Development of Bioorthogonal Molecular Tools on Gold Nanoparticles

Wilson Luo, The University of Western Ontario

Supervisor: Workentin, Mark S., The University of Western Ontario
A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Chemistry
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Abstract

This thesis describes the development of bioorthogonal chemical tools — originally designed to form bonds cleanly and selectively in living systems — on gold nanoparticles (AuNPs) as a model reactive nanomaterial template to showcase chemical modifications in a facile and robust manner. To achieve this goal, new methodologies to cleanly incorporate strained alkyne (SA) and cargo-bearing triarylphosphine derivatives onto AuNPs were developed. The protocols described herein provide well-defined reactive AuNP interfaces that undergo bioorthogonal bond-forming and breaking reactions cleanly, selectively, and rapidly to enable chemical tuning of their properties and function.

In order to circumvent the high reactivity of SAs, which hindered their incorporation onto AuNPs, a cyclopropenone-caging strategy was employed to successfully mask the SA until post-AuNP incorporation. Interfacial cyclopropenones were photochemically decarbonylated using ultraviolet A (UV-A) irradiation, which proceeded cleanly and rapidly, to afford the unmasked SA moieties. This versatile reactive AuNP template exhibited rapid reactivity with azides (via strain-promoted alkyne-azide cycloaddition, SPAAC) and nitrones (via strain-promoted alkyne-nitron cycloaddition, SPANC) to form robust covalent bonds with the partner molecule, which provides an efficient and reliable route towards derivatizing AuNP surfaces.

To further expand the scope of bioorthogonal chemistry on AuNPs, cargo-bearing triarylphosphines were used to demonstrate the release of molecules off AuNPs via the Staudinger-Bertozzi ligation (SBL) with azides. It was shown that functionalized AuNPs undergo SBL in a highly specific manner to release a Rhodamine B dye — a model cargo — from its surface. The release event was monitored from immediate turn-on of fluorescence upon treatment with an azide. Building on this “click-to-release” design, a dual-bioorthogonal molecular tool was developed to feature unprecedented versatile reactivity on AuNPs. Four bioorthogonal transformations (SPAAC, SPANC, SBL, and a modified perfluoroaryl Staudinger reaction, PFAA-SR) were deployed on AuNP surfaces to demonstrate the clean and versatile reactivity this new chemical tool offers.
In total, this thesis work describes innovative strategies to successfully incorporate bioorthogonal functionalities onto AuNPs, methods to quantitate and follow their interfacial chemistry, and the creation of versatile reactive AuNP templates that can be chemically modified with ease under mild and biocompatible conditions.

Keywords

Gold Nanoparticles, Nanomaterials, Bioorthogonal Chemistry, Click Chemistry, Interfacial Reactions, Bioconjugation, Surface Functionalization, Strained Alkyne, Strain-promoted Cycloadditions, Staudinger-Bertozzi Ligation.
Co-Authorship Statement

The work described in this thesis contains contributions from the authors and coworkers Pierangelo Gobbo, Praveen N. Gunawardene, Christopher D. McNitt, Dewey A. Sutton, Johnny Luo, Prof. Vladimir V. Popik, 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. Christopher McNitt and Dewey Sutton, supervised by Prof. Popik, synthesized and supplied the cyclopropenone starting materials. Pierangelo Gobbo conducted the TEM measurements of AuNP samples. All other experiments were performed by the author. The manuscript was written by the author, with input from Pierangelo Gobbo, Prof. Popik, and Prof. Workentin.

Chapter 3 describes a series of compounds and AuNPs that were synthesized and characterized, for the most part, by the author. Praveen Gunawardene synthesized and supplied the Rhodamine B starting material. Pierangelo Gobbo conducted the TEM measurements of AuNP samples. All other experiments were performed by the author. The manuscript was written by the author, with input and final edits from Prof. Workentin.

Chapter 4 describes a project in which all the synthesis and characterization were completed by the author. Johnny Luo took lead on the cell labelling experiments and fluorescence imaging. The manuscript was written by the author, with input from Prof. Popik and Prof. Workentin.

Chapter 5 was written by the author and edited by Prof. Mark S. Workentin.
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<tbody>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>carbon-13</td>
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<tr>
<td>$^{1}$H</td>
<td>proton</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>phosphorus-31</td>
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<tr>
<td>µL</td>
<td>microliter</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>µmol</td>
<td>micromole</td>
</tr>
<tr>
<td>AAC</td>
<td>alkyne-azide cycloaddition</td>
</tr>
<tr>
<td>AuNP</td>
<td>gold nanoparticles</td>
</tr>
<tr>
<td>AuNR</td>
<td>gold nanorods</td>
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<td>AuNS</td>
<td>gold nanostars</td>
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<td>A.U.</td>
<td>arbitrary units</td>
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<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>ADIBO</td>
<td>azadibenzocyclooctyne</td>
</tr>
<tr>
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<td>azidothymidine</td>
</tr>
<tr>
<td>BARAC</td>
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</tr>
<tr>
<td>BCN</td>
<td>bicyclononyne</td>
</tr>
<tr>
<td>BnN$_3$</td>
<td>benzyl azide</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
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<td>Bu</td>
<td>butyl</td>
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<td>CPP</td>
<td>cell penetrating peptide</td>
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<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>CuAAC</td>
<td>copper(I)-assisted alkyne-azide cycloaddition</td>
</tr>
<tr>
<td>D</td>
<td>doublet</td>
</tr>
<tr>
<td>DA</td>
<td>Diels-Alder</td>
</tr>
<tr>
<td>DAPI</td>
<td>4,6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>deuterated water</td>
</tr>
<tr>
<td>DIBAC</td>
<td>dibenzoazacyclooctyne</td>
</tr>
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<tr>
<td>DIFO</td>
<td>difluorinated cyclooctyne</td>
</tr>
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<td>DIPEA</td>
<td>$N,N$-diisopropylethylamine</td>
</tr>
<tr>
<td>DNA</td>
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</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
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</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EG</td>
<td>ethylene glycol unit</td>
</tr>
<tr>
<td>EG$_3$</td>
<td>triethylene glycol</td>
</tr>
<tr>
<td>EG$_4$</td>
<td>tetraethylene glycol</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>electrospray ionization-mass spectrometry</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>Et$_2$O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier-transform</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GFP</td>
<td>green fluorescence protein</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>hv</td>
<td>light</td>
</tr>
<tr>
<td>HRTEM</td>
<td>high resolution transmission electron microscopy</td>
</tr>
<tr>
<td>hvDIBO</td>
<td>cyclopropenone-caged dibenzocyclooctyne</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>water</td>
</tr>
<tr>
<td>HBTU</td>
<td>2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
</tbody>
</table>
IHC  immunohistochemistry
iPr  isopropyl
i-SPAAC  interfacial strain-promoted alkyne-azide cycloaddition
i-SPANC  interfacial strain-promoted alkyne-nitrone cycloaddition
IR  infrared
J  coupling constant
K  degrees Kelvin
k  rate constant
k$_2$  bimolecular rate constant
kDa  kilodalton
kJ  kilojoule
LCST  lower critical solution temperature
LDPE  low density polyethylene
m  multiplet
M  molar
Me  methyl
MHz  megahertz
min  minutes
mL  milliliter
mM  millimolar
MOFO  monofluorinated cyclooctyne
mol  mole
MWCO  molecular weight cutoff
NHS  N-hydroxysuccinimide
NIR  near-infrared
nm  nanometer
NMR  nuclear magnetic resonance
OCT  cyclooctyne
PBS  phosphate buffer solution
PDT  photodynamic therapy
PET  positron emission tomography
PFAA-SR  perfluoroaryl azide Staudinger reaction
Ph  phenyl
pNIPAM  poly(N-isopropylacrylamide)
ppm  parts per million
q  quartet
QD  quantum dots
Rf  retention factor
RGD  arginylglycylaspartic acid peptide motif
Rhod  Rhodamine B
ROS  reactive oxygen species
RSH  thiol molecule
rpm  revolutions per minute
s (in NMR data)  singlet
s  second
SBL  Staudinger-Bertozzi ligation
SERS  surface-enhanced Raman spectroscopy
SPAAC  strain-promoted alkyne-azide cycloaddition
SPANC  strain-promoted alkyne-nitrone cycloaddition
SPR  surface plasmon resonance
t  triplet
TBAF  tetrabutylammonium fluoride
TBDMS  tert-butyldimethylsilyl
TBHP  tert-butylhydroperoxide
TEM  transmission electron microscopy
TFA  trifluoroacetic acid
TGA  thermogravimetric analysis
THF  tetrahydrofuran
THPTA  tris(3-hydroxypropyltriazolylmethyl)amine
TMDIBO  tetramethoxydibenzocyclooctyne
TOAB  tetraoctylammonium bromide
UV-A  ultraviolet A
UV-vis  ultraviolet-visible
VEGF  vascular endothelial growth factor
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>XPS</td>
<td>x-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>ZE/ZR</td>
<td>leucine zipper pairs</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>wavelength</td>
</tr>
<tr>
<td>( \lambda_{\text{em}} )</td>
<td>wavelength of emission</td>
</tr>
<tr>
<td>( \lambda_{\text{ex}} )</td>
<td>wavelength of excitation</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} )</td>
<td>wavelength of maximum absorption</td>
</tr>
<tr>
<td>( \rho )</td>
<td>density</td>
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Chapter 1

1 Introduction – Bioorthogonal Chemistry for Materials Modification

1.1 Bioorthogonal Chemistry

Over the last century, chemists and biologists have shared a common interest in developing chemical tools to study biological molecules and processes within their native environment. In 2008, the Nobel Prize in Chemistry was shared between Shimomura, Chalfie, and Tsien to recognize their pioneering work that led to “the discovery and development of the green fluorescence protein, GFP.”[1-2] The development of genetically encoded GFP revolutionized the tracking and detection of native proteins in living systems; however, GFP’s large size can cause significant perturbations to a target protein’s structure — and consequently, function — and lacks amenability to biological reporters that are not genetically encoded such as lipids, glycans, and nucleic acids. To address this deficiency, an alternative tool for tagging biomolecules has emerged from the chemical biology community — the bioorthogonal chemical reporter.[3-6]

A bioorthogonal chemical reporter strategy involves two distinct steps: (1) incorporation of a unique chemical motif, often as small as a single functional group, into the target biomolecule using the living system’s own biosynthetic metabolism; (2) this motif, termed the chemical reporter (blue circle, Figure 1.1), must react selectively with a probe (yellow star) bearing the complementary chemical moiety (blue arc) while avoiding side reactions with nontarget biomolecules (grey shapes).[3] This bond-forming transformation between two components, along with its stringent requirements, is known as a bioorthogonal reaction.[4-7] A prototypical bioorthogonal reaction must satisfy most if not all of the following requirements:

1. The chemical reporter and its partner must be mutually and selectively reactive within a physiological setting (in H₂O, 37 °C, pH 6–8) while remaining inert to the myriad of functionalities found in living systems;
2. The covalent reaction should be rapid, unaided by additional reagents, to form a stable adduct with innocuous (or no) byproducts;

3. The chemical reporter and its installation process should be tolerated enzymatically and not result in the perturbation of a biomolecule’s native structure or function. As such, a small abiotic functional group that goes ‘unnoticed’ by biosynthetic pathways, such as the azide, is often employed.

![Figure 1.1](image)

**Figure 1.1.** The bioorthogonal chemical reporter strategy. A chemical reporter (blue circle) linked to a substrate (light green box) is introduced into a target biomolecule through cellular metabolism. In a second step, the reporter is covalently tagged with an exogenously delivered probe (blue arc). Both the chemical reporter and exogenous probe must avoid side reactions with nontarget biomolecules (grey shapes). Figure and caption reproduced with permission from Ref. [3].

These stringent requirements, along with the fact that nucleophiles, oxidants, and reductants are ubiquitous in living systems, means that suitable moieties for this covalent reaction are far from obvious. Despite these challenges, in the last two decades, a handful of bioorthogonal reactions have been developed.

### 1.1.1 Development of Bioorthogonal Reactions

In 2001, Sharpless introduced to the world the concept of ‘click chemistry’.[8] Although at times loosely defined, click reactions share some common characteristics[9-11]:

1. The reaction should be rapid, high yielding, and wide in scope to generate a stable adduct;

2. The transformation should proceed in simple reaction conditions and, ideally, is insensitive to water and oxygen;

3. The reaction should generate only inoffensive (or no) byproducts that, if present, can be removed without chromatography.
The fact that these characteristics are similar to that of bioorthogonal chemistry is no coincidence — bioorthogonal reactions are simply a subset of click reactions with the added constraint of biocompatibility.\textsuperscript{[11]} Therefore, the development of click and bioorthogonal reactions often occurred concurrently. An obvious starting point was the organic azide (R–N$_3$), underscored by its small size, inert nature to many chemical and biological functionalities while remaining energy-rich, and flexible reactivity.\textsuperscript{[11-13]}

1.1.1.1 Huisgen Cycloadditions to Alkynes

The potential of the organic azide to act as a dipole for forming stable bonds was popularized by Huisgen in 1963, although its discovery dates back to the late 1800s.\textsuperscript{[14]} Considered to be one of the most useful in the cycloaddition family of reactions, the cycloaddition of azides and acetylenes to give stable triazoles features high functional group tolerance and extraordinary stability towards H$_2$O and O$_2$. However, this transformation is sluggish even at elevated temperatures and affords a mixture of 1,4- and 1,5-disubstituted triazoles (Scheme 1.1).\textsuperscript{[14]} Sharpless and Meldal brought the acetylene-azide Huisgen cycloaddition back into the spotlight in 2002 with their independent reports detailing the discovery that copper(I) salts catalyze the reaction, which proceeds at room temperature and features “near perfect regioselectivity” to afford 1,4-disubstituted 1,2,3-triazoles in markedly improved yields (Scheme 1.1).\textsuperscript{[15-16]} This discovery established the Cu(I)-assisted alkyne-azide cycloaddition (CuAAC) as the click reaction benchmark and immediately led to a renaissance of Huisgen cycloadditions and their application in synthetic organic chemistry, medicinal chemistry, materials and biomaterials science. Namely, the CuAAC enabled the construction of more advanced and complex structures that were previously difficult or impossible to obtain, such as the development of diverse polymer architectures,\textsuperscript{[17-18]} biomaterials,\textsuperscript{[19]} drug discovery,\textsuperscript{[20]} and functional nanoparticles.\textsuperscript{[21]} However, its use in living systems was limited due to the cytotoxicity of excess Cu(I) that mediates the generation of reactive oxygen species (ROS), which degrades delicate biomolecules.\textsuperscript{[22-24]} Therefore, the CuAAC precluded applications wherein cells must remain viable.

Alkyne-azide cycloadditions (AACs) that are activated via other mechanisms were investigated by the synthetic community to circumvent the obstacle of Cu(I) cytotoxicity.
Ju and coworkers demonstrated that the activation of alkynes by electron-withdrawing substituents (such as an ester, 1.6) enabled the AAC to occur at ambient temperatures in water without the need of Cu(I) additives (Scheme 1.2). However, these compounds were susceptible to Michael additions with biological nucleophiles. Instead, Bertozzi and coworkers explored the activation of alkynes by means of ring strain, which later became a landmark discovery known as the strain-promoted alkyne-azide cycloaddition (or SPAAC, Scheme 1.2). Wittig and Krebs first noted such reactivity between neat cyclooctyne and phenyl azide, which “proceeded like an explosion to give a single product”. This unusual high reactivity, when compared to linear alkynes, is due to the massive bond angle deformation of the acetylene to 163° when incorporated in a cyclooctyl ring, which accounts for 18 kcal/mol of ring strain. This destabilization of the ground state provides a dramatic rate acceleration compared to unstrained alkynes. Bertozzi employed this unique reactivity to demonstrate SPAAC between 1.8 and 1.9, unaided by Cu(I), to afford an equimolar mixture of triazoles 1.10 (Scheme 1.2). The authors measured bimolecular rate constants (k₂) ranging from 10⁻⁴ to 10⁻³ M⁻¹s⁻¹ in CD₃CN, which is several orders of magnitude slower than those observed for CuAAC. Despite having only moderate rates, they successfully employed the SPAAC for selective modification of biomolecules and living cells with no negative effects on cell viability, i.e. it was bioorthogonal. This represented the onset of rapid advancement in the development of new alkyne reagents that feature increased reactivity towards azides.
Electronic activation of alkyne-azide cycloadditions

\[
\begin{align*}
\text{N}_3 & \quad \text{O} \\
\text{Et}_{1.5} & \quad \text{Et}_{1.6} \quad \text{H}_2\text{O}, \text{ r.t.} \\
\rightarrow & \quad \text{EtOOC-} \quad \text{N} \quad \text{N} \\
& \quad \text{EtOOC-} \quad \text{H}_3 \text{C} \quad \text{COOEt} \\
& \quad \text{1.7}
\end{align*}
\]

Strain-promoted alkyne-azide cycloaddition (SPAAC)

\[
\begin{align*}
\text{N}_3 & \quad \text{O} \\
\text{1.8} & \quad \text{HOOC} \\
\text{HOOC} & \quad \text{H}_2\text{O/CD}_3\text{CN}, \text{ r.t.} \\
\rightarrow & \quad \text{regioisomer} \\
& \quad \text{1.10}
\end{align*}
\]

Scheme 1.2. Early examples of AACs without the use of Cu(I) additives.

Efforts to enhance cyclooctyne reactivity towards SPAAC focused on two key design features: 1) addition of fluorine groups as electron-withdrawing substituents or 2) fusion of pendant ring systems onto the cyclooctyne core and/or sp\(^2\) hybridization of ring atoms to increase strain energy.\(^{[4,29-32]}\) Additionally, considerations regarding synthetic accessibility and availability, shelf and aqueous stability, and lipophilicity — which can lead to nonspecific “sticking” to other biomolecules and insertion into cell membranes — needed to be made.\(^{[4,29]}\) To this end, a library of cyclooctynes, all featuring chemical handles for functionalization, have been developed for use in living systems (Figure 1.2). Figure 1.2 is not meant to be exhaustive in representing all available cyclooctyne reagents in literature, but illustrative of key developments in the structural and kinetic tuning of alkynes since Bertozzi’s original cyclooctyne (1.9).\(^{[29]}\) Furthermore, although less extensively studied, electronic tuning of azide reagents was also developed to feature k\(_2\) values up to 2.0 M\(^{-1}\)s\(^{-1}\) with bicyclononyne 1.18 (Figure 1.2) in CH\(_3\)CN/H\(_2\)O, which is comparable to those observed for CuAAC.\(^{[33]}\)

The addition of alternative 1,3-dipoles to strained alkynes was also explored. Pezacki and van Delft groups independently pioneered the use of nitrones for 1,3-dipolar cycloaddition with cyclooctynes, known as the strain-promoted alkyne-nitrone cycloaddition (SPANC), to afford stable isoxazolines.\(^{[34-36]}\) The authors demonstrated rapid and clean reactivity as
Figure 1.2. Structurally modified cyclooctyne reagents for SPAAC. Figure partially reproduced with permission from Ref. [32] with 1.18 BCN structure added from Ref. [33]. The second-order rate constants are for the reaction with benzyl azide 1.8 in CH$_3$CN (1.9, 1.11, 1.12, 1.13, 1.15), methanol (1.14, 1.16, 1.17), or 3:1 CH$_3$CN/H$_2$O (1.18). OCT = cyclooctyne (1st generation); ALO = “aryl-less octyne”; MOFO = monofluorinated cyclooctyne; DIFO = difluorinated cyclooctyne; DIBO = dibenzocylooctyne; BARAC = biarylazacyclooctynone; DIBAC = dibenzoazacyclooctyne; TMDIBO = tetramethoxydibenzocylooctyne; BCN = bicyclononyne.

well as its amenability for live cell imaging and protein modification.[35-36] Although not as widely employed as its azide counterpart due to synthetic challenges and its larger size, nitrones can potentially offer additional utility due to its rapid reactivity (k$_2$ up to 60 M$^{-1}$s$^{-1}$) and their ability to bear up to three R-groups.[37]

1.1.1.2 Staudinger Ligation

It was nearly a century ago that Staudinger discovered that organic azides and trivalent phosphines undergo imination to afford iminophosphoranes (Scheme 1.3).[38-39] Now
known as the Staudinger reaction, this imination usually proceeds smoothly and quantitatively via a two-step process: initial electrophilic addition of an azide (e.g. 1.8) to a P^{III} center (e.g. 1.19) to give a phosphazide intermediate (1.20), which undergoes intramolecular rearrangement (via a 4-membered ring transition state) and N₂ elimination to afford the iminophosphorane 1.21 (Scheme 1.3).[^39-40] Bertozzi recognized several key features of this transformation that satisfy the bioorthogonality criteria. Firstly, phosphine and azide react with each other rapidly in water at room temperature in high yield. Secondly, only N₂ is produced as a byproduct. Last and most importantly, both reactive partners are abiotic and essentially unreactive towards biomolecules inside or on the surface of cells.[^41] The only drawback is that the covalent adduct, iminophosphorane 1.21, is unstable and hydrolytically cleaves to afford phosphine oxide 1.22 and amine 1.23 (Scheme 1.3). Although synthetically useful for the reduction of azides to amines,[^42-43] the Staudinger reaction did not provide the means to robustly tag chemical reporters in living systems.

Bertozzi and coworkers resolved this problem by designing a phosphine that would enable rearrangement of the unstable intermediate iminophosphorane to a stable covalent adduct. In their seminal work,[^41] the authors demonstrated that an appropriately situated electrophilic trap — a methyl ester — within the phosphine structure would capture the nucleophilic iminophosphorane via intramolecular cyclization and ultimately lead to the formation of a stable amide bond rather than hydrolysis products 1.22 and 1.23. This revision of the classic Staudinger reaction became known as the Staudinger-Bertozzi ligation (or simply Staudinger ligation) and represented the first truly bioorthogonal reaction. Soon after, Bertozzi and coworkers elucidated the mechanism of this bioorthogonal transformation (Scheme 1.3).[^40] Proceeding similarly to the Staudinger reaction for the imination steps, iminophosphorane 1.26 results from the reaction between azide 1.8 and phosphine 1.24. Unlike intermediate 1.21, which is unstable and hydrolyzes in H₂O, iminophosphorane 1.26 cyclizes intramolecularly to give the cyclic intermediate 1.27 and the methoxide anion (CH₃O⁻). In the presence of water, 1.27 hydrolyzes to afford the stable adduct 1.28 and methanol (CH₃OH). Using this new ligation strategy, Bertozzi successfully demonstrated that cell surface azides — preinstalled via the cell metabolism of synthetic azidosugars — could be used for cell surface engineering with
Scheme 1.3. Mechanistic depiction of the prototypical Staudinger reaction and Staudinger-Bertozzi ligation between benzyl azide 1.8 and triarylphosphines 1.23 and 1.26.

Further developments of the Staudinger-Bertozzi ligation (SBL) focused on accelerating the transformation. Initial efforts showed that substituents on the phenyl ring of the phosphine (1.29a-c, Scheme 1.4) had a pronounced effect on the overall rate.\textsuperscript{[40]} It was found that electron-donating substituents accelerated the SBL (i.e. rates of reaction with
benzyl azide for $1.29c > 1.29b > 1.29a$) as it favoured nucleophilic attack of the phosphine on the azide, the rate determining step. However, increased electron density on the phosphorous atom rendered the more reactive substrates to rapidly oxidize in air to phosphine oxides, which are unreactive and diminished their utility.\cite{132} Alternatively, Bertozzi demonstrated that electronic modification of the azide partner to aryl azides with varying substituents (1.31a-c, Scheme 1.4) resulted in iminophosphoranes that were too stable and exhibited slow amide formation.

Scheme 1.4. Substituent effects on the rate of the SBL.
In contrast to the reaction between benzyl azide 1.8 and phosphine 1.24, in which phosphazide formation was rate limiting, reactions between 1.24 and aryl azides 1.31a-c converted to intermediates 1.32a-c rapidly in <5 min. Instead, the intramolecular amide bond-forming step became rate limiting and required up to 48 hours for complete conversion to the ligation products (1.33a-c). Presumably, resonance stabilization of iminophosphoranes 1.32a-c reduced their reactivity and nucleophilicity relative to that generated between benzyl azide and phosphine 1.24.

Despite challenges with improving the intrinsic kinetics of the SBL, other developments to enhance their utility were made and reinforced the SBL as the bioorthogonal reaction of choice for many years. Examples include the development of “traceless”[44] and light-activated variants[45-47] that either excludes the unnatural phosphine oxide moiety from the ligated product or improves stability towards air oxidation and enables spatiotemporal resolution, respectively.

1.1.1.3 Other Bioorthogonal Reactions

Despite such stringent requirements as those outlined in bioorthogonality, the creation of SPAAC and SBL methodologies inaugurated the field of bioorthogonal chemistry and thus initiated the search for other chemical functionalities that fit these criteria. Over the last two decades, a number of bioorthogonal reactions have been successfully developed that have shown excellent biocompatibility and selectivity in living systems.[4,48-49] Lin and coworkers demonstrated a mild, photoactivated 1,3-dipolar cycloaddition through in situ generation of nitrile imines from tetrazoles using 302 nm light, which reacts rapidly with dipolarophiles to afford stable polysubstituted pyrazolines (Scheme 1.5).[50] Termed the tetrazole photoclick, the authors later demonstrated its use in the robust labelling of proteins in biological media, which yielded fluorescent pyrazoline adducts and allow for in situ monitoring.[51] Rutjes and coworkers demonstrated that azides react with oxanorbornadienes via tandem [3+2] cycloaddition – retro Diels-Alder to eliminate furan and form stable triazoles (Scheme 1.5).[52] Although it was shown to be effective for the bioconjugation of radiolabeled probes to peptides,[53] in vivo studies were never reported. Furthermore, tetrazines exhibit unique [4+2] Diels-Alder cycloadditions with strained olefins (e.g. transcyclooctene[54] and norbornene[55]) followed by rearrangement to
eliminate N₂ and form stable adducts (Scheme 1.5). This tetrazine ligation features $k_2$ values ranging from $10^1$ to $10^3$ M$^{-1}$ s$^{-1}$ and is among the fastest in bioorthogonal chemistry. In addition to those described above, new chemical ligation strategies are reported every year,[49] which offers exciting applications as these methodologies are further investigated and refined. However, among those in the bioorthogonal toolkit, the SPAAC and SBL are considered the most mature in the field and commercially relevant. Due to their high synthetic accessibility and small size, the catalog of organic azides — ranging from small molecules to large biomolecules — available commercially is by far the most extensive compared to other bioorthogonally reactive functionalities. Thus, this thesis examines these two transformations in focus.

Scheme 1.5. Prototypical representations of other bioorthogonal reactions.

1.1.2 Applications of Bioorthogonal Chemistry in Living Systems

Our understanding of living systems is refined by the chemical tools available for labelling and tracking biomolecules and their associated processes. The development of
bioorthogonal ligation strategies enabled the robust tagging of proteins, nucleic acids, lipids and glycans in real time. Since Bertozzi demonstrated cell surface glycan labeling using the SBL, many other applications have emerged in chemical biology. Several examples are highlighted below.

Boons and coworkers utilized the SPAAC reaction on metabolically installed azidosugars to track relative quantities of sialic acid in living cells that have defects in glycosylation. Their investigation revealed that cells with defects in the conserved oligomeric Golgi complex are impacted more greatly on O-glycan sialylation than galactosylation. Kim and coworkers reported the successful pretargeted labelling of tumour cells utilizing the SPAAC reaction in live mice with a $^{18}$F-labeled azide. Positron emission tomography (PET) imaging confirmed high tumor uptake of the radioprobe compared to the control, which was enabled by the in vivo SPAAC reaction. Bertozzi and coworkers reported noninvasive imaging of glycans in live developing zebrafish using in vivo SPAAC with azide-bearing fluorescent probes. Remarkably, by employing a multicolour labelling strategy to resolve temporally distinct glycan populations, the authors were able to visualize and differentiate cell-surface glycans developed in the first 60 hours post-fertilization (Figure 1.3, orange fluorescence) and those developed between 61–63 hours post-fertilization (Figure 1.3, green fluorescence). This spatiotemporal analysis of glycan expression and trafficking, enabled by rapid SPAAC reactivity, allowed for the identification of development patterns that would be undetectable with conventional molecular imaging approaches. Recently, Carrico and coworkers described a photoinitiated bioorthogonal ligation in live zebrafish. Triarylphosphines were uncaged — removal of photoprotecting group — in vivo under focused irradiation and underwent SBL with cell-surface glycans, which resulted in effective visualization of regions of interest in zebrafish larvae.

Unsurprisingly, the extensive application of bioorthogonal chemistry to form robust linkages in chemical biology quickly attracted the attention of material scientists as a viable chemical tool for the assembly of materials with more functional or complex architectures.
Figure 1.3. Brightfield (A, C, E) and fluorescence (B, D, F) images of zebrafish during development. A = frontal view; B = mouth region; C = lateral view; D = pectoral fin; E, F = embryo. Temporally distinct glycan populations resolved using two colour labelling. Scale bars in A, C, E, F = 100 μm; B, D = 10 μm. Image reproduced partially with permission from Ref. [58].

1.2 Nanomaterials Chemistry

Over the last three decades, scientists have witnessed explosive growth in the field of nanotechnology. Interest in nanomaterials — defined as materials with at least one dimension in the nanoscale — stems from their unique properties that are distinct from small molecules and bulk materials of similar composition. Perhaps more importantly, these properties change significantly with size and shape, and differently depending on the material (e.g. semiconductors vs. noble metal particles).[59] Thus, two related subfields of nanotechnology were born: 1) the synthesis of new nanostructures of different sizes and shapes and investigation of their structure-property relationships;[59] 2) the application of nanomaterials across scientific disciplines ranging from catalysis,[60-62] bioimaging and sensing,[63-65] to its appearance in commercial products.[66] The next sections focus on the latter and demonstrate how the engineering of appropriate surface functionalities on materials is often critical to their use.
1.2.1 Application of Nanomaterials

Owing to their diverse sizes, shapes, composition, and thus unique intrinsic properties, the potential applications of nanomaterials are seemingly endless. In scientific literature, the term “nano” is ubiquitous: nano-particles (or dots),\textsuperscript{[67]} clusters,\textsuperscript{[68]} rods,\textsuperscript{[69]} tubes,\textsuperscript{[70]} stars,\textsuperscript{[71]} pyramids,\textsuperscript{[72]} and sheets\textsuperscript{[73]} have been reported. Their applications utilize one or more of (but is not limited to) the following properties:

1. The material absorbs light efficiently and may be emissive or able to convert this energy into localized heat.
2. The material’s size or shape enables its components to function as nanocarriers for drugs, imaging probes, or biomolecules either on its surface or internally.
3. The material functions as a semiconductor.
4. The material features desirable macro properties such as tensile strength, high surface area, or low density (lightweight).

In addition, nanomaterials provide a platform for the integration of multiple functionalities within a single construct, which permits their use in complex environments that require specific interactions at their interface (for example, in chemical biology) or to assemble nanocomposite materials. Several such applications are highlighted below.

Jung and coworkers reported a two-step pretargeting approach to cell labelling and drug delivery using $120 \pm 30$ nm nanoemulsions — lipid vesicles — that utilize leucine zipper pairs (ZE and ZR) that have an exceptionally high binding affinity and forms a stable ZE/ZR heterodimer complex in solution (Figure 1.4).\textsuperscript{[74]} In their methodology, a ZR-containing, fluorescently-labelled antibody (4D5-FITC) was first immobilized onto the target antigen overexpressed in tumour cells (“diagnosis”); healthy cells remained unlabelled due to lack of the target antigen. Cells were then treated with either rhodamine dye- or doxorubicin-containing vesicles that display ZE (“therapy”). The authors observed successful binding of ZE-containing rhodamine-vesicles onto pretargeted tumour cells (SKBR3), which displayed the fluorescence of FITC (from antibody) and rhodamine (from vesicle). In the case of healthy cells (MCF10A), no fluorescence from
the antibody or vesicles was observed, which validated their two-step pretargeted labelling strategy. Furthermore, cell viability studies showed a significant increase in the delivery of doxorubicin using pretreatment of tumour cells with the antibody-ZE conjugate, whereas the absence of the conjugate (and thus pretargeting) led to no observed cytotoxicity of drug-containing vesicles.

