The Self-Assembly of Gold Nanoparticles Through "Click" Chemistry

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The Self-Assembly of Gold Nanoparticles Through “Click” Chemistry

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Abstract

Gold nanoparticle (AuNP) assemblies are incredibly important to modern science because of their wide span of applications in fields ranging from biology to electronics. Using “click” chemistry, we seek to develop a novel method to self-assemble AuNPs that is not restricted by environmental conditions, such as pH, temperature, and solvent. “Click” chemistry is performed using a Huisgen 1,3 dipolar cycloaddition to combine two populations of AuNPs, forming a 1,2,3-triazole in the presence of a copper(I) catalyst. The first population of AuNPs was created by functionalizing AuNPs with bis(p-sulfonatophenyl)phenylphosphine dehydrate (sppp) and performing a place exchange with amine and alkyne terminated-polyethylene glycol (NH$_2$-PEG-C$_2$), and the second population was created by functionalizing AuNPs with sppp and performing a place exchange with amine and alkyne terminated-polyethylene glycol (NH$_2$-PEG-N$_3$). Fourier transform infrared spectroscopy (FT-IR), UV-Vis spectroscopy, and dynamic light scattering (DLS) were used to characterize these populations before and after attempting coupling reactions. UV-vis showed a red-shift from 518 nm to 525 nm and 524 nm after functionalizing the nanoparticles with NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$, respectively. For population 1, we observed a +8.7 nm increase in the hydrodynamic diameter of AuNPs + sppp after adding NH$_2$-PEG-C$_2$. We did not see an increase in population 2, probably because sppp and NH$_2$-PEG-N$_3$ have about equal molecular weights. Future studies will include continual testing to prove that we have accomplished the click reaction, clicking the two populations together using electrochemistry, and using cyclic voltammetry to determine the kinetics of the reaction. We believe that we have made substantial progress towards our end goal of creating a new AuNP self-assembly method through “click” chemistry.
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Table of Contents

Abstract .........................................................................................................................i
Acknowledgements .................................................................................................ii
1. Introduction ........................................................................................................... 1
   1.1. Importance of nanoparticle assemblies ......................................................... 1
       1.1.1. Applications: Vapor sensors ................................................................. 2
       1.1.2. Applications: Biosensors ..................................................................... 3
       1.1.3. Applications: Photoelectrochemistry .................................................... 5
       1.1.4. Applications: Metal ion sensors ............................................................ 6
   1.2. Existing self-assembly methods ..................................................................... 8
       1.2.1. Biologically based self-assembly methods .......................................... 8
   1.3. Electrochemically controlled self-assembled monolayers ............................ 11
   1.4. “Click” chemistry ......................................................................................... 13
   1.5. Overview of project ....................................................................................... 15
2. Materials and methods ......................................................................................... 18
   2.1. Materials ....................................................................................................... 18
   2.2. Synthesis of 11.2 nm AuNPs ....................................................................... 18
   2.3. Functionalization of AuNPs with MeO-PEG-NH₂ ......................................... 19
   2.4. Characterization of AuNPs functionalized with MeO-PEG-NH₂ .................. 19
   2.5. Functionalization of AuNPs with sppp ......................................................... 20
   2.6. Creating two populations ............................................................................. 20
   2.7. Characterization of two populations ............................................................. 21
   2.8. Clicking together two populations ............................................................... 21
   2.9. Testing the click reaction off particle ........................................................... 22
3. Results and Discussion ......................................................................................... 23
   3.1. Functionalizing AuNPs with aminated PEG ................................................. 23
   3.2. Creating two populations ............................................................................. 27
   3.3. “Click” reaction ............................................................................................ 32
   3.4. Testing the click reaction off particle ........................................................... 37
4. Conclusions ........................................................................................................... 39
5. Appendix ............................................................................................................... 41
   5.1. Testing the surface functionalization of AuNPs .............................................. 41
6. References ............................................................................................................. 44
1. INTRODUCTION

1.1. Importance of nanoparticle assemblies

Metal nanoparticles possess several novel properties compared to bulk metals, including high extinction coefficients and high surface area to volume ratios. These properties allow them to be used in a multitude of applications. Gold nanoparticle (AuNP) assemblies in particular are of widespread interest because of their stability and electronic, magnetic, and optical properties, which can be applied to applications such as catalysis, biology and electronics. Because AuNPs are prone to aggregate, capping AuNPs with functional groups to prevent aggregation has become an important area of study for nanoscientists. AuNPs can be capped through electrostatic interactions of ions, with ligands, or through micellar formation. Electrostatic interactions and ligand capping methods allow AuNPs to be functionalized with a variety of molecules. By appropriately choosing the capping agent, AuNPs can be forced to aggregate into supramolecular structures, often through self-assembly.

AuNP self-assemblies have been created using a range of biological interactions such as DNA base-pair recognition, antibody-antigen, streptavidin/biotin, and coiled-coil peptides. Although these biologically-based methods are very useful, the environmental limitations such as pH, temperature, ionic strength, and solvent create a need for an environmentally independent self-assembly method. A more universal triggering method of self-assembly, namely, the use of “click” chemistry controlled by electrochemistry would be more independent of environmental conditions and a valuable addition to nanoscientists’ toolkit.

Electrochemical reactions can occur over a wide range of pHs, at high and low temperatures, and in many different solvents because the reactions are taking place at the surface of the electrode. This location also makes electrochemical reactions easier to control kinetically.
and more likely to occur than biological reactions that take place in the bulk of solution.\textsuperscript{10} Our project proposes the use of electrochemistry to trigger the 1,3-dipolar cycloaddition click reaction between two populations of AuNPs, catalyzed by the electrochemical reduction of Cu(II) to Cu(I), to form a 1,2,3-triazole bridge between the two populations. Once the self-assembly has formed, these AuNPs can be applied to a wide variety of applications, some of which will be described in the following sections.

1.1.1. Applications: Vapor Sensors

In order to understand the importance of AuNP assemblies, it is essential to understand their applications and properties. AuNPs’ catalytic properties allow them to be used in a wide array of sensing devices, including vapor sensors. In a study by Singh et al., In\textsubscript{2}O\textsubscript{3} nanowires functionalized with AuNPs were able to detect toxic CO gas at a much higher sensitivity than bare In\textsubscript{2}O\textsubscript{3} nanowires, as low as 0.2 ppm could be detected.\textsuperscript{11} The AuNPs were assembled onto the nanowires using a strong amine-gold interaction. The catalytic property of AuNPs increased the interaction between the reducing gas (CO) and the adsorbed oxygen, causing CO oxidation.\textsuperscript{2} A high density of AuNPs deposited on the nanowire increased the electron transfer back into the nanowire, and therefore increased the conductance of the nanowire.\textsuperscript{11} The higher sensitivity of the AuNP-coated nanowires can be used to create improved sensing devices at room temperature.

