$S(CH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)] that was stored as a film at 0°C. The typical yield of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$S(CH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)] is 80-90 mg. $^1$H NMR (CD$_2$Cl$_2$, 600 MHz): δ (ppm) 7.17 (d, 24H, J=6 Hz), 7.12 (d, 12H, J=6 Hz), 6.86 (d, 12H, J=6 Hz), 6.80 (d, 24H, J=6 Hz), 3.54 (t, 24H, J=6 Hz), 3.09 (m, 44H), 2.93 (t, 12H, J=6 Hz), 1.62 (m, 8H), 1.37-1.31 (m, 48H), 0.90 (t, 12H, J=6 Hz). HRMS (ESI[negative]) m/z calc. for C$_{144}$H$_{144}$Au$_{25}$N$_{54}$S (M$^-$): 8132.4 Da, found: 8132.9 Da. IR (ATR-IR, cm$^{-1}$): 2904, 2845, 2411, 2254, 2098, 1604, 1581, 1504. UV-Vis (Dichloromethane, 2x10$^{-4}$ mol L$^{-1}$): $\lambda_{max}$ = 682 nm, $\varepsilon$ = 1908 M$^{-1}$cm$^{-1}$, $\lambda_{max}$ = 443 nm, $\varepsilon$ = 6600 M$^{-1}$cm$^{-1}$, $\lambda_{max}$ = 404 nm, $\varepsilon$ = 7650 M$^{-1}$cm$^{-1}$.

4.6.2.9 Synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$S(CH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}$] (4.1-triazole)

To 10 mg (0.0012 mmol, 1 eq) [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$S(CH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)] in 5 mL tetrahydrofuran was added 2.5 mg (0.024 mmol, 20 eq) (Z)-cyclooct-1-ene-5-yne in 1 mL tetrahydrofuran. The solution was stirred for 10 minutes, after which the solvent and residual cyclooctyne starting material was removed by rotary evaporation. Upon removal of the solvent, a reddish brown film formed on the flask interior, which was then triturated with acetonitrile to remove residual azide-clusters, giving [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$S(CH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}$] as a reddish black film in 96% yield (12 mg).
$^1$H NMR (CD$_2$Cl$_2$, 600 MHz): $\delta$ (ppm) 7.37 (d, 24H, J=12 Hz), 7.28 (d, 12H, J=6 Hz), 7.18 (m, 36H), 5.47 (m, 24H), 5.39 (m, 12H), 3.69 (t, 24H, J=6 Hz), 3.27-3.22 (m, 36H), 3.09 (m, 56H), 2.68-2.64 (m, 36H), 2.47-2.42 (m, 72H), 1.61 (m, 8H), 1.33-1.26 (m, 48H), 0.88 (t, 12H, J=6 Hz).

MS (MALDI-TOF[negative]) $m/z$ calc. for C$_{288}$H$_{324}$Au$_{25}$N$_{54}$S (M$^-$): 10043.4 Da, found: 10048.4 Da.

IR (ATR-IR, cm$^{-1}$): 2920, 2852, 1608, 1563, 1517.

UV-Vis (Dichloromethane, 2x10$^{-4}$ mol L$^{-1}$): $\lambda_{\text{max}} = 682$ nm, $\varepsilon = 2040$ M$^{-1}$cm$^{-1}$, $\lambda_{\text{max}} = 446$ nm, $\varepsilon = 7250$ M$^{-1}$cm$^{-1}$, $\lambda_{\text{max}} = 394$ nm, $\varepsilon = 7780$ M$^{-1}$cm$^{-1}$. 

212
4.6.3 Experimental Spectra and Diagrams

4.6.3.1 Experimental Spectra and Diagrams for [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$][(CH$_3$-(CH$_2$)$_7$)$_4$N] (4.1-azido)

Figure S4.1. 600 MHz $^1$H NMR spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (4.1-azido) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvent and impurities.
**Figure S4.2.** COSY NMR spectrum of $[(\text{CH}_3-(\text{CH}_2)_7)_4\text{N}][\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{N}_3)_{18}]_{(4.1\text{-azido})}$ in $\text{CD}_2\text{Cl}_2$ at 25°C.

**Figure S4.3.** Negative ion mode ESI mass spectrum of anionic $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{N}_3)_{18}]^{1-}$. 
**Figure S4.4.** Space-filling X-ray structure diagram of \([\text{(CH}_3\text{-}(\text{CH}_2)_7\text{N})\text{Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}p\text{-C}_6\text{H}_4\text{-N}_3)_{18}}\) (4.1-azido). Yellow = gold, red = sulfur, black = carbon, green = nitrogen. Tetraoctylammonium counterion is not shown.

**Figure S4.5.** Molecular structure of \([\text{(CH}_3\text{-}(\text{CH}_2)_7\text{N})\text{Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}p\text{-C}_6\text{H}_4\text{-N}_3)_{18}}\) (4.1-azido) showing disordered tetraoctylammonium counterion. Yellow = gold, red = sulfur, black = carbon\text{\_cluster}, light blue = carbon\text{\_counterion}, green = nitrogen.
Figure S4.6. Photoluminescence spectrum of a 3 µmol/L solution of [(CH$_3$- (CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$p-C$_6$H$_4$-N$_3$)$_{18}$] (4.1-azido) in dichloromethane at 22°C, recorded with a 532 nm laser and 0.1 second exposure time.
4.6.3.2 Experimental Spectra for [(CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>N][Au<sub>25</sub>(SCH<sub>2</sub>CH<sub>2</sub>-p-C<sub>6</sub>H<sub>4</sub>-C<sub>8</sub>H<sub>10</sub>N<sub>3</sub>)<sub>18</sub>] (4.1-triazole)

![Image of NMR spectra](image)

**Figure S4.7.** 600 MHz <sup>1</sup>H NMR spectrum of [(CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>N][Au<sub>25</sub>(SCH<sub>2</sub>CH<sub>2</sub>-p-C<sub>6</sub>H<sub>4</sub>-C<sub>8</sub>H<sub>10</sub>N<sub>3</sub>)<sub>18</sub>] (4.1-triazole) in CD<sub>2</sub>Cl<sub>2</sub> at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvent and impurities.
Figure S4.8. COSY spectrum of \([\text{CH}_3-(\text{CH}_2)_7\text{N}][\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}]\) (4.1-triazole) in CD$_2$Cl$_2$ at 25°C.

Figure S4.9. Linear negative mode MALDI-TOF mass spectrum of anionic [\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}]^−.
Figure S4.10. Photoluminescence spectrum of a 3 µmol/L solution of [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-C₈H₁₀N₃)₁₈] (4.1-triazole) in dichloromethane at 22°C, recorded with a 532 nm laser and 0.1 second exposure time.
4.6.3.3 Experimental Spectra for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{10}$H$_{14}$N$_3$O)$_{18}$]. Reaction of 4.1-azido with BCN$_{exo}$-OH

**Figure S4.11.** 600 MHz $^1$H NMR spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{10}$H$_{14}$N$_3$O)$_{18}$] in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in region of relevant section of spectrum. * indicates residual protio solvents and impurities.
Figure S4.12. Linear negative mode MALDI-TOF mass spectrum of anionic [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{16}$H$_{14}$N$_3$O)$_{18}$]$^{1+}$.

Figure S4.13. UV-Vis absorption spectrum of 1x10$^{-4}$ M solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{16}$H$_{14}$N$_3$O)$_{18}$] in dichloromethane at 23°C.
Figure S4.14. Infrared spectrum of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_{10}\text{H}_{14}\text{N}_3\text{O})_{18}]$. 
4.6.3.4 Experimental Spectra for \( p \)-nitro-phenylethanethioacetate

**Figure S4.15.** \(^1\)H NMR spectrum of \( p \)-nitro-phenylethanethioacetate in CDCl\(_3\) at 25°C. * indicates residual protio solvent and impurities.

**Figure S4.16.** \(^{13}\)C{\(^1\)H} NMR spectrum of \( p \)-nitro-phenylethanethioacetate in CDCl\(_3\) at 25°C. * indicates CDCl\(_3\) solvent.
Figure S4.17. Infrared spectrum of *p*-nitro-phenylethanethioacetate.

Figure S4.18. UV-Vis absorption spectrum of 1x10^{-4} M solution of *p*-nitro-phenylethanethioacetate in dichloromethane at 23°C.
4.6.3.5 Experimental Spectra for \( p \)-ammonium-phenylethanethioacetate chloride

**Figure S4.19.** \(^1\)H NMR spectrum of \( p \)-ammonium-phenylethanethioacetate chloride in \((\text{CD}_3)_2\text{SO}\) at 25°C. * indicates residual protio solvent.

**Figure S4.20.** \(^{13}\)C\{\(^1\)H\} NMR spectrum of \( p \)-ammonium-phenylethanethioacetate chloride in \((\text{CD}_3)_2\text{SO}\) at 25°C. * indicates \((\text{CD}_3)_2\text{SO}\) solvent.
Figure S4.21. Infrared spectrum of \( p \)-ammonium-phenylethanethioacetate chloride.

Figure S4.22. UV-Vis absorption spectrum of \( 1 \times 10^{-4} \) M solution of \( p \)-ammonium-phenylethanethioacetate chloride in dichloromethane at 23°C.
4.6.3.6 Experimental Spectra for \( p \)-azido-phenylethanethioacetate

**Figure S4.23.** \(^1\)H NMR spectrum of \( p \)-azido-phenylethanethioacetate in CDCl\(_3\) at 25°C. * indicates residual protio solvent and impurities.

**Figure S4.24.** \(^{13}\)C\(^{1}\)H NMR spectrum of \( p \)-azido-phenylethanethioacetate in CDCl\(_3\) at 25°C. * indicates CDCl\(_3\) solvent.
Figure S4.25. Infrared spectrum of \textit{p-azido-phenylethanethioacetate}.

Figure S4.26. UV-Vis absorption spectrum of $1 \times 10^{-4}$ M solution of \textit{p-azido-phenylethanethioacetate} in dichloromethane at 23°C.
4.6.3.7 Experimental Spectra for \( p \)-azido-phenylethanethiol

Figure S4.27. \(^1\)H NMR spectrum of \( p \)-azido-phenylethanethiol in CD\(_2\)Cl\(_2\) at 25°C. * indicates residual protio solvent.

Figure S4.28. \(^{13}\)C\(^1\)H NMR spectrum of \( p \)-azido-phenylethanethiol in CD\(_2\)Cl\(_2\) at 25°C. * indicates CD\(_2\)Cl\(_2\) solvent.
Figure S4.29. Infrared spectrum of \textit{p-azido-phenylethanethiol}.

Figure S4.30. UV-Vis absorption spectrum of 1x10^{-4} M solution of \textit{p-azido-phenylethanethiol} in dichloromethane at 23°C.
4.6.3.8 Experimental Spectra for (Z)-5,6-dibromocyclooct-1-ene

See Section 3.6.9.2 for $^1$H NMR and $^{13}$C{$^1$H} NMR spectra for (Z)-5,6-dibromocyclooct-1-ene.

**Figure S4.31.** Infrared spectrum of (Z)-5,6-dibromocyclooct-1-ene.

**Figure S4.32.** UV-Vis absorption spectrum of 1x10^{-4} M solution of (Z)-5,6-dibromocyclooct-1-ene in dichloromethane at 23°C.
4.6.3.9 Experimental Spectra for (Z)-cyclooct-1-ene-5-yne

See Section 3.6.9.3 for $^1$H NMR and $^{13}$C($^1$H) NMR spectra for (Z)-cyclooct-1-ene-5-yne.

**Figure S4.33.** Infrared spectrum of (Z)-cyclooct-1-ene-5-yne.

**Figure S4.34.** UV-Vis absorption spectrum of $1 \times 10^{-4}$ M solution of (Z)-cyclooct-1-ene-5-yne in dichloromethane at 23°C.
4.6.4 Crystallographic Information

4.6.4.1 Data Collection and Processing

The sample was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker Kappa Axis Apex2 diffractometer at a temperature of 110 K. The unit cell dimensions were determined from a symmetry constrained fit of 9034 reflections with $4.7^\circ < 2\theta < 47.48^\circ$. The data collection strategy was a number of $\omega$ and $\phi$ scans which collected data up to $47.692^\circ (2\theta)$. The frame integration was performed using SAINT$^4$. The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS$^5$.

4.6.4.2 Structure Solution and Refinement

The structure was solved by using a dual space methodology using the SHELXT program$^6$. All Au and S atoms were obtained from the initial solution. C and N atoms were located in the subsequent difference Fourier maps. The Oct$_4$N$^+$ cation was found to exhibit disorder, residing about a crystallographic -3 site. Disordered C atoms were thus refined with partial occupancies; N-C and C-C distances were restrained (DFIX command). One of the crystallographically independent – CH$_2$CH$_2$C$_6$H$_4$N$_3$ thiolate moieties (C19-N9) also appeared to have site disorder but this could not be resolved/modelled. It was thus refined using the restraint SAME and SIMU in the software. The hydrogen atoms for the thiolate ligands were introduced at idealized positions and were allowed to ride on the parent atom. The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL program from the SHELXTL suite of crystallographic software$^7$. Graphic plots were produced using the NRCVAX program suite$^8$. Additional information and other relevant literature references can be found in the reference section of this website (http://xray.chem.uwo.ca). CCDC 1936245 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.
### 4.6.4.3 Summary of Crystal Data

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</tr>
<tr>
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</tr>
<tr>
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\( \lambda, \text{Å}, (\text{MoK}\alpha) \) 0.71073

\( \mu, (\text{cm}^{-1}) \) 16.998

Diffractometer Type Bruker Kappa Axis Apex2

Scan Type(s) phi and omega scans

Max 2\( \theta \) for data collection, \( ^\circ \) 47.692

Measured fraction of data 0.997

Number of reflections measured 208329

Unique reflections measured 5593

R\text{merge} 0.1027

Number of reflections included in refinement 5593

Cut off Threshold Expression \( I > 2\sigma(I) \)

Structure refined using full matrix least-squares using \( F^2 \)

Weighting Scheme \( w=1/[\sigma^2(Fo^2)+(0.0545P)^2+179] \) 0.3613P where \( P=(Fo^2+2Fc^2)/3 \)

Number of parameters in least-squares 400

R\text{1} 0.0515

wR\text{2} 0.1266

R\text{1} (all data) 0.0859

wR\text{2} (all data) 0.1592

GOF 1.145
Maximum shift/error 0.000

Min & Max peak heights on final $\Delta F$ Map ($e/\text{Å}$) -1.755, 2.848

Where:

$R_1 = \frac{\sum (|F_o| - |F_c|)}{\sum F_o}$

$wR_2 = \left[ \frac{\sum (w(F_o^2 - F_c^2))}{\sum (w F_o^4)} \right]^{\frac{1}{2}}$

$\text{GOF} = \left[ \frac{\sum (w(F_o^2 - F_c^2))}{(\text{No. of reflns.} - \text{No. of params.})} \right]^{\frac{1}{2}}$

4.6.5 References – Supporting Information


Chapter 5

5 Expanding the Library of Clickable Azide-Functionalized Au$_{25}$SR$_{18}$ Nanocluster Platforms: A study of Ligand Modifications on Structure, Properties and Surface Reactivity

This chapter is being submitted as a short paper and is in manuscript format. Julia Martin and Jonathan M. Wong are co-authors, along with professors Zhifeng Ding, John F. Corrigan and Mark S. Workentin.

Martin was an undergraduate thesis student under my direct co-supervision, along with my supervisor, Prof. Mark S. Workentin, who assisted in the synthesis and characterization of the three isomeric clusters and obtaining the molecular structures presented. Wong was a graduate student, who under the co-supervision of his supervisor, Prof. Zhifeng Ding, obtained the electrochemical measurements of the three isomeric clusters. Prof. John F. Corrigan was involved in obtaining and refining the molecular structures presented. The draft of the manuscript was written by the author and edited by Prof. Mark S. Workentin and Prof. John F. Corrigan.

5.1 Introduction

In contemporary nanomaterials science, ligand-protected gold nanoclusters (AuNCs) are among the most currently researched due to their superior stability and ease of synthesis compared to other noble metal nanocluster systems that tend to be more chemically sensitive. Given their synthetic accessibility, there has been extensive efforts towards understanding the fundamental conditions to produce atomically precise AuNCs, with well-defined physical and optical properties that are fingerprinted to each individual combination of surface ligand and metal configuration. It is this ability to influence the core configuration and related properties through careful alterations to reaction conditions, stoichiometries and surface ligand structure that has led to the prominent library of AuNCs currently existing, with many of the AuNCs having become promising candidates for a wide variety of applications, including in catalysis,$^{2-4}$ chemical sensing$^{5-7}$ and biomedicine.$^{8-10}$
Of the fundamental factors that can be manipulated to construct AuNCs of various sizes in an experimentally pre-defined manner, the correlation between the type and structure of the protecting surface ligand and acquired nanocluster configuration has received significant attention.\textsuperscript{11-13} Recently, there have been many examples of chemically robust N-heterocyclic carbene (NHC)-stabilized AuNCs reported.\textsuperscript{14-16} However, due to their ease of synthesis and chemical versatility, thiol-based ligands are the most common types of protecting surface ligands that are currently employed towards the construction of very stable AuNCs frameworks.\textsuperscript{17} Through protection-deprotection strategies, the thiol moiety can easily be appended to a wide variety of alkyl and aromatic ligands, and it is the ability to generate such a wide variety of thiolated ligand structures that has led to a rich and diverse library of thiolate-protected AuNC frameworks.\textsuperscript{1} Changes to the size, sterics and electronic composition of the thiol-based ligand have profound impacts on which AuNC system can be acquired.\textsuperscript{18, 19} It should also be noted that changes to experimental conditions such as reaction temperature and the ratio of Au(III) precursor to ligand play a vital role in what cluster framework is constructed, but under such similar reaction conditions, markedly different thiol-based ligands lead to distinctly different thiolated AuNC frameworks.\textsuperscript{20} It has also been demonstrated that through ligand exchange-induced size/structure transformation (LEIST), exchange of secondary thiol-based ligands onto parent AuNCs can alter the parent AuNC in a predictable manner to generate a distinct, different-sized AuNC system.\textsuperscript{21-23} There are also several thiol-based ligands, such as phenylethanethiol, that can be used to generate different-sized AuNC systems when the experimental conditions are changed.\textsuperscript{24-26}

Although such investigations have resulted in a deep understanding of the relationship between the ligand structure and acquired core configuration, there has been little research on how regio-isomerization of the thiol-based ligand impacts the acquired AuNC configuration. Jin and co-workers reported that adding \((o/m/p)\)-methylbenzenethiols (MBT) to a gold precursor, followed by size-focusing at elevated temperatures in the presence of an excess of the corresponding thiol-based ligand, led to the development of \(\text{Au}_{40}(o\text{-MBT})_{24}, \text{Au}_{104}(m\text{-MBT})_{41}\) and \(\text{Au}_{130}(p\text{-MBT})_{50}\).\textsuperscript{27} They confirmed these structures by absorption spectroscopy, mass spectrometry and thermogravimetric analysis. In the absence of such high-temperature size-focusing, Antoine and co-workers reported that, using \((o/m/p)\)-mercaptobenzoic acid (MBA), they were able to synthesize \(\text{Au}_{25}(o\text{-MBA})_{18}, \text{Au}_{25}(m\text{-MBA})_{18}\) and \(\text{Au}_{25}(p\text{-MBA})_{18}\) under kinetic control. They
confirmed these structures by absorption spectroscopy and mass spectrometry, and found that these three regioisomeric \( \text{Au}_{25}(\text{SR})_{18} \) systems had identical absorption profiles.\(^{28}\)

In addition to conferring the ability to predictably adjust the size and nuclearity of the AuNC core structure, a significant advantage for using thiol-based surface ligands is the ability to conduct ligand exchange chemistry to develop functional varieties of thiolated-AuNC frameworks having properties and applications that are dependent on the appended functionalities.\(^{29}\)

Conventional syntheses of thiolated AuNC nanoclusters involve addition of an excess of thiol-based ligand to an Au(III) precursor to generate an Au(I)-thiolate intermediate, which is subsequently reduced to generate the desired Au(0)-containing AuNC frameworks. Functional thiol-based ligands tend not to be compatible with the reducing conditions of the synthesis, and so functional AuNC systems cannot be directly accessed. However, the mild lability of the gold-sulfur bond allows for ligand exchange chemistry to be executed, in which native inert thiolated ligands in the parent system can be replaced with secondary thiol-based ligands possessing various functional substrates.\(^{30}\)

Although this strategy has found some utility, it is often limited by synthetic incompatibilities between the appended functional substrate on the ligand and the protection-deprotection strategies necessary to successfully incorporate the thiol moiety. Furthermore, ligand exchange with secondary thiol-based ligands are often not ‘orthogonal’ to the parent AuNC systems and can lead to unpredictable core variations and formation of larger undesired by-products.\(^{31, 32}\) The shortcomings of ligand exchange methodologies onto AuNCs necessitate investigations towards developing more ‘nanoorthogonal’ strategies for developing functional AuNC systems for application-based research, in which functionality can be appended to the AuNC surface in a predictable manner without altering the internal AuNC framework.