**Figure 1.4.** Left panel: Schematic representation of the two-step pretargeting approach to cell labelling using leucine zipper pairs (ZR and ZE). A fluorescently-labelled antibody (4D5-FITC) containing ZR first binds the target antigen overexpressed on the tumour cell surface; normal cell is not labelled. Vesicles displaying surface ZE recognize antibody-ZR conjugate and binds to form a stable ZE/ZR complex. Right panel: Fluorescence images of MCF10A (normal human breast epithelial cells) and SKBR3 (human breast cancer cells) after pretreatment with ZR-4D5-FITC (a, d) followed by treatment with ZE-rhodamine-vesicles (b,e). The overlay image (c,f) was generated by merging two signals, where green = FITC; red = rhodamine; blue = DAPI nuclear stain. Scale bars = 10 μm. Figures reprinted with permission from Ref. [74].

Simon and coworkers reported long-term, multicolour imaging of live cells using emissive CdSe/ZnS quantum dots (QDs).[75] The authors demonstrated that antibody-conjugated QDs could be used to specifically label extracellular proteins of interest on live HeLa cells, which enabled fluorescence imaging and tracking of their cell growth and development. Over the period of one week, they observed no effect on normal growth and development in their manipulations, which represented a non-invasive and reliable way to label live cells. Duda and coworkers more recently demonstrated a similar method using antibody-conjugated CdSe/CdZnS, CdSe/CdS, and InAs/CdZnS QDs in
mice. The authors observed high photostability, specific binding, and long circulation half-life of conjugated QDs in mice blood vessels, which offers new possibilities to study single-cell interactions and their microenvironments in live animals.

Even in the absence of drug additives, nanoconstructs can be therapeutic. Vo-Dinh and coworkers developed a protocol to prepare cell-penetrating peptide (CPP)-functionalized gold nanostars (NS) and demonstrated their use in plasmonic photothermal therapy. The authors observed that CPP-NS assemblies entered breast cancer cells significantly more than bare or PEGylated NS. In addition to enhanced intracellular delivery, they demonstrated efficient photothermolysis of tumour cells at an ultralow irradiance using an 850 nm pulsed laser, which allows for deep tissue penetration and spatially resolved ablation of cancerous tissue.

An alternative to photothermal therapy is photodynamic therapy (PDT), which utilizes nontoxic photosensitizers that, when activated by a light source, generate reactive oxygen species (ROS, $^1$O$_2$) to trigger cell death. Tan and coworkers utilized the fact that carbon nanotubes (CNTs) quench $^1$O$_2$ generation of photosensitizers that are attached to its surface to design a targeted PDT system. In their design, a photosensitizer was attached to one end of a protein-binding aptamer that wrapped onto the CNT surface (Scheme 1.6). In the absence of thrombin, the target protein, $^1$O$_2$ generation was quenched. In the presence of its target, the CNT-aptamer binding was disturbed to favour aptamer-target binding, which concomitantly liberates the photosensitizer from the CNT surface to regenerate $^1$O$_2$ generation. This represented the first targeted PDT system based on $^1$O$_2$ generation quenching on CNTs and is amenable to other aptamer-protein pairs to target different proteins of interest.

Desirable properties of nanomaterials can be combined into composite materials to further enhance their utility. Javey and coworkers fabricated CNT-hydrogel polymer composites that feature reversible thermal and optical responsivity. By utilizing poly(N-isopropylacrylamide) (pNIPAM) loaded with CNTs in hydrogels, the authors demonstrated that the abrupt strain change in the polymer hydrogel above the lower critical solution temperature (LCST) could be used to actuate the folding of low density
Scheme 1.6. Schematic of aptamer-photosensitizer-CNT complex and the regulation of $^1\text{O}_2$ generation upon target binding: (I) aptamer-photosensitizer and CNTs were mixed together to form a complex where the ssDNA aptamer is wrapped on the CNT surface, which brings the photosensitizer close to the CNT to quench $^1\text{O}_2$ generation. (II) Target binding with aptamers disturbs aptamer-CNT interactions, resulting in liberation of photosensitizer from CNT surface and thus restoration of $^1\text{O}_2$ generation. Figure reproduced with permission from Ref. [78].

Figure 1.5. Programmable cubes – a folding cube based on thermal responsive CNT-hydrogel polymer actuator. Figure reprinted with permission from Ref. [79].

Polyethylene (LDPE) strips (Figure 1.5). They observed a 5-fold enhancement in response time — time required to reach a folding angle of 90° from 180° (flat) — through the incorporation of CNTs into the polymer matrix versus without CNTs. The authors proposed that the enhanced mass transport of water molecules through CNT fluidic channels is the mechanism by which the response time is increased by CNTs. In
addition to enhanced water diffusion, CNTs provide optical responsivity to near-infrared (near-IR) irradiation. Excitation by a 785 nm laser, to which pNIPAM is transparent, resulted in hydrogel shrinking in the irradiated area due to localized heat generation from CNTs, which was followed by ultrafast (0.3 s) swelling to its original state after the laser was turned off. This conversion of thermal and optical stimuli to mechanical energy, enabled by the joint (and synergistic) properties of nanoscale materials, highlights the potential of these programmable composites as actuators in smart devices or tissue connectors in biological media.

In addition to the examples described above, new applications utilizing nanomaterials are developed every year, made possible only by the unique optical, electronic, and physical properties offered in the nanoscale.\cite{63,65,80} Such innovations, as exemplified in the applications described previously, require careful consideration and engineering of surface functionalities to determine how these materials interact with and respond to their intended environment of use. Thus, the development of functionalization methodologies that are reliable and robust is critical to the utility of nanoscale materials.

1.2.2 General Methods of Modification

Derivatization of material surfaces with target functionalities or molecules can be achieved in a many number of ways, ranging from weakly electrostatic interactions to the formation of robust, covalent bonds. This section offers a brief overview of existing methodologies that consider material composition (organic vs. metal) and their amenability towards the incorporation of chemical handles.

Self-assembly of organic monolayers onto metal surfaces has been established and well-studied for decades,\cite{81,82,83} thus offering the most obvious method of introducing further function onto metal-based nanomaterials. In addition, organic monolayers feature compatibility with a wide scope of donor ligands (N, O, S, and P-based)\cite{81,84-88} and serve to passivate and stabilize metallic colloids from aggregation. Most commonly, engineering of metal surfaces is achieved via treatment with thiol ligands carrying the desired functional group, which can range from small chemical handles (-OH, -NH₂, -COOH)\cite{89,90} to be used for further modification, to large biomolecules such as proteins...
via binding of their cysteine group(s). More recently, organic reactions are performed on terminal moieties in the monolayer to achieve the desired functionality; one popular method uses 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/N-hydroxysuccinimide (NHS) or N-Hydroxysulfosuccinimide (sulfo-NHS) to form stable amide bonds with the target molecule.

Functionalization of carbon-based nanomaterials feature greater complexity due to the inertness of their graphitic network. However, methods to chemically oxidize defect sites using harsh (extensive ultrasonic treatment in a mixture of concentrated nitric and sulfuric acid) or mild conditions (refluxing in nitric acid) can be used to generate surface -OH and -COOH, among other oxygen-containing groups, that can be elaborated into amide or ester bonds. An alternative method relies on the use of reactive species (e.g. radicals, carbenes, nitrenes) for direct insertion of functional groups into the π-conjugated carbon framework. It has been shown that direct covalent attachment of functional moieties onto CNT sidewalls strongly enhances their solubility, which improves their processability and utility.

Modification of glass or Si-based nanoparticles is most commonly achieved via treatment with trialkoxysilanes (R–Si(OR)₃) via condensation, which can be used to introduce basic chemical functionalities (e.g. amines) that can be utilized for further surface modification. An alternative method uses hydrosilylation reactions of hydride-terminated silicon surfaces with terminal alkenes and alkynes to afford alkyl- and alkenyl-substituted surfaces, respectively.

Although not exclusive, the protocols outlined above represent general methods of functionalizing nanoscale materials of different composition by using their intrinsic surface chemistry. Due to limitations with compatibility and stability, it is becoming increasingly popular to first introduce more general chemical handles followed by subsequent organic reactions on the surface, rather than employing the materials surface chemistry directly to introduce the target functionality. In alignment with our group’s interest in developing organic and photochemical tools for nanomaterial modification,
this thesis explores different methods to enable clean and reliable reactivity on gold nanoparticles as a model nanomaterial surface.

1.3 Gold Nanoparticles

For thousands of years, colloidal gold was used throughout Europe in stained glass windows of cathedrals, by the Chinese to colour vases and other ornaments, and by Indians in gold-containing ashes for medicinal use.\[^{67,103}\] It was not until recent decades that researchers began to exploit their interesting optical properties and rich surface chemistry for applications in materials science and chemical biology. This next section provides an overview of gold nanoparticles (AuNPs) to describe their properties, synthesis, characterization, applications, and methods of surface derivatization.

1.3.1 Physical and Chemical Properties

Previously (and aptly) known as monolayer-protected Au clusters, AuNPs are comprised of a metallic core of gold atoms and an organic ligand shell — usually thiolates — that passivates and stabilizes the metal core to prevent aggregation of individual nanoparticles. Although commonly depicted as spherical for simplicity, the Au core is polyhedral, characterized by a face-centered cubic lattice in which Au atoms occupy terrace, edge, and vertices (Figure 1.6).\[^{104-105}\] High resolution transmission electron microscopy (HRTEM) images of dodecanethiolate-capped AuNPs, depicted in Figure 1.6B, show individual gold atoms making up the metal core. Decahedral, icosahedral, and truncated octahedral Au core structures, among others, have been proposed and vary with the size of AuNPs.\[^{105}\]

Due to the strong affinity between sulfur and gold, combined with the lateral van der Waals and dipole interactions between thiolated ligands and their end groups,\[^{106}\] AuNPs are generally very stable and can be stored indefinitely with no consequence. However, strongly basic and acidic conditions, or high temperatures, can result in nanoparticle aggregation due to desorption of thiolates under harsh conditions.\[^{107}\] In this case, exposed core regions can coalesce to give aggregated and insoluble structures. In addition, the decomposition of AuNPs can be induced chemically by molecular iodine
(I\textsubscript{2}) or cyanide ions (CN\textsuperscript{-}),\cite{108} which allows for analysis of decomposition products to determine the original composition.

**Figure 1.6.** A) Cartoon representations of thiolate-stabilized gold nanoparticles. B) HRTEM micrographs of 4.1 nm dodecanethiolate-capped AuNPs. (a) fcc clusters, (b-c) decahedra, (d-f) multidomain. HRTEM micrographs reprinted with permission from Ref. [105].

Perhaps their most intriguing physical property, the interaction of AuNPs with light has been extensively studied and utilized. While smaller AuNPs (< 3 nm) do not exhibit a distinct maximum absorption in their ultraviolet-visible (UV-vis) spectra, larger AuNPs (8–100 nm) show a broad but detectable peak between ~510–580 nm, known as the surface plasmon resonance (SPR) band. This SPR band, caused by the collective oscillation of the free conduction electrons when interacting with incident light, is responsible for the very intense colour of AuNPs.\cite{109} The position of the SPR band depends on various parameters, with significant (and often predictable) effects of size, shape, surface chemistry, and dielectric constant of the suspending medium. For example, it has been shown that increasing the AuNP core diameter from 9 nm to 99 nm can shift $\lambda_{\text{max}}$ of the SPR band from 517 nm to 575 nm in water, respectively (Figure 1.7).\cite{109}

Moreover, altering the aspect ratio of Au colloids from quasi-spherical to rod-like nanostructures shifts the SPR band remarkably to give surface plasmon absorption
maxima between 600 and 1300 nm,[110] which falls within the optical therapeutic window where light has its maximum depth of penetration in tissue. Furthermore, AuNPs can act as “light-activated nanoscopic heaters” due to their ability to convert absorbed light energy into localized heat, termed the photothermal effect, which enabled the development of many therapeutic applications.[65]

Figure 1.7. UV-vis absorption spectra of AuNPs of various core sizes obtained in water. Figure reprinted with permission from Ref. [109].

Lastly, an important attribute of AuNPs is their low cytotoxicity and biocompatibility when their surfaces are properly engineered. Although AuNPs are typically stable under standard storage conditions or in solvent, their multitude of complex interactions within biological environments necessitate additional considerations regarding adequate biological stability. For example, the use of poly(ethylene glycol) is commonly employed as a surface ligand on AuNPs to effect hydrophilicity, increase circulatory half-life, and block the nonspecific adsorption of proteins.[111] This methodology, coupled with the design of appropriately functionalized end groups (charged molecules, biomolecules, etc.) has led to many biocompatible applications of AuNPs in medical diagnostics and therapy.
1.3.2 Synthesis of AuNPs

Since Michael Faraday observed in 1857 that “fine particles” — formed by the aqueous reduction of gold chloride by phosphorus — could be stabilized by the addition of carbon disulfides to afford a “beautiful ruby” colour, many synthetic methods following a similar strategy have been developed.\[111\] In the typical bottom-up synthesis of AuNPs, a solvated gold salt is reduced in the presence of a surface-capping ligand. Modern procedures can be classified into two major methods: the Turkevich citrate-mediated method and the Brust-Schiffrin synthesis (Scheme 1.7).

**Scheme 1.7.** Modern methods of synthesizing AuNPs. A) Turkevich method to yield 10–150 nm citrate-stabilized AuNPs. B) Brust-Schiffrin synthesis of 1–3 nm thiolate-stabilized AuNPs. TOAB = tetraoctylammonium bromide, TOA = tetraoctylammonium.

In the Turkevich method, an aqueous solution of HAuCl\(_4\) is chemically reduced at 75°C by trisodium citrate, which acts as both the reductant and stabilizing ligand. Following a two-step process of rapid nucleation then successive growth, Au(III) ions are reduced to Au(0) to afford citrate-passivated AuNPs with moderate size distribution.\[112\] The size of AuNPs produced by this method can vary from 10 to 150 nm depending on the HAuCl\(_4\):citrate ratio, reaction temperature, and order of reagents added. Weakly bound citrate ions provide water solubility and means of displacement with ligands that strongly bind Au (e.g. thiols), thus offering a method to prepare functionalized AuNPs directly.
from this “blank” template. However, AuNPs prepared this way typically feature poor stability and greater size distribution and is therefore less widely used compared to the Brust-Schiffrin synthesis.

In the Brust-Schiffrin two-phase synthesis, aqueous HAuCl$_4$ is first transferred into toluene by a phase-transfer agent (tetraoctylammonium bromide, TOAB), followed by the addition of thiol to reduce Au(III) to the Au(I)-thiol polymeric form, [(Au(I)SR)$_n$].\cite{113} Consumption of Au(III) can be evidenced by decolourization of the organic layer. Upon addition of NaBH$_4$ as a reductant, rapid conversion of [(Au(I)SR)$_n$] into AuNPs can be observed via the development of a dark brown colour corresponding to AuNPs of 1–5 nm core size. Like the Turkevich method, core size can be tuned by varying the HAuCl$_4$:RSH ratio, reaction temperature, and rate of thiol or NaBH$_4$ addition. Modifications to this method to prepare water-soluble AuNPs in a single phase, without the need of TOAB, have also been reported.\cite{114}

### 1.3.3 Characterization of AuNPs

The popularity of AuNPs as a nanoplatform can be attributed to, in part, the large number of characterization techniques available to determine their size and composition. Transmission electron microscopy (TEM) and HRTEM provides structural information regarding the size, shape, and morphology of the metal core.\cite{67,105} X-ray photoelectron spectroscopy (XPS) can be used to elucidate the oxidation state of gold atoms and those belonging to the ligand shell. Typical XPS analysis of AuNPs shows peaks corresponding to Au (4f), (4d), and (4p), C (1s), and S (2s) binding energies. Furthermore, thermogravimetric analysis (TGA) can be used to measure the mass ratio of organic ligands vs. metal as a function of mass loss over temperature.\cite{115} In the case of mixed monolayers of ligands that differ significantly in chemical structure, it is possible to resolve the mass loss of individual components via deconvolution and peak fitting of the TGA derivative plot. Lastly, dynamic light scattering (DLS) and zeta potential measurements can be used to determine their hydrodynamic radius and surface charge, thus providing insight into their stability and surface interactions in different media.\cite{115}
In addition to enhancing processability, AuNP solubility in both organic solvents and water permit their characterization via traditional techniques used for molecules. That is, nuclear magnetic resonance (NMR) spectroscopy, perhaps the most important tool in modern synthetic organic chemistry for determining chemical structure, can be used to determine AuNP purity and composition. However, the assembly of tightly-packed ligands onto the AuNP surface implies that the frequency of signals is not only determined by the chemical structure of themselves, but also by the neighbouring ligands and environment. A key characteristic of the NMR spectra of dissolved AuNPs is the presence of broad signals.\cite{116} Figure 1.8 illustrates the $^1$H NMR spectra of tiopronin and tiopronin-AuNPs obtained in D$_2$O. Several differences can be observed between the spectra: significant broadening and moderate shifting of the peaks once bound onto the AuNP core. In addition, broadening of peaks is often asymmetric and results in the appearance of peak shoulders.

![NMR spectra](image)

**Figure 1.8.** $^1$H NMR spectrum of tiopronin (a) and tiopronin-AuNPs (b) obtained in D$_2$O. Spectra reprinted with permission from Ref. [116].

The mechanism by which broadening occurs has been extensively studied and many factors can affect the degree of broadening and direction of peak shifts. First, the heterogeneity of Au-S binding sites (edge, vertex, or terrace on the polyhedral core) and the associated difference in packing density of neighbouring ligands is an important line-broadening mechanism. Secondly, slow rotational diffusion of bulky AuNPs, compared
to their analogous “free” ligand, results in additional broadening and scales with AuNP core size (i.e. larger AuNPs exhibit broader signals). As these effects are most pronounced for protons (and other nuclei) closest to the core, signals resulting from terminal functional groups that are most distant are typically sharper. Despite this broadening of signals, NMR spectra of AuNPs provide important information regarding their structure and composition, especially when compared to the spectra obtained from the corresponding unbound molecules. In fact, NMR broadening can be used to identify the presence of impurities, as unbound molecules in solution appear as sharp signals in the spectrum.

Lastly, Fourier transform-infrared (FT-IR) and UV-vis spectroscopies can be used to characterize AuNPs. FT-IR spectroscopy provides structural information regarding the functional groups present in the nanoassembly and may be useful in identifying the presence of unbound thiols due to a characteristic, albeit weak, S-H absorbance in the 2550–2600 cm⁻¹ region. As described previously, UV-vis spectroscopy is an important tool for determining Au core size. In addition, attached chromophores can be identified in their UV-vis spectrum and compared with that obtained for the unbound thiol, to independently confirm AuNP composition alongside other spectral techniques (NMR, IR).

1.3.4 Application of AuNPs

Active research surrounding their synthesis and characterization techniques enabled the widespread production of stable AuNPs of various sizes and shapes with well-defined interfaces. The simplicity of their preparation, and their commercial availability by popular vendors, allowed researchers from across disciplines to utilize them for applications in diagnostic imaging, therapeutics, and catalysis. A Web of Science search for “gold nanoparticles” results in over 95,000 published articles and review articles, with over 80,000 from the last decade alone. Several recent examples of these applications will be highlighted.

Many applications using AuNPs center around their ability to absorb light. El-Sayed and coworkers recently demonstrated the synthesis of 29 ± 3 nm AuNPs functionalized with
thiolated poly(ethylene glycol), a modified RGD peptide (known to target overexpressed receptors on cancer cells), and a nuclear localization signal (NLS) peptide (Figure 1.9). By using the combination of RGD and NLS peptides, the authors demonstrated enhanced cellular uptake and localization in the nuclear region, which was confirmed by dark field microscopy. Irradiation of internalized AuNPs using a near-IR (NIR) laser triggered photothermal heating and resulted in cell death. Surface enhanced Raman spectroscopy (SERS), conducted within a single cell, was used to monitor protein and lipid structures in real-time during the irradiation process. Thus, they presented a method to both induce and monitor the cell death mechanism within single cells in real-time using actively targeted AuNPs.

![Figure 1.9](image)

**Figure 1.9.** A) Cartoon representation of nuclear-targeted AuNPs; where PEG = poly(ethylene glycol), RGD = RGDRGDRGDRGDPGC peptide, and NLS = CGGGPKKKRKVGG peptide. B) Schematic demonstrating photothermal heating of targeted cells caused by NIR irradiation of AuNPs, leading to cell death as evidenced by dark field microscopy. Figures reproduced with permission from Ref. [121].

Gold nanoparticles also represent excellent delivery vehicles due to their rich surface chemistry, high ligand density, and biocompatibility. Dai and coworkers reported the use of 11.2 ± 0.1 nm AuNPs to carry vascular endothelial growth factors (VEGF) across the skin barrier. The authors demonstrated that negatively charged AuNPs coupled to VEGF exhibited ideal transdermal delivery efficacy, which is important for promoting angiogenesis and wound repair in skin injuries. Recently, Kanaras and coworkers reported a remarkable multiplex sensing and drug delivery system using DNA-AuNP dimers (Figure 1.10). AuNPs (15 ± 1.5 nm) featuring “sense strands” of DNA were
Figure 1.10. Multiplex sensing and drug delivery system using DNA-AuNP dimers. A) Multiplex sensing strategy: when the target mRNA binds to the sense strand, a short oligonucleotide strand is released resulting in an increase in fluorescence emission, which was previously quenched by AuNP core. B) Multiplex drug delivery strategy: when the target mRNA binds to the sense strand, intercalated drug molecules (doxorubicin or mitoxantrone) are released. As both drugs are fluorescent, and previously quenched by AuNP, release also leads to turn-on signal of emission. Reproduced from Ref. [123].

assembled into dimers via DNA hybridization of single oligonucleotides (linker strands 1 and 2, Figure 1.10) and subsequent copper-free click chemistry. The sense strands, initially hybridized to short oligonucleotides bearing a fluorophore (flare strands), can detect mRNA targets via hybridization, resulting in the release of the flare strands and “turn-on” of the previously AuNP-quenched fluorescence to give a response signal
(Figure 1.10A). The authors built on this design by demonstrating a similar sensing strategy to release intercalated anticancer drugs (doxorubicin or mitoxantrone) in live cells that carried the target mRNA (Figure 1.10B). Through cell viability studies that compared the toxicity of drug-loaded targeted AuNPs versus nontargeted AuNPs (so-called scrambled dimers), they demonstrated significant specificity of targeted AuNPs in releasing the drugs to induce cell death.

Although bulk gold is chemically inert, it is well known that AuNPs exhibit good catalytic performances. Deng and coworkers recently showed that 4 nm AuNPs supported in magnetic yolk-shell mesoporous silica microspheres can be used to catalyze the epoxidation of styrene using tert-butylhydroperoxide (TBHP) as an oxidant (Scheme 1.8). They reported excellent catalytic performance, high conversion, and recyclability of the catalyst due to the ability to isolate the nanoassemblies using an externally applied magnetic field. The authors proposed that the hollow spaces of these yolk-shell microspheres help reactants contact with highly confined AuNPs, thus leading

Scheme 1.8. Synthesis of magnetic yolk-shell mesoporous silica microspheres with supported AuNPs and its catalysis of styrene epoxidation. Scheme reproduced with permission from Ref. [125].
to greater catalytic efficiency.

In addition to those described above, new applications of AuNPs are developed every year. In nearly all cases, the choice of surface functionalities is crucial to their function as it dictates how AuNPs interact with and respond to their intended environment of use, their stability and their properties. Thus, the rich surface chemistry of AuNPs has been investigated extensively over the years to offer multiple functionalization routes.

1.3.5 Surface Derivatization of AuNPs

Although amine- and phosphine-based capping groups are feasible and have been reported, thiolates are by far the most commonly used and studied due to their high affinity for gold and tight packing to form stable monolayers. This section highlights how AuNP surface chemistry with thiols can be used to introduce the desired functionality via three routes: direct synthesis, ligand displacement, and interfacial reaction.

1.3.5.1 General Methods to Synthesize Functionalized AuNPs

As the Brust-Schiffrin synthesis of AuNPs incorporates the added thiol into its ligand shell, it represents the most direct way to introduce the desired terminal functional group onto AuNPs (Scheme 1.9A). This method, termed direct synthesis, provides access to a range of functionalities (e.g. alkyl, –OH, –NH₂, –COOH) that are compatible with the reducing conditions required for the synthesis. However, the utility of this method is limited by the fact that 1) functionalities that do not remain intact in the presence of NaBH₄ cannot be used and 2) functionalities that are susceptible to nucleophilic attack (e.g. Michael acceptors) cannot be stably obtained due to self-reactivity of the required thiol. In addition, while there are established protocols for obtaining AuNPs of discrete sizes and narrow size distribution using inert or “non-functional” (e.g. alkyl) thiols, the use of functional thiols can lead to varied sizes and broader size distribution.

To overcome the limitations with the direct synthesis route, ligand displacement reactions can be employed. In this method, inert or non-functional AuNPs are first synthesized
Scheme 1.9. Methods to derivatize AuNPs with the desired functionality. A) Direct synthesis of AuNPs using a thiol carrying the desired functionality. B) Ligand displacement onto inert or “non-functional” (e.g. alkyl or ethylene glycol) terminated AuNPs using a thiol carrying the desired functionality. C) Interfacial reaction between an intermediate reactive functionality (e.g. -COOH, azide, alkyne) on AuNPs and a reactive partner carrying the desired functionality.

using an established Brust-Schiffrin protocol. In the second step, a thiol containing the desired terminal functionality is used to displace non-functional thiols from the surface to afford a mixed monolayer (Scheme 1.9B). The overall rate and extent of displacement from the incoming thiol are affected significantly by core size, electronic charge of the AuNPs, structure of the incoming ligand, and steric effects.\[127-128\] For example, ligand displacements are initiated at AuNP surface sites where monolayer packing is less dense (and therefore more accessible), such as the edges and vertices, and diffuse into the terrace sites until an equilibrium state is reached. These parameters allow for the fine-tuning of desired ligand concentrations (functional versus non-functional) and provides greater control over the AuNP composition. Furthermore, by first using a non-functional
AuNP template of discrete size, a library of functional AuNPs can be accessed that feature the same core diameter and size distribution. This reproducibility is highly important due to the size-dependent properties associated with AuNPs. However, the issue of introducing functionalities that are incompatible with the thiol moiety persists in this method. In addition, in the presence of multiple thiol moieties (as in many peptides and proteins), it is often unpredictable where binding occurs and how the binding mode can affect the overall surface function. Lastly, due to the need for high concentrations of incoming thiol for ligand displacement to be effective, this method can be inefficient and uneconomical if the thiol ligand is expensive or difficult to synthesize.

The synthesis of thiol derivatives is often challenging or, in many cases, ineffective due to incompatibility with the desired functionality. The last and arguably most useful method overcomes this challenge by employing synthetic organic chemistry to modify the AuNP monolayer interfacially (Scheme 1.19C). Termed interfacial reactions, these transformations allow for the formation of robust covalent bonds using AuNPs as a macromolecular-type reagent to introduce the desired functionality. However, common reaction conditions such as high temperature, highly basic or acidic environments, or the use of catalysts that affect AuNP stability cannot be used. Therefore, finding reactions that proceed efficiently and cleanly under mild conditions to offer well-defined interfaces remains a challenge in the derivatization of AuNPs. Many methods have been developed and extensively used, such as EDC/NHS (or sulfo-NHS) coupling, but more recently our group has focused on utilizing click chemistry to achieve these modifications.

1.3.5.2 Interfacial Click and Bioorthogonal Reactions on AuNPs

By revisiting the criteria for a reaction to be considered a click reaction — clean, fast, selective, and high yielding reactivity under mild conditions — it becomes immediately evident why these transformations are of interest for engineering well-defined AuNP (and other material) surfaces. In particular, use of a bioorthogonal reaction features the added advantage of biocompatibility, which enables the reaction to proceed in vivo, if necessary, for the targeted delivery of AuNPs in complex biological media. This section highlights several existing methods for employing click and bioorthogonal chemistry on AuNPs.
Perhaps the most widely used in the click reaction toolkit, the CuAAC represents a fast and clean method to form covalent bonds using chemical functionalities that are small, chemically inert, and easy to introduce. Most systems employ azide-functionalized AuNPs followed by Cu(I)-mediated reaction with a terminal alkyne bearing the desired functionality (Scheme 1.10). While it has been shown that tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) and similar derivatives can be used as an additive to intercept ROS generated by excess Cu(I) during CuAAC, copper catalysts are generally cytotoxic and have limited utility in living systems. Additionally, the requirement of additives (either catalyst or stabilizing agents) compromises and weakens the cleanliness and simplicity that are key characteristics of click chemistry.

Scheme 1.10. Generalized scheme depicting alkyne-azide cycloadditions on AuNPs.

To overcome this, SPAAC can be employed, which requires no additives and generates no byproducts. For example, our group and others have demonstrated the use of strained alkyne reagents to modify azide-AuNPs. Although effective and cleaner than
CuAAC, the incorporation of strained alkynes into the target functionality can be synthetically challenging and tedious, often requiring multiple protection-deprotection steps.\textsuperscript{135} Moreover, the low commercial availability and high cost of strained alkyne reagents acts as an additional barrier to researchers who wish to explore a library of functionalities on AuNPs and other surfaces. Thus, the complementary system that utilizes alkyne-AuNPs that react with azido-bearing functionalities represents a superior method to employing SPAAC (Scheme 1.10). However, the synthesis of such alkynyl-AuNPs remains a challenge due to the instability of strained alkynes towards nucleophiles. Thus, methods of alkyne incorporation onto AuNPs require further investigation.

In addition to CuAAC and SPAAC, maleimide-based click chemistry has been extensively studied on AuNPs (Scheme 1.11). Our group has demonstrated reversible Diels-Alder and Michael addition reactivity on AuNPs in recent years and showed its applicability in the assembly of hybrid nanomaterials, bioimaging, and potential drug delivery.\textsuperscript{136-141} Due to the reversibility of maleimide chemistry, it represents an excellent platform for the development of drug delivery and sensing applications. However, maleimide reactivity features several drawbacks: 1) maleimides are susceptible to hydrolysis in basic aqueous conditions\textsuperscript{140}; 2) the use of nucleophiles as the reaction partner for Michael additions limits its use in vivo, due to the abundance of amine and thiol functionalities in biomolecules; 3) the need for high temperature or pressure for Diels-Alder reactions limits its utility in living systems or in the conjugation of sensitive molecules.

Lastly, there are no reported systems that utilize SBL — the original bioorthogonal reaction for forming stable amide bonds — on AuNPs until our group’s recent work. We successfully demonstrated that a triarylphosphine-bearing thiol ligand can be synthesized and used to reliably modify AuNPs to afford phosphine-AuNPs with a well-defined interface (Scheme 1.12).\textsuperscript{142} These AuNPs featured clean and quantitative reactivity with an azide-bearing RGD peptide to afford the first AuNP-bioconjugate synthesized using the SBL. As the SBL remains a powerful and versatile bioorthogonal transformation that
features chemical ligation and release, further investigation into its utility on AuNPs is necessary.

Scheme 1.1. Generalized scheme depicting maleimide-based click chemistry on AuNPs; Michael addition (top) and Diels-Alder cycloaddition (bottom) shown.

Scheme 1.12. Generalized scheme depicting the Staudinger-Bertozzi ligation of azides onto AuNPs.
1.4 Scope of Thesis

The reliable modification of nanomaterial surface functionalities is critical to their application as it governs how the nanoassembly interacts with and responds to the intended environment of use. This is true for AuNPs, which have found applications in drug delivery, bioimaging, photothermal therapy, and catalysis. Thus, it is of critical importance that methodologies to derivatize their surfaces under mild conditions are developed. The birth of bioorthogonal chemistry has allowed chemical biologists to reliably form bonds even in the most complex biological environments, wherein they must forfeit all control of reaction parameters such as temperature, solvent, pH, and the presence of reductants, oxidants, and nucleophiles. The careful choice of reaction partners enables fast, clean, and selective reactivity despite often unpredictable conditions. Indeed, the reliability and robustness of these chemical tools should not be excluded to applications in chemical biology. Thus, this thesis examines methods to utilize these transformations on AuNPs as a model nanomaterial.