Nanoparticle assemblies are also often used in chemical sensing as chemiresistors because of their conductive cores, ease of diffusion, and high porosity, which allows for a large uptake of analyte molecules.\textsuperscript{12} Chemiresistors measure changes in electrical resistance based on the chemical species present.\textsuperscript{2} The interparticle distances between nanoparticles, colloid size, and dielectric material between particles affect the resistance.\textsuperscript{2} A larger interparticle distance will decrease the electrical resistance of the array by lowering the medium dielectric permittivity.
Interparticle distances also have an immense effect on electronic and magnetic properties of nanoparticles. Functionalizing nanoparticles with polymers can adjust the interparticle distances, creating differences in the assembly’s porosity and changing the dielectric material between particles. The type of polymer can create multiple interaction sites and more refined selectivity so that only the analyte of interest is absorbed. An example of a nanoparticle array designed to sense a specific analyte was the use of palladium nanoparticles in a poly(\textit{p}-xylene) film to measure NH$_3$ vapor. This application of nanoparticle assemblies has led to increased sensitivity and selectivity in chemical sensing devices.

AuNP vapor sensing applications can also be used for biomedical applications, such as lung cancer research. A study by Peng et al. incorporated AuNP chemiresistors into vapor sensing as a method for lung cancer detection in exhaled breath. Gas chromatography/mass spectroscopy combined with solid-phase microextraction was used to identify 42 volatile organic compounds (VOCs) associated with lung cancer. First, Peng et al. distinguished VOCs that were biomarkers for lung cancer from VOCs found in healthy individuals. Then, they created multiple 5-nm AuNP assemblies functionalized with different organic compounds. The assemblies were effective in responding to a variety of concentrations of VOCs and had a limit of detection as low as 1–5 parts per billion. These sensors based on AuNP assemblies have the potential to provide an inexpensive, non-invasive technique for diagnosing lung cancer.

1.1.2. Applications: Biosensors

Aside from their function in vapor sensors, AuNPs can also be used to detect concentrations of biological molecules. AuNPs’ conductivity and the ability to functionalize AuNPs with many different types of molecules allow AuNPs to be used as biosensors for ammonium, glucose, proteins, DNA, and to detect respiratory diseases. The deposition
of AuNPs on an electrode was used in two different electrochemical studies to detect concentrations of ammonium and glucose. Detection of ammonium in waste water and aqueous environments is important because ammonium can destroy aquatic life. In one study, researchers looking to improve the sensitivity of an ammonium biosensor deposited multiple layers of AuNPs using C8-dithiols onto a gold electrode to improve the AlaDH enzyme’s affinity for the NH$_4^+$ ion.\textsuperscript{15} The AlaDH enzyme was covalently bonded to the AuNPs via thiol linkages, which allowed the enzyme to stick up further off the surface of the electrode and get closer to the NH$_4^+$. They found that depositing too many C8-dithiols interfered with electron transfer, but having exactly three layers of AuNPs did improve the enzyme’s affinity for NH$_4^+$.\textsuperscript{15} Similarly, in another study focusing on the detection of glucose concentrations, AuNPs were attached to the electrode’s surface to help with electron transfer between the electrode and glucose oxidase, the enzyme used to detect glucose.\textsuperscript{16} The addition of AuNPs made it easier to access the electroactive center of glucose oxidase, facilitating electron transfer between the electrode and the enzyme.\textsuperscript{16}

The ability to functionalize nanoparticles with different functional groups has the potential for DNA sensing and for the detection of respiratory diseases. In a study by Jans et al., citrate-stabilized AuNPs were functionalized with mercaptoalkanes to subsequently functionalize the AuNPs with single-stranded DNA (ssDNA) for use in DNA sensing.\textsuperscript{18} Before each step, the AuNPs were characterized using UV-visible spectroscopy (UV-Vis) and dynamic light scattering (DLS). The researchers found that AuNPs functionalized with mercaptoalkanes that included poly(ethylene oxide) (PEO) units were more stable than citrate capped AuNPs in electrolytic environments. The stability of AuNPs in electrolytic environments was tested by increasing the concentration of salt and noting the aggregation of AuNPs. AuNPs coated in ssDNA were also
found to be stable in electrolytic environments and bound specifically to their complementary DNA.\textsuperscript{18} Halfpenny et al. reviewed several studies in which AuNPs were applied to detect respiratory diseases.\textsuperscript{19} In one study, AuNPs were functionalized with antibodies to detect porcine reproductive and respiratory syndrome (PRRS) using Förster resonance energy transfer (FRET) pairs. AuNPs act as fluorescence quenchers, so researchers paired AuNPs with an organic fluorophore and used fluorescence to detect very low concentrations (the limit of detection was 1741 particles/mL) of PRRS virus (PRRSV).\textsuperscript{19} The sensor is made up of the PRRSV antibody, the Alexa Fluor 546 fluorophore, and protein A, which is conjugated to the AuNPs. When PRRSV is present, the antibody binds to PRRSV changing the formation of the sensor and putting the fluorophore close enough to the AuNP for the fluorophore to be quenched.\textsuperscript{19} These studies give a brief glimpse into AuNP assemblies’ wide variety of applications in biology.

1.1.3. Applications: Photoelectrochemistry

Several studies have also implemented AuNP assemblies to photochemical processes. The combination of AuNPs with TiO\textsubscript{2} nanoparticles’ wide band gap and photocatalytic properties have led to the application of these nanoparticle composites in increasing the photocurrent generated by a photoelectrochemical cell.\textsuperscript{20} This study used amine-functionalized N-[3-(trimethoxysilyl)propyl]ethylenediamine (EDAS) silicate sol-gel-supported TiO\textsubscript{2}-Au core-shell nanoparticles to modify an electrode used in a photoelectrochemical cell. AuNPs loaded onto the TiO\textsubscript{2} created more separation of electron and hole pairs and increased the photocurrent generation of the cell. This has led to the potential for use of AuNPs in solar cells, photocatalysis and sensors.\textsuperscript{20}

Another application of photoelectrochemistry combined AuNPs with tris(2, 2’-bipyridine) ruthenium on an electrode to use as a DNA biosensor.\textsuperscript{21} Zhang et al. used a three electrode
system and the chemiluminescence of CIPO-H₂O₂-9,10-diphenylanthracene as the light source to excite the photoelectrochemically active species Ru(bpy)₂dppz²⁺ that was intercalated within the DNA.²¹ The excited Ru(bpy)₂dppz²⁺ created a photocurrent, signaling the presence of DNA.²¹ AuNPs were deposited on the electrode to increase the sensitivity of the electrode. However, the final sensitivity of this biosensor was not very high.

1.1.4. Applications: Metal Ion Sensors

Finally, AuNPs can also be used to detect the presence of metal ions. The unique color change from a red color indicating stable AuNPs to purple or blue indicating aggregated AuNPs has been applied as a colorimetric test for sensing “spectroscopically silent” heavy metal ions which are dangerous when present in parts per million concentrations in drinking water.²²,²³ Because it is difficult to find dyes with a high enough extinction coefficient to distinguish change without instrumentation, metal nanoparticles with visible-region absorption and high extinction coefficients have been implemented in place of dyes. Hupp et al. functionalized AuNPs with 11-mercaptoundecanoic acid, which aggregated in the presence of Pb²⁺, turning a purple color. Aggregation was reversed when EDTA was added to bind the Pb²⁺, causing the solution to return to a red color. This colorimetric response also successfully detected Hg²⁺ and Cd²⁺.