To this end, we previously communicated the first synthesis of a ‘clickable’, surface-reactive \([\text{(CH}_3-(\text{CH}_2)_7\text{)}_4\text{N}]\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-N_3)_{18}\) nanocluster platform (hereafter referred to as \textit{p-azido}\(^{11}\) in current chapter (and referred to as \textit{4.1-azido} in \textbf{Chapter 4} and \textit{6.1-azido} in \textbf{Chapter 6})), using \textit{p-azidophenylethanethiol} (\text{HSCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-N_3) as the surface thiol-based ligand.\(^{33}\) This platform is capable of undergoing post-assembly modifications at all the surface azido-benzene moieties, through the cluster-surface strain-promoted alkyne-azide cycloaddition
(CS-SPAAC) reaction, in which the surface azido moieties undergo a 1,3-dipolar cycloaddition reaction with non-linear cyclooctynes to generate a surface triazole cycloadduct. Commonly utilized as a ‘bioorthogonal click reaction’ to append substrates to biologically sensitive molecular systems, the reaction between azides and cyclooctynes is highly chemoselective and occurs under mild conditions. Not only were all surface azido moieties on p-azido\textsuperscript{1} amendable to CS-SPAAC chemistry, but analysis indicated there was no change to the core framework, establishing the CS-SPAAC reaction as a “nanoorthogonal click reaction” that can be used to efficiently conduct surface modifications on chemically sensitive AuNC systems.\textsuperscript{34} To highlight the generality of this nanoorthogonal surface modification strategy, after reporting the development of p-azido\textsuperscript{1}, Kang et al. developed additional azide-modified platforms, including azide-modified Au\textsubscript{28}(SR)\textsubscript{20} and Au\textsubscript{36}(SR)\textsubscript{24}, through nanocluster transformation in which they performed ligand exchange of azido-thiophenol onto pre-synthesized nanocluster precursors.\textsuperscript{34} Similar to our observations, they reported that their systems could also do SPAAC (which we coined CS-SPAAC), but also copper-catalyzed alkyne-azide cycloaddition chemistry,\textsuperscript{34} for nanoorthogonal surface modifications of these AuNC systems.

Two regioisomeric variants of the p-azido\textsuperscript{1} platform are herein reported: the [((CH\textsubscript{3})\textsubscript{3}(CH\textsubscript{2}))\textsubscript{4}N][Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-m-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}] platform (hereafter referred to as m-azido\textsuperscript{1}), which can be made using m-azidophenylethanethiol (HSCH\textsubscript{2}CH\textsubscript{2}-m-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3}), and the [((CH\textsubscript{3})\textsubscript{3}(CH\textsubscript{2}))\textsubscript{4}N][Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-o-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}] platform (hereafter referred to as o-azido\textsuperscript{1}), which can be made using o-azidophenylethanethiol (HSCH\textsubscript{2}CH\textsubscript{2}-o-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3}) (Scheme 1). As was reported by Antoine and co-workers for their three regioisomeric forms of Au\textsubscript{25}(olml/p-MBA)\textsubscript{18} nanoclusters,\textsuperscript{28} the optical properties of these regioisomeric nanoclusters were identical. However, the electrochemical behaviour and structure showed some correlated differences as the position of the azido group was changed, while the feasibility and kinetics of the CS-SPAAC reactivity was substantially different for each isomeric variant. This provides great insight into how ligand isomerization affects structure-property relationships of Au\textsubscript{25}(SR)\textsubscript{18} nanoclusters, as well as how it affects the ability to conduct post-assembly surface modifications on Au\textsubscript{25}(SR)\textsubscript{18} systems nanoorthogonally.
5.2 Results and Discussion

In order to synthesize the three regioisomeric variants of azide-functionalized \([\text{Au}_{25}(\text{SR})_{18}]^{-}\) nanoclusters, the corresponding azide-modified thiol-based ligands were prepared according to a modified version of our previously reported procedure for the synthesis of \(p\)-azidophenylethanethiol.\(^{33}\) Whereas a four-step synthesis was previously implemented, a general three-step synthesis is herein reported that can be used to synthesize \(p\)-azidophenylethanethiol, \(m\)-azidophenylethanethiol and \(o\)-azidophenylethanethiol in an overall higher yield than the previous four-step synthesis (see Section 5.6.2 for detailed syntheses). The azide-modified \([\text{Au}_{25}(\text{SR})_{18}]^{-}\) nanocluster platforms, \(p\text{-azido}^{1}\), \(m\text{-azido}^{1}\) and \(o\text{-azido}^{1}\), were synthesized according to our

\[
\begin{align*}
\text{p-azidophenylethanethiol} & \quad \text{m-azidophenylethanethiol} & \quad \text{o-azidophenylethanethiol} \\
\text{or} & \quad \text{or} & \\
\text{or} & \\
N_1 & N_1 & N_1 \\
\begin{array}{c}
\text{SH} \\
\text{SCH}_2\text{CH}_2\text{-}p\text{-C}_6\text{H}_4\text{-N}_3
\end{array} & \\
\text{k}_2 = 0.157 \text{M}^{-1}\text{s}^{-1} & \text{k}_2 = 0.0870 \text{M}^{-1}\text{s}^{-1} & \text{k}_2 = 0.00430 \text{M}^{-1}\text{s}^{-1}
\end{align*}
\]

\[\text{[Au-SCH}_2\text{CH}_2\text{-}o/m/p\text{-C}_6\text{H}_4\text{-N}_3]_\text{n} \]

\[\text{+ NaBH}_3 \quad \text{Kinetically-Controlled Size-Focusing} \]

\[\text{[TOA][Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}p\text{-C}_6\text{H}_4\text{-N}_3)]_{\text{18}} \]

\[\text{k}_2 = 0.162 \text{ M}^{-1}\text{s}^{-1}\]

\[\text{[TOA][Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}m\text{-C}_6\text{H}_4\text{-N}_3)]_{\text{18}} \]

\[\text{k}_2 = 0.0400 \text{ M}^{-1}\text{s}^{-1}\]

\[\text{[TOA][Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}o\text{-C}_6\text{H}_4\text{-N}_3)]_{\text{18}} \]

\[\text{k}_2 = \text{N/A}\]

\[\text{+ \hspace{1cm} Kinetically-Variable CS-SPAAC} \]

\[\hspace{2.5cm} \text{Unstable towards CS-SPAAC}\]

**Scheme 5.1.** Synthesis of \([(\text{CH}_3\text{-}(\text{CH}_2)_7\text{)}_4\text{N}]\text{[Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}p\text{-C}_6\text{H}_4\text{-N}_3)]_{18}\) (\(p\text{-azido}^{1}\), black), \([(\text{CH}_3\text{-}(\text{CH}_2)_7\text{)}_4\text{N}]\text{[Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}m\text{-C}_6\text{H}_4\text{-N}_3)]_{18}\) (\(m\text{-azido}^{1}\), blue) and \([(\text{CH}_3\text{-}(\text{CH}_2)_7\text{)}_4\text{N}]\text{[Au}_{25}\text{(SCH}_2\text{CH}_2\text{-}o\text{-C}_6\text{H}_4\text{-N}_3)]_{18}\) (\(o\text{-azido}^{1}\), red). Rate of reaction \(k_2\) between AuNCs and BCN\text{ex}OH were determined under second order conditions using \(^1\text{H} \text{NMR}\) spectroscopy, and are indicated for \(p\)-azidophenylethanethiol, \(m\)-azidophenylethanethiol, \(o\)-azidophenylethanethiol, \(p\text{-azido}^{1}\) and \(m\text{-azido}^{1}\).
previously reported procedure,\textsuperscript{33} using \textit{p}-azidophenylethanol, \textit{m}-azidophenylethanol and \textit{o}-azidophenylethanol, respectively (see Section 5.6.2.12 to Section 5.6.2.14 for detailed syntheses). The general procedure involves slow reduction of HAuCl\textsubscript{4}·3H\textsubscript{2}O as the Au(III) precursor to an [-Au(I)-SR\texttextsubscript{\textit{n}}]- coordination intermediate at low temperature in the presence of one of the three azide-modified thiol-based ligands, followed by rapid reduction of the [-Au(I)-SR\texttextsubscript{\textit{n}}]- intermediate using aqueous sodium borohydride (Scheme 5.1). The negative mode electrospray ionization mass spectrometry (ESI-MS) spectrum of purified \textit{m}-azido\textsuperscript{1-} shows a large peak at 8131.9 Da (Figure S5.2), and the ESI-MS spectrum of \textit{o}-azido\textsuperscript{1-} shows a large peak at 8132.1 Da (Figure S5.4). These observed peaks are consistent with the ESI-MS spectrum for \textit{p}-azido\textsuperscript{1-} that was previously reported which contained a large peak centered at 8132.9 Da, and corresponds to the [Au\texttextsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2-}(olmlp)-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}]\textsuperscript{1-} anions of the parent nanocluster platforms (expected m/z = 8132.4 Da).

As determined by \textsuperscript{1}H NMR spectroscopy, the clusters directly prepared were anionic, with the presence of \textsuperscript{1}H NMR signals from the tetraoctylammonium counterion (see Figure S4.1, Figure S5.1 and Figure S5.3 for \textsuperscript{1}H NMR spectra). However, unlike \textit{p}-azido\textsuperscript{1-}, the anionic forms of \textit{o}-azido\textsuperscript{1-} and \textit{m}-azido\textsuperscript{1-} could not be successfully crystallized under all conditions tried. Fortunately, slow oxidation of \textit{o}-azido\textsuperscript{1-}, \textit{m}-azido\textsuperscript{1-} and \textit{p}-azido\textsuperscript{1-} in 3:1 ethanol:toluene under ambient conditions over approximately two weeks led to black needle-like crystals of the neutral cluster forming, [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2-}(olmlp)-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}]\textsuperscript{0} (hereafter referred to as \textit{o}-azido\textsuperscript{0}, \textit{m}-azido\textsuperscript{0} and \textit{p}-azido\textsuperscript{0}, respectively). The core configuration and monolayer composition of these neutral forms were confirmed by single-crystal X-ray diffraction (Figure 5.1a). It should be noted that although all other characterization and surface chemistry was conducted on the anionic forms, the structural similarities between \textit{p}-azido\textsuperscript{1-} and \textit{p}-azido\textsuperscript{0} (particularly of the monolayer composition) indicate that the molecular structures of the neutral forms provide sufficient indications as to the general molecular features of the anionic forms. Furthermore, transitioning from the anionic to the neutral form is a core transformation, and will not affect the composition of the surface monolayer that has been confirmed in the molecular structures of the neutral forms.

The \textit{o}-azido\textsuperscript{0} and \textit{p}-azido\textsuperscript{0} clusters crystallize in the triclinic space group \textit{P}1\textit{I} and the \textit{m}-azido\textsuperscript{0} cluster crystallizes in the monoclinic space group \textit{P}2\textit{I}/\textit{c}. All three molecular structures of the neutral forms comprise an internal body-centered icosahedron where the central Au atom
(Au\textsubscript{cent}) at the inversion center is ligated to twelve Au atoms at the icosahedral vertices (Au\textsubscript{vert}), with the Au\textsubscript{cent}-Au\textsubscript{vert} bond lengths being between 2.769 \textasciitilde 2.799 Å for all three structures, typical for core Au-Au bonds in Au\textsubscript{25} clusters.\textsuperscript{35-37} As observed in the molecular structure of \textit{p-azido}\textsuperscript{1}, the internal Au\textsubscript{13} cores are circumscribed by an external scaffold of six “staple motifs” (-SR-Au\textsubscript{scf}-SR-Au\textsubscript{scf}-SR-), with the Au\textsubscript{vert}-Au\textsubscript{scf} bond lengths being between 3.107 (2) to 3.334 (2) Å and Au\textsubscript{scf}-S bond lengths being between 2.294 (7) to 2.324 (7) Å, which is consistent with previously reported structures.\textsuperscript{36-38} The six staple motifs comprise two μ\textsubscript{2}-thiolate ligands in which the six central μ\textsubscript{2}-thiolate ligands occupy a distinguishable surface site (the “outer ligands”)

\textbf{Figure 5.1.} (a) \textit{Left to right}. Molecular structure of [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-p-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}]\textsuperscript{0} (\textit{p-azido}\textsuperscript{0}), [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-m-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}]\textsuperscript{0} (\textit{m-azido}\textsuperscript{0}) and [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-o-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}]\textsuperscript{0} (\textit{o-azido}\textsuperscript{0}). Au = yellow, S = red, C = black, N = green. (b) \textit{Left to right}. Comparison of core structures of \textit{p-azido}\textsuperscript{1}, \textit{p-azido}\textsuperscript{0}, \textit{m-azido}\textsuperscript{0} and \textit{o-azido}\textsuperscript{0}.
while the remaining twelve μ₂-thiolate ligands on the edges occupy a separate site (the “inner ligands”) (Figure 5.1b).

Despite these many structural similarities inherent to the molecular structures of o-azido⁰, m-azido⁰, p-azido⁰ and p-azido¹⁻, there are some noteworthy differences. Jin and co workers reported the molecular structures of anionic \([\text{Au}_{25}(\text{SCH}_{2}\text{CH}_{2} \cdot \text{C}_{6}\text{H}_{5})_{18}]^{1⁻}\) and its neutral form \([\text{Au}_{25}(\text{SCH}_{2}\text{CH}_{2} \cdot \text{C}_{6}\text{H}_{5})_{18}]^{0}\). When the \(\sigma_h\) symmetry element of the cluster cores are viewed in the \(x\)-\(y\) plane, they observed a distortion in the molecular structure of the anionic form, where one of the external sulfur atoms of the staple motifs was bent upwards and another was bent downwards out of the \(x\)-\(y\) plane. However, in the molecular structure of the neutral form this distortion was essentially not present, and the sulfur atoms were essentially coplanar. As can be seen in Figure 5.1b, a similar correlation exists when comparing the molecular structures of p-azido¹⁻ and p-azido⁰, in which the sulphur atoms are twisted out of the \(x\)-\(y\) plane in the molecular structure of the former. This structural distortion is essentially absent in the molecular structure of p-azido⁰. As was hypothesized by Jin and co workers for their structures, this can likely be attributed to the negative charge of the p-azido¹⁻ core and/or could be a steric distortion imposed by the presence of the large tetraoctylammonium counterion. Although essentially absent in the molecular structure of p-azido⁰, this structural distortion does appear in the molecular structures of o-azido⁰ and m-azido⁰ and is most pronounced in the core structure of o-azido⁰ (Figure 5.1b). This increasing molecular distortion is likely due to the steric distortion created as the azido moiety is moved closer to the internal metal core.

The electrochemistry of o-azido¹⁻, m-azido¹⁻ and p-azido¹⁻ was also investigated by means of cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The cyclic voltammograms of o-azido¹⁻, m-azido¹⁻ and p-azido¹⁻ can be found in the Supporting Information, Section 4.0. In the DPV (Figure 5.2), under the same experimental conditions, the qualitative electrochemical oxidation pattern of the three isomeric clusters looks similar to that which we have previously reported for conventional phenylethanethiol-capped \([\text{Au}_{25}(\text{SR})_{18}]^{1⁻}\) (hereafter referred to as \(\text{Au}_{25}^{1⁻}\)):⁴⁰ two adjacent quasi-reversible oxidation waves corresponding to the azido¹⁻/azido⁰ and azido⁰/azido¹⁺ oxidation state transformations. There is also an irreversible oxidation peak in the further anodic scan at around 1.000 V vs SCE that corresponds to the azido¹⁺/azido²⁺ oxidation state change (the irreversibility can be attributed to the chemical instability of the +2 form).
reduction of azido$^{1-}$ to azido$^{2-}$ was not seen in the potential window. Interestingly, there is a correlation between the formal potentials of the azido$^{1-}$/azido$^{0}$ and azido$^{0}$/azido$^{1+}$ waves and the regioisomers. As shown in Figure 5.2, the formal potentials of the azido$^{1-}$/azido$^{0}$ oxidation state change are at 0.176 V for $p$-azido$^{1-}$, 0.138 V for $m$-azido$^{1-}$ and 0.084 V for $o$-azido$^{1-}$, while that for Au$^{25,1-}$/Au$^{25,0}$ was reported by us to be 0.038 V. Similarly, the formal potentials of the second oxidation reaction of the azido$^{0}$/azido$^{1+}$ were determined to be at 0.408 V for $p$-azido$^{1+}$, 0.378 V for $m$-azido$^{1+}$ and 0.316 V for $o$-azido$^{1+}$, whereas that for Au$^{25,0}$/Au$^{25,1+}$ is 0.280 V. These suggest that the energy required to remove electrons from the regioisomers and access to different oxidation states are dependent on the existence of the azido moieties and its positions.

It is plausible that the easiness of the two oxidation reactions depends on the dielectric constants (and polarity) caused by the regioisomeric nature. The dielectric constants for para-disubstituted benzene derivatives are typically highest and those for ortho derivatives are typically the lowest, with meta derivatives having intermediate polarity. The observed tendency agrees with that of the investigation on correlation between the surface ligand structure and electrochemical behaviour of [Au$^{25}$(SR)$_{18}$]$^{1-}$ systems by Liao et al. They reported higher formal potentials for the 1-/0 and 0/1+ oxidation state changes when more polar non-isomeric surface thiolate ligands were tethered to the AuNC surface, correlating well with previous theoretical results on the same systems. Specifically, they observed the lowest formal potentials when
hexanethiolate (S-C₆H₁₃) was the ligand, intermediate formal potentials for Au²₅⁺, and the highest formal potentials when thiophenolate (S-C₆H₅) was the ligand.⁴² Thus, the current study reinforces this correlation between the electrochemical profile of [Au₂₅(SR)₁₈]¹⁻ systems and the polarity of the surface ligands, with p-azido¹⁻ having the highest formal potentials and o-azido¹⁻ having the lowest. Moreover, the formal potentials of o-azido¹⁻ is higher than those of Au²₅⁺ due to the absence of the polar azido groups in the latter. On the other hand, the three regioisomers and conventional Au²₅⁺ all have a similar potential difference value of 0.230 to 0.240 V between the first and second oxidation reactions. This difference represents charging energy or electron addition energy,⁴⁰,⁴⁴ which should not depend on the surface ligand properties.

As with the initial study using p-azido¹⁻,³³ the CS-SPAAC chemistry of the three regioisomeric clusters was explored by reacting them with excess (Z)-cyclooct-1-ene-5-yne, which was expected to transform the p-azido¹⁻ platform to the surface-modified [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-C₆H₁₀N₃)₁₈] framework (hereafter referred to as p-triazole¹⁻ in current chapter (and referred to as 4.1-triazole in Chapter 4)), the m-azido¹⁻ platform to the surface-modified [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-m-C₆H₄-C₆H₁₀N₃)₁₈] framework (hereafter referred to as m-triazole¹⁻), and the o-azido¹⁻ platform to the surface-modified [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-o-C₆H₄-C₆H₁₀N₃)₁₈] framework (hereafter referred to as o-triazole¹⁻). Each reaction was carried out as a simple mix and stir with approximately 20 equivalents of strained alkyne; subsequent purification to remove the excess strained-alkyne was accomplished through simple trituration with isopropanol, in which the triazole-modified frameworks were insoluble (see Section 5.6.2.15 to Section 5.6.2.17 for experimental details).

In the linear negative mode matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrum of the purified m-triazole¹⁻ product, there is a large peak centered at 10047.0 Da (Figure S5.9), which is similar to the peak at 10048.4 Da found in the MALDI-TOF spectrum of p-triazole¹⁻ that was previously reported (Figure S4.9). These peaks can be assigned to the parent [Au₂₅(SCH₂CH₂-(m/p)-C₆H₄-C₆H₁₀N₃)₁₈]¹⁻ anion of both surface-modified frameworks in which all surface azido moieties have undergone the CS-SPAAC reaction (expected m/z = 10043.4 Da). However, there was no peak observed in this same m/z range in the linear negative mode MALDI-TOF spectrum of o-triazole¹⁻ (Figure S5.11). Under the same experimental conditions, the MALDI-TOF spectrum of o-triazole¹⁻ showed the formation of a
wide range of products of different sizes, none of which corresponded to the expected surface-modified framework. This suggests that although o-azido\(^{-1}\) can be successfully synthesized and characterized, once the small azido moiety is modified through the CS-SPAAC reaction, the proximity of the larger triazole-moiety to the Au\(_{25}\) core results in a dismantling of the nanocluster structure.