The transposition of bioorthogonal chemistry to AuNPs began in our group only recently, with few examples demonstrating SPAAC reactivity on AuNPs and a preliminary study on SBL reactivity in our seminal work. Due to the reactivity of strained alkynes toward thiols, a reliable method for the incorporation of cylooctynes onto AuNPs has yet to be realized. In addition, SPANC reactivity on alkyne-AuNPs has not been explored. Thus, this thesis aims to address these deficiencies through:

1. The development of well-defined methodologies for the incorporation of strained alkynes and triarylphosphine derivatives onto AuNPs.
2. The exploration of combinatorial functionality of bioorthogonal moieties on AuNPs.
3. Demonstrating quantitative and qualitative determination of chemical functionalities on AuNPs.
4. Demonstrating simple, clean, and reliable reactivity on AuNPs under mild conditions.
Chapter 2, published in *Chem. Eur. J.* 2017, 23, 1052–1059, focuses on the development of cyclopropenones as a photochemical caging strategy for strained alkynes, which enabled the direct incorporation of strained alkyne precursors onto AuNPs. This chapter describes the synthesis of cyclopropenone AuNPs, their photochemical decarbonylation reactivity, and 1,3-cycloaddition reactivity. The methodology for determining AuNP composition is also outlined. A library of derivatized AuNPs was synthesized.

Chapter 3, published in *Langmuir*, 2017, 33, 1908–1913, describes the investigation of the SBL as a dual-purpose transformation on AuNPs that permits chemical ligation and release. A modified triarylphosphine thiol ligand carrying a Rhodamine B cargo was synthesized and incorporated onto AuNPs through ligand displacement. Quantitative determination of the cargo concentration on AuNPs is outlined. Finally, its reactivity towards a water soluble azide to trigger cargo release was examined and monitored via fluorescence spectroscopy. Control experiments were conducted in the absence of active phosphine and its sensitivity to displacement by glutathione was examined.

Chapter 4, published in *Bioconjugate Chem.* 2019, 30, 1140–1149, highlights the development of a dual-bioorthogonal molecular tool that permits the use of four bioorthogonal transformations interchangeably to attach, release, and replace molecules on the AuNP surface. Bioorthogonal reactivity of model molecules via two routes — “click-to-release” or “double-click” — is described. Incorporation of the molecular tool onto AuNPs, quantitative analysis, and execution of the interfacial chemistry is discussed. Finally, the use of this chemistry for the preparation of multifunctional AuNPs and their application for targeted cell labelling is examined.

Chapter 5 summarizes the conclusions of this work and provides a commentary on the contribution of this investigation to the field of materials chemistry.

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Chapter 2

2 Photo-induced Interfacial Unmasking of Strained Alkynes on Small Water Soluble Gold Nanoparticles

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2.1 Introduction

In the last decade, owing to rapid advances in nanotechnology and materials chemistry, there has been special emphasis on the modification of material surfaces. As a result, the search for efficient functionalization protocols via covalently bound mono- or multilayers has become the central focus of much research. In this regard, click chemistry is of particular relevance because of its high yields, mild reaction conditions, and ease of post-treatment.\(^\text{[1]}\) The strain-promoted alkyne-azide cycloaddition (SPAAC), designed as a tool for in vivo imaging and tracking of biomolecules,\(^\text{[2]}\) is an indispensable member of the family of bioorthogonal click reactions owing to its rapid reaction kinetics, chemoselectivity, and biocompatibility.\(^\text{[3]}\) Since its discovery, the SPAAC reaction has been used for the synthesis and/or modification of many materials, including dendrons and dendrimers,\(^\text{[4]}\) topological polymers,\(^\text{[5]}\) organo-micelles and liposomes,\(^\text{[6-7]}\) micro- and nanoparticles.\(^\text{[8-12]}\) as well as other material surfaces.\(^\text{[13-16]}\) More recently, Tsukruk and coworkers prepared branched polyhedral oligomeric silsesquioxane nanoparticles via interfacial SPAAC reaction.\(^\text{[17]}\) Yi and coworkers reported chitosan-poly(ethylene glycol) microparticles featuring azadibenzocyclooctynes (ADIBO) and their potential to undergo bioconjugation via SPAAC reaction with azide-modified proteins and antibodies to afford biosensing platforms.\(^\text{[18]}\) Gobbo et al. prepared nanomaterial hybrids by employing ADIBO-functionalized single-walled carbon nanotubes and azide-decorated gold nanoparticles.\(^\text{[19]}\) Wang et al. demonstrated the post-synthetic modification of metal-organic framework (MOF) thin films using the SPAAC reaction to achieve nearly quantitative yields and to overcome Cu(I) ion contaminants commonly found in MOFs modified with the copper-assisted alkyne-azide cycloaddition (CuAAC) reactions.\(^\text{[20]}\)
As the SPAAC reaction is becoming a popular choice of chemistry in the materials chemistry domain for building and engineering functional materials, the development of methodologies for the incorporation of reactive strained alkynes onto nanomaterial surfaces is becoming important. The evolution of strained alkyne chemistry in the last decade has led to the design of fluorinated and aryl-fused cyclooctynes that are significantly more reactive at the C–C triple bond.\textsuperscript{[21-22]} Although fast reaction kinetics are certainly desirable, this poses challenges relating to the chemistries that are suitable for the chemical attachment of strained alkynes onto material surfaces or other molecular systems, i.e. the presence of functional groups that would react with the strained alkyne (e.g. amines, thiols, azides, etc.) are prohibited. This limits the scope and utility of the SPAAC reaction on nanomaterials, especially in cases where nucleophilic groups are required to introduce the strained alkyne.

One such challenge is the incorporation of strained alkynes onto gold nanoparticles (AuNPs). Among all nanomaterials, AuNPs are often regarded as one of the most promising templates for biomedical applications such as medical diagnostics, therapeutics, and drug delivery because of their low toxicity, chemical stability, and tuneable optical and chemical properties.\textsuperscript{[23-28]} However, incorporation of strained alkynes directly onto the gold core via a thiol-bearing strained alkyne ligand is unsuitable (shown in Scheme 2.1) due to the self-reactivity of the ligand by nucleophilic attack of the thiol end onto the strained C–C triple bond.\textsuperscript{[29-31]} The Workentin group has made efforts to prepare water soluble AuNP templates that can undergo interfacial SPAAC (i-SPAAC) reaction for the facile post-synthetic modification of the gold core. Recently, we reported ADIBO-derivatized water-soluble AuNPs that were synthesized via a post-synthetic interfacial amide coupling reaction between carboxy-terminated AuNPs and an ADIBO-amine (Scheme 2.1).\textsuperscript{[32]} Although these ADIBO-modified AuNPs demonstrated efficient i-SPAAC reactivity and water solubility, their synthesis requires an interfacial coupling reaction that did not proceed to completion. This led to impurities that complicated the determination of interfacial ADIBO concentration. In addition, the loading of ADIBO moieties at the AuNP interface was governed by the number of carboxy-terminated ligands present on the AuNP surface, which had to be kept low to preserve the organic solvent solubility of the COOH-terminated AuNPs required for the
Scheme 2.1. Previous strategy towards the synthesis of strained alkyne incorporated AuNPs via interfacial amide coupling. amide coupling reaction step. This resulted in low loading of the interfacial strained alkyne. To overcome these limitations, an improved methodology must be developed to 1) simplify the preparation of strained alkyne derivatized AuNPs, 2) achieve higher loading of interfacial strained alkynes onto AuNPs and 3) reduce or eliminate the generation of impurities to provide more accurate quantitative characterization of the resulting nanomaterial.

Recently, we reported a protection-deprotection strategy of strained alkynes via the formation of a dicobalt-hexacarbonyl complex. We demonstrated that chemistry can be performed on the Co$_2$(CO)$_6$-protected bicyclononyne (BCN) for further functionalization of the complex, which can then undergo subsequent deprotection to regenerate the modified-BCN. Although this is an effective protection strategy suitable for a variety of chemistries, it was not employed for incorporation onto AuNPs due to the reactivity of alkyne-Co$_2$(CO)$_6$ complexes towards thiols. Instead, we turned our attention to cyclopropenones, a photochemical precursor of linear and cyclic alkynes. The Popik group reported a cyclopropenone-masked dibenzocyclooctyne (DIBO) that undergoes clean decarbonylation under ultraviolet A (UV-A) irradiation to afford DIBO. They’ve also demonstrated the application of these cyclooctyne precursors in the surface functionalization of patterned polymer brush films. However,
derivatization of AuNPs or Au surfaces with cyclopropenone-masked DIBO moieties has never been reported. Such masked cyclooctynes are unreactive towards azides or nucleophiles in the absence of UV light, thus making it the ideal precursor for incorporation onto AuNPs to overcome the limitations previously described.

The power of this moiety lies in its exceptional stability, clean photoreactivity, and the opportunity for spatial and temporal control of the SPAAC reaction. However, it has never been applied to gold substrates thus presenting certain challenges to executing such chemistry on these surfaces.

For the first time, we describe herein an improved methodology towards the synthesis of strained alkyne-functionalized AuNPs via the direct incorporation of a cyclopropenone-masked DIBO (hvDIBO), a strained alkyne precursor, onto AuNPs. These hvDIBO-AuNPs undergo clean and quantitative photochemical decarbonylation under UV-A irradiation to afford the final DIBO-derivatized AuNP product, which requires no further purification or post-synthetic treatment. By employing this cyclopropenone-based strategy, we were able to simplify the preparation of strained alkyne-modified AuNPs and achieve higher loading of interfacial DIBO in addition to cleaner and quantitative reactivity. DIBO-AuNPs were found to have excellent chemical stability and reacted cleanly and efficiently with various 1,3-dipoles via i-SPAAC and interfacial strain-promoted alkyne-nitrone cycloaddition (i-SPANC) reactions under mild conditions. Remarkably, hvDIBO- and DIBO-modified AuNPs display excellent solubility in both organic solvents and aqueous media despite the strong hydrophobic nature of the interfacial moieties, which provides invaluable opportunities for applications of these materials in chemical biology and materials science. More importantly, these AuNPs preserve the rapid reactivity of strained alkynes when ‘unmasked’ while offering exceptional chemical stability in its ‘masked’ form, thus providing a facile and general route towards the modification of AuNP surfaces with spatial and temporal control. For these reasons, the synthetic strategy reported here not only serves to overcome synthetic challenges towards strained alkyne-derivatized AuNPs, but offers a photoswitchable AuNP platform based on photo-‘click’ chemistry that broadens the scope of practical applications in biochemistry, medical diagnosis, drug delivery, and material sciences.
2.2 Results and Discussion

The approach chosen for the introduction of DIBO precursors onto AuNPs required the synthesis of a thiol-modified cyclopropenone-masked DIBO ligand (Scheme 2.2). Briefly, the first step involved the double Friedel-Crafts alkylation of diphenylethane derivative 1 with tetrachlorocyclopropene followed by immediate in situ hydrolysis of the intermediate dichlorocyclopropene to afford the TBS-protected cyclopropenone-masked DIBO, 2. Deprotection of 3 by tetra-N-butylammonium fluoride (TBAF) in THF followed by substitution with tosylated trityl-protected thiol 4 afforded the S-trityl protected ligand 5. The S-trityl protecting group of 5 was cleaved cleanly in a 5% trifluoroacetic acid-dichloromethane solution, leading to the desired hνDIBO-thiol ligand 6. As expected, ligand 6 was found to be remarkably stable towards self-reactivity by either nucleophilic attack or disulfide formation under standard ambient conditions. In addition, it can be stored at -20 °C and remain stable for months, thus highlighting the efficacy of this precursor strategy. Details of the ligand synthesis can be found in the Supporting Information (Section 2.6).

Scheme 2.2. Synthesis of cyclopropenone-masked DIBO bearing thiol ligand 6. (a) C₃Cl₄, AlCl₃, CH₂Cl₂, then 5% HCl(aq); (b) TBAF, THF; (c) 4, K₂CO₃, DMF; (d) TFA, TIPS, CH₂Cl₂.
hvDIBO-AuNPs were prepared through a place-exchange reaction (Scheme 2.3) of thiol 6 onto triethylene glycol monomethyl ether AuNPs (MeO-EG₃-AuNPs) in CH₂Cl₂ at room temperature. Details of their synthesis can be found in the Supporting Information (Section 2.6). The MeO-EG₃-AuNP substrate selected has a gold core diameter of 3 ± 1 nm and features excellent organic solvent and water solubility. In addition, these AuNPs are resistant to both strongly acidic⁴¹ and basic conditions,⁴² they can be heated to over 100 °C,⁴³ and can be repeatedly dried and re-dissolved in different solvents with little to no aggregation, thus making them very resilient substrates for further modification as well as interfacial organic chemistry. After 15 min of place-exchange under vigorous stirring in the dark, the solvent was evaporated and the modified AuNPs were purified from excess thiols by re-dissolving in CH₂Cl₂ (4 mL) and precipitating them by adding hexanes (22 mL) in which the AuNPs are not soluble. The supernatant (containing excess thiols and disulfides) was removed and the entire washing procedure was repeated four times overall to afford clean hvDIBO-AuNPs. It is worth noting that the amount of thiol 6 incorporated can be tuned, to an extent, by varying the place exchange reaction time and/or varying the ratio of 6:AuNP in the reaction mixture. The ratio of 6:AuNP (1:5) and reaction time was carefully chosen to allow sufficient ligand exchange to occur for characterization of the newly introduced interfacial moieties, but not such that the AuNPs lose solubility in water due to the hydrophobicity of the hvDIBO head group. For example, if either [thiol 6] or reaction time was doubled, the resulting AuNPs were rendered insoluble in water, therefore limiting its potential application in chemical biology and nanomedicine. Thus, the reaction parameters were chosen to settle on a balance between aqueous solubility and ease of characterization. However, once the AuNPs have been characterized, the interfacial exchange of 6 can be tuned depending on

![Scheme 2.3. Strategy towards the synthesis of DIBO-AuNPs.](image-url)
the specific application of the AuNPs. These AuNPs were characterized by $^1$H NMR, infrared (IR), and ultraviolet-visible (UV-vis) spectroscopy as well as by transmission electron microscopy (TEM) and thermogravimetric analysis (TGA) (see Section 2.6).

The $^1$H NMR spectrum of hvDIBO-AuNPs showed the presence of broad peaks corresponding to the newly introduced thiol ligand 6 and the triethylene glycol monomethyl ether ligand of the AuNP substrate (Figure 2.1). The broad peaks are typical of a clean AuNP sample, indicating that the washing procedure was effective in removing unbound thiols and disulfides. It is worth noting that the removal of excess thiols is of key importance to avoid any undesirable side reactivity in the later decarbonylation step. The IR spectrum of hvDIBO-AuNPs showed an intensive carbonyl absorption band at 1844 cm$^{-1}$ corresponding to the C=O of the cyclopropenone moiety, thus confirming that ligand 6 was successfully incorporated without change to the cyclopropenone structure (see Figure S2.2). The TEM analysis of hvDIBO-AuNPs also showed no significant change in gold core diameter (2.9 ± 0.5 nm) following the place-exchange reaction, which indicates that the nanoparticles are stable and do not aggregate under these conditions.

**Figure 2.1.** $^1$H NMR spectra of thiol 6 (top) and hvDIBO-AuNPs (bottom). *Denotes residual solvent peaks. Spectra were referenced against residual CHCl$_3$. 
conditions (see Figure 2.2A). In fact, they can be stored in solution (CH$_2$Cl$_2$) at -20 °C and remain stable for months.

**Figure 2.2.** TEM images of A) hvDIBO-AuNPs and B) DIBO-AuNPs.

TGA of hvDIBO-AuNPs showed that 29% of the total AuNP weight corresponds to the organic corona, i.e. MeO-EG$_3$S$^-$ and hvDIBO-EG$_4$S$^-$ (see Figure S2.4). The derivative of the TGA curve showed that there are two ligands that decompose at distinctly different temperatures (Figure S2.4): a larger component at 265°C and a small component at 375°C. By comparing to the TGA data with that of the MeO-EG$_3$-AuNP starting material, the ligand decomposing at 265 °C was assigned to MeO-EG$_3$-S$^-$,[32,42-44] whereas the ligand at 375 °C was assigned to hvDIBO-EG$_4$-S$^-$. From the combination of $^1$H NMR data, deconvolution of the TGA derivative curve, and TEM analysis, and assuming that these AuNPs are spherical and monodispersed in size, it was possible to calculate an approximate molecular formula of hvDIBO-AuNPs to be Au$_{800}$(MeO-EG$_3$-S)$_{270}$(hvDIBO-EG$_4$-S)$_{30}$. More specifically, these AuNPs contain hvDIBO-EG$_4$-S in a concentration of 0.136 μmol mg$^{-1}$ of AuNP. Details of these calculations are reported in the Supporting Information (Section 2.6).

The photoreactivity of hvDIBO-AuNPs was then investigated using UV-vis, $^1$H NMR, and IR spectroscopy. A solution of hvDIBO-AuNPs in water at a concentration of 0.25 mg/mL was irradiated with UV-A light. The UV-vis spectra of the solution before and after irradiation showed bleaching of the 331–347 nm bands corresponding to the precursor cyclopropenone, and appearance of 305–322 nm bands corresponding to the
decarbonylated strained alkyne (Figure 2.3). These values are in good correspondence with that observed in literature for similar unbound or ‘free’ DIBO molecules.\(^{[37]}\) \(^1\)H NMR analysis of the aromatic region of hvDIBO-AuNPs before and after UV irradiation indicated the disappearance of the broad peak at 7.92 ppm and a decrease in the signal at 6.90 ppm of cyclopropenone, followed by appearance of broad signals at 6.77 and 7.20 ppm corresponding to the interfacial DIBO aromatic protons (Figure 2.3). Lastly, the IR spectrum of the DIBO-AuNPs product (see Figure S2.9) showed the absence of the C=O absorption band at 1844 cm\(^{-1}\) corresponding to the cyclopropenone moiety, thus confirming quantitative decarbonylation of hvDIBO-AuNPs to afford DIBO-AuNPs with

\[\text{hvDIBO-AuNPs} \xrightarrow{hv \text{ (350 nm)}} \text{DIBO-AuNPs} \xrightarrow{BnN_3} \text{triazole1-AuNPs}\]

**Figure 2.3.** Scheme showing the photochemical deprotection of hvDIBO-AuNPs and subsequent i-SPAAC reaction with BnN\(_3\) 7. (left). UV-vis and \(^1\)H NMR data shown for (a) hvDIBO-AuNPs, (b) DIBO-AuNPs, and (c) triazole1-AuNPs (right). \(^1\)H NMR spectra were recorded in CDCl\(_3\) and referenced against residual CHCl\(_3\) (denoted by *).

\[\text{Chemical Shift (ppm)}\]

\[\text{Absorbance (A.U.)}\]

\[\text{Wavelength (nm)}\]
no significant change to the AuNP core size as confirmed by TEM (Figure 2.2B). It is worth noting that photochemical decarbonylation was found to proceed faster in more dilute solutions of hvDIBO-AuNPs, which is likely due to competitive absorption of UV light by the dark brown gold particle solution. Nevertheless, interfacial unmasking of DIBO-AuNPs was fast (<10 min) in reasonably dilute solutions of AuNPs (0.25 mg/mL solvent) and led to clean and quantitative yield of the strained alkyne derivatized AuNP product, which contained 0.136 μmol interfacial DIBO mg⁻¹ of AuNP. This is double that of ADIBO used in the previous system shown in Scheme 2.1, thus allowing for greater capitalization on the high load capacity of AuNPs. Additionally, the only byproduct generated (CO) is released as a gas, which avoids any impurities and the need for further purification of DIBO-AuNPs. More importantly, it has been shown that irradiation with UV-A light may lead to agglomerated AuNPs. However, we observed no change in AuNP core size as confirmed by TEM, thus demonstrating the overall efficacy of the cyclopropenone-based strategy employed.

The i-SPAAC reactivity of DIBO-AuNPs was investigated with benzyl azide (BnN₃) as a model molecule and monitored through UV-vis and ¹H NMR spectroscopy. BnN₃ was chosen due to its commercial availability, low cost, and use as a standard organic azide molecule for SPAAC kinetic studies in literature. When DIBO-AuNPs were treated with excess BnN₃ in CH₂Cl₂, the UV-vis absorption spectrum of the product showed bleaching of the 305–322 nm bands corresponding to the interfacial DIBO, which is consistent with quantitative formation of the triazole-AuNP product (Figure 2.3). After excess BnN₃ was removed by washes with hexanes, the ¹H NMR spectrum of the AuNPs displayed additional broad peaks in the aromatic region corresponding to the phenyl protons, and two broad singlets at 5.55 and 5.50 ppm corresponding to the benzylic protons of the two regioisomers of the interfacial triazole product (Figure 2.3). These results together confirm that the interfacial SPAAC reaction proceeds effectively and quantitatively at the interface to afford the triazole-AuNP product.

Furthermore, our ability to accurately determine the concentration of interfacial DIBO on AuNPs and the clean reactivity of these moieties with an azide counterpart allowed for investigation into the reaction kinetics of the i-SPAAC on our nanomaterial template. The
rate measurements of the i-SPAAC reaction of DIBO-AuNPs with BnN₃ were conducted by ¹H NMR spectroscopy at 25 °C in CDCl₃. Chloroform was chosen due to the high solubility of the nanoparticles in this solvent that permitted the high AuNPs concentration required for these kinetic experiments. A calculated amount of 11 mM BnN₃ solution in CDCl₃ was added to a solution of DIBO-AuNPs in CDCl₃ required to achieve an equimolar concentration of 2.2 x 10⁻³ M in both BnN₃ and interfacial DIBO. Reactions were monitored through ¹H NMR analysis by following the decay of the sharp singlet signal at 4.30 ppm corresponding to the benzylic protons of the BnN₃ starting material. These reactions were carried out in triplicate and the second-order rate constant was evaluated to be (5.3 ± 0.4) x 10⁻² M⁻¹ s⁻¹. This is consistent with bimolecular rate constants reported in literature for reactions of similar DIBO derivatives with BnN₃ in solution.[37] This is remarkable considering the markedly different chemical environment presented by the nanoparticle structure and the interfacial nature of the SPAAC reaction. In fact, previous work has showed that interfacial reactions on AuNPs can be significantly slower than the same reaction carried out in the bulk solution, where differences depend on the mechanistic nature of the interfacial reaction.[44,46] Thus, we were delighted to observe no significant change in the i-SPAAC reactivity at the AuNP interface.

Lastly, to showcase the generality of DIBO-AuNPs towards surface modification, we reacted DIBO-AuNPs with various 1,3-dipoles shown in Scheme 2.4, which include an aromatic azide (8), an aliphatic azide (9), and 3′-Azido-3′-deoxythymidine (AZT, 10), an azide-bearing drug used to prevent and treat HIV/AIDS. N-phenyl-α-phenylnitrone (11) was also used to demonstrate that DIBO-AuNPs can undergo i-SPANC in addition to the i-SPAAC, thus offering compatibility with two mutually orthogonal functional groups for AuNP modification.

The i-SPAAC/SPANC reacted AuNPs were characterized by ¹H NMR spectroscopy. In all cases, the purified AuNPs showed additional signals in their ¹H NMR spectrum corresponding to the interfacial clicked moieties (see Section 2.6). To confirm proper interfacial reactivity, all reacted AuNPs were oxidized by molecular iodine to afford a mixture of Au(I) and Au(III) complexes, which liberated the organic ligands as disulfides.
Scheme 2.4. Reaction of DIBO-AuNPs with various 1,3-dipoles.  

(Scheme 2.5). Each mixture of disulfides was characterized by ESI-MS to determine the exact mass of the disulfide molecule that is most likely to form. In all cases, the experimental m/z agreed with that calculated for each proposed disulfide, which confirmed successful SPAAC/SPANC reactivity at the interface of AuNPs (Figures S2.12-S2.15). More importantly, this study highlights the ease of use of DIBO-AuNPs as a bioorthogonal nanomaterial template from which surface modification can be achieved through simple pour and mix chemistry with various 1,3-dipoles under mild and
Scheme 2.5. Reoxidation of reacted AuNPs by molecular iodine. ESI-MS calcd for disulfides 12: C_{42}H_{58}N_5O_8S_2 [M+H]^+ 796.3665, found 796.3675; 13: C_{42}H_{58}N_5O_8S_2 [M+H]^+ 812.3614, found 812.3636; 14: C_{38}H_{57}N_3NaO_9S_2 [M+Na]^+ 786.3434, found 786.3429; 15: C_{45}H_{64}N_5O_12S_2 [M+H]^+ 930.3993, found 930.3983; 16: C_{48}H_{61}NO_9S_2 [M]^+ 859.3788, found 859.3763.

bioorthogonal reaction conditions. This is especially true in the case where AZT 10 – a structurally complex azide molecule – was used to synthesize a drug-AuNP conjugate. This efficient and easy-to-implement approach towards AuNP surface modification allows for facile synthesis of AuNP-bioconjugates, nanohybrids, and functional materials for applications across the sciences.

2.3 Conclusion

In summary, we describe an improved methodology for the introduction of strained alkyne moieties onto AuNPs for the surface modification and engineering of functional nanomaterials. By employing this cyclopropenone-based strategy, we were able to simplify the incorporation of strained alkynes onto AuNPs, achieve higher loading of interfacial strained alkynes than in our previous system, and accurately determine the concentration of interfacial DIBO in the absence of impurities that would have otherwise
been present if an interfacial amide coupling was employed instead. Furthermore, a major advantage of the method reported here is that the amount of hfDIBO (and consequently, DIBO) incorporated onto AuNPs can be tuned in a facile manner and calculated with good precision, both of which are important for potential applications in bioconjugation, medical diagnostics, or drug delivery. This methodology also represents a significant advancement in the field as it addresses one of the major challenges of employing the SPAAC reaction on AuNPs: the reactivity of the highly strained C–C triple bond towards nucleophiles, which is normally used to bind functional ligands to gold surfaces. Instead, our approach challenges the need to compromise between the favorable high reactivity of strained alkynes, and the ease of use, chemical handling and/or functionalization of strained alkynes at the interface of nanomaterials. Furthermore, cyclopropenone-masked AuNPs offer a photoswitchable AuNP platform from which spatial and temporal control of the photo- ‘click’ chemistry can be achieved, thus expanding the utility and scope of applications of the i-SPAAC reaction in materials and biomaterials sciences.

Lastly, we demonstrated fast and clean i-SPAAC/SPANC reactivity of DIBO-AuNPs with various 1,3-dipoles under mild reaction conditions. In fact, DIBO-AuNPs were even prone to modification with structurally complex azide-bearing molecules as demonstrated by the synthesis of an AZT drug-AuNP conjugate. Although the interfacial AZT-derived triazole serves only as a drug model, it highlights the simplicity with which derivatization of AuNP surfaces can be achieved. The ability to alter the surface properties of AuNPs through the atom-by-atom control provided by this versatile AuNP template will allow for one to tailor with ease the interactions of AuNPs with biological systems, materials and biomaterials to help maximize the potential of this nanomaterial.

2.4 Acknowledgements

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2.5 References


2.6 Supporting Information

General Methods.

The following reagents, unless otherwise stated, were used as received. Anhydrous deuterated chloroform (CDCl$_3$), anhydrous deuterated methanol (CH$_3$OD), tetrachloroauric acid trihydrate, sodium borohydride, $p$-Toluenesulfonyl chloride, and triisopropylsilane (TIPS) were purchased from Sigma-Aldrich. All common solvents, triethylamine (TEA), magnesium sulfate, sodium sulfate anhydrous, dry methanol, hydrochloric acid, trifluoroacetic acid, sodium hydroxide, sodium chloride were purchased from Caledon. Ethanol was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) was purchased from BDH. Flash chromatography was performed using 40-63 µm silica gel. Dialysis membranes (MWCO 6000-8000 Da) were purchased from Spectra/Por. Compound 3 was synthesized according to Chem. Commun. 2014, 50, 5307–5309. Compound 11 was synthesized according to Chem. Commun. 2008, 0, 3522–3524.

$^1$H and $^{13}$C NMR spectra were recorded on an Inova 400 or 600 MHz spectrometers using CDCl$_3$ or CH$_3$OD as solvent and were calibrated against the residual protonated solvent. Infrared spectra were recorded on a Bruker Vector 33 FTIR spectrometer. Thermogravimetric analyses (TGA) were recorded by loading the sample in a 70 µL ceramic crucible and heating from 25 °C to 750 °C at a rate of 10 °C min$^{-1}$. The experiment was run under a nitrogen flow of 70 mL min$^{-1}$ in a Mettler Toledo TGA/SDTA 851 instrument. Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and prepared by dropcasting a drop of nanoparticles solution directly onto the grid surface. The drop was then carefully removed after 30 seconds with a soft tissue. ESI-MS spectra were recorded on a Micromass LCT mass spectrometer in positive ion mode. UV-visible spectra were recorded using a Varian Cary 100 bio spectrometer and 7 mm quartz cuvettes. The nanoparticle samples were dissolved in spectroscopic grade dichloromethane. The background was automatically subtracted from each spectrum. Photolyses were conducted in a Luzchem LZC-4V photoreactor equipped with 14 UVA (350 nm) lamps.
Synthesis of hvDIBO-EG$_4$-SH ligand (6).

Scheme S2.1. Synthetic strategy for the synthesis of hvDIBO-EG$_4$-SH ligand 6.

**Synthesis of 18.** To a solution of $p$-toluenesulfonyl chloride (22.0 g, 115 mmol) in CH$_2$Cl$_2$ (200 mL) at 0 °C, was added tetra(ethylene glycol) 17 (20.3 g, 104 mmol) and dry triethylamine (15.8 g, 156 mmol). The reaction was then stirred for 2 hours at 0 °C, and left overnight at room temperature under inert atmosphere. The precipitate was filtered, and the reaction mixture was then concentrated in vacuo. The crude mixture was then purified via silica gel chromatography (hexanes: ethyl acetate 1:4) to provide monotosyl tetra(ethylene glycol) 18 (15.3 g, 42%) as a colorless oil. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.80 (d, $J = 8.3$ Hz, 2H), 7.34 (d, $J = 4.8$ Hz, 2H), 4.17 (t, $J = 4.8$ Hz, 2H), 3.73 – 3.59 (m, 14H), 2.45 (s, 3H). $^{13}$C($^1$H) NMR (CDCl$_3$, 101 MHz): $\delta$ 144.8, 132.8, 129.8, 127.8, 72.4, 70.6, 70.5, 70.3, 70.2, 69.3, 68.5, 61.5, 21.5.

**Synthesis of 19.** Triphenylmethanethiol (4.12 g, 14.9 mmol) was dissolved in a solution of EtOH/toluene (1:1, 40 mL) and NaOH (0.596 g, 14.9 mmol) in H$_2$O (8 mL) was added. To this mixture was added a solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate 18 (4.16 g, 11.9 mmol) in EtOH/toluene (1:1, 40 mL). The reaction mixture was stirred at room temperature for 18 h. Once the reaction was completed, the mixture was poured into a NaHCO$_3$ saturated solution. The organic layer was washed with NaHCO$_3$ (3x) and brine (3x). The organic
portion was then dried over MgSO₄ and concentrated in vacuo. The crude product was then purified via silica gel chromatography (hexanes: ethyl acetate 1:1 to 1:3) to provide 19 as a colorless oil (4.44 g, 82%). ¹H NMR (CDCl₃, 400 MHz): δ 7.42 – 7.40 (m, 6H), 7.28 – 7.24 (m, 6H), 7.21 – 7.17 (m, 3H), 3.69 – 3.67 (m, 2H), 3.64 – 3.60 (m, 5H), 3.58 – 3.55 (m, 4H), 3.45 – 3.43 (m, 2H), 3.29 (t, J = 6.9 Hz, 2H), 2.43 (t, J = 6.9 Hz, 2H).

¹³C{¹H} NMR (CDCl₃, 101 MHz): δ 144.9, 129.7, 128.0, 126.8, 72.7, 70.7, 70.5, 70.4, 70.2, 69.7, 66.7, 61.8, 31.7. ESI-MS calcd for C₂₇H₃₂NaO₄S [M + Na]⁺ 475.1919, found 475.1914.

Synthesis of 4. To a solution of p-toluenesulfonyl chloride (1.87 g, 9.83 mmol) in CH₂Cl₂ (100 mL) at 0 °C, was 1,1,1-triphenyl-5,8,11-trioxa-2-thiatridecan-13-ol (4.45 g, 9.83 mmol) and dry triethylamine (4.97 g, 49.1 mmol). The reaction was then stirred for 2 hours at 0 °C, and left overnight at room temperature under nitrogen. The precipitate was filtered, and the solution was concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes: ethyl acetate 1:1 to 1:3) to provide compound 4 (4.92 g, 83 %) as a colorless oil. ¹H NMR (CDCl₃, 400 MHz): δ 7.79 (d, J = 8.3 Hz, 2H), 7.42 – 7.40 (m, 6H), 7.33 – 7.31 (m, 2H), 7.29 – 7.25 (m, 6H), 7.22 – 7.18 (m, 3H), 4.13 (t, J = 4.8 Hz, 2H), 3.66 (t, J = 4.8 Hz, 2H), 3.55 – 3.51 (m, 6H), 3.44 – 3.41 (m, 2H), 3.29 (t, J = 6.9 Hz, 2H), 2.43 – 2.40 (m, 5H). ¹³C{¹H} NMR (CDCl₃, 101 MHz): δ 144.99, 144.97, 133.2, 130.0, 129.8, 128.2, 128.1, 126.9, 70.9, 70.73, 70.68, 70.3, 69.8, 69.4, 68.9, 66.8, 31.8, 21.9. ESI-MS calcd for C₃₄H₃₈NaO₆S₂ [M + Na]⁺ 629.2008, found 629.1994.