Another study used DNA-functionalized AuNPs to detect Ag(I) ions in neutral pH.²³ Because mismatched cytosine-cytosine (C-C) pairs have been found to capture Ag(I), forming C-Ag(I)-C sandwiches, this study functionalized AuNPs with unlabelled cytosine rich ssDNA and labeled sulfhydryl group C-ssDNA (i.e., HS-C-ssDNA). The C-ssDNA and HS-C-ssDNA stabilized the AuNPs, keeping the solution a red color, while the presence of Ag(I) caused aggregation of AuNPs once C-Ag(I)-C complexes formed, turning the solution purple. UV-Vis
Figure 1. Schematic illustration of the use of AuNPs functionalized with crown ethers to form crown ether-metal ion "sandwiches" to detect metal ions at low concentrations.24

In a study by Russell et al., films of monolayer protected clusters (MPCs) functionalized with crown ethers (CE) were used to detect heavy metals in ethanol and dichloromethane solutions.24 As illustrated in Figure 1, aggregation and swelling of films when CE-metal-CE sandwiches formed indicated the presence of potassium ions in solution. This was detected by a slight red shift of the surface plasmon band (SPB) in flexible films where the sandwiches made the films more compact, or a slight blue shift if the films were inflexible and AuNP cores were unable to aggregate. In aqueous solutions the films did not swell, possibly because of the insolubility of the films in aqueous solutions. This research helps explain the importance of flexibility of films in metal ion sensors. In the future, these films and the use of nanoparticle assemblies may be used to detect multiple metal ions simultaneously.

As shown by these many studies, AuNPs have a diverse range of applications and can be adapted to almost any type of sensing device. They play an important role in detecting toxins in the environment, detecting biological molecules and diseases, and as conductive material in photocatalytic devices. The flexibility of AuNPs to be used in different fields makes AuNPs valuable tools for nanoscientists.
1.2. **Existing Self-Assembly Methods**

In order to adjust the properties of AuNPs to be applied in different applications, self-assembled monolayers (SAMs) are useful methods for nanoscientists to decorate the surfaces of nanoparticles. They are composed of organic assemblies that surround metal nanoparticles and increase the number of applications to which metal nanoparticles can be applied.\(^{25}\) The strong interaction of ligands, namely sulfur and amine groups, with gold provides a method of attaching the organic assemblies to the metal. One of the most common types of SAMs, shown in Figure 2, is alkanethiols bound to the surface of gold. SAMs are easy to attach to metal nanoparticles, and increase the stability of nanoparticles in solution. SAMs also connect metal nanoparticles’ electronic and optical properties to the environment, and give nanoparticles macroscopic properties like adhesion, wetting and friction, making SAMs invaluable to nanoscientists.\(^{25}\) In our proposed project, creating a new self-assembly method through electrochemistry would broaden the environmental conditions under which SAMs can be formed so that AuNPs can be applied to new applications.

1.2.1. **Biologically Based Self-Assembly Methods**

Currently, there are several biomolecular recognition reactions that have been used to form controlled self-assemblies of AuNPs, including, streptavidin/biotin, coiled-coil peptides, DNA base-pair recognition, and antibody-antigen systems. These self-assemblies could be implemented in sensing devices such as the ones previously described and in bioengineering and medical applications. In order to properly develop our electrochemical method of self-assembly,
it is important to understand how these biologically based self-assembly methods work. Information, such as how to best attach functional groups to the AuNPs, what are limiting conditions that we want to overcome, and how to characterize self-assembled AuNPs, can be gained from these studies.

The first application of streptavidin/biotin interactions to form AuNP assemblies was designed by Connolly et al., who came up with two methods of aggregating AuNPs. The first method was to bind disulfide biotin analogue (DSBA) to the AuNPs and add in streptavidin. The second method was to first bind DSBA to streptavidin and then add in AuNPs that would chemisorb to the DSBA through thiol-gold interactions. DLS, small angle X-ray scattering, and transmission electron microscopy (TEM) were used to characterize assemblies. Another study by Stevens et al. used protein-protein interactions to form AuNP self-assemblies. They attached two different artificial peptides, one an acidic leucine zipper-like peptide and the other a basic leucine zipper-like peptide, to 8.5 nm and 53 nm AuNPs respectively, and controlled self-assembly of AuNPs using pH and temperature. The peptides were terminated with a cysteine amino acid to form covalent gold-thiol bonds between the peptide and AuNP. Combining the two populations of AuNPs at 25 °C and pH 7.4 created small self-assemblies of three to four 53 nm AuNPs surrounded by a layer of 8.5 nm AuNPs. Lowering the pH to 4.5 caused a larger, more disorganized self-assembly of many 8.5 nm AuNPs around the 53 nm AuNPs. These assemblies managed to maintain stability at 90 °C for up to 20 minutes, whereas the peptide coated 8.5 nm AuNPs started to aggregate under those conditions.

DNA-based self-assembly of AuNPs has become widely used in a variety of sensing devices. One method developed by Loweth et al., involved attachment of ssDNA to AuNPs to form homodimeric, homotrimeric, heterodimeric, and heterotrimeric assemblies of 5 nm and
10 nm AuNPs. Figure 3 demonstrates two of the three different approaches used to form the assemblies. In the first approach, dimers and trimers with zero or one break (“nick”) in the DNA were formed. In the second approach, dimers and trimers with one or two nicks were formed, and in the third approach trimers with no nicks were formed. Another DNA-based method by Mirkin et al. used oligonucleotides to reversibly form self-assemblies of AuNPs. At low temperatures (~0 °C) AuNPs would aggregate because of DNA linking of “sticky ends” between oligonucleotides. Once heated to 80 °C, the AuNPs would disperse again because the DNA would lose its “stickiness”. A color change of solution was visible changing from purple-gray at low temperatures to a dark-red solution at high temperatures.

The use of antibody/antigen recognition has also been employed to self-assemble AuNPs and Ag nanoparticles. Covalent bonds formed between anti-DNP IgE and dinitrophenyl (DNP) or anti-biotin IgG antibodies and biotin created assemblies of AuNPs or co-assemblies of Au and Ag nanoparticles. When DNP-double headed antigen was added to a red-colored solution of stable anti-DNP IgE functionalized AuNPs, after leaving the solution unstirred for four hours at

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**Figure 3.** Schematic illustration and TEM images of the use of DNA strands to form various configurations of self-assembled AuNPs. The large circles represent 10 nm AuNPs, while the small circles represent 5 nm AuNPs. Three approaches were used to form self-assemblies. A, B, and C represent the second approach forming one nick in the DNA for a dimer and two nicks in the DNA for a trimer, while D represents the first approach forming one nick in DNA for a trimer.
4 °C, a purple precipitate of aggregated AuNPs formed at the bottom and the solution turned clear. Shenton et al. used TEM to characterize the array and speculated that the IgE antibody on multiple AuNPs bound to the DNP, cross-linking AuNPs, and causing the AuNPs to aggregate. Shenton et al. also formed AuNP-AgNP assemblies by functionalizing AuNPs with IgE and functionalizing AgNPs with anti-biotin IgG antibody, then adding in an artificial antigen with both biotin and DNP antigens. Using TEM and X-ray analysis, they found that Ag and AuNPs had formed aggregated clusters, though the AuNPs seemed to aggregate more than the AgNPs.