The instability of o-azido\(^{-1}\) towards CS-SPAAC was also confirmed by \(^1\)H NMR spectroscopy, where the \(^1\)H NMR spectrum of o-triazole\(^{-1}\) (Figure S5.10) displays a general broadening of the signals compared to the \(^1\)H NMR spectrum of o-azido\(^{-1}\) (Figure S5.3). In the \(^1\)H NMR spectra of p-triazole\(^{-1}\) (Figure S4.7) and m-triazole\(^{-1}\) (Figure S5.8) there were sharp and resolvable peaks, as is generally expected in the \(^1\)H NMR spectra of Au\(_{25}\)(SR)\(_{18}\) nanoclusters. Unlike in the \(^1\)H NMR spectra of the p-triazole\(^{-1}\) and m-triazole\(^{-1}\) frameworks, there were many broad peaks in the \(^1\)H NMR spectrum of o-triazole\(^{-1}\) that could not be easily assigned to expected proton environments, further confirming that the ligand protons were no longer in expected surface environments.

The CS-SPAAC reaction of the three regioisomeric forms of the azide-modified platforms was also investigated by UV-Vis and IR spectroscopy. In the IR spectrum of p-azido\(^{-1}\), m-azido\(^{-1}\) and o-azido\(^{-1}\) (Figure 5.3a), there is a large peak observed between 2100-2117 cm\(^{-1}\) that can be assigned to the N-N stretches of the surface azido moieties. After the CS-SPAAC reaction with excess (Z)-cyclooct-1-ene-5-yn, the IR spectrum of the three triazole-modified products (Figure 5.3b) showed a total disappearance of this large peak, demonstrating that in all three cases, all the azido moieties were being consumed upon addition of the strained-alkyne, albeit that the o-azido\(^{-1}\) platform appears to undergo dismantling after the cluster-surface reaction.

The UV-Vis absorption spectra of o-azido\(^{-1}\) and m-azido\(^{-1}\) show the same absorption fingerprint as p-azido\(^{-1}\) (Figure 5.3c), with absorption peaks at ~680 nm, ~440 nm and ~400 nm, and correlates with the well-established absorption pattern of [Au\(_{25}\)(SR)\(_{18}\)]\(^{-1}\) nanoclusters, including Au\(_{28}\).\(^{45}\) In particular, the absorption at ~680 nm results from an intraband metallic transition that is explicitly associated with the anionic Au\(_{25}\) core configuration, and occurs at different absorption energies when the internal core configuration or surface ligand is significantly
In this way, this absorption peak is the main indicator in the electronic absorption pattern that the anionic azide-modified \([\text{Au}_{25}(\text{SR})_{18}]^{-1}\) core configuration has been attained. An absorption pattern similar to the parent azide-modified clusters can be seen for the \(p\)-triazole\(^{1-}\) and \(m\)-triazole\(^{1-}\) frameworks. Specifically, there is a retention of the peak at ~680 nm, indicating that the anionic Au\(_{25}\) core configuration has been retained after the CS-SPAAC reaction (Figure 5.3d). However, in the absorption spectrum of the expected \(o\)-triazole\(^{1-}\) framework (Figure 5.3d, dotted red), there is a deterioration of the pattern of electronic transitions that diverges from the expected pattern for \([\text{Au}_{25}(\text{SR})_{18}]^{-1}\) nanoclusters, with a specific disappearance of the peak at ~680 nm. In fact, the lack of distinct electronic transitions, which is a key absorption feature for ultrasmall gold nanoclusters, is consistent with the absorption behavior of larger.

Figure 5.3. (a) ATR-IR spectra of \(p\)-azido\(^{1-}\) (black), \(m\)-azido\(^{1-}\) (blue) and \(o\)-azido\(^{1-}\) (red). (b) ATR-IR spectra of \(p\)-triazole\(^{1-}\) (dotted black), \(m\)-triazole\(^{1-}\) (dotted blue) and \(o\)-triazole\(^{1-}\) (dotted red). (c) UV-Vis absorption spectra of 0.2 mM solutions of \(p\)-azido\(^{1-}\) (black), \(m\)-azido\(^{1-}\) (blue) and \(o\)-azido\(^{1-}\) (red) in dichloromethane at 23°C. (d) UV-Vis absorption spectra of 0.2 mM solutions of \(p\)-triazole\(^{1-}\) (dotted black), \(m\)-triazole\(^{1-}\) (dotted blue) and \(o\)-triazole\(^{1-}\) (dotted red) in dichloromethane at 23°C.
nanoparticles. In this way, although IR spectroscopy indicates that the surface azido moieties in o-azido are expectedly available for the CS-SPAAC reaction, UV spectroscopy provides evidence that upon total consumption the azido groups through CS-SPAAC, there is a deterioration of the [Au25(SR)18]1− structure, and leads to aggregation to form larger, polydisperse particles. The ability to synthesize [Au25(SR)18]1− nanoclusters with o-azidophenylethanethiol as a surface ligand, but modification of the azido moieties to triazole moieties leading to nanocluster deterioration, highlights the importance not only on the correlation between core nuclearity and surface ligand employed, but also the large dependence on surface ligand and nanocluster stability.

The second order rate constants (k2) with the aliphatic strained alkyne, exo-bicyclo-[6.1.0]non-4-yn-9-ol (BCNexo-OH) were estimated for the SPAAC reactivity of three thiol-based ligands, as well as the CS-SPAAC reactivity of p-azido1− and m-azido1−, using 1H NMR spectroscopy (see Section 5.6.5 for experimental details and graphs). BCNexo-OH was chosen for the kinetic study instead of (Z)-cyclooct-1-ene-5-yne because the presence of the alkene in (Z)-cyclooct-1-ene-5-yne makes the alkyne moiety more strained, and consequently more reactive, and so both the SPAAC and CS-SPAAC reactions were too fast to measure for some analytes using 1H NMR spectroscopy. It should be noted that although the exo-isomer was used for this study because it is synthesized in higher yield, the endo-isomer could have also been used and should have similar reaction kinetics.

Dommerholt and co-workers previously reported that the SPAAC reaction kinetics of azido-benzene derivatives with BCNexo-OH is dependent on the presence of either electron withdrawing or electron donating groups on the phenyl ring. Delocalization into electron withdrawing substituents accelerate the rate of reaction and electron donating substituents decelerate the rate of reaction. We herein report an additional structural feature that affects the SPAAC reaction kinetics. Specifically, the isomeric position of the substituent (for example, the mildly electron-donating ethanethiol substituent) also has a profound impact on the observed reaction kinetics. Specifically, p-azidophenylenethanethiol had the fastest reaction kinetics (k2 = 0.157 M−1.s−1), m-azidophenylenethanethiol had an intermediate reaction rate (k2 = 0.087 M−1.s−1) and o-azidophenylenethanethiol had an extremely slow reaction rate (k2 = 0.00430 M−1.s−1) (Scheme 5.1). As the electronic composition of these three structures are identical, this observed correlation in SPAAC reaction kinetics is likely due to a steric effect, rather than the electronic effect that has
been previously reported. That is, the azido moiety is most sterically (and kinetically) accessible in the \textit{para}-substituted structure, and far less accessible for SPAAC chemistry in the \textit{ortho}-substituted structure, which may have contributed to the incompatibility of the \textit{o-azido}\textsubscript{1} platform towards CS-SPAAC chemistry.

As shown in Scheme 5.1, the CS-SPAAC reaction kinetics of \textit{p-azido}\textsubscript{1} ($k_2 = 0.162 \text{ M}^{-1}\text{s}^{-1}$) was similar to the SPAAC reaction kinetics of \textit{p-azidophenylethanethiol} ($k_2 = 0.157 \text{ M}^{-1}\text{s}^{-1}$), indicating that the azido groups in \textit{p-azido}\textsubscript{1} are equally accessible as in the free thiol-based ligand. However, the CS-SPAAC reaction kinetics of \textit{m-azido}\textsubscript{1} ($k_2 = 0.0400 \text{ M}^{-1}\text{s}^{-1}$) was approximately 2 times slower than the SPAAC reaction kinetics of \textit{m-azidophenylethanal} ($k_2 = 0.0870 \text{ M}^{-1}\text{s}^{-1}$). This provides evidence that the azido groups are kinetically less reactive in \textit{m-azido}\textsubscript{1}, compared to the free thiol-based ligand, which is likely due to a more pronounced steric inaccessibility of the azido moieties when the meta-substituted thiolate ligand is tethered to the AuNC core.

5.3 Conclusions

The ability to develop functional variants of AuNC systems is of paramount importance for application-based research using these promising nanomaterial frameworks. We have developed three regioisomeric forms of azide-modified [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{1} nanocluster platforms, in which the azide moiety was appended to the para, meta and ortho phenylic positions in the phenylethanethiolate ligands, respectively, that surrounds the internal Au\textsubscript{25} core configuration, to give \textit{p-azido}\textsubscript{1}, \textit{m-azido}\textsubscript{1} and \textit{o-azido}\textsubscript{1}. The three regioisomeric clusters demonstrated near identical optical properties, exhibited subtle but correlated differences in their electrochemical behaviour, and drastic differences in their ability to undergo the CS-SPAAC reaction with excess strained alkyne. In fact, although the surface azides in the \textit{o-azido}\textsubscript{1} platform were reactive towards the surface cycloaddition reaction, analysis of the resulting product indicates that the nanocluster structure underwent deterioration during the post-assembly surface modification. The results of this study provides valuable information into the sensitive structure-ligand relationship that exists for gold nanocluster systems and extends our current knowledge on the effect of ligand
isomerization on the properties of $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ nanocluster systems and their resulting properties and reactivity.

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5.5 References


5.6 Supporting Information

5.6.1 General Materials and Methods

**Reagents and Solvents.** The following materials were used as received. 2-nitrophenethyl bromide (97%), 3-nitrophenethyl bromide (97%), potassium thioacetate (98%), zinc dust (<10\(\mu\)m, \(\geq 98\%\)), sodium azide (\(\geq 99.5\%\)), gold (III) chloride trihydrate (\(\geq 99.9\%\) trace metal basis), tetraoctyrammonium bromide (98%), sodium borohydride (\(\geq 98\%\)), 1,5-cyclooctadiene (\(\geq 99\%\)), bromine (reagent grade), potassium tert-butoxide solution (1.0M in THF), dichloromethane-d\(_2\) (CD\(_2\)Cl\(_2\), 99.5 atom %D) and dimethyl sulfoxide-d\(_6\) ((CD\(_3\))\(_2\)SO, 99.96 atom %D) were purchased from Sigma-Aldrich (Millipore Sigma). 4-nitrophenylethyl bromide was purchased from Oakwood Chemicals. Sodium chloride, sodium hydroxide pellets, methanol, tetrahydrofuran, toluene, and acetonitrile were purchased from Fischer Scientific. Technical grade ammonium chloride, magnesium sulphate, hexanes, dichloromethane, ethyl acetate, 12M hydrochloric acid, sodium nitrite, isopropanol, and pentane were purchased from Caledon. Chloroform-D\(_1\) (CDCl\(_3\),...
99.8 atom %D) was purchased from Cambridge Isotope Laboratories. Ethanol (anhydrous) was purchased from Commercial Alcohols.

Unless otherwise stated, all reactions were performed at ambient conditions.

**NMR Spectroscopy.** $^1$H and $^{13}$C{$^1$H} spectra were recorded on either a Bruker AvIII HD 400 spectrometer or Varian INOVA 600 spectrometer, as indicated. $^1$H NMR spectra are reported as δ in units of parts per million (ppm), and referenced against residual protio chloroform (7.27 ppm, s) or dichloromethane (5.32 ppm, t), as indicated. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintuplet), m (multiplet) and br (broad signal). Coupling constants are reported as a $J$ value in Hertz (Hz) according to the spectrometer frequency. The number of protons ($n$) for a given resonance is indicated as $n$H, and is based on spectral integration values. $^{13}$C{$^1$H} NMR spectra are reported as δ in units of parts per million (ppm) and referenced against the indicated deuterated solvent: chloroform-D$_1$ (77.0 ppm, t), dimethylsulfoxide-D$_6$ (39.5 ppm, septet) or dichloromethane-D$_2$ (54.0 ppm, quin), as indicated.

**Mass Spectrometry.** Electrospray ionization (ESI) mass spectra were obtained in either positive-ion or negative-ion mode using a Bruker microTOF II spectrometer. Set capillary was 4000 V, set end plate offset was -400 V, set nebulizer was 1.0 Bar and set dry heater was 100°C. To obtain the ESI spectrum of nanoclusters (obtained in negative-ion mode), a sample was dissolved in a 1:5 toluene:methanol (10mg/mL). We generally found that in order to obtain mass spectra of [Au$_{25}$SR$_{18}$] clusters, the sample solution must contain some methanol in order to minimize excessive fragmentation. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were obtained using an AB Sciex 5800 TOF/TOF system. To obtain the MALDI-TOF spectrum of nanoclusters (obtained in linear negative mode), a 1 g/L sample solution was mixed with a 10 g/L solution of trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in a ratio of 1:400 by mass. Data acquisition and data processing were respectively done using a TOF TOF Series Explore and Data Explorer (both from AB Sciex). The laser pulse rate was set to 400Hz. The mass spectrum was collected as a sum of 1000 shots.

**UV-Visible (UV-Vis) Spectroscopy.** UV-Vis absorption spectra were recorded using a Cary 5000 scan instrument using standard quartz cells (1 cm path length) with a scan range of 200-1000nm.
Samples were dissolved in the indicated solvents at the indicated concentrations. The background spectrum of the indicated solvent was subtracted internally by the software.

**Infrared (IR) spectroscopy.** Attenuated total reflectance IR (ATR-IR) spectra were recorded using a PerkinElmer Spectrum Two FT-IR spectrometer.

**Electrochemistry.** All cyclic voltammograms and differential pulse voltammograms were performed on a CHI 610A electrochemical analyzer (CH Instruments, Austin, Texas). A three-electrode system was used: the working electrode was a 2 mm diameter platinum disc inlaid in a glass (Pt) electrode; two Pt coils served as the counter and reference electrodes, respectively. Prior to each experiment, the glass electrochemical cell was cleaned in an acid bath overnight, then placed in a base bath to prevent any contamination. The platinum disc electrode was polished using 0.3 micron then 0.05 micron alumina polishing slurry (CH Instruments) until a mirror like finish was obtained. The electrode was then subjected to electrochemical cleaning by performing cyclic voltammetry in 0.1 M dilute sulfuric acid solution for 200 cycles between the cathodic and anodic potential limits. The electrode was then rinsed with ultra-pure Type-1 water and left to fully dry before use. Each electrochemical cell consisted of a 0.67mg/mL solution of gold clusters dispersed in a 1:1 anhydrous acetonitrile:benzene mixture (Sure/Seal™ acetonitrile and benzene, Sigma Aldrich), with 0.1M tetrabutylammonium perchlorate (TBAP, Sigma Aldrich) being added as the supporting electrolyte. All electrochemical cells were assembled in an inert Ar atmosphere.

### 5.6.2 Experimental Procedures

#### 5.6.2.1 Synthesis of \( p \)-nitro-phenylethanethioacetate

![Chemical structure of p-nitro-phenylethanethioacetate](image)

See Section 4.6.2.1 for detailed synthesis of \textbf{p-azido-phenylethanethioacetate}.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 8.17 (d, 2H, \( J = 8 \) Hz), 7.39 (d, 2H, \( J = 8 \) Hz), 3.15 (t, 2H, \( J = 8 \) Hz), 2.99 (t, 2H, \( J = 8 \) Hz), 2.35 (s, 3H). \(^{13}\)C NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 195.2, 147.4, 146.8, 129.5, 123.7, 35.6, 30.6, 29.7. HRMS (ESI) m/z calc. for \( C_{10}H_{11}NO_3S \) (M\(^+\)): 225.0460, found: 225.0467. IR (ATR-IR, cm\(^{-1}\)): 3025, 1680, 1597, 1513, 1448, 1411, 1340, 1280, 1129, 1096.
5.6.2.2 Synthesis of \textit{p}-azido-phenylethanethioacetate

To 13.7 g (60 mmol, 1 eq.) \textit{p}-nitro-phenylethanethioacetate in 120 mL ethanol and 120 mL distilled water was added 25.7 g (480 mmol, 8 eq) technical grade ammonium chloride, and then 24 g (360 mmol, 6 eq) zinc dust portion-wise under vigorous stirring over 5 minutes. The resulting suspension was stirred for 2 hours at room temperature, after which the solids were filtered off and washed with 50 mL of ethanol. To the supernatant was added 55 mL 12 M hydrochloric acid in 220 mL distillated water at 0°C, giving a colorless solution. To this solution was added 6.2 g (89 mmol, 1.5 eq) sodium nitrite in 130 mL distilled water dropwise over 20 minutes at 0°C under rapid stirring, resulting in a reddish solution. After stirring the solution for an additional 20 minutes at 0°C, 7.8 g (120 mmol, 2 eq) sodium azide in 130 mL distilled water was added dropwise over 15 minutes at 0°C. The solution was stirred for 20 minutes at 0°C, and then an additional 30 minutes at room temperature, after which the crude solution was extracted with dichloromethane (3 x 50 mL), dried over magnesium sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give \textit{p}-azido-phenylethanethioacetate as a pale yellow oil in 70% yield (9.2 g). $^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 7.21 (d, 2H, $J = 8$ Hz), 6.97 (d, 2H, $J = 8$ Hz), 3.10 (t, 2H, $J = 8$ Hz), 2.85 (t, 2H, $J = 8$ Hz), 2.34 (s, 3H). $^{13}$C NMR (CDCl$_3$, 400 MHz): δ (ppm) 195.6, 138.3, 136.7, 130.0, 119.1, 35.1, 30.7, 30.4. HRMS (ESI) m/z calc. for C$_{10}$H$_{14}$NOS$^+$ (M$^+$): 221.0623, found: 221.0630. IR (ATR-IR, cm$^{-1}$): 3026, 2106, 1690, 1602, 1506, 1418, 1356, 1285, 1128, 1102.

*Spectral data matches our previously reported data¹.

5.6.2.3 Synthesis of \textit{p}-azido-phenylethanethiol

See Section 4.6.2.4 for detailed synthesis of \textit{p}-azido-phenylethanethiol.

$^1$H NMR (CD$_2$Cl$_2$, 400 MHz): δ (ppm) 7.20 (d, 2H, $J = 8$ Hz), 6.98 (d, 2H, $J = 8$ Hz), 2.89 (t, 2H, $J = 8$ Hz), 2.75 (q, 2H, $J = 8$ Hz), 1.40 (t, 2H, $J = 8$ Hz). $^{13}$C NMR (CD$_2$Cl$_2$, 400 MHz): δ (ppm) 138.8, 137.4, 130.6, 119.5, 40.0, 26.5. HRMS (ESI) m/z calc. for C$_{10}$H$_{14}$NOS$^+$ (M$^+$): 179.0517, found: 179.0514. IR (ATR-IR, cm$^{-1}$): 3035, 2936, 2559, 2106, 1601, 1504, 1430, 1283, 1131.
*Spectral data matches our previously reported data\(^1\).

### 5.6.2.4 Synthesis of \(m\)-nitro-phenylethanethioacetate

![m-nitro-phenylethanethioacetate](image)

To 10.0 g (44 mmol, 1 eq.) 3-nitrophenylethyl bromide in 300 mL acetone was added 6.0 g (53 mmol, 1.2 eq.) potassium thioacetate. The resulting mixture was stirred at room temperature for 6 hours, after which the solid was removed by gravity filtration and the solution was concentrated by rotary evaporation. The resultant crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give \(m\)-nitro-phenylethanethioacetate as a yellow solid in 96% yield (9.6 g). \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) (ppm) 8.11 (m, 2H), 7.58 (m, 1H), 7.50 (m, 1H), 3.17 (t, 2H, \(J = 8\) Hz), 3.00 (t, 2H, \(J = 8\) Hz), 2.35 (s, 3H). \(^13\)C NMR (CDCl\(_3\), 400 MHz): \(\delta\) (ppm) 195.2, 143.8, 141.8, 134.9, 129.4, 123.5, 121.8, 35.4, 30.7, 29.9. HRMS (ESI) \(m/z\) calc. for C\(_{10}\)H\(_{11}\)NO\(_3\)S (M\(^+\)): 225.0460, found: 225.0489. IR (ATR-IR, cm\(^{-1}\)): 2930, 2861, 1681, 1522, 1349, 1312, 1130, 1099, 1078.