Synthesis of 5. To a solution of 3 (1.19 g, 3.70 mmol) in DMF (40 mL) was added compound 4 (3.37 g, 5.55 mmol). Next, portionwise was added K₂CO₃ (0.512 g, 3.70 mmol), then the solution was stirred and heated to 80 °C for 5 hours. The reaction was cooled to room temperature, diluted with ethyl acetate (400 mL), washed 5x with water (75 mL), brine (100 mL), and dried over MgSO₄. The organic layer was then filtered, concentrated in vacuo, and purified via silica gel chromatography (hexanes: ethyl acetate 3:1 to CH₂Cl₂: MeOH 30:1) to provide 5 (2.01 g, 72% yield) as a yellow oil. ¹H NMR (CDCl₃, 400 MHz): δ 7.95 – 7.92 (m, 2H), 7.42 – 7.40 (m, 6H), 7.29 – 7.25 (m, 6H), 7.21 – 7.18 (m, 3H), 6.90 – 6.88 (m, 4H), 4.18 (t, J = 4.7 Hz, 2H), 4.05 (t, J = 6.5, 2H), 3.87 (t,
$J = 4.7$ Hz, 2H), 3.73 – 3.71 (m, 2H), 3.65 – 3.64 (m, 2H), 3.60 – 3.57 (m, 2H), 3.46 (t, $J = 4.7$ Hz, 2H), 3.32 – 3.29 (m, 4H), 2.62 (d, $J = 10.7$ Hz, 2H), 2.43 (t, $J = 6.9$ Hz, 2H), 1.84 – 1.75 (m, 4H), 1.56 – 1.47 (m, 2H), 0.99 (t, $J = 7.4$ Hz, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): δ 162.8, 161.8, 154.0, 148.0, 148.0, 145.0, 142.6, 142.2, 136.0, 135.9, 129.8, 128.1, 126.9, 116.8, 116.6, 116.44, 116.39, 112.54, 112.45, 71.1, 70.9, 70.7, 70.4, 69.8, 69.7, 68.2, 67.9, 66.8, 37.38, 37.35, 31.9, 31.4, 19.4, 14.0. ESI-MS calcd for C$_{48}$H$_{51}$O$_6$S [M + H]$^+$ 755.3401, found 755.3401.

**Synthesis of 6.** Compound 5 (1.45 g, 1.92 mmol) was dissolved in CH$_2$Cl$_2$ (20 mL) and TFA (1.85 mL, 24.0 mmol). $^3$Pr$_3$SiH (0.866 mL, 4.23 mmol) was added and the reaction mixture was stirred at room temperature under argon for 3 hours. The reaction was concentrated in vacuo and purified via silica gel chromatography (CH$_2$Cl$_2$:MeOH 30:1) to provide 6 (0.910 g, 92%) as a yellow oil. $^1$H NMR (CDCl$_3$, 400 MHz): δ 7.94 (d, $J = 8.7$ Hz, 2H), 6.92 – 6.88 (m, 4H), 4.23 – 4.20 (m, 2H), 4.05 (t, $J = 6.5$ Hz, 2H), 3.91 – 3.88 (m, 2H), 3.76 – 3.74 (m, 2H), 3.71 – 3.59 (m, 8H), 3.33 (d, $J = 10.6$, 2H), 2.62 (d, $J = 10.7$ Hz, 2H), 2.87 – 2.72 (m, 2H), 1.84 – 1.77 (m, 2H), 1.60 (t, $J = 7.4$ Hz, 1H), 1.56 – 1.47 (m, 2H), 0.99 (t, $J = 7.4$ Hz, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): δ 162.3, 161.8, 154.0, 148.0, 142.6, 142.2, 136.0, 135.9, 116.8, 116.6, 116.43, 116.37, 112.6, 112.5, 73.1, 71.1, 70.9, 70.8, 70.4, 69.7, 68.2, 67.9, 37.39, 37.36, 31.3, 24.5, 19.4, 14.0. ESI-MS calcd for C$_{29}$H$_{37}$O$_6$S [M + H]$^+$ 513.2305, found 513.2303.

**Synthesis of MeO-EG$_3$-AuNPs.** HAuCl$_4$·3H$_2$O (1.46 g, 3.70 mmol, 1.0 eq.) was dissolved in a mixture of dry methanol (503 mL) and glacial acetic acid (83 mL). To this yellow solution was added MeO-EG$_3$-SH (2.0 g, 11 mmol, 3.0 eq.). The bright yellow solution was stirred vigorously for 1 h and the solution color faded slightly. A solution of NaBH$_4$ (1.3997 g, 37 mmol, 10.0 eq.) in nanopure H$_2$O (96 mL) was added dropwise to the reaction mixture under vigorous stirring. The mixture turned dark brown immediately. After overnight stirring at ambient temperature, the solution was concentrated and rediluted with brine. The MeO-EG$_3$-AuNPs were extracted with toluene while adding sodium chloride to the aqueous phase after each extraction to maintain the saturation. The aqueous phase was eventually colorless. The combined organic phases were then concentrated *in vacuo*. Evaporation of the solvent left a thin film of
nanoparticles which was then washed with hexanes to remove excess free thiols. The crude MeO-EG\textsubscript{3}-AuNPs were dissolved in nanopure H\textsubscript{2}O and further purified by overnight dialysis. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 3.34 (-CH\textsubscript{3}), 3.58, 3.66 (-CH\textsubscript{2}-).

**Synthesis of hvDIBO-AuNPs.** To a solution of MeO-EG\textsubscript{3}-AuNPs (120 mg) in CH\textsubscript{2}Cl\textsubscript{2} (15 mL) was added a solution of thiol 6 (24 mg, 0.05 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (15 mL) in the dark at room temperature. The mixture was stirred for 15 min then concentrated \textit{in vacuo}. The resulting AuNP film was washed with hexanes (3x) and Et\textsubscript{2}O (3x), then redissolved in 4 mL CH\textsubscript{2}Cl\textsubscript{2} and precipitated out by addition of hexanes (22 mL). The resulting suspension was placed under centrifugation at 6000 rpm for 10 min and the supernatant was removed. This washing procedure was repeated 3x and the resulting hvDIBO-AuNPs were dried \textit{in vacuo}. hvDIBO-AuNPs were stored as a solution in CH\textsubscript{2}Cl\textsubscript{2} at -20 °C.

**Synthesis of DIBO-AuNPs.** A solution of hvDIBO-AuNPs (8 mg) in CH\textsubscript{3}OH or H\textsubscript{2}O (32 mL) was irradiated with 350 nm light for 15 min. The reaction mixture was concentrated \textit{in vacuo} and the \textsuperscript{1}H NMR of the resulting AuNPs indicated quantitative decarbonylation to afford DIBO-AuNPs.

**Synthesis of triazole\textsubscript{1}-AuNPs.** To a solution of DIBO-AuNPs (15 mg) in CH\textsubscript{2}Cl\textsubscript{2} (2 mL) was added BN\textsubscript{3} (50 µL, 0.38 mmol) and left to stir for 15 min. After, the mixture was concentrated \textit{in vacuo} and the resulting film was washed with hexanes (3x) to remove excess azide. The film was remade by dissolving in CH\textsubscript{2}Cl\textsubscript{2} followed by rotary evaporation. This washing procedure was repeated two times, which afforded clean triazole\textsubscript{1}-AuNPs.

**Synthesis of triazole\textsubscript{2}-AuNPs.** To a solution of DIBO-AuNPs (10 mg) in CHCl\textsubscript{3} (400 µL) was added a 33 mM solution of p-azido anisole 8 in CH\textsubscript{2}Cl\textsubscript{2} (100 µL) and left to stir at r.t. for 4 h. After, the solvent was evaporated under reduced pressure and the reacted AuNPs were purified by dissolving in CH\textsubscript{2}Cl\textsubscript{2} (2 mL) and precipitation by hexanes (12 mL). This trituration procedure was repeated 3x, in which AuNPs were isolated each time by centrifugation at 6000 rpm for 10 min, to afford pure triazole\textsubscript{2}-AuNPs.
**Synthesis of triazole3-AuNPs.** To a solution of DIBO-AuNPs (10 mg) in CH$_2$Cl$_2$ (400 μL) was added a 33 mM solution of 3-azido-1-propanol 9 in CH$_2$Cl$_2$ (100 μL) and left to stir at r.t. for 4 h. After, the solvent was evaporated under reduced pressure and the reacted AuNPs were purified by dissolving in CH$_2$Cl$_2$ (2 mL) and precipitation by hexanes (12 mL). This trituration procedure was repeated 3x, in which AuNPs were isolated each time by centrifugation at 6000 rpm for 10 min, to afford the pure triazole3-AuNPs.

**Synthesis of triazole4-AuNPs.** To a solution of DIBO-AuNPs (10 mg) in 1:1 MeOH:CH$_2$Cl$_2$ (400 μL) was added a 33 mM solution of 3′-Azido-3′-deoxythymidine 10 in 1:1 MeOH:CH$_2$Cl$_2$ (100 μL) and left to stir at r.t. for 4 h. After, the solvent was evaporated under reduced pressure and the reacted AuNPs were purified by dialysis in MeOH overnight to afford the pure triazole4-AuNPs.

**Synthesis of isoxazoline-AuNPs.** To a solution of DIBO-AuNPs (10 mg) in CH$_2$Cl$_2$ (400 μL) was added a 33 mM solution of N-phenyl-α-phenylnitrone 11 in CH$_2$Cl$_2$ (100 μL) and left to stir at r.t. for 4 h. After, the solvent was evaporated under reduced pressure and the reacted AuNPs were purified by dissolving in CH$_2$Cl$_2$ (2 mL) and precipitation by hexanes (12 mL). This trituration procedure was repeated 3x, in which AuNPs were isolated each time by centrifugation at 6000 rpm for 10 min, to afford the pure isoxazoline-AuNPs.

**Kinetic measurements of SPAAC reactivity of DIBO-AuNPs.** Kinetic measurements using $^1$H NMR spectroscopy were performed as follows: A concentrated stock solution of DIBO-AuNPs was prepared in CDCl$_3$ to give a final concentration of 2.75 mM in interfacial DIBO. To 400 μL of this AuNP solution in a NMR tube was added 100 μL of 11 mM benzyl azide (BnN$_3$) solution to give a final equimolar concentration of 2.2 x 10$^{-3}$ M in both BnN$_3$ and interfacial DIBO. Reactions were monitored through $^1$H NMR analysis at 25 °C by following the decay of the sharp singlet signal at ~4.30 ppm corresponding to the benzylic protons of BnN$_3$. Integrations were normalized against the residual CH$_2$Cl$_2$ signal. These reactions were carried out in triplicate and the second order rate constant was calculated from a plot of 1/[BnN$_3$] (M$^{-1}$) versus time (in seconds).
Decomposition of AuNPs. For each reacted AuNP sample in CH$_3$OH, excess I$_2$ was added to decompose the AuNPs. Insoluble solids were filtered off and the filtrate containing a mixture of disulfides was submitted for ESI-MS analysis.

**Figure S2.1.** $^1$H NMR spectra of thiol ligand 6 (top) and hvDIBO-AuNPs (bottom) in CDCl$_3$. *Denotes residual protonated solvent. Spectra referenced against CHCl$_3$. 
**Figure S2.2.** Infrared spectrum of hvDIBO-AuNPs.

**Figure S2.3** UV-vis absorption spectrum of hvDIBO-AuNPs obtained in CH$_3$OH.
Figure S2.4. TGA characterization of hvDIBO-AuNPs.
Calculation of the AuNP raw formula.
From a combination of the $^1$H NMR, TGA, and TEM data, while assuming that AuNPs are perfectly spherical and monodispersed in size, it is possible to calculate an approximate raw formula for hvDIBO-AuNPs. The number of gold atoms ($N_{Au}$) can be calculated using the following formula:

$$N_{Au} = \frac{\pi \rho d^3 N_A}{6 M_{Au}}$$

Where:
- $\rho$ = density of face centered cubic (fcc) gold lattice (19.3 g cm$^{-1}$)
- $d$ = average diameter of nanoparticles in centimeters (from TEM images)
- $N_A$ = Avogadro constant
- $M_{Au}$ = mole atomic weight of gold (196.9665 g mol$^{-1}$)

The total number of thiol ligands surrounding the gold core ($N_L$) can be calculated using the following formula:

$$N_L = \frac{N_{Au} M_{Au} M_{TGA}}{(1 - M_{TGA})(M_{hvDIBO} n_{hvDIBO} + M_{MeO} n_{MeO})}$$

Where:
- $M_{TGA}$ = organic percentage from TGA
- $M_{hvDIBO}$ = molecular weight of hvDIBO-EG$_4$-S$^-$ ligand
- $n_{hvDIBO}$ = molar percentage of hvDIBO-EG$_4$-S$^-$ ligand
- $M_{MeO}$ = molecular weight of MeO-EG$_3$-S$^-$ ligand
- $n_{MeO}$ = molar percentage of MeO-EG$_3$-S$^-$ ligand

From the number of ligands per particle, the number of gold atoms per particles, and the molar percentage of the two different ligands it is possible to obtain the nanoparticles raw formula.
Figure S2.5. $^1$H NMR spectrum of DIBO-AuNPs in CDCl$_3$. *Denotes residual protonated solvent. Spectrum referenced against CHCl$_3$.

Figure S2.6. Infrared spectrum of DIBO-AuNPs.
Figure S2.7. UV-vis absorption spectrum of DIBO-AuNPs.

Figure S2.8. $^1$H NMR spectrum of triazole1-AuNPs in CDCl$_3$. *Denotes residual protonated solvent. Spectrum referenced against CHCl$_3$. 
Figure S2.9. Infrared spectrum of triazole1-AuNPs.

Figure S2.10. UV-vis absorption spectrum of triazole1-AuNPs obtained in CH₃OH.
Figure S2.11. TEM images of hvDIBO-AuNPs (left), DIBO-AuNPs (center), and triazole1-AuNPs (right).

Figure S2.12. $^1$H NMR spectrum of triazole2-AuNPs in CDCl$_3$. *Denotes residual protonated solvent. Spectrum referenced against CHCl$_3$. 
Figure S2.13. $^1$H NMR spectrum of triazole3-AuNPs in CDCl$_3$. *Denotes residual protonated solvent. Spectrum referenced against CHCl$_3$.

Figure S2.14. $^1$H NMR spectrum of triazole4-AuNPs in CDCl$_3$. *Denotes residual protonated solvent. Spectrum referenced against CHCl$_3$. 
Figure S2.15. \(^1\)H NMR spectrum of isoxazoline-AuNPs in CDCl\(_3\). *Denotes residual protonated solvent. Spectrum referenced against CHCl\(_3\).

Scheme S2.2. Re-oxidation of triazole1-AuNPs by molecular iodine. Disulfides 1A and 1B were characterized by ESI-MS. Disulfide 1A calculated 796.3665 for C\(_{42}\)H\(_{58}\)N\(_3\)O\(_8\)S\(_2\) [M+H]\(^+\), found 796.3635. Disulfide 1B calculated 1233.5769 for C\(_{70}\)H\(_{85}\)N\(_6\)O\(_{10}\)S\(_2\) [M+H]\(^+\), found 1233.5784.
Figure S2.16. ESI-MS characterization of disulfides 1A (top) and 1B (bottom).
**Scheme S2.3.** Re-oxidation of triazole2-AuNPs by molecular iodine. Disulfide 2 was characterized by ESI-MS. Disulfide 2 calculated 812.3614 for C_{42}H_{58}N_{3}O_{9}S_{2} [M+H]^+,
found 812.3636.

**Figure S2.17.** ESI-MS characterization of disulfide 2.
Scheme S2.4. Re-oxidation of triazole3-AuNPs by molecular iodine. Disulfide 3 was characterized by ESI-MS. Disulfide 3 calculated 786.3434 for C_{38}H_{57}N_{3}NaO_{9}S_{2} [M+Na]^+, found 786.3429.

Figure S2.18. ESI-MS characterization of disulfide 3.
Scheme S2.5. Re-oxidation of triazole4-AuNPs by molecular iodine. Disulfide 4 was characterized by ESI-MS. Disulfide 4 calculated 930.3993, for C_{45}H_{64}N_{5}O_{12}S_{2} [M+H]^+; found 930.3983.

![Diagram of Scheme S2.5](image.png)

**Figure S2.19** ESI-MS characterization of disulfide 4.
Scheme S2.6. Re-oxidation of isoxazoline-AuNPs by molecular iodine. Disulfide 5 was characterized by ESI-MS. Disulfide 5 calculated 859.3788 for C_{48}H_{61}NO_{9}S_{2} [M]^{+}, found 859.3763.

Figure S2.20. ESI-MS characterization of disulfides 5.
Chapter 3

A Fluorogenic Gold Nanoparticle (AuNP) Substrate: A Model for the Controlled Release of Molecules from AuNP Nanocarriers via Interfacial Staudinger-Bertozzi Ligation

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3.1 Introduction

Nanomaterials have emerged as important tools in the development of drug delivery systems due to their vast range of structures, high load capacity of surface bound and/or entrapped molecules, and their unique and tunable physical and chemical properties.[1-4] The efficacy of therapeutics, when introduced in their ‘free’ form into a biological setting, can be limited by solubility, *in vivo* stability, nonspecific bio-distribution, and multiple transport barriers.[5] In this regard, the application of gold nanoparticles (AuNPs) as a nanocarrier in drug delivery systems is a rapidly expanding field. Their inherent nontoxicity, reproducibility and scalability of their synthesis, and well-understood surface chemistry make them very attractive vehicles for the delivery of drugs, genetic materials, proteins, and small molecules.[6-7] As a result, much research has been dedicated to the development of methodologies for the controlled release of molecules from AuNP surfaces.

Nanocarriers can be loaded with the material that is destined to be released either through noncovalent or covalent incorporation. Due to the functional versatility of their monolayer chemistry, AuNPs provide an excellent platform for the design of controlled release systems. In general, AuNP-based delivery strategies rely on 1) non-covalent hydrophobic entrapment of molecules into the AuNP monolayer,[7-8] 2) photo-regulated release,[9-11] or 3) glutathione (GSH)-mediated release.[7,12] Although these systems represent effective delivery strategies, the lack of specificity and/or conjugate stability in
these ‘shotgun’-type approaches outline limits in current delivery strategies that need to be addressed. For these reasons, there remains the need to develop more efficient molecular delivery systems with greater target specificity and molecular-level control.

Despite the emergence of bioorthogonal chemistry and its use for in vivo imaging, biolabelling, and bioconjugation,[13-17] there has been little to no work done employing this family of reactions for the development of AuNP-based delivery systems. This is surprising given the widespread application of these reactions in vivo due to their high yielding nature, biocompatibility, chemoselectivity, and ability to proceed under physiological conditions.[18-19] In particular, the highly specific Staudinger-Bertozzi ligation with azides (Scheme 3.1), initially developed for cell surface engineering via amide bond formation, represents a dual-purpose transformation — chemical ligation and release — that is seldom utilized for the latter purpose.[20-21] Although this bioorthogonal ligation reaction has found applications in joining materials together covalently (e.g. bioconjugation onto nanomaterials, biolabelling with fluorescent probes, construction of mono- and multilayers on surfaces), the molecular cargo that is released is almost invariably a simple alcohol such as methanol.[22-24] However, this cargo release mechanism can be designed to carry out a function, i.e. deliver a molecular agent of interest (e.g. drug delivery in vivo, a fluorophore, a catalyst, etc.) or develop nanomaterial-based sensing platforms.

Scheme 3.1. Dual-purpose nature of the Staudinger-Bertozzi Ligation.

The union between bioorthogonal chemistry and materials chemistry is still a relatively new field. The strengths of bioorthogonal chemistry are not limited to small molecule chemistry for in vivo applications, but can be applied as a clean and fast reaction pathway towards functional material substrates. The Staudinger-Bertozzi ligation benefits from
high chemoselectivity, mild reaction conditions, as well as nitrogen gas being the only byproduct, i.e. no purification is required post-reaction.

We recently demonstrated that the Staudinger-Bertozzi ligation can be employed effectively and cleanly at the interface of organic solvent-soluble AuNPs for potential applications in bioconjugation. To further expand this technology beyond ligation onto AuNP surfaces, we set out to design a water-soluble AuNP system that fully exploits the dual-purpose nature of this bioorthogonal reaction. By employing the ester moiety as a functional handle for tethering a general cargo (drug, fluorophore, etc.), we capitalize on the alcohol release mechanism off AuNPs that occurs concomitantly with ligation via amide bond formation. The azide is becoming the most widely used tag in chemical biology because of its small size coupled with its chemical inertness towards biological functionalities. As such, a delivery strategy based on the Staudinger-Bertozzi ligation represents an extremely attractive system with high target selectivity as it offers careful and controlled release of the molecular cargo as a result of the high chemoselectivity of the nanocarrier system.

3.2 Results and Discussion

As a model for AuNP-based molecular delivery, we set out to design a system that incorporates a fluorescent dye into the AuNP monolayer. When covalently bound in close proximity to the metallic core, efficient quenching of the dye emission is expected (Figure 3.1a). This provides the baseline from which the release event can be monitored. Upon release of the dye from the nanoparticle surface into the bulk solution, fluorescence emission is turned ‘on’, thus indicating an effective release mechanism.

Rhodamine B dye was chosen for its low cost, excellent water solubility, and photostability. To incorporate this dye into the AuNP monolayer, a ligand was designed to contain three key features (Figure 3.1b): (1) a thiol head for binding to the Au surface (2) the modified triphenylphosphine moiety required for the Staudinger-Bertozzi ligation and (3) the molecular cargo bound via an ester, which is required for release.
Figure 3.1. (a) Cartoon demonstrating the design of a release system that exploits fluorescence quenching of metallic nanoparticles. (b) Design of the thiol ligand bearing the molecular cargo.

The synthesis of this thiol ligand began from commercially available 1-methyl-2-aminoterephthalate, which was transformed to phosphine 1 according to a procedure previously developed in our group (Scheme 3.2).[25] Hydrolysis of the methyl ester in 1 by a basic ethanol solution afforded the benzoic acid derivative 2, which underwent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)-mediated esterification with a Rhodamine-B alcohol derivative 3 to afford ester 4. Subsequent cleavage of the S-trityl protecting group in 4 by TFA/CH₂Cl₂ gave the target thiol ligand 5. It is worth noting that the length of thiol 5 was chosen to be long enough for the phosphine to be presented at the monolayer surface for interfacial reactivity, but short enough to observe quenching of the dye emission upon binding onto AuNPs. Thiol ligand 7, the P=O derivative of 5, which cannot undergo Staudinger-Bertozzi ligation, was also prepared to serve as a control. Details of the ligand syntheses can be found in the Supporting Information (Section 3.6).

Incorporation of thiol ligand 5 onto AuNPs was achieved via a ligand exchange reaction with HO-EG₄-AuNPs in CH₃OH overnight (Scheme 3.2). Ligand exchange represents a
**Scheme 3.2.** Synthesis of thiol ligands 5 and 7 (top) and Rhod-PPh₃-EG₄-AuNPs and Rhod-(P=O)Ph₃-EG₄-AuNPs (bottom).

mild reaction pathway towards incorporating new ligands into AuNP monolayers. The HO-EG₄-AuNPs are 5 ± 1 nm in gold core diameter and were chosen for their high water solubility (compared to MeO-EG₃-AuNPs used previously[25]) and ability to prepare them on a gram-scale. Additionally, HO-EG₄-AuNPs were found to feature exceptional
colloidal stability, where they can be stored in solution at -20°C for months with no observed aggregation. Thus, these AuNPs represent very resilient substrates for further modification as well as interfacial organic chemistry. Details of their synthesis can be found in the Supporting Information (Section 3.6).

Following place exchange, Rhod-PPh₃-EG₄-AuNPs were purified from excess thiols and disulfides via trituration with a 1:1 mixture of CH₂Cl₂/Et₂O in which the AuNPs are not soluble. It was important that any unbound or ‘free’ thiols and disulfides resulting from 5 were removed to provide a baseline of fluorescence from which we can monitor the release event. The ¹H NMR spectrum of the resulting treated AuNP sample (Figure 3.2, bottom), when compared to the HO-EG₄-AuNP starting material (Figure 3.2, top), showed additional broad peaks in the aromatic region that correspond to surface-bound ligand 5. The absence of sharp peaks in this region represents the absence of free 5 in solution, thus indicating that the washing procedure employed was effective in removing unbound thiols and disulfides. The ³¹P{¹H} NMR spectrum of Rhod-PPh₃-EG₄-AuNPs (see Figure S3.5) showed a broad signal at 41.4 ppm and a sharp signal at 33.7 ppm. As discussed previously,²⁵ the broad signal at 41.4 ppm was assigned to the Staudinger-active phosphine ligand 5. The sharp signal at 33.7 ppm is a small amount of phosphine oxide (P=O) generated from oxidation during the washing procedure. Rhod-(P=O)Ph₃-EG₄-AuNPs (Scheme 3.2), which showed one sharp resonance at 33.7 ppm in its ³¹P{¹H} NMR spectrum, were also prepared to serve as a control as it will not undergo Staudinger-Bertozzi ligation.

Thermogravimetric analysis (TGA) of Rhod-PPh₃-EG₄-AuNPs showed that 32% of the total weight is from the organic corona (see Figure S3.7). Deconvolution of the derivative plot showed that two ligands decompose at distinctly different temperatures (see Figure S3.7): a large component at 270°C and a smaller component a 375°C. By comparing to the TGA data of the HO-EG₄-AuNP starting material (See Figure S3.3), the ligand decomposing at 270 °C was assigned to HO-EG₄-S⁻ and corresponded to 83% of the organic corona by mass, whereas the ligand decomposing at 375°C was assigned to 5 and corresponded to the remaining 17%. Finally, from the deconvolution of the TGA curve, ³¹P{¹H} NMR spectrum, and the average gold core diameter (5 ± 1 nm) obtained from
transmission electron microscopy (TEM) images (see Figure S3.6), it was possible to calculate a raw formula for Rhod-PPh$_3$-EG$_4$-AuNPs: Au$_{3500}$[HO-EG$_4$-S$^-$]$_{1400}$[Rhod-PPh$_3$-EG$_4$-S$^-$]$_{40}$[Rhod-P(=O)Ph$_3$-EG$_4$-S$^-$]$_5$. More specifically, these AuNPs contain the payload to be delivered at a concentration of 0.038 μmol mg$^{-1}$ AuNP (for calculations, see Section 3.6). The concentration of 5 incorporated in this model system was chosen to strike a balance between maintaining water solubility and allowing for enough incorporation for characterization of the new functionalities. However, it is worth noting that the concentration of ligand 5 can be tuned by increasing/decreasing the ratio of 5:AuNP during the ligand exchange reaction or by increasing/decreasing reaction time as needed. This ability to tune and determine the concentration of the payload with good precision.
using the protocol reported here is of particular importance for delivery and/or sensing applications.

The final suspension of Rhod-PPh$_3$-EG$_4$-AuNPs retained only a very low level of emission, which arose from the quenching of the Rhodamine B dye’s fluorescence by proximity to the metallic core, thus providing a baseline from which the release event can be monitored. To test our goal of azide-triggered release, azide 8 was introduced to a solution of Rhod-PPh$_3$-EG$_4$-AuNPs in nanopure water and the reaction was monitored by fluorescence spectroscopy (Figure 3.3). Azide 8 was selected for its water solubility and structural resemblance to benzyl azide, a standard azide molecule employed in bioorthogonal reaction studies.$^{[20-21,25]}$ An immediate increase in fluorescence emission intensity after addition of an azide was observed. This enhancement in emission intensity indicates effective release of the dye from the nanoparticle surface into the bulk solution, reaching a plateau in 4–6 hours. It is worth noting that a high concentration of azide 8 (0.5 M) was used to trigger the release of the dye in this model study, however we’ve previously demonstrated that the Staudinger-Bertozzi ligation proceeds even at millimolar concentrations of azide on AuNPs and the same is true here.$^{[25]}

Following the addition of azide 8, the $^{31}$P{$^1$H} NMR spectrum of the reacted AuNPs showed only one component at 35 ppm corresponding to the P=O of the ligation product (and overlapped P=O from previous, see Figure S3.10),$^{[25]}$ thus confirming that the release mechanism observed here is due to the interfacial bioorthogonal reaction, which proceeded quantitatively. Therefore, the emission intensity after 4–6 hours represents full release of the cargo and the progression of the reaction can be monitored as a fraction of emission intensity.

As a control, azide 8 was also introduced to a suspension of Rhod-(P=O)Ph$_3$-EG$_4$-AuNPs, the phosphine oxide analogue to Rhod-PPh$_3$-EG$_4$-AuNPs. Due to the lack of the phosphine moiety, these AuNPs do not undergo the interfacial Staudinger-Bertozzi ligation. Even after 8 hours, no increase in fluorescence emission was observed, as expected (Figure 3.3). This further confirmed that the release mechanism is in fact by interfacial Staudinger-Bertozzi ligation in which the phosphine lone pair is required.$^{[20]}$
Figure 3.3. Top: Fluorescence emission spectra of an aqueous solution Rhod-PPh$_3$-EG$_4$-AuNPs (from bottom to top) 0 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after addition of azide 8. Bottom: Fluorescence emission intensity of AuNPs at 585 nm versus time. $\lambda_{ex} = 550$ nm. Right: Vials depicting a solution of Rhod-PPh$_3$-EG$_4$-AuNPs before and after addition of azide 8 accompanied by a scheme of the interfacial Staudinger-Bertozzi ligation reaction.

Furthermore, as stated previously, AuNP-based delivery systems are prone to ligand displacement by other thiol molecules. For example, GSH-mediated release is commonly exploited in AuNP drug delivery systems in which intracellular concentrations (1–10 mM) of GSH can trigger ligand exchange.[7] This could potentially lead to loss of specificity in which our cargo is released as a thiolate (i.e. thiolate of 5) as opposed to the intended Rhodamine B alcohol derivative shown in Figure 3.3. To test this, Rhod-PPh$_3$-EG$_4$-AuNPs were suspended in a 10 mM aqueous solution of GSH and the fluorescence emission of the solution was monitored. No significant enhancement in emission was observed (Figure 3.3) compared to the dramatic enhancement when azide 8 was employed to trigger the payload release. Thus, ligand exchange is not a major contributor to payload release. The high specificity of this nanocarrier system will allow for greater control and selectivity of the release event compared to traditional ‘shotgun’-type approaches.
3.3 Conclusion

In conclusion, to the best of our knowledge, we demonstrate for the first time a highly specific AuNP-based controlled-release system based on the bioorthogonal interfacial Staudinger-Bertozzi ligation. In our design, we exploit the efficient quenching effect of metallic nanoparticles on fluorescent dyes when they are incorporated into the surface-bound monolayer. The resulting low level of dye emission provided the baseline of fluorescence, which was used to monitor the release event. We observed – both spectroscopically and visually – an immediate ‘turn on’ of fluorescence upon introduction of an organic azide to a suspension of Rhod-PPh$_3$-EG$_4$-AuNPs, which corresponded to dye release from the nanoparticle surface. $^{31}$P{$^1$H} NMR data and control experiments independently confirmed that interfacial Staudinger-Bertozzi ligation is the mechanism of release.

Although the Staudinger-Bertozzi ligation represents a powerful organic transformation that has been extensively used in small molecule chemistry for _in vivo_ applications, it is seldom exploited for its dual-purpose nature, especially on material surfaces. In our methodology, we’ve provided a clean and simple route towards (1) chemically ligating new material onto AuNP surfaces and (2) release of a molecular cargo from AuNP nanocarriers, both of which can be achieved simultaneously within a single interfacial transformation. This, along with the ease of installation of the azide functional group in chemical biology, materials, and small molecule chemistry provides exciting opportunities for new applications of this nanomaterial-based release platform. Lastly, the molecular cargo can be designed to be a drug, fluorophore, or small molecule of interest to further expand the scope of applications both _in vivo_ and in the development of functional nanomaterials. Further studies and applications of this novel controlled-release system are currently underway.