These biologically based methods of self-assembly give us a starting point to work off of in forming a novel self-assembly method. They give us ideas ranging from techniques to cap the AuNPs to techniques to characterize the assemblies once the assemblies have been formed. Although these biologically based methods are very useful, they are limited by pH, temperature, ionic strength, and solvent. Therefore, the need for a more universal method to control the self-assembly of AuNPs has lead to the implementation of electrochemistry.

1.3. Electrochemically Controlled Self-Assembled Monolayers

The ability to control the self-assembly of AuNPs on surfaces has become an important area of research because functionalizing surfaces through a variety of methods has many applications in sensing and catalysis. One non-biologically based method for controlling the creation of SAMs is electrochemistry, in which electrodes can be decorated to undergo specific reactions at the surface of the electrode. For example, Diels-Alder reactions can be electrochemically controlled and are often used in modifying surfaces. Because the reaction takes place at the electrode’s surface, environmental conditions in the bulk of solution do not play a large role in whether or not the reaction will occur.
Several studies have been conducted using the Diels-Alder reaction to form SAMs. Chan et al. researched the electrochemical properties of SAMs with hydroquinone groups created through the Diels-Alder reaction.\(^{26}\) In one experiment illustrated in Figure 4, they examined the kinetics of the Diels-Alder reaction of cyclopentadiene with 2-mercaptobenzoquinone chemisorbed to the surface of a gold substrate.\(^{26}\) When these reactants are free in solution, this reaction usually proceeds with second-order kinetics. However, with the 2-mercaptobenzoquinone attached to the surface, this reaction proceeded as if the diene were first adsorbed through equilibrium and then reacted with the attached quinone. Their calculations determined a thermodynamic association constant for the diene adsorbing to the surface and a first-order rate constant for the cycloaddition reaction.

In another study of cyclopentadiene and benzoquinone undergoing the Diels-Alder reaction, Kwon et al. examined how steric s around the quinone affected the rate constant.\(^{27}\) The benzoquinone can undergo two reversible-reduction reactions absorbing two electrons to become hydroquinone. Therefore, cyclic voltammetry is a practical technique for studying the amounts of quinone on the surface of an electrode. Kwon et al. created a monolayer of hydroquinone-terminated alkanethiol surrounded by methyl-terminated alkanethiols. They varied the length of the hydroquinone-terminated alkanethiol, and observed how the diene reacted with the immobilized quinone. When the hydroquinone-terminated alkanethiol was longer than the surrounding hydroxyl-terminated alkanethiols, the rate constants were around 0.20 M\(^{-1}\)s\(^{-1}\). When the hydroquinone chain was shorter than the surrounding alkanethiols, the rate constant...
decreased by about 8-fold. Kwon et al. also found that the solvent composition affected the association constant for the diene, but did not affect the reaction constant. These studies using electrochemistry to control the reaction and cyclic voltammetry to measure the kinetics of the reaction will help us determine the kinetics of our reaction in the future.

1.4. “Click” Chemistry

Aside from Diels-Alder reactions, some click chemistry reactions, popularized by Sharpless, can also be controlled electrochemically. The reaction we propose to use is one of the most popular Sharpless click reactions: the Huisgen 1,3-dipolar cycloaddition of azides and alkynes, forming a 1,2,3-triazole depicted in Figure 5.28 “Click” chemistry is defined by reactions that follow a specific set of regulations. Reactions must be modular, wide in scope, produce very high yields, produce inoffensive byproducts that can be easily removed, and are stereospecific.29 In addition, the reaction must occur under simple reaction conditions, have readily available starting materials and reagents, be solventless or have a benign solvent or one that is easily separated, and have simple product isolation.29

Because click reactions are relatively easy to perform, Baskin et al. were able to modify the reaction to perform copper-free click chemistry inside living mice to study the movement of biomolecules.30 Baskin et al. implemented the bioorthogonal chemical reporting strategy to label glycans and lipids. In order to label the biomolecules in vivo, a quick, harmless reaction had to be developed combining two azide-specific reactions, the Staudinger ligation and the copper(I)-catalyzed azide-alkyne cycloaddition click reaction. The Staudinger ligation is too slow for labeling the biomolecules, and the click reaction requires the presence of Cu, which is toxic to
cells. Instead of running the click reaction in the presence of Cu, they used difluorinated
cyclooctyne (DIFO) with ring strain and electron withdrawing groups to join with the azide at a
sufficient speed without Cu present. Using this labeling technique, they were able to image
glycan movement inside a living mouse. This study gives an example of how robust the click
reaction is and the wide range of environmental conditions it can be performed in.

In another study involving the click reaction, Devaraj et al. succeeded in applying click
chemistry to modify individual electrodes’ surfaces to form SAMs terminated with a 1,2,3-
triazole. Mixed azide-terminated SAMs were formed on the surface of a pair of gold
interdigitated array (IDA) band electrodes. The electrodes were combined with 0.5 μM
ethynylferrocene and 0.5 μM copper(II)bis(bathophenanthroline)disulfonic acid. Because the
1,3-cycloaddition reaction only proceeds in the presence of Cu(I), the reaction was controlled
using electrochemistry to convert copper from its oxidized state to its reduced state, but only at
the surface of the chosen electrode. By biasing adjacent electrodes, Devaraj et al. were able to
apply −300 mV versus Ag|AgCl|saturated NaCl reference electrode to one electrode ensuring the
presence of Cu(I) at the surface of that electrode. On the adjacent electrode, they left the
potential at +250 mV so that only Cu(II) was present at the surface. Figure 6 depicts a schematic
diagram of their reaction forming the 1,2,3-triazole. This technique can be applied to the
chemical modification of multielectrode arrays, and demonstrates the ability to control the click
reaction using electrochemistry and apply the reaction to specific locations.
1.5. Overview of Project

Similar to Devaraj et al., this project seeks to use electrochemistry to control the formation of a 1,2,3-triazole by reducing Cu(II) to Cu(I). The click reaction was chosen based on its ease of use, as seen by its application *in vivo*, and its ability to undergo electrochemistry. We hope to form the 1,2,3-triazole between two populations of AuNPs. Figure 7 illustrates population 1, which will be functionalized with amine and alkyne terminated-poly(ethyleneglycol) (NH$_2$-PEG-C$_2$). The structure of the commercially obtained NH$_2$-PEG-C$_2$ (MW=3000 Da) is shown in Figure 8.
Population 2, shown schematically in Figure 9, will be functionalized with amine and azide terminated poly(ethyleneglycol) (NH$_2$-PEG-N$_3$). The structure of the commercially obtained NH$_2$-PEG-N$_3$ (MW=530 amu) is shown in Figure 10.

![Figure 9](image_url)  
**Figure 9.** Population 2, gold nanoparticle functionalized with amino and azide terminated PEG.

The two populations will then be combined in the presence of Cu(II). A negative potential will be applied to the electrode to reduce the copper catalyst, creating Cu(I) at the surface of the electrode and “clicking” the two populations of AuNPs together to form a 1,2,3-triazole. A schematic illustration of the reaction is outlined in Figure 11. The copper catalyst reacts with alkyne, creating an electrophilic species to which the azide bonds.