### 5.6.2.5 Synthesis of \(m\)-azido-phenylethanethioacetate

![m-azido-phenylethanethioacetate](image)

To 7.9 g (35 mmol, 1 eq.) \(m\)-nitro-phenylethanethioacetate in 70 mL ethanol and 70 mL distilled water was added 15.0 g (280 mmol, 8 eq) technical grade ammonium chloride, and then 13.8 g (210 mmol, 6 eq) zinc dust portion-wise under vigorous stirring over 5 minutes. The resulting suspension was stirred for 2 hours at room temperature, after which the solids were filtered off and washed with 30 mL of ethanol. To the supernatant was added 32 mL 12 M hydrochloric acid in 140 mL distilled water at 0°C, giving a colorless solution. To this solution was added 3.7 g (53 mmol, 1.5 eq) sodium nitrite in 75 mL distilled water dropwise over 20 minutes at 0°C under rapid stirring, resulting in a reddish solution. After stirring the solution for an additional 20 minutes at 0°C, 4.6 g (70 mmol, 2 eq) sodium azide in 75 mL distilled water was added dropwise over 15 minutes at 0°C. The solution was stirred for 20 minutes at 0°C, and then an additional 30 minutes at room temperature, after which the crude solution was extracted with dichloromethane (3 x 50 mL), dried over magnesium sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give \(m\)-azido-phenylethanethioacetate as a pale yellow oil in 75% yield (5.8 g). \(^1\)H NMR (CDCl\(_3\), 400 MHz):
δ (ppm) 7.29 (t, 1H, J = 8 Hz), 7.01 (d, 1H, J = 8 Hz), 6.90 (m, 2H), 3.12 (t, 2H, J = 8 Hz), 2.87 (t, 2H, J = 8 Hz), 2.35 (s, 3H). 13C NMR (CDCl3, 400 MHz): δ (ppm) 195.2, 141.6, 139.8, 129.5, 124.9, 118.9, 116.9, 35.3, 30.3, 29.9. HRMS (ESI) m/z calc. for C10H14NOS+ (M+): 221.0623, found: 221.0652.

IR (ATR-IR, cm⁻¹): 2927, 2858, 2405, 2102, 1686, 1586, 1487, 1445, 1288, 1132.

5.6.2.6 Synthesis of m-azido-phenylethanethiol

To 5.8 g (26 mmol, 1 eq.) m-azido-phenylethanethioacetate in 250 mL deoxygenated methanol was added 40 mL (40 mmol, 1.5 eq.) of deoxygenated 1 M sodium hydroxide in water and the solution was stirred for 20 minutes at room temperature under argon, resulting in a yellow solution. Next, 80 mL (80 mmol, 3 eq.) of deoxygenated 1 M hydrochloric acid in water was added. The solution was stirred for 15 minutes at room temperature under argon, resulting in a pale yellow solution, after which distilled water (150 mL) was added and the product was extracted with dichloromethane (3 x 50 mL), dried over magnesium sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give m-azido-phenylethanethiol as a pale yellow oil in 92% yield (4.7 g). 1H NMR (CD2Cl2, 400 MHz): δ (ppm) 7.30 (t, 1H, J = 8 Hz), 6.98 (d, 1H, J = 8 Hz), 6.90 (m, 2H), 2.91 (t, 2H, J = 8 Hz), 2.78 (q, 2H, J = 8 Hz), 1.40 (t, 2H, J = 8 Hz). 13C NMR (CD2Cl2, 400 MHz): δ (ppm) 142.7, 140.7, 130.3, 125.9, 119.8, 117.6, 40.5, 26.3. HRMS (ESI) m/z calc. for C10H14NOS+ (M+): 179.0517, found: 179.0532. IR (ATR-IR, cm⁻¹): 2931, 2849, 2105, 1586, 1487, 1445, 1288, 1132.

5.6.2.7 Synthesis of o-nitro-phenylethanethioacetate

To 9.6 g (44 mmol, 1 eq.) 2-nitrophenylethyl bromide in 280 mL acetone was added 5.7 g (50 mmol, 1.2 eq.) potassium thioacetate. The resulting mixture was stirred at room temperature for 6 hours, after which the solid was removed by gravity filtration and the solution was concentrated by rotary evaporation. The resultant crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give o-nitro-phenylethanethioacetate as a yellow solid in 99% yield (9.5 g). 1H NMR (CDCl3, 400 MHz): δ (ppm) 7.96 (dd, 1H, J1 = 8 Hz, J2 = 1 Hz), 7.57 (dt, 1H, dd, 1H, J1 = 8 Hz, J2 = 1 Hz),
7.43 (m, 2H), 3.19 (m, 4H), 2.34 (s, 3H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 195.5, 149.2, 134.8, 133.1, 132.6, 127.8, 124.9, 33.3, 30.6, 29.4. HRMS (ESI) $m/z$ calc. for C$_{10}$H$_{11}$NO$_3$S (M$^+$): 225.0460, found: 225.0463. IR (ATR-IR, cm$^{-1}$): 3062, 2930, 2862, 1686, 1522, 1345, 1129.

5.6.2.8 Synthesis of o-azido-phenylethanethioacetate

To 5.1 g (23 mmol, 1eq.) o-nitro-phenylethanethioacetate in 45 mL ethanol and 45 mL distilled water was added 9.8 g (184 mmol, 8 eq) technical grade ammonium chloride, and then 9.1 g (138 mmol, 6 eq) zinc dust portion-wise under vigorous stirring over 5 minutes. The resulting suspension was stirred for 2 hours at room temperature, after which the solids were filtered off and washed with 50 mL of ethanol. To the supernatant was added 20 mL 12 M hydrochloric acid in 80 mL distilled water at 0°C, giving a colorless solution. To this solution was added 2.4 g (35 mmol, 1.5 eq) sodium nitrite in 50 mL distilled water dropwise over 20 minutes at 0°C under rapid stirring, resulting in a reddish solution. After stirring the solution for an additional 20 minutes at 0°C, 3.0 g (46 mmol, 2 eq) sodium azide in 50 mL distilled water was added dropwise over 15 minutes at 0°C. The solution was stirred for 20 minutes at 0°C, and then an additional 30 minutes at room temperature, after which the crude solution was extracted with dichloromethane (3 x 50 mL), dried over magnesuim sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give o-azido-phenylethanethioacetate as a pale yellow oil in 43% yield (2.2 g). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 7.29 (dt, 1H, $J_1$ = 8 Hz, $J_2$ = 1 Hz), 7.21 (dd, 1H, $J_1$ = 8 Hz, $J_2$ = 1 Hz), 7.15 (dd, 1H, $J_1$ = 8 Hz, $J_2$ = 1 Hz), 7.09 (dt, 1H, $J_1$ = 8 Hz, $J_2$ = 1 Hz), 3.12 (t, 2H, $J$ = 8 Hz), 2.85 (t, 2H, $J$ = 8 Hz), 2.34 (s, 3H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 195.7, 138.2, 131.1, 130.9, 128.1, 124.7, 118.1, 31.2, 30.6, 29.2. HRMS (ESI) $m/z$ calc. for C$_{10}$H$_{14}$NOS$^+$ (M$^+$): 221.0623, found: 221.0648. IR (ATR-IR, cm$^{-1}$): 3025, 2928, 2118, 1688, 1581, 1488, 1283, 1132.

5.6.2.9 Synthesis of o-azido-phenylethanethiol

To 2.2 g (10 mmol, 1 eq.) o-nitro-phenylethanethioacetate in 160 mL deoxygenated methanol was added 15 mL (15 mmol, 1.5 eq.) of deoxygenated 1 M sodium hydroxide in water and the solution was stirred for 20 minutes at room temperature under argon,
resulting in a yellow solution. Next, 30 mL (30 mmol, 3 eq.) of deoxygenated 1 M hydrochloric acid in water was added. The solution was stirred for 15 minutes at room temperature under argon, resulting in a pale yellow solution, after which distilled water (100 mL) was added and the product was extracted with dichloromethane (3 x 25 mL), dried over magnesium sulphate and concentrated using rotary evaporation. The crude residue was purified by flash column chromatography (2:1 hexanes:dichloromethane) to give o-azido-phenylethanethiol as a pale yellow oil in 90% yield (1.6 g). $^1$H NMR (CD$_2$Cl$_2$, 400 MHz): $\delta$ (ppm) 7.28 (dt, 1H, $J_1 = 8$ Hz, $J_2 = 1$ Hz), 6.98 (m, 2H), 7.08 (dt, 1H, $J_1 = 8$ Hz, $J_2 = 1$ Hz), 2.86 (t, 2H, $J = 8$ Hz), 2.73 (q, 2H, $J = 8$ Hz), 1.40 (t, 2H, $J = 8$ Hz). $^{13}$C NMR (CD$_2$Cl$_2$, 400 MHz): $\delta$ (ppm) 138.8, 131.9, 131.5, 128.6, 125.2, 118.8, 36.5, 25.2. HRMS (ESI) m/z calc. for C$_{10}$H$_{14}$NOS$^+$ (M$^+$): 179.0517, found: 179.0539. IR (ATR-IR, cm$^{-1}$): 2980, 2930, 2116, 1580, 1487, 1450, 1282, 1155.

5.6.2.10 Synthesis of (Z)-cyclooct-1-ene-5-yne

See Section 3.6.3.3 for detailed synthesis of (Z)-cyclooct-1-ene-5-yne.

$^1$H NMR (CD$_2$Cl$_2$, 400 MHz): $\delta$ (ppm) 5.85 (m, 2H), 2.56 (m, 4H), 2.14 (m, 2H). $^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 131.9, 100.8, 31.2, 19.5. HRMS (ESI) m/z calc. for C$_8$H$_{10}$(M$^+$): 106.0783, found: 106.0782. IR (ATR-IR, cm$^{-1}$): 3008, 2924, 2850, 1635, 1450, 1315, 1207.

*Spectral data matches our previously reported data$^1$.

5.6.2.11 Synthesis of BCN$_{exo}$-OH

*Synthesized according to Dommerholt et al.$^2$ See Section 2.6.2.6 for characterization data.
5.6.2.12 Synthesis of [(CH$_3$-(CH$_2$)$_7$N)[Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$)$_{18}$] ($p$-azido$^1$)

See Section 4.6.2.8 for detailed synthesis of [(CH$_3$-(CH$_2$)$_7$N)[Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$)$_{18}$] ($p$-azido$^1$). The yield of was 26% atomic Au basis.

$^1$H NMR (CD$_2$Cl$_2$, 600 MHz): $\delta$ (ppm) 7.17 (d, 24H, $J = 6$ Hz), 7.12 (d, 12H, $J = 6$ Hz), 6.86 (d, 12H, $J = 6$ Hz), 6.80 (d, 24H, $J = 6$ Hz), 3.54 (t, 24H, $J = 6$ Hz), 3.09 (m, 44H), 2.93 (t, 12H, $J = 6$ Hz), 1.62 (m, 8H), 1.37-1.31 (m, 48H), 0.90 (t, 12H, $J = 6$ Hz). HRMS (ESI [negative]) $m/z$ calc. for C$_{144}$H$_{144}$Au$_{25}$N$_5$S$_4$ (M$^-$): 8132.4 Da, found: 8132.9 Da. IR (ATR-IR, cm$^{-1}$): 2904, 2845, 2411, 2254, 2098, 1604, 1581, 1504. UV-Vis (Dichloromethane, 2x10$^{-4}$ mol L$^{-1}$): $\lambda_{\text{max}}$ = 682 nm, $\varepsilon$ = 1908 M$^{-1}$cm$^{-1}$, $\lambda_{\text{max}}$ = 443 nm, $\varepsilon$ = 6600 M$^{-1}$cm$^{-1}$, $\lambda_{\text{max}}$ = 404 nm, $\varepsilon$ = 7650 M$^{-1}$cm$^{-1}$.

*Spectral data matches our previously reported data$^1$.

5.6.2.13 Synthesis of [(CH$_3$-(CH$_2$)$_7$N)[Au$_{25}$(SCH$_2$CH$_2$-$m$-C$_6$H$_4$-N$_3$)$_{18}$] ($m$-azido$^1$)

To a yellow solution of 230 mg (0.58 mmol) tetrachloroauroic(III) acid trihydrate in 24 mL tetrahydrofuran was added 350 mg (1.1 eq, 0.64 mmol) tetraoctylammonium bromide at a stirring speed of 600 rpm. The solution was stirred for 15 minutes at room temperature and 30 minutes at 0$^\circ$C, resulting in a dark red solution. The stirring was reducing to 80 rpm, and to the red solution was added an ice-cold solution of 630 mg (6 eq. 3.5 mmol) of $m$-azido-phenylethanethiol in 1.2 mL tetrahydrofuran over 4 minutes at 0$^\circ$C at 80 rpm. The resulting solution was stirred at 0$^\circ$C for 1 hour at 80 rpm, then at room temperature for 3 hours at 80 rpm, then at room temperature for 1 hour at 600 rpm. Over the stirring period, the solution turned from dark red to yellow within the first hour, and once the temperature was increased to room temperature and stirred for four hours, it turned a very pale-yellow color. After the stirring period, the pale-yellow solution was cooled to 0$^\circ$C for 15 minutes.
at 600 rpm, after which an ice-cold, freshly prepared solution of 220 mg (10 eq, 5.8 mmol) sodium borohydride in 4.5 mL milli-Q water was added over approximately 15-20 seconds at 600 rpm, resulting in bubbling and an immediate color change from pale yellow to black. The neck of the flask was covered with a cap, and a light stream of argon was passed over the top of the flask, and the solution was stirred for 18 hours at room temperature at 600 rpm. After the overnight reaction, there was white precipitate on the side of the flask, and the solution turned from black to a reddish-brown color. The crude solution was filtered through glass wool, and the tetrahydrofuran was removed by rotary evaporation. The crude residue was dissolved in 50 mL toluene and extracted with 200 mL milli-Q water. The organic phase was removed, and the toluene was removed by rotary evaporation. To the crude brownish-red oil was added approximately 20 mL isopropanol, resulting in the formation of small insoluble black solids. The solids were collected by filtering the suspension through glass wool in a funnel. NOTE: the solution may have to be filtered more than once to collect all black solids. The glass wool was washed thoroughly with isopropanol to fully remove residual thiols and disulfides. After the isopropanol washes, 10 mL acetonitrile was added, resulting in dissolution of most of the black solids to give reddish brown filtrate. The acetonitrile was removed by rotary evaporation, resulting in a film on the flask wall. The film was washed again with isopropanol, and then re-dissolved in acetonitrile and filtered once more through glass wool. Removal of the acetonitrile by rotary evaporation gave a reddish-black oily film of purified [(CH₃(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-m-C₆H₄-N₃) (m-azido₁⁺) that was stored as a film at 0°C. The yield of m-azido₁⁺ was 38% atomic Au basis. ¹H NMR (CD₂Cl₂, 600 MHz): δ (ppm) 7.17 (t, 6H, J = 12 Hz), 7.10 (t, 12H, J = 12 Hz), 6.96 (m, 30H), 6.83 (m, 12H), 6.74 (m, 12H), 3.56 (m, 24H), 3.12 (m, 44H), 2.95 (t, 12H, J = 12 Hz), 1.62 (m, 8H), 1.33 (m, 48H), 0.89 (t, 12H, J = 6 Hz). HRMS (ESI [negative]) m/z calc. for C₁₄₄H₁₄₄Au₂₅N₅₄S (M⁻): 8132.4 Da, found: 8131.9 Da. IR (ATR-IR, cm⁻¹): 2938, 2920, 2848, 2390, 2107, 1601, 1583. UV-Vis (Dichloromethane, 2x10⁻⁴ mol L⁻¹): λₘₐₓ = 682 nm, ε = 1940 M⁻¹cm⁻¹, λₘₐₓ = 444 nm, ε = 6620 M⁻¹cm⁻¹, λₘₐₓ = 406 nm, ε = 7660 M⁻¹cm⁻¹.
5.6.2.14 Synthesis of [(CH$_3$-(CH$_2$)$_7$)_4N][Au$_{25}$(SCH$_2$CH$_2$-o-C$_6$H$_4$-N$_3$)$_{18}$] (o-azido$^1$)

To a yellow solution of 244 mg (0.62 mmol) tetrachloroauric(III) acid trihydrate in 27 mL tetrahydrofuran was added 373 mg (1.1 eq, 0.68 mmol) tetraoctylammonium bromide at a stirring speed of 600 rpm. The solution was stirred for 15 minutes at room temperature and 30 minutes at 0°C, resulting in a dark red solution. The stirring was reducing to 80 rpm, and to the red solution was added an ice-cold solution of 660 mg (6 eq, 3.7 mmol) of o-azido-phenylethanol in 1.2 mL tetrahydrofuran over 4 minutes at 0°C at 80 rpm. The resulting solution was stirred at 0°C for 1 hour at 80 rpm, then at room temperature for 3 hours at 80 rpm, then at room temperature for 1 hour at 600 rpm. Over the stirring period, the solution turned from dark red to yellow within the first hour, and once the temperature was increased to room temperature and stirred for four hours, it turned a very pale-yellow color. After the stirring period, the pale-yellow solution was cooled to 0°C for 15 minutes at 600 rpm, after which an ice-cold, freshly prepared solution of 234 mg (10 eq, 6.2 mmol) sodium borohydride in 4.8 mL milli-Q water was added over approximately 15-20 seconds at 600 rpm, resulting in bubbling and an immediate color change from pale yellow to black. The remainder of the protocol was identical to that used to synthesize m-azido$^2$ (see Section 5.6.2.13). The yield of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-o-C$_6$H$_4$-N$_3$)$_{18}$] was 47% atomic Au basis.

$^1$H NMR (CD$_2$Cl$_2$, 600 MHz): δ (ppm) 7.35 (d, 12H, $J = 12$ Hz), 7.21 (m, 12H), 7.13 (m, 12H), 7.08 (d, 6H, $J = 12$ Hz), 6.96 (m, 12H), 6.91 (m, 18H), 3.57 (t, 24H, $J = 12$ Hz), 3.07 (m, 44H), 2.95 (t, 12H, $J = 12$Hz), 1.62 (m, 8H), 1.32 (m, 48H), 0.89 (t, 12H, $J = 12$ Hz). HRMS (ESI [negative]) $m/z$ calc. for C$_{144}$H$_{144}$Au$_{25}$N$_{54}$S (M$^-$): 8132.4 Da, found: 8132.1 Da. IR (ATR-IR, cm$^{-1}$): 2964, 2933, 2875, 2117, 1578. UV-Vis (Dichloromethane, 2x10$^{-4}$ mol L$^{-1}$): $\lambda_{\text{max}}$ = 681 nm, $\varepsilon$ = 1982 M$^{-1}$cm$^{-1}$, $\lambda_{\text{max}}$ = 442 nm, $\varepsilon$ = 6690 M$^{-1}$cm$^{-1}$, $\lambda_{\text{max}}$ = 405 nm, $\varepsilon$ = 7600 M$^{-1}$cm$^{-1}$.
5.6.2.15 Synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}]$ (p-triazole$^1$)

See Section 4.6.2.9 for detailed synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}]$ (p-triazole$^1$).

$^1$H NMR (CD$_2$Cl$_2$, 600 MHz): δ (ppm) 7.37 (d, 24H, J=12 Hz), 7.28 (d, 12H, J=6 Hz), 7.18 (m, 36H), 5.47 (m, 24H), 5.39 (m, 12H), 3.69 (t, 24H, J=6 Hz), 3.27-3.22 (m, 36H), 3.09 (m, 56H), 2.68-2.64 (m, 36H), 2.47-2.42 (m, 72H), 1.61 (m, 8H), 1.33-1.26 (m, 48H), 0.88 (t, 12H, J=6 Hz). MS (MALDI-TOF [negative]) m/z calc. for C$_{288}$H$_{324}$Au$_{25}$N$_{54}$S (M$^-$): 10043.4 Da, found: 10048.4 Da. IR (ATR-IR, cm$^{-1}$): 2920, 2852, 1608, 1563, 1517. UV-Vis (Dichloromethane, 2 x 10$^{-4}$ mol L$^{-1}$): $\lambda_{max}$ = 682 nm, $\varepsilon$ = 2040 M$^{-1}$ cm$^{-1}$, $\lambda_{max}$ = 446 nm, $\varepsilon$ = 7250 M$^{-1}$ cm$^{-1}$, $\lambda_{max}$ = 394 nm, $\varepsilon$ = 7780 M$^{-1}$ cm$^{-1}$.

*Spectral data matches our previously reported data$^1$.