3.4 Acknowledgement

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3.5 References


3.6 Supporting Information

**General Methods.** The following reagents, unless otherwise stated, were used as received. Triethylene glycol monomethyl ether, tetaethylene glycol, 1-methyl-2-aminoterephthalate, sodium azide, triphenylmethanethiol, triphenylphosphine, anhydrous deuterated chloroform (CDCl₃), anhydrous deuterated methanol (CD₃OD), deuterium oxide (D₂O), tetrachloroauric acid trihydrate, sodium borohydride, p-Toluenesulfonyl chloride, triisopropylsilane (TIPS), N,N-Diisopropylethylamine (DIEA), O-Benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate (HBTU), and Rhodamine B were purchased from Sigma-Aldrich. All common solvents, triethylamine (TEA), magnesium sulfate, sodium sulfate anhydrous, potassium iodide, sodium sulphite, sodium bicarbonate, dry methanol, hydrochloric acid, trifluoroacetic acid, sodium hydroxide, and sodium chloride were purchased from Caledon. Palladium(II) diacetate trimer and diphenylphosphine were purchased from Alfa Aesar. Ethanol was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) and sodium nitrite were purchased from BDH. Dialysis membranes (MWCO 6000-8000 Da) were purchased from Spectra/Por. 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate and compound 1 were synthesized from 1-methyl-2-aminoterephthalate according to a previously established protocol in our group (Org. Biomol. Chem. 2015, 13, 4605–4612). Compound 3 was synthesized according to Org. Lett. 2003, 5, 3245–3248.

¹H, ¹³C and ³¹P NMR spectra were recorded on an Inova 400 or 600 MHz spectrometers using CDCl₃, CD₃OD, or D₂O as solvent and were calibrated against the solvent or using H₃PO₄ as a standard. Thermogravimetric analyses (TGA) were recorded by loading the sample in a 70 μL ceramic crucible and heating from 25 °C to 750 °C at a rate of 10 °C min⁻¹. The experiment was run under a nitrogen flow of 70 mL min⁻¹ in a Mettler Toledo TGA/SDTA 851 instrument. Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and prepared by dropcasting a drop of nanoparticles solution directly onto the grid surface. The drop was then carefully removed after 30 seconds with a soft tissue. ESI-MS spectra were recorded
on a Micromass LCT mass spectrometer. Fluorescence spectroscopic studies were performed on a Photon Technology International (PTI) fluorimeter.

**Synthesis of compound 2.** A solution of methyl 2-(diphenylphosphino)-4-((1,1,1-triphenyl-5,8,11-trioxo-2-thiatridecan-13-yl)carbamoyl)benzoate 1 (834 mg, 1.07 mmol) in argon purged 1 M NaOH solution in 95% ethanol (120 mL) was prepared under an argon atmosphere and stirred vigorously at r.t. for 90 min. After, the mixture was acidified with 1 M HCl(aq) (200 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers was dried over Na$_2$SO$_4$ and concentrated _in vacuo_. The residue was purified by column chromatography on silica gel using 1:6 iPrOH:CH$_2$Cl$_2$ as the eluent to afford 2 (610 mg, 73%) as a pale yellow solid. $^1$H NMR (CDCl$_3$, 400 MHz): δ 8.10 (dd, $J$ = 8.2 Hz, 3.8 Hz, 1H), 7.74 (br, 1H), 7.41 – 7.16 (m, 26H), 6.56 (br, 1H), 3.62 – 3.48 (m, 10H), 3.42 (t, $J$ = 4.4 Hz, 2H), 3.27 (t, $J$ = 6.8 Hz, 2H), 2.40 (t, $J$ = 7.0 Hz, 2H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): δ 169.2, 166.5, 144.8, 142.7, 142.5, 137.7, 137.2, 137.1, 135.3, 135.2, 134.0, 133.9, 132.8, 131.5, 129.6, 128.9, 128.6, 128.5, 127.8, 126.6, 70.5, 70.4, 70.2, 70.1, 69.6, 66.6, 39.8, 31.6. $^{31}$P{$^1$H} NMR (CDCl$_3$, 162 MHz): δ -4.04 (s). ESI-MS calcd for C$_{47}$H$_{47}$NO$_6$PS$^+$ [M + H]$^+$ 784.2862, found 784.2876.

**Synthesis of compound 4.** To a solution of 2 (400 mg, 0.51 mmol), 3 (407 mg, 0.60 mmol), and HBTU (378 mg, 0.99 mmol) in MeCN (28 mL) was added DIEA (0.24 mL, 1.43 mmol) at r.t. under an argon atmosphere. The resulting solution was stirred for 18 h. After, the solution was concentrated _in vacuo_ and the residue was purified by column chromatography on silica gel using 1:6 iPrOH:CH$_2$Cl$_2$ as the eluent to afford 4 (720 mg, 97%) as a purple solid. $^1$H NMR (CDCl$_3$, 400 MHz): δ 8.06 (dd, $J$ = 7.8 Hz, 3.7 Hz, 1H), 7.78 (dd, $J$ = 7.8 Hz, 2.0 Hz, 1H), 7.70 – 7.63 (m, 2H), 7.56 – 7.49 (m, 1H), 7.43 – 7.16 (m, 29H), 6.94 (dd, $J$ = 9.4 Hz, 2.7 Hz, 2H), 6.76 (d, $J$ = 2.3 Hz, 2H), 6.54 (t, $J$ = 5.1 Hz, 1H), 4.71 (t, $J$ = 6.6 Hz, 2H), 3.66 – 3.48 (m, 18H), 3.45 – 3.40 (m, 2H), 3.40 – 3.30 (m, br, 4H), 3.26 (t, $J$ = 7.0 Hz, 2H), 2.40 (t, $J$ = 7.0 Hz, 2H), 2.33 – 2.14 (m, br, 6H), 1.71 (t, $J$ = 7.0 Hz, 2H), 1.30 (t, $J$ = 7.2 Hz, 12H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): δ 167.2, 166.3, 166.2, 157.6, 155.9, 155.5, 144.6, 140.9, 140.6, 137.2, 137.2, 137.1, 136.7, 136.6, 135.3, 133.8, 133.6, 132.9, 132.0, 131.81, 131.76, 130.6, 130.5, 130.0, 129.9, 129.7, 129.4, 128.7, 128.5, 128.4, 127.7, 127.4, 126.7, 126.5, 113.9, 113.6, 96.1, 70.3, 70.2,
70.0, 69.9, 69.4, 66.4, 64.1, 63.5, 54.3, 45.9, 39.7, 31.5, 29.5, 25.5, 12.4. \(^{31}\text{P}\text{--}^{1}\text{H}\) NMR (CDCl\textsubscript{3}, 162 MHz): \(\delta -4.32\) (s). ESI-MS calcd for C\textsubscript{82}H\textsubscript{90}N\textsubscript{5}O\textsubscript{8}PS\textsubscript{2}\textsuperscript{2+} [M + H]\textsuperscript{2+} 1335.6242, found 1335.6227.

**Synthesis of compound 5.** To a solution of 4 (50 mg, 0.03 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (18 mL) was added TFA (0.6 mL, 7.8 mmol) dropwise at 0 °C under an argon atmosphere followed by addition of \(^3\text{Pr}_3\text{SiH} (16 \mu\text{L}, 0.08 \text{mmol}). \text{Ar}(g)\) was bubbled through the reaction mixture for an additional 15 min. The flask was sealed and left to stir at 0 °C to r.t. in the dark for 2 h. After, the solution was concentrated in vacuo and the residue was purified by column chromatography on silica gel using 1:6 \(^3\text{PrOH}:\text{CH}_2\text{Cl}_2\) then 1:4 MeOH:CH\textsubscript{2}Cl\textsubscript{2} as the eluent to afford 5 (31 mg, 75%) as a purple solid. \(^1\text{H}\) NMR (CD\textsubscript{3}OD, 400 MHz): \(\delta 8.02\) (dd, \(J = 8.2\) Hz, \(J = 3.5\) Hz, 1H), 7.81 (dd, \(J = 8.2\) Hz, \(J = 2.0\) Hz, 1H), 7.79 – 7.73 (m, 2H), 7.67 – 7.61 (m, 1H), 7.55 – 7.49 (m, 1H), 7.46 – 7.41 (m, 1H), 7.39 – 7.19 (m, 12H), 7.05 (dd, \(J = 9.6\) Hz, \(J = 2.5\) Hz, 2H), 6.95 (d, \(J = 2.3\) Hz, 2H), 4.13 (t, \(J = 6.2\) Hz, 2H), 3.71 – 3.50 (m, 20H), 3.46 (t, \(J = 5.5\) Hz, 2H), 3.37 (s, br, 4H), 2.58 (t, \(J = 6.2\) Hz, 2H), 2.32 (t, \(J = 7.0\) Hz, 2H), 2.22 (s, br, 4H), 1.70 (quintet, \(J = 7.0\) Hz, 2H), 1.28 (t, \(J = 7.0\) Hz, 12H). \(^{13}\text{C}\text{--}^{1}\text{H}\) NMR (CD\textsubscript{3}OD, 101 MHz): \(\delta 169.4, 169.3, 168.0, 159.4, 157.3, 157.0, 142.1, 141.9, 139.2, 139.1, 138.8, 138.7, 136.6, 135.3, 135.1, 134.8, 133.6, 132.3, 131.8, 131.7, 131.5, 131.4, 130.3, 129.9, 129.0, 128.1, 115.5, 115.0, 97.5, 74.2, 71.73, 71.66, 71.4, 71.2, 70.5, 64.9, 64.4, 56.9, 55.7, 47.0, 41.2, 31.7, 31.2, 26.0, 25.4, 24.8, 13.1. \(^{31}\text{P}\text{--}^{1}\text{H}\) NMR (CD\textsubscript{3}OD, 162 MHz): \(\delta -4.34\) (s). ESI-MS calcd for C\textsubscript{63}H\textsubscript{76}N\textsubscript{5}O\textsubscript{8}PS\textsubscript{2}\textsuperscript{2+} [M + H]\textsuperscript{2+} 1093.5147, found 1093.5127.

**Synthesis of compound 6.** To a solution of 4 (57 mg, 0.04 mmol) in CHCl\textsubscript{3} (5 mL) was added 10 mL 3% H\textsubscript{2}O\textsubscript{2}(aq) while stirring. The mixture was heated to 45 °C and left to stir for 3 h. After, the reaction mixture was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10 mL) and the combined organic fractions were dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated to give 6 as a purple solid (58 mg, quant. yield), which was used in the next step without further purification. \(^1\text{H}\) NMR (CDCl\textsubscript{3}, 400 MHz): \(\delta 8.09 – 8.02\) (m, 2H), 7.86 (dd, \(J = 7.8\) Hz, \(J = 3.1\) Hz, 1H), 7.69 – 7.15 (m, 31H), 7.07 (t, \(J = 5.5\) Hz, 1H), 6.92 (dd, \(J = 9.8\) Hz, 2.7 Hz, 2H), 6.75 (d, \(J = 2.3\) Hz, 2H), 3.93 (t, \(J = 6.6\) Hz, 2H), 3.65 – 3.51 (m, 18H), 3.43 – 3.28 (m, 6H), 3.25 (t, \(J = 6.6\) Hz, 2H), 2.38 (t, \(J = 7.0\) Hz, 2H), 2.28 – 2.10 (m, br, 6H),
1.53 (m, 2H), 1.29 (t, $J = 7.3$ Hz, 12H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 167.3, 166.7, 165.6, 157.7, 156.2, 155.6, 144.7, 138.4, 136.6, 136.5, 135.5, 133.4, 133.3, 133.2, 131.9, 131.8, 130.6, 130.5, 130.1, 129.8, 129.6, 128.5, 128.3, 127.8, 127.5, 126.6, 114.1, 113.8, 96.1, 70.5, 70.3, 70.2, 70.1, 69.5, 69.4, 66.5, 64.0, 54.3, 53.4, 53.0, 52.3, 47.6, 46.0, 41.7, 40.0, 31.6, 29.7, 25.1, 12.6. $^{31}$P{$^1$H} NMR (CDCl$_3$, 162 MHz): $\delta$ 31.2 (s).

ESI-MS calcd for C$_{82}$H$_{89}$N$_5$O$_9$PS$^+$ [M]$^+$ 1350.6113, found 1350.6115.

**Synthesis of compound 7.** To a solution of 6 (50 mg, 0.03 mmol) in CH$_2$Cl$_2$ (20 mL) was added TFA (1.4 mL, 18 mmol) dropwise at 0 °C under an argon atmosphere followed by addition of iPr$_3$SiH (20 μL, 0.10 mmol). Ar(g) was bubbled through the reaction mixture for an additional 15 min. The flask was sealed and left to stir at 0 °C to r.t. in the dark for 2 h. After, the solution was concentrated in vacuo and the residue was purified by column chromatography on silica gel using 1:6 iPrOH:CH$_2$Cl$_2$ then 1:4 MeOH:CH$_2$Cl$_2$ as the eluent to afford 7 (29 mg, 80%) as a purple solid. 

$^1$H NMR (CD$_3$OD, 600 MHz): $\delta$ 8.69 (t, $J = 5.5$ Hz, 1H), 8.14 (d, $J = 8.2$ Hz, 1H), 8.01 (dd, $J = 7.6$ Hz, $J = 3.5$ Hz, 1H), 7.86 (m, 1H), 7.80 – 7.77 (m, 2H), 7.73 (m, 1H), 7.80 – 7.50 (m, 11H), 7.26 (d, $J = 9.4$ Hz, 2H), 7.07 (dd, $J = 9.4$ Hz, $J = 2.3$ Hz, 2H), 6.97 (d, $J = 2.3$ Hz, 2H), 4.04 (t, $J = 5.9$ Hz, 2H), 3.71 – 3.59 (m, 26H), 3.14 – 2.78 (m, br, 6H), 2.59 (t, $J = 6.4$ Hz, 2H), 1.86 (s, br, 2H), 1.30 (t, $J = 7.6$ Hz, 12H). $^{13}$C{$^1$H} NMR (CD$_3$OD, 150 MHz): $\delta$ 169.4, 168.2, 168.2, 168.1, 159.4, 157.4, 156.9, 139.92, 139.86, 138.6, 138.4, 136.0, 135.3, 135.2, 133.9, 133.9, 133.7, 133.3, 133.1, 133.0, 132.6, 132.5, 132.3, 132.23, 132.15, 132.1, 132.0, 131.7, 131.5, 130.2, 130.1, 129.0, 119.8, 116.9, 115.5, 115.0, 97.5, 74.2, 71.74, 71.66, 71.4, 71.2, 70.5, 64.9, 64.4, 55.7, 49.2, 47.1, 41.3, 39.5, 25.4, 24.8, 13.0. $^{31}$P{$^1$H} NMR (CD$_3$OD, 243 MHz): $\delta$ 33.8 (s). ESI-MS calcd for C$_{63}$H$_{75}$N$_5$O$_9$PS$^+$ [M]$^+$ 1108.5018, found 1108.5041.
Scheme S3.1. Synthesis of sodium 4-(azidomethyl)benzoate 8.

**Synthesis of compound 8.** To a suspension of 4-(bromomethyl)benzoic acid (2.0 g, 9.3 mmol) in MeCN (30 mL) was added NaN₃ (800 mg, 12.3 mmol). The mixture was heated to 85°C and stirred under reflux for 14 h. After, the solvent was removed by rotary evaporation and CH₂Cl₂ (50 mL) was added followed by extraction by H₂O (50 mL x 2) to remove unreacted NaN₃. The organic phase was dried over Na₂SO₄ and concentrated. The residue was moved forward to the next step without any further purification.

The residue was dissolved in 4 mL acetone and added to a stirring solution of NaOH(aq) (247 mg, 6.17 mmol) in H₂O (10 mL). The resulting mixture was stirred vigorously at room temperature for 18 h. After, the remaining solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford 8 as a white solid (926 mg, 50%). ¹H NMR (CDCl₃, 400 MHz): δ 7.86 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 4.44 (s, 2H).

Scheme S3.2. Synthesis of HO-EG₄-SH thiol ligand 9.

**Synthesis of compound 9.** To a solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5 g, 14.6 mmol) in acetone (75 mL) was added potassium thioacetate (1.71 g, 15.0 mmol) while stirring. The solution was heated to 50 °C and stirred under reflux for 18 h. After, the solid was
removed by gravity filtration and the filtrate was concentrated in vacuo. The crude product was purified by column chromatography using 3:1 CH₂Cl₂:acetone as the eluent to afford 9 as a yellow oil (3.03 g, 84%). ¹H NMR (CDCl₃, 400 MHz): δ 3.74 (m, 2H) 3.67 (m, 12H) 3.10 (t, J = 12 Hz, 2H), 2.34 (s, 3H). ¹³C{¹H} NMR (CDCl₃, 101 MHz): δ 195.5, 72.5, 70.61, 70.45, 70.32, 70.23, 69.7, 61.7, 30.5, 28.8. CI-HRMS: calcd for C₁₀H₂₀O₅S [M+H⁺]: 253.1031, found 253.1105.

**Synthesis of compound 10.** To a solution of 8 (3.03 g, 0.012 mmol) in anhydrous CH₃OH (20 mL) was added 12 mL of 1 M NaOH in EtOH under an argon atmosphere while stirring. In a separate flask, argon was bubbled through a 2 M HCl(aq) solution. After 20 min, 12 mL of HCl(aq) was added to the reaction flask and left to react for 5 min. After, the product was extracted into CH₂Cl₂ (20 mL x 5). The combined organic fractions were dried over MgSO₄ and concentrated in vacuo. The crude product was purified by column chromatography using 2:1 CH₂Cl₂:acetone as the eluent to afford 9 as a pale yellow oil (2.38 g, 94%). ¹H NMR (CDCl₃, 400 MHz): δ 3.73 (m, 2H) 3.68 (m, 12H) 2.70 (m, 2H), 2.39 (bs, 1H), 1.63 (t, J = 16 Hz, 1H). ¹³C{¹H} NMR (CDCl₃, 101 MHz): δ 72.89, 72.47, 70.63, 70.48, 70.33, 70.17, 61.7, 24.2. CI-HRMS: calcd for C₈H₁₉NO₃S [M+H⁺]: 211.0926, found 211.0996.

**Synthesis of HO-EG₄-AuNPs.** HAuCl₄·3H₂O (1.4564 g, 3.7 mmol, 1.0 eq.) was dissolved in a mixture of dry methanol (503 mL) and glacial acetic acid (83 mL). To this yellow solution was added HO-EG₄-SH (10) (2.3 g, 11 mmol, 3.0 eq.). The bright yellow solution was stirred vigorously for 1 h and the solution color faded slightly. A solution of NaBH₄ (1.3997 g, 37 mmol, 10.0 eq.) in nanopure H₂O (96 mL) was added dropwise to the reaction mixture under vigorous stirring. The mixture turned dark brown immediately. After overnight stirring at ambient temperature, the solution was concentrated in vacuo. The resulting film of AuNPs was washed with toluene (3x) and the film was remade by dissolving the AuNPs in methanol followed by rotary evaporation to remove the solvent. This washing procedure was repeated 6 times. Next, the crude HO-EG₄-AuNPs were dissolved in nanopure water and centrifuged at 6000 rpm for 10 min to remove insoluble solid impurities. The crude HO-EG₄-AuNPs were then further purified by overnight dialysis in H₂O.
Figure S3.1. $^1$H NMR spectrum of HO-EG$_4$-AuNPs in CD$_3$OD. *Denotes residual protium solvent. Spectrum referenced against residual CD$_3$OH.

Figure S3.2. TEM images of HO-EG$_4$-AuNPs.
Figure S3.3. TGA characterization of HO-EG₄-AuNPs.

Synthesis of Rhod-PPh₃-EG₄-AuNPs. To a solution of HO-EG₄-AuNPs (84 mg) in MeOH (5 mL) was added a solution of thiol (5) (27 mg, 0.02 mmol) in MeOH (5 mL) under an argon atmosphere in the dark. The resulting mixture was stirred vigorously for 18 h in the dark. After, the solution was concentrated in vacuo and the resulting film was washed with argon purged 1:1 CH₂Cl₂:Et₂O until washings were nearly colorless. The film was remade by dissolving in MeOH and concentrating in vacuo. The washes were then repeated until the washings were no longer colored or fluorescent under a UV lamp.
**Figure S3.4.** $^1$H NMR spectrum of Rhod-PPh$_3$-EG$_4$-AuNPs in CD$_3$OD. *Denotes residual protium solvent. Spectrum referenced against residual CD$_3$OH.

**Figure S3.5.** $^{31}$P{$^1$H} NMR spectrum of Rhod-PPh$_3$-EG$_4$-AuNPs in CD$_3$OD. Spectrum referenced against H$_3$PO$_4$ as a standard.
Figure S3.6. TEM images of Rhod-PPh₃-EG₄-AuNPs.

Figure S3.7. TGA characterization of Rhod-PPh₃-EG₄-AuNPs.
Synthesis of Rhod-P(=O)Ph₃-EG₄-AuNPs (control). To a solution of HO-EG₄-AuNPs (21 mg) in MeOH (5 mL) was added a solution of thiol (7) (9 mg, 7 μmol) in MeOH (2.5 mL) under an argon atmosphere in the dark. The resulting mixture was stirred vigorously for 18 h in the dark. After, the solution was concentrated in vacuo and the resulting film was washed with argon purged 1:1 CH₂Cl₂:Et₂O until washings were nearly colorless. The film was remade by dissolving in MeOH and concentrating in vacuo. The washes were then repeated until the washings were no longer colored or fluorescent under a UV lamp.

![NMR spectrum](image)

**Figure S3.8.** ¹H NMR spectrum of Rhod-(P=O)Ph₃-EG₄-AuNPs in CD₃OD. *Denotes residual protium solvent. Spectrum referenced against residual CD₃OH.
Figure S3.9. $^{31}$P$\{^1$H$\}$ NMR spectrum of Rhod-(P=O)Ph$_3$-EG$_4$-AuNPs in CD$_3$OD. Spectrum referenced against H$_3$PO$_4$ as a standard.

Calculation of the nanoparticles raw formula

From the deconvolution of the TGA derivative (see Figure S3.7, bottom) it is possible to calculate the weight amount of template ligand (HO-EG$_4$-S$^-$) and phosphine ligand 5 per milligram of Rhod-PPPh$_3$-EG$_4$-AuNP. A fraction of ligand 5 is present as phosphine oxide (12 % from the $^{31}$P$\{^1$H$\}$ NMR spectrum). It is possible to calculate the amount, in grams, of oxidized phosphine ligand ($mg_{P=O}$) and active phosphine ($mg_P$) per milligram of AuNP from the following equations:

$$mg_P = \frac{M_{TGA(P+P=O)} \cdot MW_P \cdot n_{\%P}}{MW_P \cdot n_{\%P} + MW_{P=O} \cdot n_{\%P=O}}$$

$$mg_{P=O} = M_{TGA(P+P=O)} - mg_P$$

Where:

$M_{TGA(P+P=O)}$ = mass of phosphine ligand (P: + P=O) per milligram of AuNP from TGA

$MW_P$ = molecular weight of phosphine ligand

$MW_{P=O}$ = molecular weight of phosphine oxide ligand
\( n_{\%P} \) = molar percentage of phosphine ligand from \(^{31}\text{P}\{\text{H}\}\) NMR

\( n_{\%P=O} \) = molar percentage of phosphine oxide ligand from \(^{31}\text{P}\{\text{H}\}\) NMR (normalized to 1)

Knowing the molecular weights of the three different ligands (HO-EG\(_4\)-S\(^-\); thiolate of 5; oxidized thiolate of 5), it is possible to know the molar percentage of each ligand per milligram of nanoparticles.

The number of gold atoms \((N_{Au})\) can be calculated from the following formula\(^3\):

\[
N_{Au} = \frac{\pi \rho d^3 N_A}{6 M_{Au}}
\]

Where:

\( \rho \) = density of face centered cubic (fcc) gold lattice (19.3 g cm\(^{-1}\))

\( d \) = average diameter of nanoparticles in centimeters (from TEM images)

\( N_A \) = Avogadro constant

\( M_{Au} \) = mole atomic weight of gold (196.9665 g mol\(^{-1}\))

This is assuming that the AuNPs are spherical and that their size is monodispersed.

The total number of ligands \((N_L)\) can be calculated using the following formula:

\[
N_L = \frac{N_{Au} M_{Au} M_{TGA}}{(1 - M_{TGA})(M_{WP} n_{\%P} + M_{W_{OH}} n_{\%OH} + M_{WP=O} n_{\%P=O})}
\]

Where:

\( M_{TGA} \) = organic percentage from TGA

\( M_{W_{OH}} \) = molecular weight of HO-EG\(_4\)-S\(^-\) ligand

\( n_{\%OH} \) = molar percentage of HO-EG\(_4\)-S\(^-\) ligand

From the number of ligands per particle, the number of gold atoms per particles, and the molar percentage of the three different ligands it is possible to obtain the nanoparticles raw formula.
Fluorescence spectroscopic studies of Rhod-PPh₃-EG₄-AuNPs. To a suspension of Rhod-PPh₃-EG₄-AuNPs (10 mg) in 116.7 μL nanopure H₂O was added 50 μL of 1.727 M sodium 4-(azidomethyl)benzoate 8 in nanopure H₂O. The reaction was monitored by fluorescence spectroscopy. After every stated interval, 5 μL of the mixture was diluted by a factor of 1.5 x 10³ and fluorescence emission and excitation spectra of these solutions were recorded. λₑₓ = 550 nm, λₑ𝐦 = 600 nm.

Figure S3.10. ³¹P{¹H} NMR spectrum of Rhod-PPh₃-EG₄-AuNPs in D₂O after treatment with sodium 4-(azidomethyl)benzoate. Spectrum referenced against H₃PO₄ as a standard.

Fluorescence spectroscopic studies of Rhod-P(=O)Ph₃-EG₄-AuNPs. To a suspension of Rhod-(P=O)Ph₃-EG₄-AuNPs (10 mg) in 116.7 μL nanopure H₂O was added 50 μL of 1.727 M sodium 4-(azidomethyl)benzoate 8 in nanopure H₂O. The reaction was monitored by fluorescence spectroscopy. After every stated interval, 5 μL of the mixture was diluted by a factor of 1.5 x 10³ and fluorescence emission and excitation spectra of these solutions were recorded. λₑₓ = 550 nm, λₑ𝐦 = 600 nm.
Fluorescence spectroscopic studies of Rhod-PPh$_3$-EG$_4$-AuNPs in GSH. To a suspension of Rhod-PPh$_3$-EG$_4$-AuNPs (10 mg) in 116.7 μL nanopure H$_2$O was added 50 μL of 33.3 mM GSH in nanopure H$_2$O to give a final concentration of 10 mM GSH$_{(aq)}$. The reaction was monitored by fluorescence spectroscopy. After every stated interval, 5 μL of the mixture was diluted by a factor of 1.5 x 10$^3$ and fluorescence emission and excitation spectra of these solutions were recorded. $\lambda_{ex} = 550$ nm, $\lambda_{em} = 600$ nm.
Chapter 4

4 Dual-Bioorthogonal Molecular Tool: “Click-to-Release” and “Double-Click” Reactivity on Small Molecules and Material Surfaces

Reprinted with permission from Bioconjugate Chem. 2019, 30, 1140–1149, with minor formatting changes to maintain consistency throughout thesis. Figure S4.X refers to Supporting Information (Section 4.6).

4.1 Introduction

The ability to cleanly and selectively form and break chemical bonds in complex, biological media is an important tool in the fields of chemical biology, medicine, and increasingly nanotechnology as it enables selective (and often, site-specific) manipulation of biomolecules, cells, particles and surfaces. The development of bioorthogonal chemistries has enabled researchers to monitor biomolecular dynamics and processes in their native environment, i.e. in vivo, due to several key features: (1) these reactions proceed in physiological conditions in water and neutral pH; (2) they form robust covalent bonds; and (3) they are chemoselective and bioorthogonal, i.e. they are not perturbed by native biological functionalities (nucleophiles, electrophiles, reducing or oxidizing conditions). As a result, many widely used bioorthogonal ligation strategies have been developed over the last two decades: the Staudinger-Bertozzi ligation (SBL), strain-promoted alkyne-azide cycloaddition (SPAAC), strain-promoted alkyne-nitrone cycloaddition (SPANC), tetrazene ligation, and tetrazole ‘photo-click’ to name a few.

More recently, there has been a focus on the development of ‘click-to-release’ strategies, wherein a bioorthogonal ligation reaction results in the concomitant release of a drug, fluorophore, or small molecule of interest. Several of these systems involve an intramolecular Diels-Alder (DA) click, which is designed to trigger the release of carbon monoxide (CO) or sulfur dioxide (SO₂) as a prodrug. Pluth and coworkers designed a tetrazene click-based system that releases carbonyl sulfide (COS), which is enzymatically...
hydrolyzed to hydrogen sulfide (H$_2$S) — a biologically relevant gasotransmitter — by carbonic anhydrase.$^{[11]}$ Other strategies that enable more complex payloads to be released have also been developed. Wang and coworkers demonstrated a cascade prodrug system in which an inverse-electron demand DA click and subsequent lactonization resulted in the release of CO, a drug, and fluorescent byproduct.$^{[12]}$ Chatterjee and coworkers reported a chemoselective rapid azo-coupling reaction between 5-hydroxyindoles and aromatic diazonium ions.$^{[13]}$ Although this ligation strategy does not result in concomitant release of a payload, the otherwise stable azo-linkage can be “unclicked” by treatment with dithionite to afford a release product. Robillard and coworkers demonstrated a tetrazene ligation in which the 1,4-dihydropyridazine product undergoes an electron cascade mechanism to extrude CO$_2$ and the carbamate-installed payload.$^{[14-15]}$ Recently, Taran and coworkers reported a novel [3+2] cycloaddition between iminosydnones and strained cyclooctynes, in which the cycloaddition product undergoes unimolecular fragmentation to afford a ligation product and a release product.$^{[16]}$ Although effective, many of these click-to-release strategies face challenges with incomplete conversion, use of additional harsh exogenous reagents to “unclick”, or suffer from limitations regarding synthetic accessibility or amenability of the desired cargo.

Our group has recently revisited the SBL — a classic in the field of bioorthogonal chemistry as a powerful transformation for amide bond formation through organic azides — as a click-to-release strategy off gold nanoparticles (AuNPs) by utilizing an electrophilic ester as a cargo scaffold.$^{[17]}$ Prior to this, the dual-purpose nature (chemical ligation and release) of this highly specific transformation was seldom recognized or utilized. Florent and coworkers demonstrated a SBL-based release strategy that featured rearrangement of the intermediate aza-ylide to a phenol anion, which subsequently underwent a 1,6-quinone methide rearrangement and hydrolysis to liberate the phosphine oxide, CO$_2$, a drug, and urea byproduct (Scheme 4.1a).$^{[18]}$ They reported yields approaching 90%, but this multicomponent system remains synthetically complex and lacks the ease-of-use offered by the traditional SBL. In contrast to their work, where the azide is destined to be liberated as a urea, in our design, retaining the ‘click’ aspect of the SBL was desired. Instead, we demonstrated installation of the cargo via the ester electrophilic trap (Scheme 4.1b), which is liberated as an alcohol following the classic

SBL mechanism. This methodology worked effectively as demonstrated by the tethering and release of a Rhodamine B dye from a AuNP nanocarrier.\textsuperscript{[17]}

However, as in many existing click-to-release strategies, synthetic accessibility and amenability remains a real challenge. These systems typically require arduous synthetic pathways that must be taken for each payload of interest to be suitable for a specific application. Instead, we propose a single dual-bioorthogonal molecular tool that can
undergo multiple controlled bioorthogonal reaction pathways, which can be applied to small molecules and material surfaces (Scheme 4.1c). In this design, we envisioned an electrophilic ester-bound strained cyclooctyne that is capable of covalently “catching” a general payload via 1,3-dipolar cycloaddition (SPAAC depicted, SPANC can be employed as well). The resulting triazole- or isoxazoline-tethered cargo can then be liberated via SBL “click-to-release” with an exogenous azide molecule. Additionally, we propose a secondary “double-click” reaction sequence that can be employed for multiple ligations within the single moiety (Scheme 4.1c). Sundhoro et al. recently reported a modified Staudinger reaction between aryl phosphines and perfluoroaryl azides (abbreviated PFAA-SR) that affords a uniquely stable iminophosphorane “click” product that doesn’t undergo the cyclization and hydrolysis steps of typical SBL reactions.\textsuperscript{[19-20]} We utilize the PFAA-SR as a secondary click reaction and alternative to click-to-release, which provides unprecedented versatility and full molecular control of bond-forming and bond-breaking reactions within a small molecule or material surface system. We successfully demonstrate this dual-bioorthogonal click-to-release and double-click reactivity and its implementation on a model material (AuNPs) for cell-surface labelling.