![Figure 11](image_url)  
**Figure 11.** Scheme of electrochemically triggered click reaction in the presence of a copper (I) catalyst between Population 1, alkyne functionalized AuNPs (in blue), and Population 2, azide functionalized AuNPs (in red), forming a 1,4-disubstituted 1,2,3-triazole.
The overall goal of this project is to create a new, more universal triggering method, namely electrochemistry, to form AuNP self-assemblies. The objectives of this project are:

- create populations 1 and 2
- use CuSO$_4$ and sodium ascorbate to click the two populations together; and
- use cyclic voltammetry to reduce Cu(II) to Cu(I) to catalyze the click reaction.

We are using cyclic voltammetry (CV) to reduce the Cu(II) to Cu(I). This technique is advantageous because we can use it to monitor the rate of catalyst formation by determining how much of the oxidized or reduced form of copper is present. Using spectroscopy, this rate can also be correlated with the rate of AuNP aggregate formation to changes in the surface plasmon resonance band. CV was used in previous work to examine the kinetics of electrochemically induced Diels-Alder reactions involving an immobilized dienophile, where a first-order rate constant was determined.$^{25,26}$ Real-time progress of the reaction producing 1,2,3-triazole-terminated SAMs on the surface of one out of a pair of gold IDA band electrodes has also been accomplished using CV.$^{10}$

The successful completion of this project will add a useful tool to nanoscientists’ toolkit by providing the ability to self-assemble AuNPs under many environmental conditions. Understanding how this project works is the first step in understanding potential new electrochemical triggering methods. Additionally, using electrochemistry as the triggering method will provide nanoscientists with a way to monitor their reactions (using CV), as well as apply the self-assembled AuNPs to a plethora of new applications.
2. MATERIALS AND METHODS

2.1. Materials

For all sample preparations and experiments, purified “nanopure” water was used. Water was purified using Barnstead Easypure II from Thermoscientific to 18.2 MΩ cm resistivity. Potassium gold (III) chloride and sodium citrate tribasic dehydrate were purchased from Sigma-Aldrich. Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (sppp) was obtained from Strem Chemicals. MeO-PEG-NH$_2$ (MW=5300 Da) or Amino and Methoxy Terminated Poly(ethylene oxide) was obtained from Polymer Source. NH$_2$-PEG-N$_3$ (MW=530 amu) or O-(2-Aminoethyl)-O’-(2-azidoethyl)nonaethylene glycol was purchased from Sigma-Aldrich and NH$_2$-PEG-C$_2$ (MW=3000 Da) or α-Amino-ω-propargylacetamido poly(ethylene glycol) was purchased from IRIS Biotech GmbH. SH-PEG-COOH (MW=2600 Da) or α-Thiol ω-Carboxy Terminated PEG was purchased from Polymer Source. Copper sulfate, Pentahydrate was purchased from BDH, and L(+)-Ascorbic acid Sodium salt was purchased from Fluka. Aqua regia solution made up of 3:1 v/v solution of 10.8 M HCl (37% purity) from VWR International and 15.7 M HNO$_3$ (69% purity) from EMD Chemicals was used to clean all glassware in contact with AuNPs. For characterization of AuNPs using DLS, a 3 mL syringe with Luer-Lok™ tip from National Scientific Company and 25 mm syringe filter with 0.45 μm cellulose acetate membrane from VWR International were used.

2.2. Synthesis of 11.2 nm AuNPs

The 11.2 nm AuNPs synthesis method described by Flynn and Gewirth was followed.$^{31}$ All glassware was cleaned with aqua regia and soaked in nanopure overnight. During the synthesis, the glassware was wrapped in aluminum foil to minimize light. A 125 mL round bottom flask filled with 50.0 mL of 1.00 mM solution of potassium tetrachloroaurate (III) was
heated to reflux at 100 °C for approximately 10–15 minutes and vigorously stirred. Once the solution reached 100 °C, 5 mL of 38.8 mM sodium citrate tribasic solution was added to the flask. The solution refluxed for an hour and a color change of the solution from clear to black to wine red was visible within the first ten minutes. Solution was cooled for thirty minutes and stored at room temperature in the dark.

2.3. Functionalization of AuNPs with MeO-PEG-NH₂

To 4.00 mL of AuNPs, 8.0 mg MeO-PEG-NH₂ was added. The solution was stirred overnight in the dark. In order to remove excess MeO-PEG-NH₂, the solution was centrifuged using a Sorvall® SS-34 rotor at 7,649 g (8,000 rpm) for 30 minutes at 10 °C. The supernatant was removed using a Pasteur pipet and the the pellet was resuspended in 10.0 mL of nanopure water. The solution was sonicated using a Branson Sonifer 250 from VWR Scientific to ensure thorough mixing. This centrifugation process was repeated once more.

2.4. Characterization of AuNPs Functionalized with MeO-PEG-NH₂

Ultraviolet-visible spectra (UV-vis) of all AuNP solutions were obtained using a Varian Cary 500 Scan UV-Visible-NIR spectrophotometer. Absorbance values were collected from 350–800 nm using a 1-cm path-length plastic cuvette. All solutions were diluted 1:10 in water. Prior to spectra acquisition, data were collected for a baseline of water.

Dynamic light scattering (DLS) was used to measure the hydrodynamic diameter of particles using a Malvern Zetasizer Nano ZS running Zetasizer Software 6.01. Samples were filtered using 3 mL syringe with Luer-Lok™ tip and a 25 mm syringe filter with 0.45 μm cellulose acetate membrane. One mL of sample was transferred to a 1-cm path-length plastic fluorescence cuvette. Polydispersity index and particle hydrodynamic diameter size were collected from the “Number Particle Size Distribution” report. Results were calculated as a mean
value of 13 measurements taken three times. The instrument was calibrated for accuracy of size measurements using Zeta Potential Transfer Standard solution composed of 280-310 nm polystyrene beads from Malvern.

Fourier transform-infrared (FT-IR) spectroscopy was performed to test for the presence of PEG on AuNPs using a Perkin Elmer Spectrum One FT-IR spectrometer equipped with a Perkin-Elmer Universal ATR sampling accessory. A 10 mL sample of the AuNPs were centrifuged at 34,540 g (17,000 rpm) for 20 minutes at 10 °C. The supernatant was removed and a drop of the pellet was pipetted onto the ATR crystal. For each spectrum, 128 scans were taken over a range of 650-4000 cm\(^{-1}\) at a resolution of 2.00 cm\(^{-1}\). A background of water was used. As controls, spectra of the supernatant and concentrated MeO-PEG-NH\(_2\) in water were obtained.

2.5. Functionalization of AuNPs with sppp

To 10.0 mL of AuNPs, 1.0 mg of sppp was added. The solution was stirred overnight in the dark. In order to remove excess sppp, the solution was centrifuged at 34,540 g (17,000 rpm) for 20 minutes at 10 °C. The supernatant was removed and the pellet was resuspended in 10.0 mL of nanopure. The solution was then sonicated, and this centrifugation process was repeated once more.

2.6. Creating Two Populations

To make a 2 mg/mL solution of NH\(_2\)-PEG-N\(_3\), 16.0 μL of a 500 mg/mL NH\(_2\)-PEG-N\(_3\) in ethanol stock solution was added to 4.00 mL of AuNPs functionalized with sppp. To make a 2 mg/mL solution of NH\(_2\)-PEG-C\(_2\), 48.0 μL of a stock solution of 500 mg/3 mL of NH\(_2\)-PEG-C\(_2\) was added to 4.00 mL of AuNPs functionalized with sppp. Both mixtures were stirred overnight in the dark. To remove excess NH\(_2\)-PEG-N\(_3\) and excess NH\(_2\)-PEG-C\(_2\), the solutions were centrifuged at 17,210 g (12,000 rpm) for 20 minutes at 10 °C. The supernatant of each sample
was pipetted off, and the pellet was resuspended in 4.00 mL of nanopure. Each solution was sonicated. The centrifugation process was completed a total of two times.