5.6.2.16 Synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}]$ (m-triazole$^1$)

To 10 mg (0.0012 mmol, 1 eq) [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-N$_3$)] (m-azido$^1$) in 5 mL tetrahydrofuran was added 2.5 mg (0.024 mmol, 20 eq) (Z)-cyclooct-1-ene-5-yne in 1 mL tetrahydrofuran. The solution was stirred for 10 minutes, after which the solvent and residual cyclooctyne starting material was removed by rotary evaporation. Upon removal of the solvent, a reddish brown film formed on the flask interior, which was then triturated with acetonitrile to remove residual azide-clusters, giving [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-m-C$_6$H$_4$-C$_8$H$_{10}$N$_3$)$_{18}]$ as a reddish black film. $^1$H NMR (CD$_2$Cl$_2$, 600 MHz): δ (ppm) 7.26-7.06 (m, 72H), 5.44 (m, 12H),
5.40 (m, 24H), 3.59 (t, 18H, J = 12 Hz), 3.15-2.95 (m, 90H), 2.70 (t, 36H, J = 12 Hz), 2.42 (m, 72H), 1.59 (m, 8H), 1.33-1.26 (m, 48H), 0.87 (t, 12H, J = 6Hz). MS (MALDI-TOF [negative]) m/z calc. for C_{288}H_{324}Au_{25}N_{54}S (M^-): 10043.4 Da, found: 10047.0 Da. IR (ATR-IR, cm^{-1}): 2912, 1704, 1662, 1607. UV-Vis (Dichloromethane, 2 x 10^{-4} mol L^{-1}): λ_{max} = 680 nm, ε = 2100 M^{-1} cm^{-1} , λ_{max} = 442 nm, ε = 7350 M^{-1} cm^{-1} , λ_{max} = 399 nm, ε = 7890 M^{-1} cm^{-1}.

5.6.2.17 Synthesis of [(CH_{3}-(CH_{2})_{7})_{4}N][Au_{25}(SCH_{2}CH_{2}-o-C_{6}H_{4}-C_{8}H_{10}N_{3})_{18}] (o-triazole\textsuperscript{1-})

To 10 mg (0.0012 mmol, 1 eq) [(CH_{3}-(CH_{2})_{7})_{4}N][Au_{25}(SCH_{2}CH_{2}-o-C_{6}H_{4}-N_{3})] (o-azido\textsuperscript{1-}) in 5 mL tetrahydrofuran was added 2.5 mg (0.024 mmol, 20 eq) (Z)-cyclooct-1-ene-5-yne in 1 mL tetrahydrofuran. The solution was stirred for 10 minutes, after which the solvent and residual cyclooctyne starting material was removed by rotary evaporation. Upon removal of the solvent, a reddish brown film formed on the flask interior, which was then triturated with acetonitrile to remove residual azide-clusters. The film was not very soluble in dichloromethane, particulates could be seen in solution, indicating the sample had undergone some aggregation.
5.6.3 Experimental Spectra and Diagrams

5.6.3.1 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$N_3$)$_{18}$] ($p$-azido$^1$)

See Section 4.6.3.1 for $^1$H NMR spectrum and ESI-MS spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$N_3$)$_{18}$] ($p$-azido$^1$).

5.6.3.2 Experimental Spectra and Diagrams for [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$m$-C$_6$H$_4$-$N_3$)$_{18}$] ($m$-azido$^1$)

![Figure S5.1](image)

**Figure S5.1.** 600 MHz $^1$H NMR spectrum of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$m$-C$_6$H$_4$-$N_3$)$_{18}$] (m-azido$^1$) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvent and impurities.
Figure S5.2. Negative ion mode ESI mass spectrum of anionic [Au$_{25}$(SCH$_2$CH$_2$-$m$-C$_6$H$_4$-N$_3$)$_1$].
5.6.3.3 Experimental Spectra and Diagrams for \([\text{((CH}_3\text{-})\text{(CH}_2\text{)}_7\text{)}_4\text{N}]\text{[Au}_{25}\text{(SCH}_2\text{CH}_2\text{-o-C}_6\text{H}_4\text{-N}_3)_{18}}\) (\(o\text{-azido}^{1-}\))

Figure S5.3. 600 MHz \(^1\text{H NMR spectrum of [((CH}_3\text{-})\text{(CH}_2\text{)}_7\text{)}_4\text{N}]\text{[Au}_{25}\text{(SCH}_2\text{CH}_2\text{-o-C}_6\text{H}_4\text{-N}_3)_{18}}\) (\(o\text{-azido}^{1-}\)) in CD\(_2\text{Cl}_2\) at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvent and impurities.
Figure S5.4. Negative ion mode ESI mass spectrum of anionic [Au$_{25}$(SCH$_3$CH$_2$-o-C$_6$H$_4$-N$_3$)$_1$.}
5.6.3.4 Experimental Diagrams for $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{p-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($\text{p-azido}^0$)

Figure S5.5. Space-filling X-ray structure diagram of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{p-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($\text{p-azido}^0$). Au = yellow, S = red, C = black, N = green.

5.6.3.5 Experimental Diagrams for $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($\text{m-azido}^0$)

Figure S5.6. Space-filling X-ray structure diagram of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}]$ ($\text{m-azido}^0$). Au = yellow, S = red, C = black, N = green.
5.6.3.6 Experimental Diagrams for \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4-\text{N}_3)_{18}]\) (\(o\)-azido\(^0\))

**Figure S5.7.** Space-filling X-ray structure diagram of \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4-\text{N}_3)_{18}]\) (\(o\)-azido\(^0\)). Au = yellow, S = red, C = black, N = green.
5.6.3.7 Experimental Spectra and Diagrams for \([(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}])\) (p-triazole$^{1-}$)

See Section 5.6.3.2 for $^1$H NMR spectrum and linear negative mode MALDI-TOF mass spectrum of \([(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}])\) (p-triazole$^{1-}$).

5.6.3.8 Experimental Spectra and Diagrams for \([(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}])\) (m-triazole$^{1-}$)

Figure S5.8. 600 MHz $^1$H NMR spectrum of \([(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}])\) (m-triazole$^{1-}$) in CD$_2$Cl$_2$ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvent and impurities.
Figure S5.9. Linear negative mode MALDI-TOF mass spectrum of anionic \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-m-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}]^{1-}\).
5.6.3.9 Experimental Spectra and Diagrams for [\((\text{CH}_3-\text{CH}_2)_7\text{N}\)[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}] (\text{o-triazole}^{1-})

Figure S5.10. 600 MHz $^1$H NMR spectrum of [\((\text{CH}_3-\text{CH}_2)_7\text{N}\)[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}] (\text{o-triazole}^{1-}) in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvents.
Figure S5.11. Linear negative mode MALDI-TOF mass spectrum of anionic \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-o-C_6\text{H}_4-C_8\text{H}_{10}\text{N}_3)_{18}]\).
5.6.3.10 Experimental Spectra for $p$-nitro-phenylethanethioacetate

See Section 4.6.3.4 for $^1$H NMR, $^{13}$C $^1$H NMR and IR spectra of $p$-nitro-phenylethanethioacetate.

5.6.3.11 Experimental Spectra for $p$-azido-phenylethanethioacetate

See Section 4.6.3.6 for $^1$H NMR, $^{13}$C $^1$H NMR and IR spectra of $p$-azido-phenylethanethioacetate.

5.6.3.12 Experimental Spectra for $p$-azido-phenylethanethiol

See Section 4.6.3.7 for $^1$H NMR, $^{13}$C $^1$H NMR and IR spectra of $p$-azido-phenylethanethiol.
5.6.3.13 Experimental Spectra for \( m \)-nitro-phenylethanethioacetate

**Figure S5.12.** \(^1\)H NMR spectrum of \( m \)-nitro-phenylethanethioacetate in CDCl\(_3\) at 25°С. * indicates residual protio solvent and impurities.

**Figure S5.13.** \(^{13}\)C\{\(^1\)H\} NMR spectrum of \( m \)-nitro-phenylethanethioacetate in CDCl\(_3\) at 25°С. * indicates CDCl\(_3\) solvent.
Figure S5.14. Infrared spectrum of *m*-nitro-phenylethanethioacetate.
5.6.3.14 Experimental Spectra for m-azido-phenylethanethioacetate

**Figure S5.15.** $^1$H NMR spectrum of *m-azido-phenylethanethioacetate* in CDCl$_3$ at 25°C. * indicates residual protio solvent and impurities.

**Figure S5.16.** $^{13}$C($^1$H) NMR spectrum of *m-azido-phenylethanethioacetate* in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
Figure S5.17. Infrared spectrum of $m$-azido-phenylethanethioacetate.
5.6.3.15 Experimental Spectra for $m$-azido-phenylethanethiol

**Figure S5.18.** $^1$H NMR spectrum of $m$-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent.

**Figure S5.19.** $^{13}$C($^1$H) NMR spectrum of $m$-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.
Figure S5.20. Infrared spectrum of \textit{m}-azido-phenylethanethiol.
5.6.3.16 Experimental Spectra for o-nitro-phenylethanethioacetate

**Figure S5.21.** $^1$H NMR spectrum of *o-nitro-phenylethanethioacetate* in CDCl$_3$ at 25°C. * indicates residual protio solvent and impurities.

**Figure S5.22.** $^{13}$C($^1$H) NMR spectrum of *o-nitro-phenylethanethioacetate* in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
Figure S5.23. Infrared spectrum of \textit{o-nitro-phenylethanethioacetate}. 
5.6.3.17 Experimental Spectra for o-azido-phenylethanethioacetate

Figure S5.24. $^1$H NMR spectrum of o-azido-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates residual protio solvent and impurities.

Figure S5.25. $^{13}$C{$^1$H} NMR spectrum of o-azido-phenylethanethioacetate in CDCl$_3$ at 25°C. * indicates CDCl$_3$ solvent.
**Figure S5.26.** Infrared spectrum of \textit{o-azido-phenylethanedithioacetate}. 
5.6.3.18 Experimental Spectra for o-azido-phenylethanethiol

Figure S5.27. $^1$H NMR spectrum of o-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent and impurities.

Figure S5.28. $^{13}$C{${^1}$H} NMR spectrum of o-azido-phenylethanethiol in CD$_2$Cl$_2$ at 25°C. * indicates CD$_2$Cl$_2$ solvent.
Figure S5.29. Infrared spectrum of o-azido-phenylethanol.
5.6.4 Electrochemical Graphs

5.6.4.1 Cyclic Voltammetry (CV) Graph of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] ($p$-azido$^1$)

Figure S5.30. Cyclic voltammetry (CV) graph of 0.1 mM solution of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] ($p$-azido$^1$) in 1:1 acetonitrile:benzene. Supporting electrolyte for CV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP).
5.6.4.2 Cyclic Voltammetry (CV) Graph of \([(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}] \text{ (m-azido}^{1-})\]

**Figure S5.31.** Cyclic voltammetry (CV) graph of 0.1 mM solution of \([(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{m-C}_6\text{H}_4\text{-N}_3)_{18}] \text{ (m-azido}^{1-})\] in 1:1 acetonitrile:benzene. Supporting electrolyte for CV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP).
5.6.4.3 Cyclic Voltammetry (CV) Graph of \([(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4\text{-N}_3)_{18}] \text{ (o-azido}^+\text{)})

Figure S5.32. Cyclic voltammetry (CV) graph of 0.1 mM solution of \([(\text{CH}_3-(\text{CH}_2)_7\text{N})\text{[Au}_{25}(\text{SCH}_2\text{CH}_2-o-\text{C}_6\text{H}_4\text{-N}_3)_{18}] \text{ (o-azido}^+\text{)}) in 1:1 acetonitrile:benzene. Supporting electrolyte for CV measurements was 0.1 M tetra-n-butylammonium perchlorate (TBAP).
5.6.5 Kinetic Measurements

5.6.5.1 General Experimental Details

Estimated rate constants for \textit{p-azidophenylethanethiol}, \textit{m-azidophenylethanethiol} and \textit{o-azidophenylethanethiol}, \textit{p-azido} and \textit{m-azido} were determined under second order conditions \( (k_2) \) in deuterated dichloromethane at 25°C using \(^1\)H NMR spectroscopy.

In order to estimate \( k_2 \) values for \textit{p-azidophenylethanethiol}, \textit{m-azidophenylethanethiol} and \textit{o-azidophenylethanethiol}, stock solutions of the thiols and BCN\textsubscript{exo}-OH were first prepared and then equimolar quantities of each were added to an NMR tube. To prepare the stock solution of each thiol, 10 mg of each thiol was dissolved in 3 mL of deuterated dichloromethane, from which 0.3 mL (containing 0.1 mg (0.58 \( \mu \text{mol, 1 equivalent} \)) thiol) was transferred to an NMR tube. A \( t_0 \) (time zero) \(^1\)H NMR spectrum was taken of this sample containing only the azide-modified thiol. To prepare the stock solution of BCN\textsubscript{exo}-OH, 8.7 mg of BCN\textsubscript{exo}-OH was dissolved in 1000 \( \mu \text{L} \) deuterated dichloromethane, from which 10 \( \mu \text{L} \) (containing 0.087 mg (0.58 \( \mu \text{mol, 1 equivalent} \)) BCN\textsubscript{exo}-OH) was added to the NMR tube containing the thiol sample, and \(^1\)H NMR spectra were acquired over pre-determined time intervals according to the speed of the reaction.

In order to estimate \( k_2 \) values for \textit{p-azido} and \textit{m-azido}, a solution was prepared by dissolving 5 mg (0.58 \( \mu \text{mol, 1 equivalent} \)) of each Au\textsubscript{25} sample in 0.3 mL deuterated dichloromethane in an NMR tube. A \( t_0 \) (time zero) \(^1\)H NMR spectrum was taken of this sample containing only the azide-modified Au\textsubscript{25} sample. A stock solution of BCN\textsubscript{exo}-OH was prepared by dissolving 16 mg of BCN\textsubscript{exo}-OH in 100 \( \mu \text{L} \) deuterated dichloromethane, from which 10 \( \mu \text{L} \) (containing 1.6 mg (10.6 \( \mu \text{mol, 18 equivalents} \)) BCN\textsubscript{exo}-OH) was added to the NMR tube containing the Au\textsubscript{25} sample, and \(^1\)H NMR spectra were acquired over pre-determined time intervals according to the speed of the reaction.

Second order rate constants were determined by plotting the \( 1/\text{[azide]} \) versus time, where the slope gives the rate constant \( (k_2) \). In order to determined \([\text{azide}]\) at each time interval, in each time zero NMR spectrum, the ratio of a signal integral in the parent azide (either in the free thiol or Au\textsubscript{25} sample) to the signal integral of residual protio \( \text{CH}_2\text{Cl}_2 \) in the sample was determined, to give the ‘time zero azide signal’. After addition of BCN\textsubscript{exo}-OH to each sample, the ratio of the
integral of this decreasing signal in each acquired $^1$H NMR spectra versus the integral of the initial ‘time zero azide signal’ was calculated and multiplied by the initial concentration of azide at time zero (0.0019 M) to give [azide] at each time interval. Experiments were performed in duplicate.

5.6.5.2 Kinetic Measurements for $p$-azidophenylethanethiol

![Graph](image)

**Figure S5.33.** Second order kinetics graph for $p$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 1).

![Graph](image)

**Figure S5.34.** Second order kinetics graph for $p$-azidophenylethanethiol with BCN$_{exo}$-OH (Trial 2).
5.6.5.3 Kinetic Measurements for \textit{m}-azidophenylethanethiol

\textbf{Figure S5.35.} Second order kinetics graph for \textit{m}-azidophenylethanethiol with BCN_{exo}-OH (Trial 1).

\textbf{Figure S5.36.} Second order kinetics graph for \textit{m}-azidophenylethanethiol with BCN_{exo}-OH (Trial 2).
5.6.5.4 Kinetic Measurements for \( o \)-azidophenylethanolthiol

**Figure S5.37.** Second order kinetics graph for \( o \)-azidophenylethanolthiol with BCN\(_{exo}\)OH (Trial 1).

**Figure S5.38.** Second order kinetics graph for \( o \)-azidophenylethanethiol with BCN\(_{exo}\)OH (Trial 2).
5.6.5.5 Kinetic Measurements for [(CH$_3$-(CH$_2$)$_7$)N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$)$_{18}$] ($p$-azido$^{1-}$)

![First graph](image1)

**Figure S5.39.** Second order kinetics graph for $p$-azido$^{1-}$ with BCN$_{ex}$-OH (Trial 1).

![Second graph](image2)

**Figure S5.40.** Second order kinetics graph for $p$-azido$^{1-}$ with BCN$_{ex}$-OH (Trial 2).
5.6.5.6 Kinetic Measurements for $\left[\text{(CH}_3\text{-CH}_2\right)_7\text{N}\left]\text{Au}_{25}\text{(SCH}_2\text{CH}_2\text{-m-C}_6\text{H}_4\text{-N}_3\right)_18\] (m-azido$^1$)

**Figure S5.41.** Second order kinetics graph for $m$-azido$^1$ with BCN$_{\text{exo-OH}}$ (Trial 1).

**Figure S5.42.** Second order kinetics graph for $m$-azido$^1$ with BCN$_{\text{exo-OH}}$ (Trial 2).
5.6.6 Crystallographic Information

5.6.6.1 Data Collection and Processing

The sample \( p\)-azido\(^0\), \( m\)-azido\(^0\) and \( o\)-azido\(^0\) were crystallized out of a 1:4 mixture of toluene:ethanol over two weeks at ambient conditions. A crystal of each sample was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. All X-ray measurements were made on a Bruker Kappa Axis Apex2 diffractometer at a temperature of 110 K.

The unit cell dimensions for \( p\)-azido\(^0\) were determined from a symmetry constrained fit of 9989 reflections with \( 4.42^\circ < 2\theta < 39.64^\circ \). The data collection strategy was a number of \( \omega \) and \( \phi \) scans which collected data up to \( 39.778^\circ \) (20). The unit cell dimensions for \( o\)-azido\(^0\) were determined from a symmetry constrained fit of 9919 reflections with \( 5.92^\circ < 2\theta < 49.22^\circ \). The data collection strategy was a number of \( w \) and \( j \) scans which collected data up to \( 49.458^\circ \) (20). The unit cell dimensions for \( o\)-azido\(^0\) were determined from a symmetry constrained fit of 9821 reflections with \( 4.86^\circ < 2\theta < 58.52^\circ \).

The frame integration was performed using SAINT.\(^3\) The resulting raw data were scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS.\(^4\)

5.6.5.2 Structure Solution and Refinement

The structures for \( p\)-azido\(^0\), \( m\)-azido\(^0\) and \( o\)-azido\(^0\) was solved by using a dual space methodology using the SHELXT program.\(^5\) All non-hydrogen atoms were obtained from the initial solution. The hydrogen atoms were introduced at idealized positions and were allowed to ride on the parent atom. The structural model was fit to the data using full matrix least-squares based on \( F^2 \). The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL program from the SHELXTL suite of crystallographic software.\(^6\) For all structures, the aryl-azide groups exhibited significant disorder and were refined using available restraints in the SHELXTL software. Graphic plots were produced using the Mercury program suite. Additional information and other relevant literature references can be found in the reference section of this website (http://xray.chem.uwo.ca).
5.6.6.3 Summary of Crystal Data for [(CH$_3$-(CH$_2$)$_7$)N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^+$)

See Section 4.6.4.3 for crystallographic summary of [(CH$_3$-(CH$_2$)$_7$)N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^+$).