4.2 Results and Discussion

Design and Synthesis of Dual-Action Molecular Tool.

The design of a dual-bioorthogonally reactive moiety needed to address several considerations: (i) stability of the strained cyclooctyne under various synthetic conditions; (ii) relative reactivity kinetics between cyclooctyne and phosphine towards an azide, as cycloaddition via SPAAC should proceed first; and (iii) its amenability towards incorporation onto surfaces to expand its applicability in materials science and nanotechnology. To this end, we employed a photoactivated dibenzocyclooctyne (hvDIBO) moiety as our cyclooctyne precursor of choice for its exceptional stability and ability to liberate CO to generate the parent dibenzocyclooctyne (DIBO) upon irradiation with UVA light.\textsuperscript{[21]} We previously demonstrated its utility and versatility on AuNPs, in which interfacial DIBO reacted cleanly and effectively with various 1,3-dipoles,\textsuperscript{[22]} thus making it the perfect candidate for covalently tethering a general cargo. Additionally, bimolecular rate constants illustrate at least a 10-fold preference for SPAAC between DIBO\textsuperscript{[21]} and
benzyl azide (BnN$_3$) versus SBL between a triarylphosphine$^{[20]}$ and BnN$_3$, which we envisioned would be key in enabling cycloaddition to proceed first, as per our design for the click-to-release pathway outlined in Scheme 4.1c. We designed the multicomponent ligand 5 (Scheme 4.2, top) to bear several key features: 1) a hvDIBO moiety tethered by an ester electrophilic trap, where CO liberation can be spatiotemporally controlled and allows for covalent cargo catching via SPAAC/SPANC of the resulting cyclooctyne; the limited lifetime of strained alkynes in aqueous solutions and their incompatibility with nucleophilic thiols make this photo-caging strategy necessary; 2) triaryl phosphine moiety capable of undergoing either SBL to trigger click-to-release or PFAA-SR for ‘double-click’ reactivity; and 3) a thiol moiety as a representative functional handle for materials modification or small molecule manipulation. Thiols are highly amenable to materials incorporation, e.g. metallic surfaces (2D or nanoparticles) via S-M (where M = metal) bond formation$^{[23]}$ or polymers and organic nanoscale materials via thiol-ene or thiol-Michael addition click reactions.$^{[24-25]}$

The synthesis of 5 was achieved via a convergent approach (Scheme 4.2, top). Cyclpropenone 1$^{[26]}$ was alkylated with 3-bromo-1-propanol to give alcohol 2, which underwent DCC-mediated coupling with phosphine 3$^{[17]}$ to afford ester 4 in moderate yields. Cleavage of the S-trityl protecting group of 4 by trifluoroacetic acid in CH$_2$Cl$_2$ gave thiol 5 in excellent yield. Prescher and coworkers recently reported a new bioorthogonal ligation that proceeds efficiently between cyclopropenones and triarylphosphines bearing nucleophiles (–OH, –NH$_2$, and –SH),$^{[27]}$ which drew attention to 5 potentially being self-reactive in this manner. However, we observed no bioorthogonal self-reactivity, likely due to the dibenzocyclooctyl-fused cyclopropenone offering additional steric protection, rendering the cyclopropenone sterically and conformationally unavailable to the phosphine. As the dibenzocyclooctyne was photocaged, we also observed no self-reactivity via attack from the nucleophilic thiol. Thiol 5 is stable for months under standard storage conditions at -20°C in the freezer.

**Click-to-release and double-click reactivity.**

Upon confirming the synthetic accessibility of the ligand 5, we investigated the dual-bioorthogonal reactivity of 4, which lacks the nucleophilic –SH of 5 and avoids undesired
Scheme 4.2. Synthesis of multicomponent thiol ligand 5 (top) and multi-bioorthogonal reactivity of compound 4 (bottom).

d-self-reactivity upon alkyne generation. Irradiation of a solution of 4 in CH$_3$OH at 22°C with 14 x 8 W UVA (350 nm) lamps afforded alkyne 6 cleanly within minutes (Scheme 4.2, bottom). Upon decarbonylation, $^1$H NMR spectral analysis showed disappearance of the signal at 7.93 ppm and a decrease in the 6.88 ppm signal followed by appearance of signals at 7.20 ppm and 6.74 ppm (Figure 4.1A). The Fourier transform infrared (FT-IR) spectrum of 6 showed disappearance of the characteristic C=O absorption band (1843 cm$^{-1}$) resulting from the cyclopropenone in 4. These results are consistent with generation of
Figure 4.1. Spectroscopic analysis of small molecules 4, 6, 7a/7b, 9, 11a/11b, and 13a/13b. (A) $^1$H NMR spectra obtained in CDCl$_3$ and referenced to residual CHCl$_3$. Blue arrows (↑) designate expected signal decrease and red arrows (↓) designate expected signal increase resulting from decarbonylation. Blue highlight (●) designates $^1$H from ethylene bridge of cycloctyl ring. Red highlight (■) designates –C($^1$H)$_3$ from butyl group. Green star (*) designates triazole-derived benzylic $^1$H and blue star (★) designates isoxazoline Cα-$^1$H. (B) UV-Vis spectroscopic analysis obtained in CH$_3$OH. (C) $^{31}$P{$^1$H} NMR spectra obtained in CDCl$_3$ and referenced to H$_3$PO$_4$. *Inset obtained in DMSO-d$_6$.

the alkyne species.$^{[20]}$ Addition of 1 equiv. of BnN$_3$ to 6 in CH$_2$Cl$_2$ (1.70 mM, equimolar) provided the cycloaddition products 7a and 7b as a pair of inseparable regioisomers within 8 h. $^{31}$P{$^1$H} NMR analysis of the crude mixture showed no reaction at the phosphine center (i.e. no conversion of P: to P=O via SBL), which indicated reaction selectivity of SPAAC over SBL, as envisioned. The $^1$H NMR spectrum of 7a/7b provided clear evidence of regioisomerism (Figure 4.1A): proton environments in proximity of the triazole-phosphine moiety showed split signals for 7a and 7b. Namely, the –CH$_3$ of the
butyl group of 7a and 7b can be seen as two distinct triplets at 0.99 and 0.95 ppm. In addition, the ethylene bridge of 6 (3.19 and 2.45 ppm) appears as four broad signals at 3.25, 2.98, 2.83, and 2.68 ppm in 7a/7b, characteristic of triazole formation. Lastly, triazole-bound benzylic protons at 5.54 ppm are diagnostic for successful SPAAC with BnN₃.[22] Cycloaddition of 6 with nitrones can also be achieved with greater precision due to the orthogonality of SPANC versus SBL. Addition of nitrone 12 to alkyne 6 in CH₂Cl₂ (0.65 mM, equimolar) provided quantitative conversion to isoxazolines 13a and 13b within 8 h. As with 7a/7b, the ¹H NMR spectrum of 13a/13b showed distinct peaks for each regioisomer as well as a diagnostic Cα–H of isoxazolines at 4.97 ppm (Figure 4.1A).

Reactivity of the hvDIBO moiety can also be monitored via UV-vis spectroscopy, in which 4 displays a characteristic absorption band at 331–347 nm (Figure 4.1B).[21] Decarbonylation of 4 to give 6 results in a hypsochromic shift to a 302–320 nm absorption band. Upon cycloaddition with BnN₃ or nitrone 12 (Scheme 4.2, bottom), we observed bleaching of the 302–320 nm absorption, which is consistent with consumption of the alkyne species via SPAAC and SPANC, respectively (Figure 4.1B).[21]

Treatment of a solution of 7a/7b in CH₂Cl₂ with excess BnN₃ and H₂O resulted in liberation of 8a/8b cleanly via SBL click-to-release within 16 h (Scheme 4.2, bottom). Isomers 8a and 8b could be separated and isolated alongside the click product 9, thus illustrating the efficacy of the dual-bioorthogonal click-to-release strategy outlined in Scheme 4.1c. Similarly, treatment of isoxazolines 13a/13b with BnN₃ under similar conditions resulted in liberation of 14a/14b as a pair of inseparable isomers and the click product 9. Furthermore, we tested the reactivity of phosphines 7a and 7b towards perfluoroaryl azide 10 as an alternate reaction pathway to click-to-release (Scheme 4.2, bottom). Remarkably, we observed rapid and clean conversion to iminophosphoranes 11a and 11b within minutes. The iminophosphorane linkage of 11a/11b proved to be exceptionally robust under various conditions, including being left in water-containing solvent for weeks and being heated to 80°C in DMSO with no hydrolysis or fragmentation observed. Thus, this represents a robust dual-bioorthogonal double-click strategy that, in addition to click-to-release, provides opportunities for bioorthogonal multiplex labelling.[28,29]
**31P NMR Investigation.**

One key feature offered by this dual-bioorthogonal moiety is the ability to track the reaction species by 31P NMR spectroscopy, thus allowing for ease of analysis even in complex mixtures (Figure 4.1C). Upon decarbonylation of 4 to 6, we observe no change in the 31P environment (-4.33 ppm), as expected. However, treatment of 6 with BnN3 resulted in two equal phosphine signals at -4.32 and -4.36 ppm belonging to regioisomers 7a and 7b. Similarly, treatment of 6 with nitrone 12 results in a spectrum with phosphine signals at -4.28 and -4.30 ppm belonging to regioisomers 13a and 13b. Upon triggered release by SBL, this regioisomeric information is lost (and passed on to 8a/8b and 14a/14b, respectively) as both yield the click product 9, which displays only a single 31P signal at 31.9 ppm corresponding to the phosphine oxide.[30] Furthermore, when employing the double-click pathway, generation of the iminophosphorane products 11a/11b from 7a/7b results in a spectrum that shows a single triplet ($J_{PF} = 4.5$ Hz) at 11.51 ppm in CDCl3, a chemical shift that is characteristic of iminophosphoranes.[19] Although we did not observe two distinct regioisomers for 11a/11b in CDCl3 (as in 7a/7b), obtaining the spectrum in DMSO-d6 allowed us to resolve the two species with triplet signals at 11.73 and 11.67 ppm. These results highlight a key advantage of this dual-bioorthogonal system in that the ability to monitor reaction species and progress with diagnostic 31P signals represents an extremely powerful and useful tool, especially as researchers utilize bioorthogonal chemistry with structurally complex molecules (enzymes, proteins, antibodies, etc.) — as they often do — rendering traditional spectroscopic analyses unfeasible or difficult to interpret.

**AuNP Incorporation and Implementation.**

After confirming the click-to-release and double-click functionality of 4, we investigated the efficacy of this dual-bioorthogonal tool on the surface of AuNPs as a model material substrate. For the last two decades, interest in metal-free click chemistry on surfaces has increased dramatically due to their ease-of-use for fabricating multifunctional materials and biosensors in a clean, bottom-up approach.[31] We envisioned that the highly versatile moiety demonstrated here would find useful application on material surfaces. Thus, we incorporated thiol 5 into the monolayer of 5 nm hydroxyl-terminated AuNPs (HO-EG4-AuNPs) via a ligand displacement reaction. These HO-EG4-AuNPs were synthesized via
a modified Brust-Schiffin method, where HAuCl$_4$·3H$_2$O is reduced by NaBH$_4$ in the presence of hydroxyl-terminated thiol (tetraethylene, HO-EG$_4$-SH) to afford nanoparticles with Au core diameter of 5 ± 1 nm, as confirmed by TEM. These AuNPs represent an ideal model substrate due to their amphiphilicity (high solubility in both water and organic solvents), ease-of-characterization by spectroscopy (NMR, UV-vis, and IR), and offers an inert and stable nanoscale platform for our investigation. Functionalization of 5 onto AuNPs was achieved via a stirring solution of 5 and HO-EG$_4$-AuNPs in CH$_2$Cl$_2$/CH$_3$OH at 22°C for 16 h (Scheme 4.3), followed by trituration of a AuNP film (prepared by rotary evaporation, see Supporting Information) by 2:1 Et$_2$O:CH$_2$Cl$_2$ to remove excess 5, displaced thiols and disulfides. The derivatized AuNPs, hvDIBO-PPh$_3$-EG$_4$-AuNPs, were characterized by $^1$H and $^{31}$P{$^1$H} NMR, UV-vis, and IR spectroscopy as well as thermogravimetric analysis to confirm successful incorporation of 5.

The $^1$H NMR spectrum of hvDIBO-PPh$_3$-EG$_4$-AuNPs showed characteristic broad peaks that correspond to ligand 5 (Figure S4.36) and a broad signal at ~40 ppm in the $^{31}$P{$^1$H} NMR spectrum typical of phosphine-based monolayers on AuNPs (Figure S4.39). UV-vis spectroscopy showed a characteristic absorption band at 331–347 nm corresponding to the hvDIBO moiety of AuNP-bound 5 (Figure S4.40). Lastly, thermogravimetric analysis (TGA) provided proof of two ligands decomposing at distinctly different temperatures (250 and 375°C, see Figure S4.35) corresponding to the hydroxyl-terminated ligand and ligand 5 at a ratio of 47:53, respectively. From deconvolution of the TGA curve, the ligand concentration of 5 was determined to be 0.367 μmol mg$^{-1}$ AuNP. This data also allows us to determine ligand density based on Au core size (TEM), organic content of AuNPs (TGA curve), and bilayer composition (TGA curve deconvolution). The AuNP raw formula was calculated to be Au$_{3500}$[HO-EG$_4$-S]$_{685}$[hvDIBO-PPh$_3$-EG$_4$-S]$_{760}$, which assumes that particles are spherical and monodispersed (see Supporting Information for calculations). This stoichiometric information can be utilized to carefully control the amount of cargo that is to be immobilized on the nanocarrier surface. Together, these results confirm successful incorporation of ligand 5 into the AuNP monolayer.
Scheme 4.3. Synthesis of AuNPs and ‘click-to-release’ on AuNPs via SPAAC/SPANC and SBL. Reaction conditions: a) 1 equiv. BnN\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}; b) excess BnN\textsubscript{3}, H\textsubscript{2}O, CH\textsubscript{2}Cl\textsubscript{2}/CH\textsubscript{3}OH; c) nitrone 12, CH\textsubscript{2}Cl\textsubscript{2}.

A solution of \textit{hvDIBO-PPh\textsubscript{3}-EG\textsubscript{4}}-AuNPs in methanol decarbonylated cleanly upon photoirradiation (UVA) within minutes to afford DIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs (Scheme 4.3). As with the conversion of 4 to 6, we observed new signals in the \textsuperscript{1}H NMR spectrum at 7.19 ppm and 6.72 ppm corresponding to interfacial DIBO (Figure S4.36) as well as a hypsochromic shift to 302–320 nm in UV-vis absorption (Figure S4.40). In addition, the FT-IR spectrum of DIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs showed the disappearance of the characteristic C=O absorption band at 1843 cm\textsuperscript{-1} resulting from the cyclopropenone (Figure S4.37). DIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs were then evaluated for their ability to
interfacially tether a cargo via cycloaddition by treatment with BnN\textsubscript{3} and nitrone \textbf{12} separately as models (Scheme 4.3). In both cases, characteristic peaks in the \textsuperscript{1}H NMR spectra of treated AuNPs provided clear evidence of triazole or isoxazoline formation (Figure S4.38). Namely, benzylic protons at 5.54 ppm and C\textsubscript{α}–H proton at 4.97 ppm are consistent with the analogous compounds \textbf{7a/7b} and \textbf{13a/13b}, respectively, and are diagnostic of effective cycloaddition between DIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs and the respective cargo. This was also seen by UV-vis spectroscopy, in which the treated AuNPs showed bleaching of the 302-320 nm absorption that is consistent with consumption of the interfacial alkyne species (Figure S4.36).

Throughout irradiation and subsequent cycloaddition of hvDIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs, \textsuperscript{31}P\{\textsuperscript{1}H\} NMR analysis displayed no significant changes in chemical shift (Figure S4.39). Although spectral broadening was observed after cycloaddition, this is likely due to the regioisomerism of cycloadducts and is consistent with that observed in \textbf{7a/7b} and \textbf{13a/13b}. When treated with excess BnN\textsubscript{3} to trigger click-to-release (Scheme 4.3), the \textsuperscript{31}P\{\textsuperscript{1}H\} NMR spectra of both release experiments (from (benzyl)triazole-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs and isoxazoline-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs) displayed a single sharp signal at 37.9 ppm corresponding to the phosphine oxide click product formed by interfacial SBL, benzylamide-P(=O)Ph\textsubscript{3}-EG\textsubscript{4}-AuNPs (Figure S4.39).\textsuperscript{[17,27]} Trituration of the AuNP samples with 2:1 EtOAc/hexanes after click-to-release to remove unbound molecules revealed the presence of the release products \textbf{8a/8b} and \textbf{14a/14b} from their respective AuNPs (Figures S4.41 and S4.42), thus confirming the successful implementation of this dual-bioorthogonal click-to-release system on a material surface. Importantly, this click-to-release system represents a powerful pseudo “transclick” reaction on materials as it exchanges functionality from the primary click product (green sphere, Scheme 4.3) to a secondary click functionality from the incoming azide (blue star, Scheme 4.3), which occurs readily and cleanly at room temperature. This is advantageous to existing transclick methodologies in which high temperatures (>100\textdegree C)\textsuperscript{[32]} or UV light\textsuperscript{[33]} is needed to initiate the exchange reaction.

To further illustrate the utility and ease-of-use of this molecular tool on a surface, we employed the double-click pathway on hvDIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs to prepare a
multifunctional AuNP template. However, we opted to subject hvDIBO-PPh₃-EG₄-AuNPs to PFAA-SR first, followed by photouncaging and subsequent SPAAC. Unlike click-to-release, in which the method requires that the cargo be installed first via SPAAC/SPANC then released by SBL, the double-click features two bond-forming reactions that are not restricted to a specific sequence; i.e., it can proceed via photouncaging, SPAAC/SPANC, then PFAA (as in Scheme 4.2) or PFAA-SR, photouncaging, then SPAAC/SPANC (as in Scheme 4.4) to achieve the same result. To highlight this flexibility, hvDIBO-PPh₃-EG₄-AuNPs were treated with a perfluoroaryl azido Rhodamine B derivative (17, see Supporting Information) to afford the interfacial iminophosphorane product, hvDIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs, via the modified Staudinger reaction (Scheme 4.4). Excess 17 was removed by dialysis in CH₃OH/H₂O for 2 days to ensure that no unbound molecules remained. After purification, we observed a significant shift in the $^{31}$P{¹H} NMR spectrum from a broad signal at ~40 ppm to a sharp signal at 11.6 ppm corresponding to the iminophosphorane (Figure S4.44).¹¹⁹ UV-vis spectroscopy also confirmed the presence of AuNP-bound rhodamine, which showed the characteristic absorbance of Rhodamine B at 560 nm as well as the 331–347 nm absorption band typical of hvDIBO in the spectrum (Figure S4.46). This highlights two key aspects of this double-click strategy: (1) the hvDIBO moiety allows for temporal control of the photoclick, thus allowing the PFAA-SR click to truly proceed orthogonally and (2) this double-click strategy can therefore be done in reverse order, i.e. PFAA-SR and SPAAC/SPANC can be utilized interchangeably as the primary or secondary click, thus offering a higher degree of modularity and control to the multifunctionalization reaction sequence.

A solution of hvDIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs decarbonylated cleanly in methanol to liberate the alkyne, which was treated with a commercially available azide-PEG3-biotin conjugate (CAS 875770-34-6, Sigma Aldrich) to afford the triazole via SPAAC (Scheme 4.4). Excess biotin was removed by dialysis in CH₃OH/H₂O to provide clean (biotin)-triazole-P(=N-Rhodamine)Ph₃-EG₄-AuNPs. Effective secondary functionalization with biotin was confirmed by UV-vis spectroscopy, which showed a hypsochromic shift from 331–347 nm absorption to 302–320 nm upon irradiation, followed by bleaching of the 302–320 nm absorption to indicate triazole formation with
Scheme 4.4. Double-click reactivity of AuNPs and reoxidation of (biotin)-triazole-P(=N-Rhodamine)Ph3-EG4-AuNPs by molecular I₂. ESI-MS calcd for C₁₁₆H₁₄₂F₄N₁₂O₂₀PS₃⁺ [M]⁺ 2225.92992, found 2225.93351; difference -1.61 ppm. Values for m/z main peaks were enlarged for clarity (full figure available in Supporting Information). Reaction conditions: a) perfluoroaryl azido Rhodamine 17 (see Supporting Information), CH₂Cl₂/CH₃OH; b) hν (350 nm), CH₃OH, 6 min; c) azide-PEG3-biotin conjugate, CH₃OH.

Biotin (Figure S4.46). Remarkably, the iminophosphorane proved to be robust through multiple overnight dialysis steps and irradiation with UVA light as indicated by ³¹P{¹H} and ¹⁹F{¹H} NMR analysis (Figures S4.44 and S4.45). It is worth noting that these transformations (SPAAC, SPANC, SBL, and PFAA-SR) were all found to proceed quantitatively even on AuNPs. For interfacial SPAAC/SPANC, bleaching of the 302–320 nm absorption band was followed by UV-vis spectroscopy until the alkyne species was fully consumed. For SBL and PFAA-SR, disappearance of the broad signal at ~40 ppm in the ³¹P{¹H} NMR spectrum (P: of active phosphine) was monitored until completion and concurrently with the appearance of a sharp signal at 37.9 ppm for SBL (P=O of ligated phosphine oxide) or 11.6 ppm for PFAA-SR (P=N of iminophosphorane).
To further confirm successful formation of the double-clicked product, (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs were reoxidized by molecular iodine to afford a mixture of Au(I) and Au(III) complexes, which liberated organic ligands as disulfides (Scheme 4.4).\textsuperscript{[22,34]} The mixture of disulfides was examined by ESI-MS to determine the exact mass of the disulfide molecule most likely to form. The experimental m/z matched with that calculated for the proposed disulfide 19, thus confirming successful implementation of this double-click strategy on AuNPs. This ability to implement two robust click reactions on a surface using structurally complex substrates (e.g. dyes, drugs, and other biologically relevant macromolecules) in a highly controlled and orthogonal manner — enabled by a single moiety — represents a powerful tool for preparing multifunctional materials under mild and biocompatible reaction conditions.

**AuNP Targeting on Human Fibroblast Cells.**

Lastly, we aim to demonstrate the utility of (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs — which contains both a targeting and an imaging agent — as a proof-of-concept model for AuNP-based pretargeted delivery. Due to the high affinity of biotin to bind avidin and streptavidin, the avidin-biotin system is widely used in immunohistochemistry (IHC), enzyme-linked immunosorbent assay, and molecular biology.\textsuperscript{[35]} We modified human fibroblast cells that contain vimentin — the epitope — via common IHC protocols to display avidin on the cell surface (see cartoons, Figure 4.2).\textsuperscript{[36]} Briefly, cell samples were fixed and incubated with a primary goat anti-vimentin antibody, washed, then treated with a secondary biotinylated donkey anti-goat IgG antibody. A solution of avidin in PBS was then introduced for binding to the fixed cells, which contained available binding sites for AuNP recognition. Although Rhodamine B emission is known to quench on metallic particle surfaces,\textsuperscript{[17,37]} we envisioned that residual emission would provide sufficient contrast for visualization by fluorescence microscopy. Thus, we incubated avidin-modified human fibroblast cells with (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs followed by 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining. Unbound AuNPs and DAPI were washed with PBS in triplicate with each step. Separately, control experiments in which either AuNP-bound biotin or avidin was excluded from the protocol were carried out. Visualization by fluorescence microscopy ($\lambda_{ex} = 532$ nm) confirmed successful targeting of (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs.
**Figure 4.2.** Fluorescence microscopy ($\lambda_{ex} = 532$ nm) of human fibroblast cells (A–D) accompanied by schematic cartoon of IHC protocol. White arrow indicates cytosol, where labeling would occur. (A) Human fibroblast cells incubated with secondary antibody bearing Alexa Fluor 568 (B) Human fibroblast cells incubated with avidin and (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs. (C) Human fibroblast cells incubated with avidin and hvDIBO-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs. (D) Human fibroblast cells incubated with only (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs, no avidin. Scale bar = 50 μm.

Towards avidin-modified cells (Figure 4.2B), which gave similar results to the positive control (Figure 4.2A). In the absence of AuNP-bound biotin — i.e. when hvDIBO-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs were incubated instead — we observed no significant binding (Figure 4.2C). Similarly, when avidin was excluded, non-specific binding of (biotin)-triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs was not observed (Figure 4.2D). Although IHC imaging has now become routine, efforts to combine chemical imaging and IHC to develop new imaging technologies are ongoing.$^{[36]}$ This proof-of-concept model for AuNP-based pretargeted delivery of a fluorophore highlights 1) the ease-of-use
of this dual-bioorthogonal molecular tool on a surface, and 2) its applicability in the
development of multifunctional materials via a single moiety in a facile manner.

4.3 Conclusion

In this work, we report the design, synthesis, and implementation of a dual-bioorthogonal
molecular tool that features clean reactivity under mild conditions. It was demonstrated
both on small molecules and a material surface that this tool enables researchers to
interchangeably utilize four bioorthogonal transformations (SPAAC, SPANC, SBL, and
PFAA-SR) to attach, release, and replace functionalities in a facile and highly modular
manner. Importantly, the applicability of the click-to-release and double-click reaction
pathways — based on simple yet highly specific recognition events — is complemented
and reinforced by the widespread use and commercialization of organic and biologically
relevant azides. Additionally, this dual-bioorthogonal moiety represents a highly versatile
tool that provides multiple avenues for implementing and monitoring the attachment or
release of molecules even in complex mixtures. Specifically, the ability to employ $^{31}$P
NMR spectroscopy to monitor reaction species and progress via diagnostic $^{31}$P signals
represents a key advantage when utilizing structurally complex reaction partners that
would render traditional spectroscopic analyses either unfeasible or difficult to interpret.
The dual-bioorthogonal molecular tool described herein thus enables the design and
production of molecular and material systems with unique, modular, and tunable
dynamic properties. This was demonstrated for metallic nanoparticles, as exemplified
with the preparation of multifunctional AuNPs and pretargeted delivery into human
fibroblast cells, but we believe this moiety and its associated chemistries will become
useful for many applications in materials science and nanotechnology. Additional
development of applications of this dual-bioorthogonal moiety on surfaces is currently
underway.

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4.5 References


4.6 Supporting Information

Material and Methods.

All reagents, unless otherwise stated, were purchased from Sigma-Aldrich and used as received. All common solvents, triethylamine (TEA), sodium sulfate anhydrous, and trifluoroacetic acid were purchased from Caledon. Benzyl azide was purchased from Alfa Aesar. Ethanol was purchased from Commercial Alcohols. Dialysis membranes (MWCO 6000-8000 Da) were purchased from Spectra/Por. Compound 1 was synthesized according to a literature procedure (Chem. Commun. 2014, 50, 5307–5309). Compound 3 was synthesized from 1-methyl-2-aminoterephthalate according to a previously established protocol in our group (Org. Biomol. Chem. 2015, 13, 4605–4612). Azides 10 and 16 were synthesized according to procedures developed by Sunhoro et al (Angew. Chem. Int. Ed. 2017, 56, 12117–12121). Rhodamine B derivative 15 was prepared from a previous protocol (Synlett 2015, 26, 1169–1174). Azide-PEG3-biotin conjugate 18 was purchased from Sigma-Aldrich.

$^1$H, $^{13}$C{$^1$H}, $^{31}$P{$^1$H}, and $^{19}$F{$^1$H} NMR spectra were recorded on Varian INOVA 400 (or 600) or Bruker AvIII HD 400 spectrometers using CDCl₃ or CD₃OD as solvent. FT-IR spectra were recorded using an attenuated total reflectance (ATR) attachment using a Bruker Vector 33 FT-IR spectrometer. Ultraviolet (UV)-visible spectra were recorded using a Varian Cary 300 Bio spectrometer. Thermogravimetric analyses (TGA) were recorded by loading the sample in a 70 μL ceramic crucible and heating from 25ºC to 750ºC at a rate of 10 ºC min⁻¹. The experiment was run under a nitrogen flow of 70 mL min⁻¹ in a Mettler Toledo TGA/SDTA 851 instrument. Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and prepared by dropcasting a drop of nanoparticles solution directly onto the grid surface. The drop was then carefully removed after 30 seconds with a soft tissue. ESI-MS spectra were recorded on a Micromass LCT mass spectrometer. Photolyses were conducted in a Luzchem LZC-4V photoreactor equipped with 14 UVA (350 nm) 8 watt lamps. Fluorescence spectroscopic studies were performed on a Photon Technology International (PTI) fluorimeter. Fluorescence microscopy was performed using a Nikon
ECLIPSE Ti2 Inverted Research Microscope. Palmar fascia tissue samples were resected from patients with Dupuytren’s disease undergoing surgery at the Roth McFarlane Hand and Upper Limb clinic in London, Ontario, Canada. All patients were provided written informed consent and tissue samples were collected with the approval of the University of Western Ontario Research Ethics Board for Health Sciences Research involving Human Subjects. Primary fibroblasts were derived from surgically resected palmar fascia tissues of Dupuytren’s disease patients. Fibroblast cultures were incubated in Dulbecco’s Modified Eagle Medium (D-MEM) supplemented with 10% fetal bovine serum albumin (Invitrogen), 1% L-glutamine and antibiotic-antimycotic solution (Sigma-Aldrich). Cell cultures were serially passaged at confluence up to 6 passages for analysis; otherwise, cells were discarded.

**Immunocytochemistry**

Primary fibroblasts were split and 1 x 10^5 cells were plated into each well on 8 well μ-slide (Cat. # 80826, Ibidi) and incubated for 72 hours at 37 °C. Cells were then washed, fixed with 4% paraformaldehyde at room temperature for 10 minutes, thoroughly washed and permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature and blocked with serum free Background Sniper for 10 minutes. Primary goat polyclonal vimentin antibody was incubated over night at 4 °C followed by incubation with biotinylated donkey anti-goat IgG (H + L) antibody at room temperature for 45 minutes. Wells were washed with PBS and incubated with avidin at room temperature for 15 minutes. After, avidin-labeled fibroblast cells were incubated with a PBS solution of AuNPs (0.23 mg AuNP mL^-1) at room temperature (15 minutes) for binding. Wells were washed 3 times with PBS, DAPI stained, and visualized under a fluorescence microscope. In two separate control experiments, either avidin or AuNP-bound biotin was excluded from the procedure.

**Synthesis of compound 2.** To 1 (0.132 g, 0.412 mmol) and K_2CO_3 (0.285 g, 2.06 mmol) in a flask was added CH_3CN (10 mL) followed by 3-bromopropanol (0.19 mL, 2.06 mmol) at r.t. while stirring. The resulting mixture was heated to 80 °C and stirred for 16 h. After, CH_3CN was removed by rotary evaporation and replaced with 2:1 CH_2Cl_2/CH_3OH (10 mL). Solids were removed by gravity filtration and the filtrate was
concentrated by evaporation in vacuo. The crude product was purified by column chromatography on silica gel using 10% CH$_3$OH in CH$_2$Cl$_2$ as the eluent to afford compound 2 as a white solid (0.116 g, 74%). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.93 (d, $J = 9$ Hz, 1H), 7.88 (d, $J = 8.5$ Hz, 1H), 6.90 – 6.84 (m, 4H), 4.20 (t, $J = 6$ Hz, 2H), 4.04 (t, $J = 6.5$ Hz, 2H), 3.89 (t, $J = 6$ Hz, 2H), 3.73 – 3.24 (m, 2H), 2.65 – 2.53 (m, 2H), 2.47 (s, OH), 2.08 (tt, $J = 6$ Hz, $J = 6$ Hz, 2H), 1.84 – 1.75 (m, 2H), 1.56 – 1.46 (m, 2H), 0.99 (t, $J = 7.5$ Hz, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 162.1, 161.8, 153.8, 147.8, 142.2, 141.9, 135.8, 135.7, 116.4, 116.23, 116.17, 116.1, 112.33, 112.32, 68.0, 65.5, 59.6, 37.12, 37.11, 32.0, 31.2, 19.2, 13.8. FT-IR (ATR, $\nu$, cm$^{-1}$): 3382, 2955, 2937, 2873, 1845, 1605, 1557, 1501, 1486, 1471, 1456, 1432, 1360, 1319, 1319, 1276, 1251, 1179, 1167, 1141, 1094, 1063, 1025. ESI-MS calcd for C$_{24}$H$_{27}$O$_4$+ [M + H]$^+$ 379.19093, found 379.19035; difference +1.5 ppm.