2.7. Characterization of Two Populations

UV-vis and DLS techniques as described in section 2.4 were used to characterize all AuNP solutions. FT-IR spectroscopy was performed to test for the presence of PEG on both populations of AuNPs. Samples of 4.00 mL of the AuNPs functionalized with NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$ were centrifuged at 17,210 g (12,000 rpm) for 20 minutes at 10 °C. The supernatant was removed and a drop of the pellet was pipetted onto the ATR crystal. For each spectrum, 256 scans were taken over a range of 650-4000 cm$^{-1}$ at a resolution of 2.00 cm$^{-1}$. A background of water was used. As controls, spectra of solid NH$_2$-PEG-N$_3$ and NH$_2$-PEG-C$_2$ were obtained by pipetting a droplet of the stock solution onto the ATR crystal and letting the solvent evaporate. For these spectra, a background of air was used and 256 scans were taken at the same settings as above.

2.8. Clicking Together Two Populations

To click the two populations together, 2.00 mL of AuNPs functionalized with NH$_2$-PEG-C$_2$, 2.00 mL of NH$_2$-PEG-N$_3$, 4.00 μL of 1.0 M CuSO$_4$ and 4.00 μL of 1.0 M sodium ascorbate were combined in a glass vial and stirred. Three control solutions were made by adding 4.00 μL of 1.0 M CuSO$_4$ and 4.00 μL of 1.0 M sodium ascorbate to 4.00 mL of control solutions. The control solutions were bare AuNPs, AuNPs + sppp, and AuNPs + SH-PEG-COOH place exchanged with sppp. All solutions were sonicated and UV-vis and DLS were used to characterize the particles. FT-IR was run on the click reaction pellet using a background of water and averaging 256 scans. Before characterization, the sample was sonicated and 1.00 mL of the
sample was centrifuged using an Eppendorf Centrifuge 5415R for 15 minutes, 24 °C, at 13.2 rpm. The supernatant was removed and a small amount of pellet was dotted onto the ATR crystal.

2.9. Testing the Click Reaction Off Particle

To prove that the click reaction did occur, a solution of 2.00 mL of water, 48.0 μL of NH₂-PEG-C₂ stock solution, 16.0 μL of NH₂-PEG-N₃, 4.00 μL of 1.0 M CuSO₄ stock solution and 4.00 μL of 1.0 M sodium ascorbate stock solution were combined and stirred for 2 hours. EDTA was added to solution to make it a 1.0 mM EDTA solution and the solution was left stirring for 45 minutes. AuNPs + sppp were centrifuged at 17,210 g (12,000 rpm), the supernatant was removed and the AuNPs + sppp were resuspended in 2.00 mL of water. To the off particle clicked solution, 2.00 mL of the resuspended AuNPs + sppp were added. The complete solution stirred overnight in the dark. UV-vis and DLS were used to characterize the resulting solution.

A click control reaction was made as follows. To 5.00 mL of AuNPs + sppp, 60.0 μL of NH₂-PEG-C₂ stock solution and 20.0 μL of NH₂-PEG-N₃ stock solution was added and stirred overnight to perform a place exchange reaction. To remove excess NH₂-PEG-N₃ and excess NH₂-PEG-C₂, the solution was centrifuged at 26,890 g (15,000 rpm) for 20 minutes at 10 °C. The supernatant of each sample was pipetted off, and the pellet was resuspended in 5.00 mL of nanopure. Each solution was sonicated and the centrifugation process was completed a total of two times. The AuNPs + NH₂-PEG-C₂ + NH₂-PEG-N₃ were characterized using UV-vis and DLS. To 4.00 mL of the AuNPs + NH₂-PEG-C₂ + NH₂-PEG-N₃ solution, 4.00 μL of 1.0 M sodium ascorbate stock solution was added and the whole solution was stirred overnight in the dark. UV-vis and DLS were used to characterize the solution.
3. RESULTS AND DISCUSSION

3.1. Functionalizing AuNPs with Aminated PEG

In order to create gold nanoparticles functionalized with either NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$, we wanted to first demonstrate that PEG with a terminal amine would adsorb to the surface of AuNPs. Other studies using proteins have shown that amine groups have a relatively strong covalent interaction with the AuNPs.$^{32,33}$ To test the ease of functionalization and stability, we functionalized AuNPs with MeO-PEG-NH$_2$ following the procedure described in section 2.3.

UV-vis, DLS, and FT-IR spectroscopy are valuable tools to characterize the MeO-PEG-NH$_2$ functionalized AuNPs. IR spectroscopy provides us with the most direct evidence to confirm the successful functionalization of AuNPs with MeO-PEG-NH$_2$. IR spectra of concentrated MeO-PEG-NH$_2$ in water, AuNPs functionalized with MeO-PEG-NH$_2$ and the supernatant of the AuNPs + MeO-PEG-NH$_2$ are shown in Figure 12. In the concentrated MeO-PEG-NH$_2$ sample, the strongest peaks are from N-H bending of primary amines at 1638 cm$^{-1}$ (usually seen at 1650–1580 cm$^{-1}$) and a strong C-O-C asymmetrical stretching band at 1082 cm$^{-1}$ (usually ~1100 cm$^{-1}$).$^{34}$ The expected primary amine N-H stretching peak (represented by two peaks between 3500–3300 cm$^{-1}$) is obscured by the broad O-H peak from water in the region from 3600–3200 cm$^{-1}$.$^{34}$ The IR spectrum of the supernatant, the top layer of solution that was removed from the AuNPs functionalized with MeO-PEG-NH$_2$ after centrifuging, does not have any peaks. Therefore, the signatures we see with the AuNPs and MeO-PEG-NH$_2$ present, as indicated by the overlapping peaks at 1640 cm$^{-1}$ and 1100 cm$^{-1}$, are a result of PEG bound to the AuNPs and not from free PEG in solution. The IR spectrum of AuNPs with MeO-PEG-NH$_2$ has an additional peak representing the alkyl C-H stretch at 2920 cm$^{-1}$ that is barely visible in the
spectrum of concentrated MeO-PEG-NH₂. This is perhaps caused by the orientation of the MeO-PEG-NH₂ on the AuNPs. Since the amine group is adsorbed to the AuNPs, the methyl group is on the outer surface of the nanoparticles, which allows the IR to pick up the methyl signal more easily. Whereas, in contrast, the MeO-PEG-NH₂ in water could be in any orientation, so it is more difficult for the IR to pick up the CH₃ signal. Furthermore, the large amount of water interacting with the PEG probably overshadows the signal from the methyl group.

![FT-IR spectra](image)

**Figure 12.** FT-IR spectra of the AuNPs functionalized with MeO-PEG-NH₂ (on the y-axis to the left), supernatant of AuNPs functionalized with MeO-PEG-NH₂ (on the y-axis to the left), and concentrated MeO-PEG-NH₂ in water (on the y-axis to the right).