5.6.6.4 Summary of Crystal Data for [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (p-azido$^0$)

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Number of parameters in least-squares 3412

R₁ 0.0467

wR₂ 0.0911

R₁ (all data) 0.0965

wR₂ (all data) 0.1102

GOF 1.021

Maximum shift/error 0.001

Min & Max peak heights on final ΔF Map (e⁻/Å) -1.550, 1.852

Where:

\[ R₁ = \frac{\Sigma (|F_0| - |F_c|)}{\Sigma F_0} \]

\[ wR₂ = \left[ \frac{\Sigma (w(F_o^2 - F_c^2)^2)}{\Sigma w F_o^4} \right]^\frac{1}{2} \]

\[ GOF = \left[ \frac{\Sigma (w(F_o^2 - F_c^2)^2)}{(\text{No. of reflns.} - \text{No. of params.})} \right]^\frac{1}{2} \]

5.6.6.5 Summary of Crystal Data for \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-m-\text{C}_6\text{H}_4-\text{N}_3)_{18}] (m-\text{azido}^0)\)

Formula \(\text{C}_{144}\text{H}_{144}\text{Au}_{25}\text{N}_{54}\text{S}_{18}\)

Formula Weight (g/mol) 8132.37

Crystal Dimensions (mm) 0.052 \times 0.032 \times 0.019

Crystal Color and Habit black prism
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Max 2θ for data collection, ° 49.458

Measured fraction of data 0.994

Number of reflections measured 133112

Unique reflections measured 15188

$R_{\text{merge}}$ 0.1770

Number of reflections included in refinement 15188

Cut off Threshold Expression $I > 2\sigma(I)$

Structure refined using full matrix least-squares using $F^2$

Weighting Scheme $w=1/[\sigma^2(Fo^2)+(0.0273P)^2]$

where $P=(Fo^2+2Fc^2)/3$

Number of parameters in least-squares 1010

$R_1$ 0.0399

$wR_2$ 0.0762

$R_1$ (all data) 0.0766

$wR_2$ (all data) 0.0838

GOF 0.857

Maximum shift/error 0.001

Min & Max peak heights on final $\Delta F$ Map ($e/\text{Å}$) -2.019, 2.641

Where:

$R_1 = \Sigma (|F_o| - |F_c|) / \Sigma F_o$
\[ wR^2 = \left[ \frac{\Sigma(w(F^2 - F_c^2)^2)}{\Sigma(wF^4)} \right]^{\frac{1}{2}} \]

\[ GOF = \left[ \frac{\Sigma(w(F^2 - F_c^2)^2)}{(\text{No. of reflns.} - \text{No. of params.})} \right]^{\frac{1}{2}} \]

### 5.6.6.6 Summary of Crystal Data for \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-\text{o}-\text{C}_6\text{H}_4-\text{N}_3)_{18}] (\text{o-azido}^0)\)

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Min and Max 2θ for cell determination, °

4.86, 58.52

Z

1

F(000)

3649

ρ (g/cm³)

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λ, Å, (MoKα)

0.71073

μ, (cm⁻¹)

19.914

Diffractometer Type

Bruker Kappa Axis Apex2

Scan Type(s)

phi and omega scans

Max 2θ for data collection, °

58.736

Measured fraction of data

0.998

Number of reflections measured

118552

Unique reflections measured

25346

Rmerge

0.0531

Number of reflections included in refinement

25346

Cut off Threshold Expression

I > 2σ(I)

Structure refined using

full matrix least-squares using F²

Weighting Scheme

w=1/[σ²(Fo²)+(0.0350P)²+10.8644P] where P=(Fo²+2Fc²)/3

Number of parameters in least-squares

1505
309

R₁ 0.0341

wR₂ 0.0769

R₁ (all data) 0.0658

wR₂ (all data) 0.0859

GOF 1.069

Maximum shift/error 0.008

Min & Max peak heights on final ΔF Map (e'/Å) -1.999, 2.244

Where:

R₁ = \frac{\sum |F_o| - |F_c|}{\sum F_o}

wR₂ = \sqrt{\frac{\sum w(F_o^2 - F_c^2)^2}{\sum (w F_o^4)}}

GOF = \sqrt{\frac{\sum w(F_o^2 - F_c^2)^2}{(\text{No. of reflns.} - \text{No. of params.})}}

5.6.7 References – Supporting Information


3. Bruker-AXS, SAINT version 2013.8, 2013, Bruker-AXS, Madison, WI 53711, USA

5. Sheldrich, G.M., Acta Crystallographica Section A. 2015, A71, 3-8


Chapter 6

6 Expanding the Frontiers of Ultrasmall Gold Nanocluster Surface Composition through Cluster-Surface Click Chemistry: A Ferrocenyl-Modified Au$_{25}$(SR)$_{18}$ Nanocluster

This chapter is being submitted as a short paper and is in manuscript format. Jonathan M. Wong is a co-author, along with professors Zhifeng Ding, John F. Corrigan and Mark S. Workentin.

Wong was a graduate student, who under the co-supervision of his supervisor, Prof. Zhifeng Ding, obtained the electrochemical measurements. The draft of the manuscript was written by the author and edited by Prof. Mark S. Workentin and Prof. John F. Corrigan.

6.1 Introduction

In recent years, monolayer-protected metallic nanomaterials have emerged as prominent frameworks within the fast-paced realm of nanomaterial research, whose functional complexity can be tuned through changes to the surface composition while retaining the properties of the internal core framework. Thiolate-protected gold nanomaterials are amongst the most widely-studied, largely due to the stability of core gold-gold interactions and ease-of-synthesis of structurally diverse thiol ligands, both of which facilitates not only their synthesis, but applications in settings which tend to be incompatible with other metallic or intermetallic frameworks. Distinguished by their larger size, functionally complex thiolate-protected gold nanoparticles (AuNPs) have found extensive use in a wide variety of biomedical applications$^{1-3}$. However, when the smaller atomically precise gold nanocluster (AuNC) size regime ($<$2nm) is accessed, discrete core electronic characteristics manifest molecular-type features that distinguishes these thiolate-protected AuNCs. Not only are they in smaller size, but their unique molecule-like properties (which are not observed with larger AuNPs) are contingent on the core framework and surface thiolate composition$^4,5$. Furthermore, unlike their larger counterparts, gold nanoclusters can be synthesized in an atomically predictable and definable manner, permitting analysis by techniques that are largely reserved for small molecules such as X-ray crystallography and $^1$H NMR spectroscopy. This has yielded an enormous understanding of the bonding features within the
framework and surface monolayer\textsuperscript{6, 7} and how their structure correlates with their properties\textsuperscript{8, 9}. Overall, these characteristics have rendered thiolate-protected gold nanoclusters very prominent nanomaterials in a variety of applications such as chemical sensing\textsuperscript{10-12}, catalysis\textsuperscript{13-17}, nanomedicine\textsuperscript{18-21} and optical imaging\textsuperscript{22-24}.

To date there exists an extensive library of atomically precise thiolate-protected gold nanoclusters, whose core framework and surface composition can be tuned through very sensitive changes to ratio of gold (III) precursor to thiol, the structure of the thiol ligand and reaction conditions\textsuperscript{6}. The notoriously sensitive relationship between thiol structure and acquired core configuration has imparted their popular nickname: the so-called “magic clusters”\textsuperscript{6}. For this reason, the vast majority of magic clusters are synthesized using small inert thiol ligands, as more complex and functional thiol ligands are incompatible with direct synthetic strategies and does not lead to the desired core configuration. Application-based research on magic clusters is primarily reliant on place exchange chemistry, in which native inert ligands are replaced with functional thiol ligands. However, such strategies are limited by inabilitys to establish total exchange of native thiol ligands and synthetic difficulties in developing functionally complex thiolated substrates, which often requires difficult protection/deprotection strategies. Cluster rearrangements are also observed\textsuperscript{6, 25, 26}. Given the significant application potential for thiolate-protected gold nanoclusters, these limitations of place exchange strategies warrant investigations to explore new methods for introducing functional complexity onto the nanocluster surface.

In contrast to direct syntheses and place exchange strategies towards introducing functional complexity on the nanocluster surface, we recently presented the first atomically precise [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{1} thiolate-protected gold nanocluster platform with a reactive azide moiety appended to each of the 18 surface ligands, [(CH\textsubscript{3}-(CH\textsubscript{2})\textsubscript{7})\textsubscript{4}N][Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}-p-C\textsubscript{6}H\textsubscript{4}-N\textsubscript{3})\textsubscript{18}] (hereafter referred to as 6.1-azido in current chapter (and referred to as 4.1-azido in Chapter 4 and p-azido\textsuperscript{0} in Chapter 5)\textsuperscript{27}. The novelty of this strategy is that this platform is capable of undergoing post-assembly surface modifications in which the surface azide groups orthogonally and chemoselectively reacts with strained-alkynes through the cluster-surface strain-promoted alkyne-azide cycloaddition (CS-SPAAC), without perturbing the internal core configuration. Unlike with place exchange chemistry, since all surface azide moieties are amenable to this cluster-surface reaction, it is possible to incorporate functional substrates with an appended strained-alkyne
moiety to all surface ligand simply by using a slight excess of the strained-alkyne coupled substrate. This presents an exciting new avenue towards developing functional varieties of AuNC systems by transforming the surface monolayer through a high-yielding method that is dependent only on the creation of functional substrates possessing the complementary strained-alkyne moiety. For our seminal study, we demonstrated CS-SPAAC chemistry by reacting 6.1-azido with an inert symmetrical strained-alkyne, (Z)-cyclooct-1-ene-5-yne. To demonstrate the true power of utilizing post-assembly CS-SPAAC chemistry, we herein report the first example of a [Au$_{25}$SR$_{18}$]$_1^-$ nanocluster with ferrocenyl moieties on all the surface ligands, by reacting 6.1-azido with a ferrocene-coupled strained-alkyne. We show that all surface ligands are amendable to the incorporation of the electroactive ferrocenyl moieties through CS-SPAAC, which is amongst the largest functional Au$_{25}$SR$_{18}$ nanocluster systems reported to date.

6.2 Results and Discussion

The first step in synthesizing azide-modified [Au$_{25}$(SR)$_{18}$]$^1$ clusters is the synthesis of the corresponding thiol ligand, $p$-azido-phenylethanethiol (HSCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$), which was carried out according to our previous established procedure$^{27}$ with one major modification. Specifically, the 4-step procedure was reduced to a 3-step procedure, which at the same time gives a higher overall yield of $p$-azido-phenylethanethiol (see Section 5.6.2 for detailed synthesis). The synthesis of 6.1-azido was carried out according to our previously reported procedure without modifications$^{27}$ (see Section 4.6.2.8 for detailed synthesis). The $^1$H NMR, IR and UV spectroscopic data and electrospray-ionization mass spectral data for 6.1-azido was consistent with the data from our first report (see Section 4.6.3.1 for spectra of 6.1-azido)

In order to synthesize a ferrocene-coupled strained-alkyne, ferrocene-carboxylic acid was coupled to $exo$-bicyclo[6.1.0]non-4-yn-9-ol (BCN$_{exo}$-OH) in the presence of N,N’-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to give “ferrocene-BCN$_{exo}$”. Although kinetically less reactive, the aliphatic BCN was the strained-alkyne chosen for this study because it is more stable than other benzoannulated strained-alkynes (such as dibenzocyclooctyne, DBCO), can be synthesized on gram scale, and has minimal reactivity with thiols$^{28-30}$. As with (Z)-cyclooct-1-ene-5-yne, the reaction between 6.1-azido and a slight excess of ferrocene-BCN$_{exo}$ is a simple mix and stir at room temperature, transforming 6.1-azido into the
ferrocenated [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{21}$H$_{22}$FeN$_3$O$_2$)$_{18}$] (hereafter referred to as 6.1-ferrocenyl) (Scheme 6.1) Removal of excess ferrocene-BCN$_{exo}$ was accomplished through simple trituration with 2-propanol. The linear negative mode matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum of the purified product contained a well-resolved peak at 14,662.1 Da that can be assigned to the parent [Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{21}$H$_{22}$FeN$_3$O$_2$)$_{18}$]$^-$ anion of the 6.1-ferrocenyl cluster (expected m/z = 14,562 Da).

Scheme 6.1. Synthetic approach to the preparation of fully-ferrocenated [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-C$_{21}$H$_{22}$FeN$_3$O$_2$)$_{18}$] (6.1-ferrocenyl) through CS-SPAAC between [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-p-C$_6$H$_4$-N$_3$)$_{18}$] (6.1-azido) and ferrocene-BCN$_{exo}$.

The completion of the CS-SPAAC reaction can easily be evaluated using IR spectroscopy. The IR spectrum of 6.1-azido (Figure 6.1a, black) contains a large stretch at 2098 cm$^{-1}$ that can be attributed to N-N stretches of the azide moiety, which disappears entirely upon quantitative consumption of all surface azide groups, as can be seen in the IR spectrum of 6.1-ferrocenyl (Figure 6.1a, red). Furthermore, there is a signal at 1703 cm$^{-1}$ in the IR spectrum of 6.1-ferrocenyl that results from C=O stretches at the carbamate linkage, which is also present in the IR spectrum of ferrocene-BCN$_{exo}$. 
The UV-Vis absorption spectrum of **6.1-azido** (Figure 6.1b, black) is nearly identical to the observed pattern of absorption maxima as other thiolate-protected Au$_{25}$(SR)$_{18}$ clusters, there are absorption maxima at 682, 443 and 404 nm. The absorption maxima at 682 nm has been previously assigned to an intraband metallic transition specifically within an Au$_{25}$ core framework, and any difference in the observed energy of this transition suggests the presence of a different core framework other than Au$_{25}$. As can be seen in the UV-Vis absorption spectrum of **6.1-ferrocenyl** (Figure 6.1b, red), both the energy and intensity of the transition at 682 nm remains after the CS-SPAAC reaction, indicating the retention of the Au$_{25}$ core framework. Similar to the UV-Vis absorption spectrum of **6.1-azido**, there are also transitions at 405 and 445 nm in the UV-Vis absorption spectrum of **6.1-ferrocenyl**. However, the absorption intensity of the transition at 445 nm is larger in **6.1-ferrocenyl**, which can be attributed to the newly incorporated surface ferrocene moieties that have their own low intensity d-d transition at 445 nm, which can also be seen in the UV-Vis absorption spectrum of ferrocene-BCN$_{exo}$.

**Figure 6.1c** (black) shows the $^1$H NMR spectrum of **6.1-ferrocenyl** in deuterated dichloromethane at 23°C. As with the $^1$H NMR spectrum of **6.1-azido**, there are signals arising from the tetraoctylammonium counterion at 0.87 (-CH$_3$), 1.25 (-CH$_2$), 1.31 (-CH$_2$) and 3.09 ppm (N$^+$-CH$_2$), verifying that the anionic core configuration of **6.1-ferrocenyl** predominates and minimal oxidation has occurred after the CS-SPAAC reaction (see Figure S6.1 for full $^1$H NMR spectrum). As in our first study in which (Z)-cycloct-1-ene-5-yne and bicyclo[6.1.0]non-4-yn-9-ol were used, the CS-SPAAC reaction between **6.1-azido** and ferrocene-BCN$_{exo}$ leads to a distinct change in the chemical environment of the aromatic ligand protons. In **6.1-azido**, each of the two binary sets of aromatic protons produce two signals in a 2:1 ratio that correlates with the 2:1 ratio of chemically inequivalent inner ligand to outer ligand ratio on the cluster surface, with the twelve inner ligands producing doublet signals at 6.80 and 7.12 ppm and the six outer ligands producing doublet signals at 6.86 and 7.17 ppm. As seen in **Figure 6.1c**, after the CS-SPAAC reaction, these two sets of doublets disappear entirely, and there are new sets of doublets at 7.36 and 7.15 ppm from the twelve inner ligands and another set of doublets at 7.29 and 7.14 ppm from the six outer ligands. The appearance of these new sets of doublets and their associated chemical shifts are the same regardless of the cyclooctyne structure, and hence can be used as an NMR fingerprint to evaluate the success and completion of the CS-SPAAC reaction.
After the CS-SPAAC reaction between 6.1-azido and ferrocene-BCNexo, there are also new sets of signals between 0.82 and 3.70 ppm that correspond to the eight membered ring. In our first study in which (Z)-cyclooct-1-ene-5-yne was reacted with 6.1-azido, the symmetrical and achiral nature of the strained-alkyne eliminated the common problem of creating two isomers after the SPAAC reaction, and so these signals could be resolved in a 2:1 ratio that corresponded with the expected inner:outer ligand ratio. However, when chiral strained-alkynes are used, such as BCNexo, two isomers form in what is typically an inequivalent ratio. Consequently, the signals that correspond to the protons in the eight membered ring and the methylene protons do not appear in a distinguishable 2:1 ratio. The incorporation of the ferrocene-BCNexo to the cluster surface is accompanied by two new signals at 4.05 ppm and 3.99 ppm in a 2:1 ratio, which can be attributed to the α-methylene protons in ferrocene-BCNexo. There are also new triplets at 4.36 and 4.75 ppm and a new singlet at 4.16 ppm that corresponds to the cyclopentadienyl protons of the newly incorporated ferrocenyl rings. However, unlike with all the other surface protons that are closer to the cluster surface, the cyclopentadienyl protons are further away from the cluster surface and therefore have a lower chemical shift.
the central core, and produce signals in a 2:1 ratio due to the presence of the two distinguishable ligand environments, the cyclopentadienyl signals from the surface ferrocenyl groups appear as one signal instead of two. This indicates an interplay between the observed chemical inequivalence of the surface protons and the proximity to the central metallic core. Chemical inequivalence in the surface ligands is generated for the more proximal ligand components, but the ferrocenyl moieties are far enough away from the central core that the inner to outer ligand distinction becomes inconsequential, and the surface ferrocenyl become effectively magnetically equivalent.

The electrochemistry of 6.1-azido and 6.1-ferrocenyl was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using our previously reported conditions\textsuperscript{31}. The CV spectra of 6.1-azido and 6.1-ferrocenyl can be found in the Supporting Information (Figure S5.30 and Figure S6.7, respectively). In the DPV for 6.1-azido (Figure 6.2a), two redox waves can be observed with formal potentials of 0.178 V and 0.405 V (vs. saturated calomel electrode (SCE)) in the potential range between 0.2 and 1.2 V. These two redox waves can be attributed to the conversion between Au\textsubscript{25}\textsuperscript{1-}/Au\textsubscript{25}\textsuperscript{0} (0.178 V) and Au\textsubscript{25}\textsuperscript{0}/Au\textsubscript{25}\textsuperscript{1+} (0.405 V), and the quasi-reversible nature of these waves in the anodic (black) and cathodic (red) scans is indicative of the stability of the -1, 0 and +1 forms of the Au\textsubscript{25} nanoclusters. These are the benchmark peaks for the Au\textsubscript{25}SR\textsubscript{18} core configuration. It should be noted that the formal potential of these waves for our azido-phenylethanethiolate protected Au\textsubscript{25} nanocluster system differ from our previously reported values for the conventional phenylethanethiolate-protected Au\textsubscript{25} nanocluster system, where the Au\textsubscript{25}\textsuperscript{1-}/Au\textsubscript{25}\textsuperscript{0} redox event has a formal potential of 0.038 V and the Au\textsubscript{25}\textsuperscript{0}/Au\textsubscript{25}\textsuperscript{1+} redox event has a formal potential of 0.280 V.\textsuperscript{31} This signifies the effect of the surface ligand structure on the electrochemical behaviour of the central gold core. There is another half-wave in the anodic scan with a potential of 1.062 V which is caused by the Au\textsubscript{25}\textsuperscript{1+}/Au\textsubscript{25}\textsuperscript{2+} redox event. Due to the chemical instability of the +2 form, this half-wave has limited reversibility in the cathodic scan. We were unable to observe a Au\textsubscript{25}\textsuperscript{1-}/Au\textsubscript{25}\textsuperscript{2-} redox event in either the cathodic or anodic scans, indicating that the surface azide-modified ligands make the -2 form of the cluster chemically inaccessible.
In the DPV of 6.1-ferrocenyl (Figure 6.2b) there is a large quasi-reversible wave with a formal potential of 0.409 V (vs. SCE) which can be attributed to all surface ferrocenyl moieties undergoing a redox event. There is also a half-wave in the anodic scan with a formal potential of 0.172 V which is likely caused by the Au$_{25}^{1-}$/Au$_{25}^{0}$ redox event in the central core. Given that the regeneration of the parent -1 form of the nanocluster during the cathodic scan requires an approximate 20 electron transfer process into both the 18 surface ferrocenyl and internal gold core, the irreversibility of Au$_{25}^{1-}$/Au$_{25}^{0}$ wave is not surprising. Looking at the area under the half-waves at 0.172 V and 0.413 V in the anodic scan, the area of the half-wave from the expected 18 electron transfer process within the surface ferrocenyl moieties is approximately 20 times larger than the Au$_{25}^{1-}$/Au$_{25}^{0}$ single electron process within the cluster core, further indicating that all surface ferrocenyl moieties are electrochemically accessible.

6.3 Conclusions

In order to highlight the power of utilizing post-assembly surface modifications towards the development of functionally complex nanocluster systems, the development of the first example of a fully ferrocenated [Au$_{25}$(SR)$_{18}$]$^{1-}$ (6.1-ferrocenyl) nanocluster framework is herein reported, which is the largest [Au$_{25}$(SR)$_{18}$]$^{1-}$ nanocluster currently reported. This study not only demonstrates
that all surface azido groups in 6.1-azido are accessible for CS-SPAAC, but that all surface ferrocene moieties are electrochemically accessible for pseudo-reversible redox chemistry. This demonstrates that CS-SPAAC chemistry not only bestows the ability to modify the surfaces of [Au$_{25}$(SR)$_{18}$]$^{1-}$ in a nanoorthogonal manner (i.e. without altering the parent nanocluster structure), but can be done with substrates that retain their properties after incorporation. We are currently exploring other functional substrates to incorporate onto the surface [Au$_{25}$(SR)$_{18}$]$^{1-}$ for more directed applications.