**Synthesis of compound 4.** To a flask containing 2 (0.120 g, 0.317 mmol), 3 (0.206 g, 0.264 mmol), DCC (0.065 g, 0.317 mmol), and DMAP (0.007 g, 0.0573 mmol) under an Ar(g) atmosphere was added dry THF (25 mL) at r.t.. The resulting mixture was stirred for 16 h. After, solids were removed by filtration and the filtrate was concentrated by evaporation in vacuo. The crude product was purified by column chromatography on silica gel using EtOAc as the eluent to afford compound 4 as a pale yellow solid (0.160 g, 53%). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 8.02 (dd, $J = 8$ Hz, $J = 3.5$ Hz, 1H), 7.88 (d, $J = 9$ Hz, 1H), 7.87 (d, $J = 8$ Hz, 1H), 7.74 (dd, $J = 8$ Hz, $J = 2$ Hz, 1H), 7.43 – 7.07 (m, 26H), 6.88 – 6.74 (m, 4H), 6.50 (t, $J = 5$ Hz, NH), 4.33 (t, $J = 6$ Hz, 2H), 4.00 (t, $J = 6.5$ Hz, 2H), 3.94 (t, $J = 6$ Hz, 2H), 3.60 – 3.42 (m, 10H), 3.41 – 3.34 (m, 2H), 3.31 – 3.23 (m, 2H), 3.22 (t, $J = 6.5$ Hz, 2H), 2.65 – 2.48 (m, 2H), 2.36 (t, $J = 6.5$ Hz, 2H), 2.04 (tt, $J = 6$ Hz, $J = 6$ Hz, 2H), 1.80 – 1.70 (m, 2H), 1.53 – 1.41 (m, 2H), 0.96 (t, $J = 7.5$ Hz, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 166.33, 166.31, 166.2, 162.0, 161.4, 153.6, 147.6, 144.7, 142.3, 141.9, 140.9, 140.6, 137.4, 137.1, 137.0, 136.7, 135.6, 135.5, 133.8, 133.6, 132.8, 130.72, 130.69, 129.5, 128.9, 128.6, 128.5, 127.8, 126.7, 126.5, 116.43, 116.2, 116.1, 116.06, 112.20, 112.17, 70.4, 70.3, 70.1, 70.0, 69.5, 69.4, 67.9, 66.5, 64.5, 62.1, 39.7, 37.05, 37.03, 31.5, 31.0, 28.3, 19.1, 13.7. $^{31}$P{$^1$H} NMR (CDCl$_3$, 162 MHz): $\delta$ -4.34 (s). FT-IR (ATR, $\nu$, cm$^{-1}$): 3321, 3055, 2957, 2931, 2870, 1843, 1716, 1652, 1600, 1561, 1486, 1471, 1443, 1435, 1351, 1319, 1276, 1249, 1165, 1183, 1130, 1094, 1055, 1027.
ESI-MS calcd for C_{71}H_{71}NO_{9}PS^{+} [M + H]^{+} 1144.45872, found 1144.46015; difference -1.25 ppm.

**Synthesis of compound 5.** To a solution of 4 (0.100 g, 0.0872 mmol) in CH_{2}Cl_{2} (24 mL) was added trifluoroacetic acid (1.995 g, 17.4 mmol) and triisopropylsilane (0.042 g, 0.265 mmol) under an Ar(g) atmosphere while stirring at r.t. The resulting mixture was stirred for 30 min and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography on silica gel using 7% CH_{3}OH in EtOAc as the eluent to afford compound 5 as a pale yellow oil (0.074 g, 94%). 1H NMR (CDCl_{3}, 400 MHz): δ 8.08 (dd, J = 8 Hz, J = 3.5 Hz, 1H), 7.98 – 7.90 (m, 2H), 7.80 (dd, J = 8 Hz, J = 2 Hz, 1H), 7.40 – 7.22 (m, 11H), 6.93 – 6.79 (m, 4H), 6.48 (s, br, NH), 4.38 (t, J = 6 Hz, 2H), 4.05 (t, J = 6.5 Hz, 2H), 4.00 (t, J = 6 Hz, 2H), 3.68 – 3.49 (m, 14H), 3.39 – 3.24 (m, 2H), 2.64 (dt, J = 8 Hz, J = 6.5 Hz, 2H), 2.68 – 2.53 (m, 2H), 2.09 (tt, J = 2 Hz, J = 2 Hz, 2H), 1.86 – 1.75 (m, 2H), 1.56 (t, J = 8 Hz, SH), 1.56 – 1.46 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H). 13C{^1}H NMR (CDCl_{3}, 101 MHz): δ 166.4, 166.2, 162.1, 161.5, 153.8, 147.8, 142.2, 141.8, 141.0, 140.7, 137.4, 137.2, 137.1, 136.8, 135.8, 133.9, 133.7, 132.9, 130.8, 128.9, 128.7, 128.6, 116.3, 116.2, 116.1, 116.0, 112.3, 112.2, 72.8, 70.5, 70.4, 70.1, 70.05, 69.5, 67.9, 64.5, 62.1, 39.8, 37.0, 31.1, 28.3, 24.1, 19.1, 13.7. 31P{^1}H NMR (CDCl_{3}, 162 MHz): δ -4.34 (s). FT-IR (ATR, ν, cm⁻¹): 3335, 3054, 2955, 2933, 2870, 1843, 1716, 1652, 1600, 1557, 1505, 1471, 1434, 1354, 1319, 1275, 1248, 1165, 1115, 1055, 1026. ESI-MS calcd for C_{52}H_{57}NO_{9}PS^{+} [M + H]^{+} 902.34917, found 902.34774; difference +1.58 ppm.

**Synthesis of compound 6.** A solution of 4 (0.060 g, 0.0523 mmol) in CH_{3}OH was irradiated in a Luzchem (LZC-4V) photoreactor equipped with 14 UVA (350 nm) lamps for 3 min at r.t. The solution was concentrated in vacuo and purified by column chromatography on silica gel using 2:1 EtOAc:hexanes as the eluent to afford compound 6 as a pale yellow oil (0.058 g, 99%). 1H NMR (CDCl_{3}, 400 MHz): δ 8.07 (dd, J = 8 Hz, J = 3.5 Hz, 1H), 7.77 (dd, J = 8 Hz, J = 2 Hz, 1H), 7.45 – 7.15 (m, 28H), 6.89 (d, J = 2 Hz, 1H), 6.84 (d, J = 2 Hz, 1H), 6.77 (dd, J = 8 Hz, J = 2 Hz, 1H), 6.71 (dd, J = 8 Hz, J = 2 Hz, 1H), 6.40 (t, J = 5 Hz, NH), 4.37 (t, J = 6 Hz, 2H), 3.99 (t, J = 6.5 Hz, 2H), 3.94 (t, J = 6 Hz, 2H), 3.65 – 3.48 (m, 10H), 3.46 – 3.39 (m, 2H), 3.28 (t, J = 7 Hz, 2H), 3.24 –
3.13 (m, 2H), 2.51 – 2.39 (m, 2H), 2.41 (t, J = 6.5 Hz, 2H), 2.06 (tt, J = 6 Hz, J = 6 Hz, 2H), 1.84 – 1.74 (m, 2H), 1.57 – 1.46 (m, 2H), 0.99 (t, J = 7.5 Hz, 3H). 13C{1H} NMR (CDCl₃, 101 MHz): δ 166.4, 166.2, 158.7, 158.1, 154.8, 144.7, 141.1, 140.8, 137.3, 137.2, 137.1, 137.07, 136.9, 133.9, 133.7, 132.8, 130.8, 130.76, 129.5, 128.9, 128.7, 128.6, 127.8, 126.7, 126.6, 126.58, 116.7, 116.6, 116.4, 111.8, 110.5, 110.2, 70.5, 70.4, 70.2, 70.1, 69.6, 69.5, 67.7, 66.6, 64.3, 62.4, 39.8, 36.61, 36.58, 31.6, 31.3, 28.4, 19.2, 13.8. 31P{1H} NMR (CDCl₃, 162 MHz): δ -4.33 (s). FT-IR (ATR, ν, cm⁻¹): 3336, 3054, 2955, 2929, 2870, 1716, 1661, 1609, 1562, 1559, 1489, 1471, 1444, 1434, 1387, 1335, 1277, 1264, 1250, 1232, 1183, 1154, 1108, 1055. ESI-MS calcd for C₇₀H₇₁NO₈PS⁺ [M + H]⁺ 1116.46380, found 1116.46207; difference +1.55 ppm.

**Synthesis of compounds 7a and 7b.** To a solution of 6 (0.057 g, 0.0511 mmol) in CH₂Cl₂ (2 mL) was added a solution of benzyl azide (0.0068 g, 0.0511 mmol) in CH₂Cl₂ (1 mL) at r.t. while stirring. The resulting solution was stirred for 8 h then concentrated in vacuo. The crude product was purified by column chromatography on silica gel using EtOAc as the eluent to afford a 1:1 mixture of compounds 7a and 7b as a pale yellow oil (0.056 g, 88%). 1H NMR (CDCl₃, 400 MHz): δ 8.11 – 8.00 (m, 1H), 7.82 – 7.74 (m, 1H), 7.50 – 7.16 (m, 29H), 7.14 – 7.03 (m, 2H), 7.01 – 6.94 (m, 2H), 6.84 – 6.60 (m, 4H), 6.54 – 6.43 (m, NH), 5.64 – 5.43 (m, 2H), 4.42 – 4.27 (m, 2H), 4.02 – 3.81 (m, 4H), 3.67 – 3.47 (m, 10H), 3.47 – 3.38 (m, 2H), 3.34 – 3.20 (m, 3H), 3.05 – 2.91 (m, 1H), 2.90 – 2.76 (m, 1H), 2.73 – 2.61 (m, 1H), 2.47 – 2.36 (m, 2H), 2.13 – 1.96 (m, 2H), 1.81 – 1.68 (m, 2H), 1.55 – 1.40 (m, 2H), 1.04 – 0.92 (m, 3H). 13C{1H} NMR (CDCl₃, 101 MHz): δ 166.41, 166.39, 166.37, 166.3, 166.2, 160.0, 159.5, 158.7, 158.2, 146.8, 146.6, 144.7, 143.2, 143.1, 141.0, 140.7, 139.1, 139.0, 137.4, 137.3, 137.22, 137.18, 137.1, 137.07, 136.9, 135.6, 133.9, 133.7, 133.6, 132.9, 132.8, 130.8, 130.2, 129.6, 128.9, 128.9, 128.6, 128.56, 128.0, 127.8, 127.4, 127.3, 126.8, 126.7, 126.6, 122.7, 122.3, 118.7, 118.2, 116.4, 116.3, 115.6, 115.5, 112.5, 112.4, 112.2, 112.1, 70.5, 70.4, 70.2, 70.1, 69.6, 69.5, 67.7, 67.5, 66.6, 64.2, 64.1, 62.43, 62.38, 52.1, 39.8, 36.5, 32.9, 31.6, 31.3, 31.2, 28.4, 19.2, 13.8. 31P{1H} NMR (CDCl₃, 162 MHz): δ -4.32 (s), -4.35 (s). FT-IR (ATR, ν, cm⁻¹): 3361, 3061, 2955, 2924, 2853, 1733, 1698, 1683, 1661, 1652, 1646, 1608, 1568, 1558, 1539, 1516, 1489, 1455, 1437, 1379, 1313, 1280, 1245, 1162, 1120, 1104, 1087, 1061.
Synthesis of compounds 8a, 8b, and 9. To a solution of compounds 7a and 7b (0.020 g, 0.0160 mmol) in CH$_2$Cl$_2$ (3 mL) was added H$_2$O (0.1 mL) and benzyl azide (0.0197 g, 0.160 mmol) at r.t. while stirring. The resulting mixture was stirred for 16 h then concentrated in vacuo. The crude products were purified by column chromatography on silica gel using 3:1 EtOAc as the eluent to afford compounds 8a (Rf = 0.55) and 8b (Rf = 0.45), then 10% CH$_3$OH in CH$_2$Cl$_2$ to elute compound 9.

Compound 8a (yellow oil, 3 mg, 78%): $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.46 (d, $J$ = 8.5 Hz, 1H), 7.31 – 7.24 (m, 3H), 7.12 – 7.06 (m, 2H), 6.98 (m, $J$ = 8 Hz, 1H), 6.81 (d, $J$ = 2.5 Hz, 1H), 6.76 (dd, $J$ = 5 Hz, $J$ = 2.5 Hz, 1H), 6.74 (dd, $J$ = 5 Hz, $J$ = 2.5 Hz, 1H), 6.66 (d, $J$ = 2.5 Hz, 1H), 5.62 – 5.45 (m, 2H), 4.12 (dt, $J$ = 6 Hz, $J$ = 2.5 Hz, 2H), 3.92 (t, $J$ = 6.5 Hz, 2H), 3.34 – 3.20 (m, 1H), 3.05 – 2.91 (m, 1H), 2.89 – 2.77 (m, 1H), 2.74 – 2.61 (m, 1H), 2.05 (tt, $J$ = 6 Hz, $J$ = 6 Hz, 2H), 1.77 – 1.69 (m, 2H), 1.69 – 1.62 (s, br, OH), 1.52 – 1.41 (m, 2H), 0.95 (t, $J$ = 7.5 Hz, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 159.6, 158.7, 146.8, 143.3, 139.0, 135.7, 133.6, 132.9, 130.3, 128.7, 128.1, 127.4, 122.4, 118.8, 116.4, 115.6, 112.5, 112.2, 67.5, 65.6, 60.2, 52.1, 36.5, 32.9, 31.9, 31.3, 19.2, 13.8. FT-IR (ATR, $\nu$, cm$^{-1}$): 3355, 3064, 3031, 2954, 2930, 2872, 1607, 1574, 1515, 1489, 1472, 1455, 1430, 1393, 1361, 1345, 1335, 1280, 1175, 1118, 1103, 1062, 1028, 1008. ESI-MS calcd for C$_{30}$H$_{24}$N$_{3}$O$_3$+ [M + H]$^+$ 484.26002, found 484.25875; difference +2.62 ppm.

Compound 8b (yellow oil, 3.5 mg, 90%): $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.47 (d, $J$ = 8.5 Hz, 1H), 7.31 – 7.23 (m, 3H), 7.12 – 7.06 (m, 2H), 6.97 (d, $J$ = 8.5 Hz, 1H), 6.81 – 6.75 (m, 2H), 6.73 (dd, $J$ = 8.5 Hz, $J$ = 2.5 Hz, 1H), 6.68 (d, $J$ = 2.5 Hz, 1H), 5.63 – 5.45 (m, 2H), 4.09 (t, $J$ = 6 Hz, 2H), 3.95 (dt, $J$ = 6.5 Hz, $J$ = 2 Hz, 2H), 3.88 – 3.80 (m, 2H), 3.33 – 3.20 (m, 1H), 3.05 – 2.91 (m, 1H), 2.89 – 2.78 (m, 1H), 2.73 – 2.61 (m, 1H), 2.02 (tt, $J$ = 6 Hz, $J$ = 6 Hz, 2H), 1.81 – 1.71 (m, 2H), 1.55 – 1.45 (m, 2H), 0.99 (t, $J$ = 7 Hz, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 160.0, 158.3, 146.7, 143.1, 139.2, 135.7, 133.8, 133.0, 130.2, 128.7, 128.0, 127.4, 122.9, 118.3, 116.4, 115.6, 112.5, 112.2, 67.7,
65.6, 60.5, 52.1, 36.6, 32.9, 31.9, 31.2, 19.2, 13.8. FT-IR (ATR, ν, cm⁻¹): 3363, 3065, 3032, 2955, 2929, 2872, 1607, 1577, 1575, 1558, 1516, 1489, 1471, 1455, 1429, 1362, 1345, 1306, 1281, 1257, 1243, 1208, 1176, 1163, 1119, 1105, 1062. ESI-MS calcd for C₃₀H₃₄N₃O₃⁺ [M + H]⁺ 484.2600, found 484.2586.; difference +2.9 ppm.

**Compound 9 (yellow oil, 13 mg, 92%)**: ¹H NMR (CDCl₃, 400 MHz): δ 9.10 (t, J = 5.5 Hz, NH), 8.06 (dd, J = 8 Hz, J = 3.5 Hz, 2H), 7.96 (dt, J = 8 Hz, J = 1.5 Hz, 1H), 7.73 – 7.57 (m, 7H), 7.55 – 7.46 (m, 4H), 7.45 – 7.38 (m, 6H), 7.33 – 7.18 (m, 13H), 6.80 (t, J = 5.5 Hz, NH), 4.12 (d, J = 5.5 Hz, 2H), 3.65 – 3.51 (m, 10H), 3.48 – 3.42 (m, 2H), 3.29 (t, J = 7 Hz, 2H), 2.41 (t, J = 6.5 Hz, 2H). ¹³C{¹H} NMR (CDCl₃, 101 MHz): δ 166.53, 166.49, 165.4, 144.7, 143.4, 143.3, 137.4, 135.7, 135.6, 132.9, 132.7, 132.59, 132.56, 132.0, 131.9, 131.8, 131.7, 131.0, 130.8, 130.2, 129.9, 129.8, 129.6, 128.9, 128.8, 128.5, 128.0, 127.9, 127.2, 126.6, 70.5, 70.4, 70.3, 70.1, 69.6, 69.5, 66.6, 44.1, 39.9, 31.6. ³¹P{¹H} NMR (CDCl₃, 162 MHz): δ 34.91 (s). FT-IR (ATR, ν, cm⁻¹): 3274, 3055, 3030, 2921, 2866, 1656, 1652, 1645, 1598, 1557, 1538, 1486, 1455, 1438, 1349, 1307, 1277, 1265, 1252, 1142, 1118, 1100, 1070, 1028. ESI-MS calcd for C₅₄H₅₃N₂NaO₆PS⁺ [M + Na]⁺ 911.32596, found 911.32537; difference +0.65 ppm.

**Synthesis of compounds 11a and 11b.** To a solution of compounds 7a and 7b (0.020 g, 0.0160 mmol) in CH₂Cl₂ (3 mL) was added perfluoroaryl azide 10³ (0.005 g, 0.020 mmol) at r.t. while stirring. A temporary change from a colorless solution to a yellow solution was observed. Within several minutes, it returned to a colorless solution. After 20 min, the solvent was evaporated in vacuo. The crude products were purified by column chromatography on silica gel using EtOAc as the eluent to afford a 1:1 mixture of compounds 11a and 11b as a colorless oil (0.022 g, 93%). ¹H NMR (CDCl₃, 400 MHz): δ 8.26 – 8.14 (m, 1H), 8.06 – 7.99 (m, 1H), 7.91 – 7.81 (m, 1H), 7.74 – 7.62 (m, 4H), 7.55 – 7.36 (m, 13H), 7.32 – 7.16 (m, 12H), 7.14 – 7.06 (m, 2H), 7.03 – 6.83 (m, 2H), 6.83 – 6.56 (m, 4H), 5.64 – 5.45 (m, 2H), 4.05 – 3.88 (m, 4H), 3.88 – 3.84 (m, 3H), 3.79 – 3.52 (m, 12H), 3.46 – 3.40 (m, 2H), 3.34 – 3.21 (m, 3H), 3.06 – 2.91 (m, 1H), 2.90 – 2.78 (m, 1H), 2.74 – 2.62 (m, 1H), 2.40 (t, J = 6.5 Hz, 2H), 1.87 – 1.67 (m, 4H), 1.56 – 1.41 (m, 2H), 1.03 – 0.92 (m, 3H). ¹³C{¹H} NMR (CDCl₃, 101 MHz): δ 166.42, 166.39, 166.31, 166.29, 165.3, 165.2, 160.0, 159.4, 158.7, 158.2, 146.8, 146.6, 144.7, 143.2, 143.1, 139.2,
139.0, 138.2, 138.1, 138.13, 138.0, 136.82, 136.78, 136.71, 136.67, 135.7, 135.6, 133.83, 133.76, 133.7, 133.6, 132.9, 132.2, 132.13, 132.0, 131.9, 130.89, 130.86, 130.8, 130.7, 130.4, 130.33, 130.30, 130.2, 129.80, 129.76, 129.6, 128.8, 128.7, 128.65, 128.6, 128.0, 127.8, 127.4, 127.3, 126.6, 122.8, 122.4, 118.7, 118.2, 116.3, 115.6, 112.5, 112.3, 112.2, 112.1, 70.5, 70.33, 70.27, 70.1, 69.52, 69.45, 67.7, 67.5, 66.6, 64.0, 63.8, 62.82, 62.80, 52.22, 52.20, 52.1, 40.1, 36.51, 36.49, 32.90, 32.88, 31.6, 31.3, 31.2, 29.7, 27.9, 19.2, 13.8.

$^{31}$P{$^1$H} NMR (CDCl$_3$, 162 MHz): $\delta$ 11.51 (t, $J_{PF} = 4.5$ Hz). $^{31}$P{$^1$H} NMR (DMSO-$d_6$, 162 MHz): $\delta$ 11.73 (t, $J_{PF} = 4.5$ Hz), 11.67 (t, $J_{PF} = 4.5$ Hz).

$^{19}$F{$^1$H} NMR (CDCl$_3$, 377 MHz): $\delta$ -143.1 (m), -152.8 (m). FT-IR (ATR, $\nu$, cm$^{-1}$): 3335, 3059, 3031, 2952, 2929, 2871, 1722, 1661, 1637, 1608, 1557, 1519, 1516, 1486, 1435, 1413, 1361, 1347, 1254, 1206, 1171, 1107, 1062, 1015. ESI-MS calcd for C$_{85}$H$_{81}$F$_4$N$_5$O$_{10}$PS$^+$ [M + H]$^+$ 1470.53779, found 1470.53946; difference -1.14 ppm.

**Synthesis of compound 12.** To a solution of 4-formylbenzonitrile (0.150 g, 1.14 mmol) in CH$_3$CN (20 mL) was added $N$-methylhydroxylamine hydrochloride (0.196 g, 2.35 mmol, 2.35 mmol) and Et$_3$N (0.65 mL, 4.69 mmol) at r.t. while stirring. After, Na$_2$SO$_4$ (0.100 g) was added to the stirring reaction mixture to absorb the H$_2$O byproduct. The mixture was stirred at r.t. for 16 h. Solids were removed by filtration and the filtrate was concentrated by evaporation in vacuo. The crude product was purified by column chromatography on silica gel using EtOAc as the eluent to afford compound 12 as a white solid (0.145 g, 79%). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 8.31 (d, $J = 8.5$ Hz, 2H), 7.69 (d, $J = 8.5$ Hz, 2H), 7.46 (s, 1H), 3.93 (s, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 134.2, 133.4, 132.2, 128.2, 118.4, 113.0, 55.0. FT-IR (ATR, $\nu$, cm$^{-1}$): 3165, 3086, 3039, 2991, 2933, 2223, 1585, 1549, 1442, 1421, 1402, 1396, 1318, 1275, 1267, 1261, 1184, 1166, 1096, 947. ESI-MS calcd for C$_{95}$H$_{81}$F$_4$N$_5$O$_{10}$PS$^+$ [M + H]$^+$ 1470.53779, found 1470.53946; difference -1.14 ppm.

**Synthesis of compounds 13a and 13b.** To a solution of 6 (0.029 g, 0.0260 mmol) in CH$_2$Cl$_2$ (4 mL) was added nitrone 12 (0.005 g, 0.0312 mmol) at r.t. while stirring. The resulting mixture was stirred for 4 h and the solvent was removed by evaporation in vacuo. The crude product was purified by column chromatography on silica gel using 2:1 EtOAc:hexanes as the eluent to afford a 1:1 mixture of compounds 13a and 13b as a pale
yellow oil (0.032 g, 96%). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 8.09 – 8.00 (m, 1H), 7.80 – 7.72 (m, 1H), 7.60 – 7.52 (m, 2H), 7.52 – 7.16 (m, 29H), 7.06 – 6.98 (m, 1H), 6.74 – 6.56 (m, 4H), 6.47 – 6.40 (m, 1H), 5.02 – 5.01 (m, 1H), 4.37 – 4.28 (m, 2H), 3.95 – 3.82 (m, 4H), 3.66 – 3.47 (m, 10H), 3.47 – 3.39 (m, 2H), 3.36 – 3.23 (m, 3H), 3.13 (s, 3H), 3.08 – 2.96 (m, 1H), 2.96 – 2.85 (m, 1H), 2.76 – 2.66 (m, 1H), 2.41 (t, $J = 6.5$ Hz, 2H), 2.07 – 1.96 (m, 2H), 1.78 – 1.64 (m, 2H), 1.52 – 1.40 (m, 2H), 1.00 – 0.92 (m, 3H). $^{13}$C{$^1$H} NMR (CDCl$_3$, 101 MHz): $\delta$ 166.38, 166.37, 166.3, 159.2, 158.7, 158.1, 157.6, 146.9, 146.7, 146.6, 144.7, 142.64, 142.56, 141.1, 141.0, 140.79, 140.75, 140.65, 137.35, 137.33, 137.2, 137.1, 136.9, 133.9, 133.7, 132.8, 132.3, 131.31, 131.28, 130.80, 130.78, 129.6, 129.0, 128.9, 128.7, 128.6, 127.8, 126.9, 126.8, 126.6, 126.0, 125.7, 124.0, 123.6, 119.9, 119.5, 118.9, 116.2, 116.1, 115.1, 115.0, 112.5, 112.4, 112.3, 112.2, 111.0, 108.4, 108.2, 79.8, 70.5, 70.4, 70.2, 70.1, 69.6, 69.5, 67.5, 66.6, 64.1, 62.5, 62.4, 46.8, 39.8, 36.80, 36.77, 32.93, 32.90, 31.6, 31.3, 31.2, 28.4, 28.3, 19.2, 19.1, 13.81, 13.77. $^{31}$P{$^1$H} NMR (CDCl$_3$, 162 MHz): $\delta$ -4.29 (s), -4.30 (s). FT-IR (ATR, $\nu$, cm$^{-1}$): 3331, 3054, 2957, 2929, 2870, 2227, 1716, 1661, 1605, 1505, 1471, 1444, 1435, 1393, 1347, 1278, 1241, 1173, 1161, 1110, 1055. ESI-MS calcd for C$_{79}$H$_{78}$N$_3$NaO$_9$PS$^+$ [M + Na]$^+$ 1298.50941, found 1298.50959; difference -0.14 ppm.

**Synthesis of compounds 14a and 14b, and 9.** To a solution of compounds 13a and 13b (0.012 g, 0.0094 mmol) in CH$_2$Cl$_2$ (2 mL) was added a solution of benzyl azide (0.0125 g, 0.0939 mmol) in CH$_2$Cl$_2$ (1 mL) followed by H$_2$O (0.1 mL) at r.t. while stirring. The resulting mixture was stirred for 8 h and concentrated in vacu. Excess benzyl azide was removed by washing with hexanes. The resulting crude residue was purified by column chromatography on silica gel using EtOAc to elute 14a and 14b as a 1:1 mixture (Rf = 0.8) followed by 10% CH$_3$OH in CH$_2$Cl$_2$ to elute 9.

**Compound 14a/14b mixture (colorless oil, 4.2 mg, 88%):** $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.57 (d, $J = 8.5$ Hz, 2H), 7.46 (d, $J = 8.5$ Hz, 2H), 7.42 (dd, $J = 8.5$ Hz, $J = 3.5$ Hz, 1H), 7.02 (dd, $J = 8.0$ Hz, $J = 5.0$ Hz, 1H), 6.74 – 6.61 (m, 4H), 5.01 (s, 1H), 4.10 – 4.04 (m, 2H), 3.90 (t, $J = 6.5$ Hz, 2H), 3.84 (dt, $J = 6.5$ Hz, $J = 6.0$ Hz, 2H), 3.65 (t, $J = 6.5$ Hz, OH), 3.36 – 3.25 (m, 1H), 3.13 (s, 3H), 3.07 – 2.97 (m, 1H), 2.95 – 2.86 (m, 1H), 2.76 – 2.66 (m, 1H), 2.07 – 1.97 (m, 2H), 1.78 – 1.67 (m, 2H), 1.53 – 1.40 (m, 2H), 0.99
- 0.92 (m, 3H). $^{13}$C\{$^1$H\} NMR (CDCl$_3$, 101 MHz): $\delta$ 159.2, 158.8, 158.1, 157.7, 146.9, 146.7, 146.6, 142.7, 142.6, 140.8, 140.7, 132.3, 131.4, 131.3, 129.1, 129.0, 128.8, 126.9, 124.2, 123.6, 120.0, 119.5, 118.9, 116.1, 115.11, 115.07, 112.5, 112.3, 112.2, 111.0, 108.4, 108.2, 79.9, 77.2, 67.5, 65.6, 65.5, 60.5, 60.3, 46.8, 36.8, 32.9, 31.93, 31.87, 31.3, 31.2, 19.23, 19.18, 13.83, 13.79. FT-IR (ATR, $\nu$, cm$^{-1}$): 3396, 3035, 2956, 2930, 2872, 2227, 1671, 1605, 1569, 1505, 1471, 1456, 1429, 1398, 1343, 1279, 1241, 1175, 1128, 1109, 1063. ESI-MS calcd for C$_{32}$H$_{34}$N$_2$NaO$_4^+$ [M + Na]$^+$ 533.24163, found 533.24131; difference +0.60 ppm.

**Compound 9 (yellow oil, 7.5 mg, 90\%):** Characterizations matched data from previous release experiment with compounds 7a/7b.

![Scheme S4.1. Synthesis of perfluoroaryl azide 17.](image)

**Synthesis of compound 17.** To a solution of 16 (0.100 g, 0.301 mmol) in CH$_2$Cl$_2$ (15 mL) was added a solution of Rhodamine B derivative 15 (0.226 g, 0.361 mmol) and Et$_3$N (0.061 g, 0.602 mmol) in CH$_2$Cl$_2$ (5 mL) at r.t. while stirring. The resulting solution was stirred in the dark for 3 h. After, the solvent was removed by evaporation in vacuo. The resulting crude residue was purified by column chromatography on silica gel using 10% CH$_3$OH in CH$_2$Cl$_2$ as the eluent to afford 17 as a dark purple solid (0.190 g, 75\%). $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ 7.86 – 7.71 (m, 3H), 7.57 – 7.50 (m, 1H), 7.33 – 7.22 (m, 2H), 7.13 – 7.04 (m, 2H), 6.99 – 6.92 (m, 2H), 3.85 – 3.53 (m, 12H), 1.33 (t, $J = 7.0$ Hz, 12H). $^{13}$C\{$^1$H\} NMR (CD$_3$OD, 101 MHz): $\delta$ 169.5, 162.9, 159.3, 157.3, 156.8, 147.1 – 146.8 (m), 144.6 – 144.3 (m), 143.3 – 143.0 (m), 140.9 – 140.5
(m), 135.7, 133.1, 132.5, 131.9, 131.7, 131.5, 128.9, 123.1 – 122.8 (m), 115.5, 114.9, 112.9, 97.5, 47.0, 44.3, 39.7, 13.0. $^{19}$F-$^{1}$H NMR (CD$_3$OD, 377 MHz): δ -76.8 (s), -143.1 – -143.3 (m), -153.7 – -154.0 (m). FT-IR (ATR, v, cm$^{-1}$): 3392, 2978, 2932, 2872, 2718, 2125, 1645, 1584, 1529, 1486, 1411, 1393, 1334, 1273, 1246, 1197, 1178, 1159, 1131, 1073. ESI-MS calcd for C$_{39}$H$_{38}$F$_{4}$N$_{7}$O$_{3}$ $^{+}$ 728.2972, found 728.2993; difference -2.86 ppm.

**Figure S4.1.** $^1$H NMR spectrum of compound 2 obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.
**Figure S4.2.** $^{13}$C\{\textsuperscript{1}H\} NMR spectrum of compound 2 obtained in CDCl$_3$ and referenced to CDCl$_3$.