Measuring the surface plasmon resonance (SPR) band of AuNPs through absorption of a sample is a common technique used to examine how the surface of AuNPs has been modified. Absorption of light by the nanoparticle causes excitation of electrons in the conduction band of the metal nanoparticle, creating an oscillating electron density; these oscillations are called the SPR. SPR is what gives metal nanoparticles a high extinction coefficient, which in turn leads to their strong color (wine red for AuNPs) in colloidal solutions. The size, shape, composition,
and aggregation of nanoparticles affect the peak maximum and peak bandwidth.\textsuperscript{35} In addition to these parameters, the surface functionalization of the nanoparticle also influences the SPR band. Specifically, functionalization or aggregation of the AuNPs causes a red-shift and band broadening resulting from interference in the surface plasmon electrons’ oscillations, namely, a dampening of their oscillations.\textsuperscript{35}

For bare AuNPs, the expected absorption maximum is 518 nm with a narrow bandwidth.\textsuperscript{31} In Figure 13, the bare AuNPs are seen in a narrow peak at a wavelength of 520 nm, which in further trials was reproducible with typical wavelength maxima of 518 nm to 520 nm. The UV-vis spectra in Figure 13 illustrate the red-shift from 520 nm to 522 nm upon functionalization with MeO-PEG-NH$_2$ (MW=5300). The broadness of the peak did not change as calculated from the full width at half maximum (FWHM) values, also known as the plasmon bandwidth, which measured 80 nm for both the bare AuNPs and for AuNPs + MeO-PEG-NH$_2$. Link et al. found that the plasmon bandwidth is inversely proportional to the radius of the particle for sizes smaller than 20 nm, but for particles larger than 25 nm in radius, the bandwidth increases with increasing size of the radius due to extrinsic size effects.\textsuperscript{35}

In a study where 60 nm AuNPs were coated with thiol-terminated PEG (HS-PEG) (MW=5000), researchers saw minimal changes in absorption spectra (<1 nm shift in SPR band) and similar bandwidths after functionalization.\textsuperscript{37} Similarly, our UV-vis spectra for AuNPs + MeO-PEG-NH$_2$ shifted between 0 to +2 nm and had no change in bandwidth. However, another study in which 20 nm citrate-capped AuNPs were functionalized with 11-mercaptopoundecanoic acid (MUA), researchers saw a +5 nm red-shift from 520 nm to 525 nm.\textsuperscript{38} Since the HS-PEG is more similar to our MeO-PEG-NH$_2$ than MUA, the small shift in peak absorbance from functionalizing AuNPs with MeO-PEG-NH$_2$ is a reasonable result.
DLS is the third major analytical technique we use to characterize AuNPs by measuring the hydrodynamic diameter of AuNPs in solution through light scattering and Brownian motion. Light is directed into a small volume of sample and fluctuations in scattered light intensity caused by particle motion is measured and correlated to particle size. The homogeneity of particle sizes in solution is measured through the polydispersity index (PDI). Having a low PDI is desirable, because a low PDI means that the particles are all uniform in size and shape. A high PDI would indicate particle aggregation or non-uniform functionalization of AuNPs with PEG.

Figure 13 shows a unimodal distribution of particles from DLS, signifying that the red-shift in the UV-vis spectrum is the result of uniform adsorption of MeO-PEG-NH$_2$ to the surface of the AuNPs, rather than particle aggregation. The initial DLS measurement of the diameter of bare AuNPs was 10.7 nm and the PDI was 0.25. After functionalizing with MeO-PEG-NH$_2$, we saw an increase in hydrodynamic diameter to 24.1 nm and the PDI remained low at 0.23. The +13.4 nm to +15.4 nm increase we saw in replicate trials after functionalizing with the MeO-PEG-NH$_2$ (MW=5300) is comparable to literature values for 20 nm AuNPs functionalized with
PEG1500 and PEG5000. Liu et al. saw a +9.5 nm and +18.2 nm increase in diameter for citrate capped AuNPs starting at 21.8 nm and increasing to 31.3 nm for PEG1500 and 40.0 nm for PEG5000, respectively. The results we saw after coating AuNPs with MeO-PEG-NH₂ were similar to work done by our collaborators coating AuNPs with SH-PEG-biotin (MW=3400), who saw an increase of +15.6 nm in diameter after functionalization.

![Figure 14](image.png)

**Figure 14.** Particle size distribution for AuNPs + MeO-PEG-NH₂. Data was collected from “Number PSD” report. A peak is shown at a particle diameter of 24.1 nm.

### 3.2. Creating Two Populations

IR spectra of both populations of functionalized nanoparticles and solid NH₂-PEG-C₂ and NH₂-PEG-N₃ were taken. Figure 15 shows IR spectra of solid NH₂-PEG-C₂ and of population 1. For population 1, we expected to see the alkyne represented by a sharp peak at 3300 cm⁻¹ (≡C-H) and medium peak at 2100–2140 cm⁻¹ (C ≡ C). The peaks in our population 1 spectra were a broad peak from 3200–3600 cm⁻¹ (O-H stretch from water), a small peak at 2330 cm⁻¹, and a peak at 1640 cm⁻¹ possibly from scissoring of the amine. On the solid NH₂-PEG-C₂, two peaks indicating PEG appeared at 2880 cm⁻¹ (R-CH₂ stretching) and 1095 cm⁻¹ (C-O-C assymetrical stretching). The same possible NH₂ peak at 1640 cm⁻¹ and a broad, multi-component peak at 3450 cm⁻¹ indicating N-H stretching also appeared. Our collaborators have taken IR spectra of AuNPs + sppp and saw a series of small signature peaks at 1500 cm⁻¹ from C-C bonds of the benzene ring. As seen in Figure 15, our AuNPs + NH₂-PEG-C₂ do not have identifiable sppp...
peaks. Unfortunately, the water peak on the population 1 spectrum covers up the signature peak of N-H stretching usually seen from 3500–3400 cm$^{-1}$. However, the overlapping peaks at 1640 cm$^{-1}$ and $\sim$1100 cm$^{-1}$ are suggestive that we have successfully attached NH$_2$-PEG-C$_2$ to the nanoparticles.

Figure 16 shows IR spectra of solid NH$_2$-PEG-N$_3$ and population 2. The IR spectra of the solid NH$_2$-PEG-N$_3$ had most of the expected peaks, including a peak at 2100 cm$^{-1}$ that could be the azide, and two peaks signifying PEG at 2865 cm$^{-1}$ and 1100 cm$^{-1}$. The peaks from PEG are so strong that they overshadow the small peak at 3400 cm$^{-1}$ representing the N-H stretch. The population 2 spectrum was very noisy, and the only distinguishable peak is from 3400–3100 cm$^{-1}$, an O-H stretch from water. There may be some peaks around 2200–2000 cm$^{-1}$, but they could not be identified because of the noise. Since the sample was taken in a solution of water, the high noise may be attributed to the low concentration of sample and high concentration of water. In order to increase our signal to noise ratio, we would have to increase the number of scans. For both azide spectra, the amine peaks (usually at 3500–3400 cm$^{-1}$ and 1640–1560 cm$^{-1}$) were hard to find. In the solid NH$_2$-PEG-N$_3$ spectrum, the signal from PEG is so strong that it obscured the N-H peaks, and in the population 2 spectrum the N-H stretch is covered up by water. Overall, the IR spectra are somewhat inconclusive in helping us identify if we have successfully created population 2.
Figure 15. FT-IR spectra of solid NH$_2$-PEG-C$_2$ (y-axis on right, black) and AuNPs functionalized with sppp and place exchanged with NH$_2$-PEG-C$_2$ (y-axis on left, red).