6.4 Acknowledgements

This work was funded by NSERC-DG and the University of Western Ontario. We thank D. Hairsine (MS Facility), K Jurcic (MALDI-MS Facility – Biochemistry).

6.5 References


6.6 Supporting Information

6.6.1 General Methods and Reagents

\textit{Reagents and Solvents.} The following materials were used as received. Potassium thioacetate (98%), zinc dust (<10\textmu m, \geq 98%), sodium azide (\geq 99.5%), gold (III) chloride trihydrate (\geq 99.9% trace metal basis), tetraoctylammonium bromide (98%), sodium borohydride (\geq 98%), 1,5-
cyclooctadiene (≥99%), bromine (reagent grade), potassium tert-butoxide solution (1.0M in THF) and dichloromethane-D$_2$ (CD$_2$Cl$_2$, 99.5 atom %D) were purchased from Sigma-Aldrich (Millipore Sigma). 4-nitrophenylethyl bromide was purchased from Oakwood Chemicals. Sodium chloride, sodium hydroxide pellets and tetrahydrofuran were purchased from Fischer Scientific. Technical grade ammonium chloride, magnesium sulphate, hexanes, dichloromethane, ethyl acetate, 12M hydrochloric acid, di-ethyl ether, dimethyl sulfoxide, sodium nitrite, methanol, toluene, isopropanol, acetonitrile and pentane were purchased from Caledon. Chloroform-D$_1$ (CDCl$_3$, 99.8 atom %D) was purchased from Cambridge Isotope Laboratories. Ethanol (anhydrous) was purchased from Commercial Alcohols.

Unless otherwise stated, all reactions were performed at ambient conditions.

NMR Spectroscopy. $^1$H and $^{13}$C{$^1$H} spectra were recorded on either a Bruker AvIII HD 400 spectrometer or Varian INOVA 600 spectrometer, as indicated. $^1$H NMR spectra are reported as δ in units of parts per million (ppm), and referenced against residual protio chloroform (7.27 ppm, s), dimethylsulfoxide (2.50 ppm, quin) or dichloromethane (5.32 ppm, t), as indicated. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintuplet), m (multiplet) and br (broad signal). Coupling constants are reported as a $J$ value in Hertz (Hz) according to the spectrometer frequency. The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. $^{13}$C{$^1$H} NMR spectra are reported as δ in units of parts per million (ppm) and referenced against the indicated deuterated solvent: chloroform-D$_1$ (77.0 ppm, t) or dichloromethane-D$_2$ (54.0 ppm, quin).

Mass Spectrometry. Electrospray ionization (ESI) mass spectra were obtained in either positive-ion or negative-ion mode using a Bruker microTOF II spectrometer. To obtain the ESI spectrum of [Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$N_3$)$_{18}$]$^-$ nanoclusters (obtained in negative-ion mode), a sample was dissolved in 1:5 toluene:methanol (10mg/mL). We generally found that in order to obtain mass spectra of [Au$_{25}$SR$_{18}$] clusters, the sample solution must contain some methanol in order to minimize excessive fragmentation. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were obtained using an AB Sciex 5800 TOF/TOF system. To obtain the MALDI-TOF spectrum of [Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-$C_8$H$_{10}$N$_3$)$_{18}$]$^-$ nanoclusters (obtained in linear negative mode), a 1g/L sample solution was mixed with a 10g/L solution of trans-2-[3-(4-
tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in a ratio of 1:400 by mass. Data acquisition and data processing were respectively done using a TOF TOF Series Explore and Data Explorer (both from AB Sciex). The laser pulse rate was set to 400Hz. The mass spectrum was collected as a sum of 1000 shots.

**UV-Visible (UV-Vis) Spectroscopy.** UV-Vis absorption spectra were recorded using a Cary 5000 scan instrument using standard quartz cells (1cm path length) with a scan range of 200-1000nm. Samples were dissolved in the indicated solvents at the indicated concentrations. The background spectrum of the indicated solvent was subtracted internally by the software.

**Infrared (IR) spectroscopy.** Attenuated total reflectance IR (ATR-IR) spectra were recorded using a PerkinElmer Spectrum Two FT-IR spectrometer.

**Electrochemistry.** All cyclic voltammograms and differential pulse voltammograms were performed on a CHI 610A electrochemical analyzer (CH Instruments, Austin, Texas). A three-electrode system was used: the working electrode was a 2 mm diameter platinum disc inlaid in a glass (Pt) electrode; two Pt coils served as the counter and reference electrodes, respectively. Prior to each experiment, the glass electrochemical cell was cleaned in an acid bath overnight, then placed in a base bath to prevent any contamination. The platinum disc electrode was polished using 0.3 micron then 0.05 micron alumina polishing slurry (CH Instruments) until a mirror like finish was obtained. The electrode was then subjected to electrochemical cleaning by performing cyclic voltammetry in 0.1 M dilute sulfuric acid solution for 200 cycles between the cathodic and anodic potential limits. The electrode was then rinsed with ultra-pure Type-1 water and left to fully dry before use. Each electrochemical cell consisted of a 0.67mg/mL solution of gold clusters dispersed in a 1:1 anhydrous acetonitrile:benzene mixture (Sure/Seal™ acetonitrile and benzene, Sigma Aldrich), with 0.1M tetrabutylammonium perchlorate (TBAP, Sigma Aldrich) being added as the supporting electrolyte. All electrochemical cells were assembled in an inert Ar atmosphere.
6.6.2 Experimental Procedures

6.6.2.1 Synthesis of \( p \)-nitro-phenylethanethioacetate

See Section 4.6.2.1 for detailed synthesis and characterization data of \( p \)-nitro-phenylethanethioacetate.

6.6.2.2 Synthesis of \( p \)-azido-phenylethanethioacetate

See Section 5.6.2.2 for detailed synthesis and characterization data of \( p \)-azido-phenylethanethioacetate.

6.6.2.3 Synthesis of \( p \)-azido-phenylethanethiol

See Section 4.6.2.4 for detailed synthesis and characterization data of \( p \)-azido-phenylethanethiol.
6.6.2.4 Synthesis of \[\text{[(CH}_3\text{-}(\text{CH}_2)_7\text{N}] [\text{Au}_{25} (\text{SCH}_2\text{CH}_2\text{-p-C}_6\text{H}_4\text{-C}_8\text{H}_{10}\text{N}_3)_{18}] \]
(6.1-azido)

See Section 4.6.2.8 for detailed synthesis and characterization data of \[\text{[(CH}_3\text{-}(\text{CH}_2)_7\text{N}] [\text{Au}_{25} (\text{SCH}_2\text{-p-C}_6\text{H}_4\text{-C}_8\text{H}_{10}\text{N}_3)_{18}] \] (6.1-azido).

6.6.2.5 Synthesis of BCN\(_{\text{exo}}\)-OH

Successfully synthesized according to Dommerholt et al.\(^1\) See Section 2.6.2.6 for characterization data.

6.6.2.6 Synthesis of Ferrocene-BCN\(_{\text{exo}}\)

To a solution of 0.52 g (2.3 mmol, 1 eq) ferrocenecarboxylic acid and 0.14 g (1.2 mmol, 0.5 eq) 4-dimethylaminopyridine in 25 mL dichloromethane was added 0.58 g (2.8 mmol, 1.2 eq) N,N'-dicyclohexylcarbodiimide in 5 mL dichloromethane. The resulting solution was stirred for 10 minutes, after which 0.42 g (2.8 mmol, 1.2 eq) BCN\(_{\text{exo}}\)-OH in 5 mL dichloromethane was added. The solution was stirred for 5 hours, after which the solvent was removed by rotary evaporation and the resulting crude residue was purified by flash column chromatography (100% dichloromethane) to give ferrocene-BCN\(_{\text{exo}}\) as a light orange solid in 72% yield (0.6 g). \(^1\)H NMR (CD\(_2\)Cl\(_2\), 400 MHz): \(\delta\) (ppm) 4.77 (t, 2H, \(J = 4\) Hz), 4.39 (t, 2H, \(J = 4\) Hz), 4.19 (s, 5H), 4.11 (d, 2H, \(J = 8\) Hz), 2.43 (m, 2H), 2.27 (m, 2H), 2.14 (m, 2H), 1.42 (m, 2H), 0.80 (m, 3H). \(^{13}\)C NMR (CD\(_2\)Cl\(_2\), 400 MHz): \(\delta\) (ppm) 171.9, 99.2, 72.3, 71.8, 70.6, 70.3, 68.7, 33.9, 24.3, 23.6, 21.8. HRMS (ESI) \(m/z\) calc. for C\(_{21}\)H\(_{22}\)FeO\(_2\) (M\(^+\)): 362.0969, found: 362.0982. IR
6.6.2.7 Synthesis of Ferrocene-Triazole-Thioacetate

To a solution of 0.11 g (0.30 mmol, 1.0 eq) ferrocene-BCN$_{exo}$ in 5 mL dichloromethane was added 0.080 g (0.36 mmol, 1.2 eq) $p$-azido-phenylethanethioacetate in 1 mL dichloromethane. The resulting solution was stirred for 4 hours, after which the solvent was evaporated and the resulting crude residue was purified by gradient column chromatography (100% dichloromethane to tetrahydrofuran) to give ferrocene-triazole-thioacetate as a light orange oil in 93% yield (0.15 g). $^1$H NMR (CD$_2$Cl$_2$, 400 MHz): δ (ppm) 7.34 (d, 2H, $J = 8$ Hz), 7.27 (d, 2H, $J = 8$ Hz), 4.77 (t, 2H, $J = 4$ Hz), 4.36 (t, 2H, $J = 4$ Hz), 4.16 (s, 5H), 4.06 (d, 2H, $J = 8$ Hz), 3.19 (m, 1H), 3.11 (m, 2H), 2.91 (m, 3H), 2.83 (m, 1H), 2.61 (m, 1H), 2.48 (m, 1H), 2.37 (m, 1H), 2.31 (s, 3H), 1.42 (m, 1H), 1.36 (m, 1H), 0.96 (m, 3H). HRMS (ESI) m/z calc. for C$_{21}$H$_{22}$FeO$_2$ (M$^+$): 540.1408, found: 540.1421.

6.6.2.8 Synthesis of [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-C$_{21}$H$_{22}$FeN$_3$O$_2$)$_{18}$] (6.1-ferrocenyl)

To 50 mg (0.0060 mmol, 1 eq) [(CH$_3$-(CH$_2$)$_7$)$_4$N][Au$_{25}$(SCH$_2$CH$_2$-$p$-C$_6$H$_4$-N$_3$)] (6.1-azido) in 10 mL tetrahydrofuran was added 2.6 mg (0.0072 mmol, 1.2 eq) ferrocene-BCN$_{exo}$ in 1 mL tetrahydrofuran. The solution was stirred for 2 hours, after which the solvent was removed by
rotary evaporation. Upon removal of the solvent, a reddish brown film formed on the flask interior, which was then triturated with isopropanol to remove residual staring materials, giving [(CH_3-(CH_2)_n)N][Au_25(SCH_2CH_2-p-C_6H_4-C_21H_22FeN_3O_2)_18] (6,1-ferrocenyl) as a reddish black film in 96% yield (12 mg). ^1H NMR (CD_2Cl_2, 600 MHz): δ (ppm) 7.37 (d, 24H, J = 6 Hz), 7.28 (d, 12H, J = 6 Hz), 7.14 (d, 36H, J = 6 Hz), 4.75 (t, 36H, J = 3 Hz), 4.36 (t, 36H, J = 3 Hz), 4.16 (s, 90H), 4.05 (m, 18H), 3.98 (m, 18H), 3.70 (m, 18H), 3.30 (m, 18H), 3.24 (m, 18H), 3.09 (m, 54H), 2.78 (m, 36H), 2.48 (m, 36H), 2.23 (m, 18H), 1.59 (m, 8H), 1.38 (m, 18H), 1.28 (m, 48H), 0.99 (m, 18H), 0.90 (m, 18H), 0.87 (t, 12H, J = 6 Hz), 0.83 (m, 18H). MS (MALDI-TOF[negative]) m/z calc. for C_522H_540Au_25Fe_18N_54S_18 (M^−): 14653.2 Da, found: 14662.1 Da. IR (ATR-IR, cm⁻¹): 3107, 3066, 2959, 2859, 1702, 1515, 1456, 1376, 1273, 1134. UV-Vis (Dichloromethane, 2x10⁻⁴ mol L⁻¹): λ_max = 679 nm, ε = 1715 M⁻¹cm⁻¹, λ_max = 443 nm, ε = 8888 M⁻¹cm⁻¹, λ_max = 405 nm, ε = 8597 M⁻¹cm⁻¹.
6.6.3 Experimental Spectra and Diagrams

6.6.3.1 Experimental Spectra for [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-C₈H₁₀N₃)₁₈] (6.1-azido)

See Section 4.6.3.1 for ¹H NMR spectrum and ESI-MS spectrum of [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-N₃)₁₈] (6.1-azido⁺).

6.6.3.2 Experimental Spectra for [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-C₂₁H₂₂FeN₃O₂)₁₈] (6.1-ferrocenyl)

Figure S6.1. 600 MHz ¹H NMR spectrum of [(CH₃-(CH₂)₇)₄N][Au₂₅(SCH₂CH₂-p-C₆H₄-C₂₁H₂₂FeN₃O₂)₁₈] (6.1-ferrocenyl) in CD₂Cl₂ at 25°C. Red insets are zoom-in regions of relevant sections of spectrum. * indicates residual protio solvent and impurities.
Figure S6.2. Linear negative mode MALDI-TOF mass spectrum of anionic \([\text{Au}_{25}(\text{SCH}_2\text{-CH}_2\text{-p-C}_6\text{H}_4\text{-C}_2\text{H}_2\text{FeN}_3\text{O}_2)]^{1-}\).
6.6.3.3 Experimental Spectra for Ferrocene-BCN<sub>exo</sub>

**Figure S6.3.** $^1$H NMR spectrum of ferrocene-BCN<sub>exo</sub> in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent and impurities.

**Figure S6.4.** $^{13}$C($^1$H) NMR spectrum of ferrocene-BCN<sub>exo</sub> in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent.
Figure S6.5. Infrared spectrum of ferrocene-BCN$_{exo}$. 
6.6.3.4 Experimental Spectra of Ferrocene-Triazole-Thioacetate

**Figure S6.6.** $^1$H NMR spectrum of ferrocene-triazole-thioacetate in CD$_2$Cl$_2$ at 25°C. * indicates residual protio solvent.
6.6.4 Electrochemical Graphs

6.6.4.1 Cyclic Voltammogram (CV) of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}]]$ (1-azido)

See Section 5.6.4.1 for CV spectrum of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_8\text{H}_{10}\text{N}_3)_{18}]]$ (6.1-azido).

6.6.4.2 Cyclic Voltammogram (CV) of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_{21}\text{H}_{22}\text{FeN}_3\text{O}_2)_{18}]]$ (6.1-ferrocenyl)

![Cyclic voltammogram](image)

Figure S6.7. Cyclic voltammogram of a 0.1 mM solution of $[(\text{CH}_3-(\text{CH}_2)_7\text{N})[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_{21}\text{H}_{22}\text{FeN}_3\text{O}_2)_{18}]]$ (6.1-ferrocenyl) in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.
6.6.4.3 Cyclic Voltammogram (CV) of $p$-azido-phenylethanethioacetate

![Cyclic Voltammogram](image)

**Figure S6.8.** Cyclic voltammogram of a 1 mM solution of $p$-azido-phenylethanethioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.

6.6.4.4 Differential Pulse Voltammogram (DPV) of $p$-azido-phenylethanethioacetate

![Differential Pulse Voltammogram](image)

**Figure S6.9.** Differential pulse voltammogram of a 1 mM solution of $p$-azido-phenylethanethioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.
6.6.4.5 Cyclic Voltammogram (CV) of Ferrocene-Triazole-Thioacetate

**Figure S6.10.** Cyclic voltammogram of a 3 mM solution of ferrocene-triazole-thioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.

6.6.4.6 Differential Pulse Voltammogram (DPV) of Ferrocene-Triazole-Thioacetate

**Figure S6.11.** Differential pulse voltammogram of a 3 mM solution of ferrocene-triazole-thioacetate in 1:1 acetonitrile:benzene, containing 0.1 M TBAP as the supporting electrolyte, with a scan rate of 100 mV/s. The arrows show the potential scanning direction.
6.6.5 References – Supporting Information

Chapter 7

7 Contributions and Future Perspectives

7.1 Contributions

Application-based research using polydisperse gold nanoparticles (AuNPs) and atomically precise gold nanoclusters (AuNCs) is contingent on synthetic strategies in which the composition of their surface structure can be modified in a reliable and efficient manner. As outlined in Section 1.2.4 and Section 1.3.5, there exists a delicate and modifiable relationship between the exhibited properties of these popular nanomaterials and the overall structure of the framework (including the surface structure), with small changes to the surface structure leading to well-defined changes to these structure-property relationships.\(^1\)\(^,\)\(^2\) In addition, the ability to tether functional substrates onto the surfaces of AuNPs and AuNCs renders these nanomaterial frameworks very promising platforms in a variety of applications such as biotechnology, nanomedicine and catalysis.\(^3\)\(^,\)\(^4\)

The traditional synthetic method for derivatizing the surfaces of AuNPs and AuNCs is ligand exchange, in which template frameworks (possessing inert thiolated ligands) are mixed with functional thiolated ligands (possessing the desired structure or functionality). Although ligand exchange strategies have found great utility, there are several problems associated with them: (1) It is often difficult to establish effective ligand exchange (2) it is difficult to tether reactive thiol groups to sensitive substrates and (3) changes to the ligand structure can alter the internal core framework (which is especially problematic with exchange strategies on the more chemically sensitive AuNC systems) (Section 1.4).

This thesis describes the use of two popular “bioorthogonal click reactions” (the strain-promoted alkyne-azide cycloaddition (SPAAC) reaction and strain-promoted alkyne-nitrone cycloaddition (SPANC) reaction) as an alternative and more reliable approach for derivatizing the surface structures of polydisperse AuNPs and atomically precise AuNCs, without the limitations inherent to ligand exchange strategies (Scheme 7.1). Of the bioorthogonal reactions developed to date, the SPAAC and SPANC reactions are among the most popular, because: (1) the two reactive partners are chemically stable (2) the two reactive partners chemoselectively react
with each other in the presence of other reactive moieties (3) can be performed at ambient, physiological conditions and (4) have fast and adjustable reaction kinetics.

The goal of this thesis is to transpose these advantageous characteristics of SPAAC and SPANC from the biologically sensitive settings in which they are primarily conducted to the chemically sensitive AuNP and AuNC surface settings. This approach not only allows for clean, quantitative and well-defined modifications to the surface corona, but by eliminating the necessity of risky ligand exchanges at the core, it also provides a means to orthogonally link functional substrate reporters to the AuNP/AuNC surfaces without altering the chemically sensitive core structures. In this way, this thesis demonstrates that the SPAAC and SPANC reactions are not only “bioorthogonal click reactions” but can also be classified as “nanoorthogonal click reactions”, allowing for nanoorthogonal and efficient surface modifications of AuNPs and AuNCs while retaining the internal parent frameworks.

Our group has previously explored the use of SPAAC and SPANC on the surfaces of AuNPs, in which azide-terminated, strained-alkyne-terminated, and nitrone-terminated AuNP
platforms have been reported, each of which could undergo interfacial SPAAC chemistry with the complementary reactive partner for nanoorthogonal surface modifications. We have also demonstrated that other bioorthogonal click reactions, such as the Staudinger-Bertozzi ligation and maleimide-thiol reaction, can be performed nanoorthogonally on AuNP surfaces. In our previous strained-alkyne-terminated AuNP platform, the benzoannulated strained-alkyne, dibenzocyclooctyne (DBCO), was incorporated onto the AuNP surface. Although this represented an exciting new approach for nanoorthogonal surface modifications of AuNPs, the main limitation was the instability of the benzoannulated DBCO moiety (see Section 1.5.5).

To address this limitation, Chapter 2 describes the development of an AuNP platform in which the less stable benzoannulated DBCO moieties were replaced with the more stable aliphatic strained-alkyne, \textit{exo}-bicyclo[6.1.0]nonyne (BCN\textit{exo}) (hereafter referred to as “AuNP-BCN”). The incorporation of the interfacial BCN\textit{exo} moieties was confirmed by $^1$H NMR and X-ray photoelectron spectroscopies. Interfacial SPAAC (I-SPAAC) and interfacial SPANC (I-SPANC) were demonstrated using a library of azides and nitrones, respectively. For the first time, it was shown that alterations to the kinetic profiles of SPAAC and SPANC (see Section 1.5.5) could be transposed to the AuNP-BCN platform, with more electron-deficient dipoles undergoing more rapid interfacial cycloaddition chemistry than their electron-rich counterparts (Scheme 7.2). In competition experiments between equimolar amounts of an azide, a nitrone and AuNP-BCN, exclusive reactivity with one dipolar species over the other was also demonstrated, which was dependent on the electronic composition of the dipolar species (Scheme 7.2).