**Figure S4.3.** $^1$H NMR spectrum of compound 4 obtained in CDCl$_3$ and referenced to residual CHCl$_3$. 
**Figure S4.4.** $^{31}\text{P}^{\{1\text{H}\}}$ NMR spectrum of compound 4 obtained in CDCl$_3$ and referenced to H$_3$PO$_4$.

**Figure S4.5.** $^{13}\text{C}^{\{1\text{H}\}}$ NMR spectrum of compound 4 obtained in CDCl$_3$ and referenced to CDCl$_3$. 
Figure S4.6. $^1$H NMR spectrum of compound 5 obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.

Figure S4.7. $^{31}$P{$^1$H} NMR spectrum of compound 5 obtained in CDCl$_3$ and referenced to H$_3$PO$_4$. 
Figure S4.8. $^{13}$C{\(^{1}H\)} NMR spectrum of compound 5 obtained in CDCl\(_3\) and referenced to CDCl\(_3\).

Figure S4.9. \(^{1}H\) NMR spectrum of compound 6 obtained in CDCl\(_3\) and referenced to residual CHCl\(_3\).
**Figure S4.10.** $^{31}$P{$^1$H} NMR spectrum of compound 6 obtained in CDCl$_3$ and referenced to H$_3$PO$_4$.

**Figure S4.11.** $^{13}$C{$^1$H} NMR spectrum of compound 6 obtained in CDCl$_3$ and referenced to CDCl$_3$. 
Figure S4.12. $^1$H NMR spectrum of compounds 7a/7b obtained in CDCl$_3$ and referenced to residual CHCl$_3$.

Figure S4.13. $^{31}$P{$^1$H} NMR spectrum of compounds 7a/7b obtained in CDCl$_3$ and referenced to H$_3$PO$_4$. 
**Figure S4.14.** $^{13}$C($^1$H) NMR spectrum of compounds 7a/7b obtained in CDCl$_3$ and referenced to CDCl$_3$.

**Figure S4.15.** $^1$H NMR spectrum of compound 8a obtained in CDCl$_3$ and referenced to residual CHCl$_3$. 
Figure S4.16. $^{13}$C\{$^1$H\} NMR spectrum of compound 8a obtained in CDCl$_3$ and referenced to CDCl$_3$.

Figure S4.17. $^1$H NMR spectrum of compound 8b obtained in CDCl$_3$ and referenced to residual CHCl$_3$. 
Figure S4.18. $^{13}$C($^1$H) NMR spectrum of compound 8b obtained in CDCl$_3$ and referenced to CDCl$_3$.

Figure S4.19. $^1$H NMR spectrum of compound 9 obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.
Figure S4.20. $^{31}$P{\textsuperscript{1}H} NMR spectrum of compound 9 obtained in CDCl\textsubscript{3} and referenced to H\textsubscript{3}PO\textsubscript{4}.

Figure S4.21. $^{13}$C{\textsuperscript{1}H} NMR spectrum of compound 9 obtained in CDCl\textsubscript{3} and referenced to CDCl\textsubscript{3}.
Figure S4.22. $^1$H NMR spectrum of compounds 11a/11b obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.

Figure S4.23. $^{31}$P{$^1$H} NMR spectrum of compounds 11a/11b obtained in CDCl$_3$ and referenced to H$_3$PO$_4$. 
**Figure S4.24.** $^{13}$C{^1}H NMR spectrum of compounds 11a/11b obtained in CDCl$_3$ and referenced to CDCl$_3$.

**Figure S4.25.** $^1$H NMR spectrum of compound 12 obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.
Figure S4.26. $^{13}\text{C} \{^1\text{H}\}$ NMR spectrum of compound 12 obtained in CDCl$_3$ and referenced to CDCl$_3$.

Figure S4.27. $^1\text{H}$ NMR spectrum of compounds 13a/13b obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.
Figure S4.28. $^{31}\text{P}\{\text{H}\}$ NMR spectrum of compounds 13a/13b obtained in CDCl$_3$ and referenced to H$_3$PO$_4$.

Figure S4.29. $^{13}\text{C}\{\text{H}\}$ NMR spectrum of compounds 13a/13b obtained in CDCl$_3$ and referenced to CDCl$_3$. 
Figure S4.30. $^1$H NMR spectrum of compounds 14a/14b obtained in CDCl$_3$ and referenced to residual CHCl$_3$. *Denotes residual solvent signal.

Figure S4.31. $^{13}$C($^1$H) NMR spectrum of compounds 14a/14b obtained in CDCl$_3$ and referenced to CDCl$_3$. 

**Figure S4.32.** $^1$H NMR spectrum of compound 17 obtained in CD$_3$OD and referenced to residual CD$_3$OH.

**Figure S4.33.** $^{13}$C{$^1$H} NMR spectrum of compound 17 obtained in CD$_3$OD and referenced to CD$_3$OD.
Figure S4.34. $^{19}\text{F}\{^{1}\text{H}\}$ NMR spectrum of compound 17 obtained in CD$_3$OD and referenced against trifluoroacetic acid.

**Synthesis of HO-EG$_4$-AuNPs.** HAuCl$_4$·3H$_2$O (1.4564 g, 3.7 mmol, 1.0 eq.) was dissolved in a mixture of dry methanol (503 mL) and glacial acetic acid (83 mL). To this yellow solution was added HO-EG$_4$-SH (10) (2.3 g, 11 mmol, 3.0 eq.). The bright yellow solution was stirred vigorously for 1 h and the solution color faded slightly. A solution of NaBH$_4$ (1.3997 g, 37 mmol, 10.0 eq.) in nanopure H$_2$O (96 mL) was added dropwise to the reaction mixture under vigorous stirring. The mixture turned dark brown immediately. After overnight stirring at ambient temperature, the solution was concentrated *in vacuo*. The resulting film of AuNPs was washed with toluene (3x) and the film was remade by dissolving the AuNPs in methanol followed by rotary evaporation to remove the solvent. This washing procedure was repeated 6 times. Next, the crude HO-EG$_4$-AuNPs were dissolved in nanopure water and centrifuged at 6000 rpm for 10 min to remove insoluble solid impurities. The crude HO-EG$_4$-AuNPs were then further purified by overnight dialysis.
Synthesis of hvDIBO-PPh$_3$-EG$_4$-AuNPs. To a solution of thiol 5 (0.060 g, 0.0665 mmol) in 1:1 CH$_2$Cl$_2$:CH$_3$OH (6 mL) was added a solution of HO-EG$_4$-AuNPs$^2$ in 1:1 CH$_2$Cl$_2$:CH$_3$OH (6 mL) under an Ar(g) atmosphere and vigorous stirring at r.t. The resulting solution was stirred for 16 h. After, solvents were removed by rotary evaporation to deposit a film of AuNPs inside a 500 mL round bottom flask. This film was washed 3x by trituration with 2:1 Et$_2$O:CH$_2$Cl$_2$ to remove excess 5, displaced thiols and disulfides. The AuNP film was remade by dissolving in 1:1 CH$_2$Cl$_2$:CH$_3$OH followed by rotary evaporation. This washing procedure was repeated 4x to afford clean hvDIBO-PPh$_3$-EG$_4$-AuNPs (dark brown solid, 0.065 g).

**Calculation of nanoparticle raw formula.**

The number of gold atoms ($N_{Au}$) can be calculated from the following formula$^2$:

$$N_{Au} = \frac{\pi \rho d^3 N_A}{6 M_{Au}}$$

Where:

$\rho$ = density of face centered cubic (fcc) gold lattice (19.3 g cm$^{-1}$)

$d$ = average diameter of nanoparticles in centimeters (from TEM images)

$N_A$ = Avogadro constant

$M_{Au}$ = mole atomic weight of gold (196.9665 g mol$^{-1}$)

This is assuming that the AuNPs are spherical and that their size is monodispersed.

The total number of ligands ($N_L$) can be calculated using the following formula:

$$N_L = \frac{N_{Au} M_{Au} M_{TGA}}{(1 - M_{TGA})(MW_P n_{%P} + MW_{OH} n_{%OH})}$$

Where:

$M_{TGA}$ = organic percentage from TGA

$MW_{OH}$ = molecular weight of HO-EG$_4$-S$^-$ ligand

$n_{%OH}$ = molar percentage of HO-EG$_4$-S$^-$ ligand

From the number of ligands per particle, the number of gold atoms per particles, and the molar percentage of the three different ligands it is possible to obtain the nanoparticles raw formula.
Quantitative analysis of hvDIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs.

![Graph of thermogravimetric analysis and derivative plot](image)

Mass of AuNP analyzed: 2.8487 mg  
Organic mass loss: 1.1395 mg

![Chemical structures A and B](image)

Area = 0.0125  
Proportion = 17.3% by mass  
Mass of A = 0.1971 mg  
MW\textsubscript{A} = 209.28 g mol\textsuperscript{-1}  
n\textsubscript{A} = 0.942 μmol  
[A] = 0.331 μmol / mg AuNP

Area = 0.0596  
Proportion = 82.7% by mass  
Mass of B = 0.9424 mg  
MW\textsubscript{B} = 901.03 g mol\textsuperscript{-1}  
n\textsubscript{B} = 1.046 μmol  
[B] = 0.367 μmol / mg AuNP

**Figure S4.35.** Thermogravimetric analysis (left) and derivative plot (right) of hvDIBO-PPh\textsubscript{3}-EG\textsubscript{4}-AuNPs.
Figure S4.36. $^1$H NMR spectra of thiol 5, hvDIBO-PPh$_3$-EG$_4$-AuNPs, and DIBO-PPh$_3$-EG$_4$-AuNPs obtained in CDCl$_3$ and referenced to residual CHCl$_3$. Blue arrows designate expected shifts from hvDIBO-PPh$_3$-EG$_4$-AuNPs to DIBO-PPh$_3$-EG$_4$-AuNPs after photochemical decarbonylation.
Figure S4.37. FT-IR spectra of hvDIBO-PPh$_3$-EG$_4$-AuNPs (left) and DIBO-PPh$_3$-EG$_4$-AuNPs (right) acquired on a PerkinElmer Spectrum Two FTIR spectrometer with an attenuated total reflectance (ATR) attachment and a single reflection diamond.
Figure S4.38. $^1$H NMR spectra of 13a/13b, isoxazoline-PPh$_3$-EG$_4$-AuNPs, 7a/7b, and (benzyl)triazole-PPh$_3$-EG$_4$-AuNPs obtained in CDCl$_3$ and referenced to residual CHCl$_3$. Dashed lines indicate correlation between AuNPs and the analogous ‘free’ unbound molecules.
Figure S4.39. $^{31}$P{$^1$H} NMR spectra of thiol 5 and AuNPs obtained in CDCl$_3$ and referenced to H$_3$PO$_4$. 
Figure S4.40. UV-vis spectra of AuNPs obtained in CH$_3$OH.

**Synthesis of DIBO-PPh$_3$-EG$_4$-AuNPs.** A solution of hvDIBO-PPh$_3$-EG$_4$-AuNPs (0.059 g) in 1:1 CH$_2$Cl$_2$:CH$_3$OH (450 mL) was irradiated in a Luzchem photoreactor equipped with 14 x 8 W UVA (350 nm) lamps for 3 min at r.t. while stirring vigorously. The solvents were removed by rotary evaporation to afford DIBO-PPh$_3$-EG$_4$-AuNPs (dark brown solid, 0.059 g).

**Synthesis of (benzyl)triazole-PPh$_3$-EG$_4$-AuNPs.** To a solution of DIBO-PPh$_3$-EG$_4$-AuNPs (0.032 g, estimated 11.74 μmol interfacial DIBO) in CH$_2$Cl$_2$ was added a solution of benzyl azide in CH$_2$Cl$_2$ (1 mL, 11.7 mM) at r.t. while stirring. The resulting solution was stirred for 16 h. The solvent was removed by rotary evaporation to afford (benzyl)triazole-PPh$_3$-EG$_4$-AuNPs (dark brown solid, 0.033 g).
Synthesis of isoxazoline-PPh$_3$-EG$_4$-AuNPs. To a solution of DIBO-PPh$_3$-EG$_4$-AuNPs (0.028 g, estimated 10.28 μmol interfacial DIBO) in CH$_2$Cl$_2$ was added a solution of nitrone 12 in CH$_2$Cl$_2$ (1 mL, 20.6 mM) at r.t. while stirring. The resulting solution was stirred for 16 h. The solvent was removed by rotary evaporation to afford isoxazoline-PPh$_3$-EG$_4$-AuNPs (dark brown solid, 0.030 g).

Release experiment for (benzyl)triazole-PPh$_3$-EG$_4$-AuNPs. To a solution of (benzyl)triazole-PPh$_3$-EG$_4$-AuNPs (0.015 g) in 1:1 CH$_2$Cl$_2$:CH$_3$OH (1 mL) was added H$_2$O (0.1 mL) and a solution of benzyl azide in CH$_2$Cl$_2$ (0.5 mL, 0.11 M, ~10 equiv.) at r.t. The mixture was left for 16 h. After, the solvents were removed by rotary evaporation and resulting AuNP film was washed with 2:1 EtOAc:hexanes (3x). The film was remade by dissolving in 1:1 CH$_2$Cl$_2$:CH$_3$OH followed by rotary evaporation. This washing procedure was repeated 2x and the washes were combined, concentrated, and analyzed by $^1$H NMR spectroscopy.

![Figure S4.41. $^1$H NMR spectral overlay of 8a/8b and 2:1 EtOAc:hexanes washes from release experiment with (benzyl)triazole-PPh$_3$-EG$_4$-AuNPs. All spectra obtained in CDCl$_3$ and referenced to residual CHCl$_3$ (7.27 ppm). Signals at 1.26 and 0.88 ppm correspond to residual hexanes.](image-url)
**Release experiment for isoxazoline-PPh₃-EG₄-AuNPs.** To a solution of isoxazoline-PPh₃-EG₄-AuNPs (0.015 g) in 1:1 CH₂Cl₂:CH₃OH (1 mL) was added H₂O (0.1 mL) and a solution of benzyl azide in CH₂Cl₂ (0.5 mL, 0.11 M, ~10 equiv.) at r.t. The mixture was left for 16 h. After, the solvents were removed by rotary evaporation and resulting AuNP film was washed with 2:1 EtOAc:hexanes (3x). The film was remade by dissolving in 1:1 CH₂Cl₂:CH₃OH followed by rotary evaporation. This washing procedure was repeated 2x and the washes were combined and analyzed by ¹H NMR spectroscopy.

![1H NMR spectral overlay of 14a/14b and 2:1 EtOAc:hexanes washes from release experiment with isoxazoline-PPh₃-EG₄-AuNPs. All spectra obtained in CDCl₃ and referenced to residual CHCl₃ (7.27 ppm). Signals at 1.26 and 0.88 ppm correspond to residual hexanes.](image)

**Figure S4.42.** ¹H NMR spectral overlay of 14a/14b and 2:1 EtOAc:hexanes washes from release experiment with isoxazoline-PPh₃-EG₄-AuNPs. All spectra obtained in CDCl₃ and referenced to residual CHCl₃ (7.27 ppm). Signals at 1.26 and 0.88 ppm correspond to residual hexanes.

**Synthesis of hvDIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs.** To a solution of hvDIBO-PPh₃-EG₄-AuNPs (0.038 g, estimated 13.95 μmol interfacial phosphine) in 1:1 CH₂Cl₂:CH₃OH (2 mL) was added azide 17 (0.035 g, 41.8 μmol) and left to stir for 16 h at r.t. After, solvent was evaporated and crude AuNPs were dialyzed in 70% CH₃OH in H₂O over 2 days (solvent changed 4x) until washings no longer became pink. Solvent was then removed by rotary evaporation to afford hvDIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs (dark brown-purple solid, 0.037 g).
Synthesis of DIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs. A solution of hvDIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs (0.023 g) in 1:1 CH₂Cl₂:CH₃OH (200 mL) was irradiated in a Luzchem photoreactor equipped with 14 x 8 W UVA (350 nm) lamps for 6 min at r.t. while stirring vigorously. The solvents were removed by rotary evaporation to afford DIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs (dark brown-purple solid, 0.023 g).

Synthesis of (biotin)triazole-P(=N-Rhodamine)Ph₃-EG₄-AuNPs. To a solution of DIBO-P(=N-Rhodamine)Ph₃-EG₄-AuNPs (0.019 g, estimated 6.97 μmol interfacial DIBO) in CH₃OH (1 mL) was added azide-PEG3-biotin conjugate 18 (0.006 g, 13.5 μmol) at r.t. while stirring. This mixture was stirred for 6 h and the solvent was removed by rotary evaporation. Crude AuNPs were dialyzed in 50% CH₃OH in H₂O overnight (solvent changed 3x) and the clean AuNP solution was concentrated in vacuo to afford (biotin)triazole-P(=N-Rhodamine)Ph₃-EG₄-AuNPs (dark brown-purple solid, 0.022 g).

Figure S4.43. ¹H NMR spectral overlay of multifunctional AuNPs obtained in CD₃OD and referenced to residual CD₃OH (3.31 ppm).
Figure S4.44. $^{31}\text{P}\left\{^1\text{H}\right\}$ NMR spectral overlay of multifunctional AuNPs obtained in CD$_3$OD and referenced to H$_3$PO$_4$.

Figure S4.45. $^{19}\text{F}\left\{^1\text{H}\right\}$ NMR spectral overlay of multifunctional AuNPs obtained in CD$_3$OD and referenced to CFCl$_3$. 
**Figure S4.46.** UV-Vis spectroscopic analysis of multifunctional AuNPs in CH$_3$OH.
**Figure S4.47.** ESI-MS analysis of disulfide 19.

**Figure S4.48.** TEM images of a) hvDIBO-PPh$_3$-EG$_4$-AuNPs, b) benzylamide(P=O)Ph$_3$-EG$_4$-AuNPs, and c) (biotin)triazole-P(=N-Rhodamine)Ph$_3$-EG$_4$-AuNPs.
Figure S4.49. Additional images at 60x magnification of fibroblast cells labeled with multifunctional AuNPs. Scale bar = 100 μm.
Chapter 5

5 Contributions of the Studies and Outlook

5.1 Contributions of the Studies

The ability to reliably engineer target functionalities onto material surfaces to program and tune their properties and reactivity in different environments represents an important challenge in nanomaterials chemistry. This thesis examines the use of two prototypical bioorthogonal reactions — originally designed to form bonds cleanly and selectively in complex biological environments — as a chemical tool to effectively derivatize gold nanoparticles (AuNPs) as a model nanomaterial. In addition to their ease-of-synthesis and processability, AuNPs feature high stability, rich surface chemistry, and characterizability by various techniques (e.g. NMR, IR, and UV-Vis spectroscopies, TGA, TEM) that permit the precise determination of their composition and monitoring of their interfacial chemistry. Furthermore, their widespread use in diagnostic imaging, therapeutics, and catalysis makes AuNPs an interesting and relevant nanoplatform to investigate.

Specifically, this work describes:

1. New methodologies to cleanly incorporate strained alkyne (SA) and cargo-bearing triarylphosphine derivatives onto AuNPs to afford well-defined reactive interfaces that undergo bioorthogonal transformations.
2. A new method to release molecules off AuNP nanocarriers, initiated by a highly specific reaction between phosphines and azides.
3. Methods to quantitatively and qualitatively determine these chemical functionalities on AuNPs and monitor interfacial reactivity of their monolayers.
4. An innovative strategy towards deploying multiple bioorthogonal reactions independently on AuNPs via a single ligand, enabled by a combinatorial approach using phosphine and strained alkyne reactivity.
5. New approaches for simple, clean, reliable, and versatile reactivity on AuNPs under mild conditions to provide surfaces that are derivatized with target molecules.
The strain-promoted cycloaddition of 1,3-dipoles (e.g. azides and nitrones) onto alkynes is one of the most important reactions in the click and bioorthogonal toolkit. It offers a rapid, clean (no byproduct), and selective route towards forming robust covalent bonds. However, the incorporation of SAs into AuNP monolayers is nontrivial; the strained C–C triple bond is susceptible to thiol-yne addition, thus a SA-bearing thiol ligand cannot be stably obtained and used. Chapter 2 addresses this deficiency through the use of cyclopropenones, which feature exceptional stability and inertness towards thiols, as a photochemical cyclooctyne precursor (Scheme 5.1). A cyclopropenone-bearing thiol ligand was synthesized and allowed for direct incorporation of the cyclooctyne precursor into the AuNP monolayer via ligand displacement. It was shown that the interfacial cyclopropenone decarbonylates quantitatively (and rapidly, <10 min) under UV irradiation to uncage dibenzocylooctyne, which reacts with various azides and a nitrone to afford a library of derivatized AuNPs. Kinetic investigation of the interfacial SPAAC between benzyl azide and dibenzocylooctyne-AuNPs revealed no significant difference

**Scheme 5.1.** Generalized overview of AuNP surface derivatization by employing a cyclopropenone-precursor strategy followed by interfacial SPAAC and SPANC with azides and nitrones, respectively. Shapes represent a general functionality of interest.
in reaction rate relative to the analogous reaction of unbound molecules. Modified AuNPs were decomposed by I$_2$ and the decomposition products were analyzed by mass spectrometry to further confirm the presence of products resulting from successful SPAAC and SPANC reactivity. This work represents an important advancement to AuNP derivatization as it enables the incorporation of SA precursors directly into their monolayer — without the aid of additional reagents or generation of byproducts — followed by clean and rapid “shine & click” chemistry.

The Staudinger-Bertozzi ligation (SBL), as its name suggests, is a powerful chemical tool to form bonds between two abiotic functionalities: azide and phosphine. However, its ability to liberate an alcohol in the process is seldom utilized or recognized. Thus, Chapter 3 serves to reinforce the SBL as a viable dual-purpose transformation (chemical ligation and release) to demonstrate the release of molecules off AuNPs, triggered only by a highly specific interaction with azides. The electrophilic ester trap, almost invariably a methyl ester, was synthetically modified to tether a Rhodamine B dye as a model cargo (Scheme 5.2). This cargo-bearing phosphine was successfully incorporated into AuNP monolayers via a terminal thiol. Upon treatment with a water-soluble benzyl azide

![Scheme 5.2](image_url)

**Scheme 5.2.** Demonstrative release of molecules off AuNP surfaces using the SBL as a bioorthogonal cleavage reaction. Shapes represent a general functionality of interest.
variant, modified AuNPs underwent SBL to afford the ligated AuNP product and liberated a cargo — in this case, a Rhodamine B alcohol. Due to the choice of cargo, the release process could be monitored by fluorescence spectroscopy: as AuNP-bound dye molecules are released from the surface, their previously quenched emission is recovered (“turn-on”), which can be observed visually and spectroscopically. A control experiment using the analogous phosphine oxide (P=O) modified AuNPs (which do not undergo SBL) exhibited no release upon treatment with azide. Similarly, no significant release via ligand displacement was observed when AuNPs were dispersed in intracellular concentrations (1–10 mM) of glutathione. Thus, it was shown that the SBL represents the only significant release mechanism among the conditions examined. This work is the first time the SBL is utilized on a material surface to release molecules, enabled only by a highly specific transformation. To facilitate the visualization of the release process, a fluorescent dye was investigated as the cargo; however, the ester linkage is easily synthetically accessible and represents a versatile chemical handle for tethering different cargos such as drugs or biomolecules. Therefore, this work underscores the SBL as a versatile chemical tool for tailoring AuNP surface functionalities via ligation and release mechanisms.

The versatility of the SBL for chemical ligation and release on materials led to a major shift in this thesis work to focus on addressing the challenge of multifunctionality — wherein a single molecule or material can perform multiple chemical functions (or reactions) independently. Multifunctionality on AuNPs can be achieved using multiple functional thiols concurrently. However, the ligand ratios of the resulting multi-mixed monolayers are difficult to control and quantitate, which hinders our understanding of the final AuNP composition. To address this challenge, a single thiol ligand bearing multiple functionalities can be employed. Chapters 2 and 3 demonstrated reliable methodologies for incorporating SAs and cargo-carrying phosphines onto AuNPs to afford well-defined mixed monolayers of known ligand concentrations. Chapter 4 extends this development via a combinatorial approach: a cyclooctyne precursor tethered via an electrophilic ester trap to the triarylphosphine (Scheme 5.3). This cyclooctyne, once uncaged, represents a “reactive tether” that enables a cargo to be immobilized onto AuNPs interfacially (via SPAAC or SPANC with azides and nitrones, respectively) without having to
Scheme 5.3. Generalized overview of highly modular reactivity on AuNPs. Bioorthogonal ligation and cleavage reactions can be used interchangeably to afford reliable and clean derivatization of AuNP surfaces. Shapes represent a general functionality of interest.

 synthetically introduce it in the thiol synthesis stage. Consequently, stability of the cargo towards thiols, which was necessary in Chapter 3, is not necessitated in this new design. This dual-bioorthogonal molecular tool exhibits the bioorthogonal reactivity of both phosphine and SA functionalities: SPAAC or SPANC via the alkyne, and SBL or the modified perfluoroaryl azide Staudinger reaction (PFAA-SR) via the phosphine. This unique design enables two reaction pathways: “click-to-release”, which proceeds via SPAAC/SPANC then SBL, or “double-click”, which proceeds via SPAAC/SPANC then PFAA-SR (or reverse order).

A thiol ligand bearing this multifunctional moiety was synthesized and incorporated into the AuNP monolayer successfully. Despite its structural complexity, reactivity at each site can be carefully monitored via NMR (¹H and ³¹P), IR, and UV-vis spectroscopies to understand the AuNP composition at each step. Both click-to-release and double-click strategies were successfully employed on the modified AuNPs. Biotin- and Rhodamine B
dye-modified AuNPs (obtained via double-click) were used to demonstrate targeted labelling of human fibroblast cells. This work represents significant advancement in AuNP derivatization for two reasons: 1) it offers a modular and highly controlled method to attach, release, and replace molecules as well as access multifunctional AuNPs from a single template, and 2) it constitutes an important combinatorial prototype that is key to achieving multifunctionality on materials in a controlled manner. As outlined in Sections 1.2.1 and 1.3.4, applications of nanomaterials typically require multiple functionalities (e.g. targeting agent, imaging agent, therapeutics, or biomolecules) on their surface to be effective, which highlights the importance of reliable and controlled derivatization methodologies. The combination of bioorthogonal moieties that react in a modular and mutually orthogonal manner overcomes the challenges associated with multifunctionality and provides access to materials that are complex but still well-defined.

Overall this thesis work examines ways in which AuNP monolayers can be modified effectively using two prototypical bioorthogonal reactions. Indeed, surface derivatization of AuNPs has been achieved for decades — with varying levels of success — using direct ligand binding approaches or other interfacial organic reactions (e.g. EDC/NHS coupling). However, the resulting mixed monolayers are often ill-defined with respect to their chemical composition and ligand concentrations. Instead, this work provides the means to modify AuNP monolayers using reactions that proceed cleanly, rapidly, selectively, and to completion under mild conditions (Scheme 5.4, Route A). The underlying objective is two-fold: 1) conserving the AuNP core diameter and size distribution during modification and 2) precisely determining and conserving the concentration of target functionalities within the AuNP monolayer in each step. Although seemingly trivial, the control of these two parameters can be highly consequential. Properties of AuNPs are significantly affected by their size and shape, while their ligand composition governs their interactions and activity inside different environments. Existing applications that use derivatized AuNPs demonstrate that their methods work — e.g. adding a targeting agent increased cellular uptake or the release of a drug off AuNP monolayers led to decreased cell viability — but rarely investigate how well it works. Continued advancement in nanotechnology using AuNPs (and other materials) will require systematic investigations where the determination of precise ligand
Scheme 5.4. Conceptual scheme depicting the advantages of utilizing interfacial bioorthogonal chemistry on AuNPs (Route A) versus other organic reactions (Route B). Concentrations is important to answer questions such as: How does varying the concentration of surface peptides affect cellular uptake? If the drug loading in the monolayer was increased/decreased to X mmol mg\(^{-1}\) AuNP, will it have an effect on cell viability? Using other organic reactions, which suffer from the generation of byproducts, incomplete or side reactivity, need for high temperature or pressure, use of a catalyst, or incompatibility with in vivo use, can lead to AuNPs of changed core diameter, greater size distribution, presence of impurities (both AuNP-bound and unbound), and ill-defined monolayers that hinder systematic investigations (Scheme 5.4, Route B). The AuNPs reported in this work represent not only a versatile platform on which surface modifications can be achieved, but allows for careful tuning, determination, and conservation of AuNP size and ligand concentrations due to the advantages associated with bioorthogonal reactivity. Lastly, widespread commercialization of organic azides — ranging from small molecules to large biomolecules — will result in further ease in achieving surface modifications even by non-experts using the AuNPs reported in this thesis.
5.2 Outlook

The methodologies described in this thesis serve only as a stepping stone towards the realization of multifunctional nanomaterials with well-defined interfaces. The transposition of bioorthogonal chemistry to materials science is a relatively young field, where existing methodologies are more application-oriented with less emphasis on investigating the derivatized interface. Thus, future work in this field should address one of the following two challenges:

1. **Continuing to demonstrate and expand the viability of bioorthogonal reactions on nanomaterials to achieve controlled modifications.** The bioorthogonal toolkit is extensive and prolific, where new reactions are reported every year, each with their own advantages with respect to stability of reactants, reaction kinetics, orthogonality to other bioorthogonal reactions, and biocompatibility. Methodologies for incorporating these chemical functionalities onto materials, as well as quantifying them, will be an important and necessary advancement in nanotechnology to fully understand their composition-function relationship.

2. **Developing other combinatorial approaches to deploying bioorthogonal chemistry on materials.** The engineering of nanomaterials that can perform multiple chemical functions (or reactions) independently is challenging, with emphasis on the difficulties associated with controlling and fine-tuning the incorporation of multiple target functionalities concurrently on a single material. As demonstrated in Chapter 4, the combination of bioorthogonal chemical functionalities that are mutually orthogonal can serve to overcome these challenges via a modular approach wherein each bioorthogonal reaction is executed independently and sequentially to afford a well-defined multifunctional interface.

With respect to the work specifically described in this thesis, our group’s short-term goals include expanding the methodologies developed here to other nanomaterials. Initially, AuNPs were investigated due to their rich surface chemistry, high stability, and characterizability by various techniques that permitted careful monitoring of their monolayer composition and reactivity. Next steps include transposing and adapting these
bioorthogonal strategies to other nanomaterials, e.g. Au nanorods (AuNRs), carbon-based nanoparticles (or carbon dots), and carbon nanotubes (CNTs). Compared to AuNPs, AuNRs exhibit higher absorbance of near-infrared (NIR) light, which features greater utility for therapeutic applications involving photothermal heating and fluorescence imaging.\[^{2-3}\] In addition, we wish to further expand the application of bioorthogonal chemistry onto carbon-based nanomaterials, which are far less studied. Carbon dots feature low toxicity and exciting emissive properties, while CNTs are effective nanocarriers for drugs and biomolecules and has potential for photothermal therapy.\[^{4-5}\] However, due to their general inertness, well-defined modifications on carbon dots and CNTs are challenging to achieve. Our group has recently started investigating these methods on CNTs, using protocols we previously developed for introducing chemical functionality.\[^{6-7}\] Lastly, new click-to-release and double-click strategies that involve different bioorthogonal chemical functionalities will be further explored.

5.3 References

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Curriculum Vitae

Name: Wilson Luo

Post-secondary Education and Degrees:
University of Windsor
Windsor, Ontario, Canada
2010-2014 B.Sc.

The University of Western Ontario
London, Ontario, Canada
2014-2019 Ph.D.

Honours and Awards:
CSC Travel Award
2017

Lipson-Baines Award in Chemistry
2017

Natural Science and Engineering Research Council (NSERC)
Canada Graduate Scholarship – Doctoral Level
2017-2019

Ontario Graduate Scholarship (OGS)
2017-2018
(Declined due to NSERC)

ASPIRE Award
2017

Ontario Graduate Scholarship (OGS)
2016-2017

Ontario Graduate Scholarship (OGS)
2015-2016

University of Windsor
Outstanding Scholars Honorarium
2011-2014

University of Windsor
Outstanding Scholars Award
2010-2014
University of Windsor
Entrance Scholarship
2010-2014

Ontario Government
Queen Elizabeth Aiming for the Top Scholarship
2010-2012

**Related Work**
Teaching Assistant – CHEM 3373, 2213, 2223

**Experience**
The University of Western Ontario
2014-2016

**Publications:**


Gobbo, P.; Gunawardene, P.; Luo, W.; Workentin, M. S. *Synlett* 2015, 26, 1169–1174.