Having established that I-SPAAC could be conducted to modify the surfaces of AuNPs, our group subsequently reported the development of a nitrone-functionalized AuNP platform which was capable of undergoing nanoorthogonal I-SPANC with complementary strained-alkynes. For this prototype study, the nitrone chosen possessed an electron-rich methyl group on the N\textalpha of the nitrone moiety and another electron-rich alkyl group on the C\textalpha of the nitrone moiety, which had slow reaction kinetics due to the two electron donating groups (see Section 1.5.5). Chapter 3 sought to explore the development of a more reactive nitrone-functionalized AuNP platform for more efficient and rapid surface modifications. This chapter describes the first synthesis of nitrones possessing highly electron-deficient pyridinium groups on the C\textalpha of the nitrone moiety, which resulted in substantial improvements to the kinetic profile of the SPANC.
reaction.\textsuperscript{12} The kinetics of these “pyridinium-nitrone” could be modulated by changing the $\text{Na}$ substituents, where an electron-rich anisole substituent resulted in kinetic deceleration, and an electron-deficient benzonitrile substituent resulting in a substantial kinetic acceleration (Scheme 7.3). In fact, density functional theory indicates that LUMO energy of this latter nitrone (“$\text{C}#$-pyridinium-$\text{Na}$-cyanophenyl nitrone”) has a similar energy to the HUMO energy of BCN, giving rise to the fastest cycloaddition reaction between a nitrone and BCN currently reported.\textsuperscript{12} To translate this rapid cycloaddition chemistry onto the surface of AuNPs, a thiolated ligand possessing the pyridinium-nitrone moiety was pursued, which could be incorporated to the AuNP surface using either a direct synthetic approach or ligand exchange strategy. However, unlike in our previously reported prototype study, the high reactivity of the pyridinium-nitrone moiety made it incompatible with the thiol moiety and the necessary deprotection of the thiol, preventing the development of such a thiolated ligand.
To date, our group has reported many examples of nanoorthogonal modifications of AuNPs using a variety of bioorthogonal click reactions. Although AuNPs have shown to be promising candidates for a variety of applications, atomically precise AuNCs have also shown great promise for application-based research. However, whereas the AuNP core frameworks are more rigid due to their larger size, and their internal structure is less sensitive to surface alterations, the relationship between the surface structure and metallic framework of monodisperse AuNCs is much more sensitive. Small structural changes to the protecting ligand can lead to larger changes to the nuclearity and configuration of the internal metallic framework of AuNCs. In this way, ligand exchange strategies at the metallic core are particularly problematic with AuNC systems, and so nanoorthogonal click chemistry at the surface corona (instead of at the metallic core) serves as a promising alternative to contemporary strategies to reliably derivatize the surface structures of AuNCs.

**Scheme 7.3. Chapter 3.** (a) Development of highly reactive pyridinium-nitrone for rapid and tunable SPANC chemistry. (b) Proposed synthesis for the development of pyridinium-nitrobenzyl-functionalized AuNP platform. However, the necessary thiolated-ligand possessing the terminal pyridinium-nitrobenzyl moiety could not be isolated.
To demonstrate nanoorthogonal click chemistry on AuNCs, the $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ framework was chosen, because it features superior chemical stability and ease of synthesis compared to other popular atomically precise AuNC systems. **Chapter 4** describes the first example of an azide-functionalized $[\text{Au}_{25}((\text{SCH}_2\text{CH}_2-p-C_6\text{H}_5-N_3)_{18})^{1-}]$ platform that was capable of undergoing nanoorthogonal cluster-surface SPAAC (CS-SPAAC) chemistry (Scheme 7.4). This was not only the first example of an $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ platform whose surface could be modified through nanoorthogonal click chemistry, but the first example of an AuNC system that could be modified using such a strategy. This platform was characterized by UV, IR, $^1$H NMR spectroscopies, mass spectrometry, and its molecular structure was confirmed by single crystal X-Ray diffraction. Quantitative consumption of all surface azides after the CS-SPAAC reaction with a small strained-alkyne, $($Z$)$-cyclooct-1-ene-5-yne, was also confirmed using the same spectroscopic techniques as before, although the molecular structure of this modified framework could not be acquired. This previously unreported nanoorthogonal approach represents an exciting new paradigm for efficient and reliable surface modifications of $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ frameworks with functional substrate reporter without changing the internal metallic nuclearity, for application-based research using $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ frameworks.

![Scheme 7.4](image_url)

**Scheme 7.4.** Chapter 4. Development of azide-functionalized $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ platform for nanoorthogonal CS-SPAAC chemistry for efficient and reliable surface modifications.

Having developed the azide-modified platform reported in **Chapter 4**, the relationship between the isomeric position of the azide moiety on the surface phenyl ligands and the related properties and reactivity were explored. **Chapter 5** describes the synthesis of two isomeric forms of the $[\text{Au}_{25}((\text{SCH}_2\text{CH}_2-p-C_6\text{H}_5-N_3)_{18})^{1-}]$ platform: the $[\text{Au}_{25}((\text{SCH}_2\text{CH}_2-m-C_6\text{H}_5-N_3)_{18})^{1-}]$ platform...
and the [Au25(SCH2CH2-o-C6H5-N3)18]1− platform (Figure 7.1). These previously unreported [Au25(SR)18]1− isomers were confirmed by UV and IR spectroscopy and mass spectrometry. Slow oxidation of the three anionic forms led to the neutral forms of the AuNCs forming, whose molecular structures were confirmed using single crystal X-ray diffraction. The CS-SPAAC chemistry was explored using the small strained-alkyne, (Z)-cycloct-1-ene-5-yne. As with the [Au25(SCH2CH2-p-C6H5-N3)18]1− platform, all the surface azides in the [Au25(SCH2CH2-p-C6H5-N3)18]1− platform were consumed while retaining the internal framework. Kinetic analysis indicates that the [Au25(SCH2CH2-p-C6H5-N3)18]1− platform had a higher reaction rate with BCNexo-OH compared to [Au25(SCH2CH2-m-C6H5-N3)18]1−, which is likely due to a steric effect. For the [Au25(SCH2CH2-o-C6H5-N3)18]1− platform, spectroscopic analysis and mass spectrometry indicate that the CS-SPAAC reaction is not nanoorthogonal to this azide-modified AuNC system, and the [Au25(SR)18]1− configuration appears to deteriorate after the surface reaction. This emphasizes the sensitive relationship between the structure of the protecting ligand and the configuration (and integrity) of the internal metallic framework.

**Figure 7.1.** Chapter 5. Investigation of isomeric effects of the azide position on the surface ligands in azide-functionalized [Au25(SR)18]1− platforms, and related properties, structure and surface reactivity.
Having developed the three clickable platforms and establishing that the $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_5-\text{N}_3)_{18}]^{1-}$ platform is the most reactive, **Chapter 6** focuses on incorporating a model functional substrate reporter to the surface of this azide-modified $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ platform. For this prototype study, a strained-alkyne-modified ferrocenyl moiety was nanoorthogonally tethered to the external interface of $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_5-\text{N}_3)_{18}]^{1-}$ through CS-SPAAC to give the $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_4-\text{C}_2\text{H}_2\text{FeN}_3\text{O}_2)_{18}]^{1-}$ framework (Scheme 7.5), which was confirmed by IR and $^1\text{H}$ NMR spectroscopy and mass spectrometry. UV-Vis spectroscopy demonstrates that the absorption fingerprint of ferrocenated AuNC changes due to the presence of the newly incorporated surface ferrocenyl groups. Most importantly, due to the two different stable charge states of each of the 18 surface ferrocene moieties, the electrochemical pattern of the ferrocenated AuNC possesses a large peak that is attributed to the surface ferrocenyl moieties. This not only confirms the incorporation of the ferrocenyl moieties, but the analysis signifies that all surface ferrocenyl groups are electrochemically active.

![Scheme 7.5](image)

**Scheme 7.5. Chapter 6. Development of ferrocene-modified $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ framework through nanoorthogonal CS-SPAAC chemistry.**

### 7.2 Future Perspectives

#### 7.2.1 General Comments

The research presented in this thesis examines conducting “nanoorthogonal” surface modifications of polydisperse AuNPs and atomically precise $[\text{Au}_{25}(\text{SR})_{18}]^{1-}$ AuNCs using two of the most
popular bioorthogonal click reactions. Whereas ligand exchange chemistry has found utility for surface derivatization, the practicality of this approach is primarily limited by (1) inefficient and/or ineffective exchanges at core that leads to poorly defined mixed surface systems and (2) ligand exchanges at the core can lead to alterations to the size, shape and nuclearity of the internal core structure, again leading to a poorly defined system. Furthermore, if multi-functional systems are required (e.g. one possessing a targeting agent and therapeutic agent for more targeted drug delivery than that described in Section 1.2.4 and Section 1.3.5), ligand exchange strategies prevents well-defined systems from being developed, which prevents conclusive and systematic analyses of how the application-based responses are related to the system being used.

The work presented in this thesis seeks to mitigate all the limitations of ligand exchange chemistry and presents nanoorthogonal strategies for clean, efficient, and high-yielding surface modifications of AuNPs and the \([\text{Au}_{25}(\text{SR})_{18}]\) system using the chemoselective and atom-efficient SPAAC and SPANC reactions. One particular advantage is that the methodologies presented herein navigates around the necessity of synthesizing thiolated ligands possessing functional substrates. This is dually restrictive by the incompatibility that often exists between the thiol moiety and desired substrate, as well as synthetic incompatibilities between protection/deprotection protocols of the thiol moiety and the desired substrate. Due to the comparative chemical stability of the reactive partners in SPAAC and SPANC, incorporation of the azide/nitrone moiety or the strained-alkyne moiety onto ligands possessing the desired substrate can be accomplished via substitution chemistry, in the absence of protection/deprotection strategies. This not only permits greater synthetic accessibility of functional varieties of AuNP and \([\text{Au}_{25}(\text{SR})_{18}]\) systems but also expands the range of the types and sizes of functional substrates that can be incorporated to their surfaces that cannot achieved via ligand exchange chemistry with thiolated ligands.

The research presented primarily explores prototype investigations in which quantitative consumption of the reactive groups are accomplished using model reactive partners. For example, in Chapter 6, the surface of the azide-functionalized \([\text{Au}_{25}(\text{SCH}_2\text{CH}_2-p-\text{C}_6\text{H}_5-\text{N}_3)_{18}]\) platform was quantitatively modified with the ferrocenyl substrate via nanoorthogonal CS-SPAAC to enhance the electrochemical response of the \([\text{Au}_{25}(\text{SR})_{18}]\) system. However, another general advantage of the methodologies presented is that they can be used to more reliably develop
multifunctional systems using multiplexes of functional reactive partners, since surface modifications is reliant on well-defined chemical transformations rather than poorly defined and inefficient exchange equilibria. In this way, future work can be directed towards semi-incorporation of property-altering substrates such as ferrocene, while also concurrently being able to incorporate other substrates to the surfaces of AuNPs and the [Au_{25}(SR)_{18}]^{1-} framework that can either further tune and alter the structure-property relationships (e.g. further enhance electrochemical responses) or target them for specific applications (e.g. incorporation of biomolecules or targeting agents for intracellular responses).

As outlined in Chapter 3, although a thiolated ligand possessing a pyridinium-nitrone moiety could not be achieved to incorporate this reactive moiety to the AuNP surface, our group is interested in translating the rapid reaction kinetics of pyridinium-nitrone to the surfaces of other materials systems that do not require the development of thiolated ligands. In particular, we have explored surface derivatization of carbonaceous materials (such as carbon nanotubes and glassy carbon electrodes), using the SPAAC reaction and diazarine-alkene reactions. Although these strategies represented a clean and reliable way to incorporate property-altering substrates to their surfaces to create hybrid carbonaceous nanomaterial systems, their efficiency was limited by slow reaction kinetics (using the SPAAC reaction) and necessity for photolytic conditions (using the diazarine-alkene reactions). To this end, execution of the rapid SPANC chemistry reported in Chapter 3 using pyridinium-nitrone serves as a more efficient derivatization method for carbonaceous materials.

7.2.2 Future Perspectives for AuNPs

Our group has previously explored the use of many bioorthogonal click reactions for surface modifications of AuNPs, including the Staudinger-Bertozzi ligation and thiol-maleimide reactions. The research presented in Chapter 2 expands upon our group’s toolbox for more efficient surface modifications of AuNPs that can be achieved via ligand exchange chemistry. In particular, the kinetically modifiable SPAAC and SPANC chemistry onto AuNP-BCN presented in Chapter 2 represents a very practical strategy towards developing multi-functional AuNP systems using a kinetically-directed approach, in which nitrone- and azide-functionalized substrates can be incorporated to the surface in a kinetically predictable manner. Our group is now interested in
translating such surface modifications through this kinetically-directed approach to ‘self-sorting’ applications, which encompasses the hierarchal construction of complex nanomaterial systems from simpler nanomaterial building blocks. Conventional self-sorting methodologies are reliant on chemically orthogonal ‘host/guest information’ between building blocks, in which building blocks possess reactive groups (host) on their surfaces that are known to react exclusively with complementary reactive groups (guest) on the surfaces of other building blocks. However, as presented in Chapter 2, the ability to kinetically isolate surface reactivity exclusively to one reactive partner in the presence of another, presents a new potential self-sorting paradigm, which is now reliant on well-defined ‘kinetic information’ that is inherent to the electronic composition of the azide or nitrone species on other building blocks.

7.2.3 Future Perspectives for AuNCs

Whereas application-based research using AuNPs is quite mature, the more recent discovery of AuNCs renders their application-based research field quite underdeveloped, and there is an ongoing pursuit in contemporary literature to explore how these promising nanomaterial systems can be exploited in different scientific disciplines (see Section 1.3.5). Having developed an extensive toolbox of surface derivatization strategies for AuNP surfaces using nanoorthogonal click chemistry, our group has now become interested in exploring such strategies on the surfaces of AuNCs to similarly derivatize their surfaces with functional reporter substrates. As their configurations are more reminiscent of molecular species compared to larger plasmonic AuNP structures, the structure of AuNCs is very sensitive to the structure of the protecting surface ligand. This poses a particular challenge to derivatize their surfaces, as ligand exchange at the core with non-native ligands very often leads to either deterioration of the internal core or a change in its nuclearity and size. For this reason, nanoorthogonal click chemistry serves as a promising new paradigm to conduct derivatization on the external interface of AuNCs (instead of relying on risky exchanges at the core) to create functional varieties of these nanomaterial systems.

In Chapter 4, the first example of an AuNC system (specifically the [Au25(SR)18]1− system) capable of undergoing post-assembly surface chemistry was reported, in which the surface of this azide-functionalized AuNC platform can be modified nanoorthogonally via CS-SPAAC chemistry. Having developed three isomeric forms of this platform in Chapter 5, and
authenticating that the para-isomer is the most reactive, a functional ferrocene reporter was incorporated to the surface of this platform in Chapter 6 to create a prototypical functional variety of the [Au25(SR)18]1- system that had an enhanced electrochemical response. The establishment of this foundation for nanoorthogonal surface modifications of the [Au25(SR)18]1- system set in this thesis inaugurates a new and previously unrealized scope for surface derivatization of this nanomaterial system with functional substrates that up to this point could not be incorporated using other strategies. As outlined in Section 1.3.5, the [Au25(SR)18]1- system is a promising candidate for applications such as drug delivery, bioimaging and catalysis, requiring surface derivatization with therapeutic agents, fluorophores, and structurally complex organic functionalities, respectively. Such applications have so far relied on risky ligand exchanges to incorporate such functionality to the [Au25(SR)18]1- surface to tailor their structure-property relationships and surface compositions to the desired application. The nanoorthogonal approaches for surface derivatization presented herein presents an alternative and more reliable method to

Figure 7.2. Development of functional [Au25(SR)18]1- systems using nanoorthogonal CS-SPAAC chemistry for applications in drug delivery, bioimaging and catalysis.
incorporate such functional substrates (Figure 7.2), without risking the integrity and configuration of the internal structure, which broadens the range of applications that the \([\text{Au}_{25}\text{(SR)}_{18}]^{1-}\) system can be used for. Furthermore, having established a prolific nanoorthogonal toolbox for surface modifications of AuNPs using the assortment of bioorthogonal click reactions available to date, future work will be directed towards transposing this toolbox to the \([\text{Au}_{25}\text{(SR)}_{18}]^{1-}\) system. Described in Section 1.5.2, bioorthogonal click reactions such as the Staudinger-Bertozzi ligation, the trans-cyclooctene-tetrazine ligation and azide-oxanorbornadiene cycloaddition reactions also serve as promising candidates for the modification of \([\text{Au}_{25}\text{(SR)}_{18}]^{1-}\) without the necessity of risky ligand exchange strategies.

7.3 References


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A.2 Curriculum Vitae

EDUCATION

**Doctor of Philosophy** *(Organic Chemistry)*  
*University of Western Ontario, London, Ontario*  
2021

**Bachelor of Science** *(Honors Specialization in Chemistry and Biochemistry)*  
*The University of Western Ontario, London, Ontario*  
2015

- Relevant Courses – Organic Chemistry, Inorganic Chemistry, Physical Chemistry, Analytical Chemistry, Quantum Chemistry, Biochemistry, Genetics, Molecular Biology, Biological Macromolecules, Cell Biology

**Ontario College Diploma** *(Chemical Laboratory Technology)*  
*Seneca College, York, Ontario*  
2012

- President’s Honor List
- Relevant Courses: General Chemistry, Organic Chemistry, Applied Inorganic Chemistry, Chemical Instrumentation, Biology, Biochemistry, Physics, Pharmaceutical Analysis, Techniques in Analytical Chemistry, Introductory Microbiology, Industrial Microbiology

**Ontario Secondary School Diploma**

*Crescent High School, Toronto, Ontario*

**GCE Ordinary Level Diploma**

*Saltus Grammar School, Hamilton, Bermuda*

RESEARCH EXPERIENCE

**Organic Chemistry Summer Research Scholar**  
*University of Western Ontario – Mark Workentin Research Group*  
Apr 2015 – Aug 2015

**Organic Chemistry Pre-Thesis Summer Research Scholar**  
Apr 2014 – Aug 2014
University of Western Ontario – Mark Workentin Research Group

- Responsible for assisting PhD candidate and member of Workentin research group, Pierangelo Gobbo.
- Projects involved in: Rhodamine (fluorescent dye) synthesis and potential nanomaterial applications.

Organic Chemistry Volunteer Research Assistant  
May 2013 – Apr 2014
University of Western Ontario – Mark Workentin Research Group

- Responsible for assisting PhD candidate and member of Workentin research group, Mahdi Hesari.
- Projects involved in: BODIPY (fluorescent dye) synthesis and potential nanomaterial applications, diazarine synthesis and potential applications.
- Independently responsible for synthetic procedures assigned and applications in gold nanoparticle and graphene research.

Organic Chemistry Laboratory Technician  
Jan 2011 – Oct 2011
AGAT Laboratories

- Responsible for gathering samples from incoming clients and distributing samples to appropriate laboratory sections.
- Responsible for government-mandated CCME determinations of hydrocarbon levels in both water and soil samples sent in from clients.
- Responsible for proper disposal of waste materials and glassware.
- Responsible for dispersal of incoming materials and solvents to appropriate laboratory sections.

SCHOLARSHIPS AND ACADEMIC HONOURS

Ontario Graduate Scholarship 2018
Dean’s Honor List 2015
PRESENTATIONS

Canadian Society for Chemistry (CSC) Conference June 2019

- Oral Presentation: “Synthesis of Bicyclononyne-Functionalized Gold Nanoparticles for Variable and Competitive Interfacial Strain-Promoted Alkyne-Azide Cycloaddition”

Physical Organic Mini-Symposium (POMS) Conference Nov 2017

- Oral Presentation: “Kinetically Modifiable Interfacial Strain-Promoted Alkyne/Nitrone Cycloaddition for Tunable Modifications of Gold Nanoparticles”
- Awarded 1st Prize for Oral Presentation

Canadian Society for Chemistry (CSC) Conference May 2017

- Oral Presentation: “TheClickable Gold Nanoclusters: An Exciting New Chapter in Au_{25}SR_{18} Chemistry”

PUBLICATIONS


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General Laboratory Safety and Hazardous Waste Management 2014

Comprehensive WHIMIS Certification