Figure 16. FT-IR spectra of NH$_2$-PEG-N$_3$ (y-axis on right, black) and AuNPs functionalized with sppp and place exchanged with NH$_2$-PEG-N$_3$ (y-axis on left, red).
UV-vis spectra of both populations were taken at each step of the functionalization process. Figure 17 shows spectra of bare AuNPs, AuNPs functionalized with sppp, and AuNPs functionalized with either NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$. The initial SPR band of bare AuNPs was 518 nm, which shifted to 521 nm upon functionalization with sppp. Previous results have shown that capping AuNPs with sppp increases the stability of AuNPs in solution. In population 1, we saw a further shift to 525 nm after the place exchange between sppp and NH$_2$-PEG-C$_2$. Population 2 shifted to 524 nm after the place exchange with NH$_2$-PEG-N$_3$. In further trials these shifts were reproducible for population 1 with typical wavelength maxima of 524 nm to 525 nm and for population 2 with typical wavelength maxima of 522 nm to 524 nm. These shifts seem to agree with the literature. Both populations were created from the same AuNPs, which were subsequently functionalized by sppp for stability. The FWHM measured 84 nm for both bare AuNPs and AuNPs + sppp. The plasmon bandwidth decreased slightly to 82 nm for population 1 and increased slightly to 86 nm for population 2. Since all the samples were diluted by a factor of 10, we calculated a percent yield of the AuNPs after functionalization. For the AuNPs functionalized with sppp, we had a 95% retention rate, for population 1 we had a 71% retention rate, and for population 2 we had a 66% retention rate.

Since the red-shifts we saw are not larger than +14 nm, we know that the red-shifts were not caused by particle aggregation. The red-shifts indicate that the NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ have adsorbed to the surface of the AuNPs causing the SPR band to change and increasing the peak wavelength. The three red-shifts are about where we expect them to be according to Li et al., who saw red-shifts of +5 nm upon functionalizing 20 nm AuNPs with MUA (MW=218 amu), because MUA adsorbed to the surface of the AuNPs forming a dielectric monolayer. Similarly the +7 nm and +6 nm red-shifts we saw from the functionalization of NH$_2$-PEG-C$_2$ and
NH₂-PEG-N₃, which have a much higher MW than the MUA, give us hope that we have also successfully adsorbed NH₂-PEG-C₂ and NH₂-PEG-N₃ to the surface of our AuNPs.

We used DLS to monitor the change in nanoparticle size after addition of sppp, NH₂-PEG-C₂, and NH₂-PEG-N₃. Table 1, which summarizes these results, shows a +0.8 nm increase in the AuNPs’ diameter after functionalizing with sppp and a +8.7 nm increase from the bare AuNPs after the place exchange with NH₂-PEG-C₂. We did not see a change after the place exchange between sppp and NH₂-PEG-N₃ because the NH₂-PEG-N₃ is about the same size as sppp (MW=500), as compared to the NH₂-PEG-C₂ (MW=3000). Since we are functionalizing the whole surface of the AuNP, or both “sides” of the AuNP, the change in diameter is twice that of the expected size of one monolayer of the functionalizing molecule. This indicates that the length of the NH₂-PEG-C₂ is about 4 nm in solution. The diameter of the AuNPs did not change significantly after the place exchange with NH₂-PEG-N₃. This is possibly because the NH₂-PEG-
N₃, having only ten EG repeating units, is roughly the same size as the sppp. The increase in diameter after functionalizing with NH₂-PEG-C₂ is close to the expected literature values of +9.5 nm for functionalizing 20 nm AuNPs with PEG₁₅₀₀ and +18.2 nm for functionalizing AuNPs with PEG₅₀₀₀.⁴⁰ Our collaborators work also showed an increase in diameter of +7.3 nm with SH-PEG-OH (MW=2600), which also supports our diameter measurement.⁴¹ The low PDI values, seen in Table 1, indicate that our samples were uniformly dispersed and did not aggregate. Overall, the DLS data further strengthens our belief that we have successfully functionalized AuNPs with NH₂-PEG-C₂ and NH₂-PEG-N₃.

Table 1. DLS and SPR band shifts of AuNPs and AuNPs functionalized with sppp and following a place exchange reaction between sppp and NH₂-PEG-C₂. All samples were passed through a 0.22 μm cellulose acetate filter before readings were taken.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Δλ (nm)</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs</td>
<td>0</td>
<td>11.6</td>
<td>0.06</td>
</tr>
<tr>
<td>AuNPs + sppp</td>
<td>3</td>
<td>12.4</td>
<td>0.20</td>
</tr>
<tr>
<td>AuNPs + sppp + NH₂-PEG-C₂</td>
<td>7</td>
<td>20.3</td>
<td>0.39</td>
</tr>
<tr>
<td>AuNPs + sppp + NH₂-PEG-N₃</td>
<td>6</td>
<td>12.3</td>
<td>0.20</td>
</tr>
</tbody>
</table>

3.3. Click Reaction

The click reaction and three controls were made following the procedure in section 2.8. Prior to addition of the copper catalyst, all solutions were wine red in color. The control solutions were expected to stay a wine red color, while the color of the click reaction solution was expected to change. The first two controls, bare AuNPs and AuNPs functionalized with SH-PEG-COOH, remained a wine red color. However, upon adding CuSO₄ to the control solution with AuNPs and sppp, the solution turned a midnight blue. This color change is possibly caused by the copper complexing to the phosphine groups of sppp. Optical images of the sample and three controls: bare AuNPs, AuNPs place exchanged with sppp and SH-PEG-COOH, and AuNPs functionalized with sppp after the click reaction are shown in Figure 18. The three controls
remained largely unchanged with only the sppp functionalized sample indicating a visual difference. In contrast, we saw a significant change in the click reaction solution. The solution turned clear and the nanoparticles precipitated to the bottom of the vial. We believe that the aggregation was the result of the formation of triazole rings between two AuNPs.

**Figure 18.** Solutions of “click” reaction and controls. Three controls were tested by combining CuSO₄ and sodium ascorbate with **A**) Plain AuNPs **B**) AuNPs functionalized with sppp followed by a place exchange of SH-PEG-COOH **C**) AuNPs functionalized with sppp. **D**) The “click” reaction was performed by combining both populations of AuNPs with CuSO₄ and sodium ascorbate. All solutions had a final concentration of 1 mM CuSO₄ and 1 mM sodium ascorbate.

After optical images of the click controls and click reaction were taken, the solutions were sonicated and characterized using UV-vis spectroscopy. As shown in Figure 19, the control solution of bare AuNPs did not have a red-shift, which is what we expected because there was nothing in solution to adsorb to the surface of the AuNPs. The FWHM of bare AuNPs was 84 nm, and only increased to 88 nm after the copper catalyst was added. Before adding the copper catalyst, the maximum absorbance wavelength of the control sample AuNPs + SH-PEG-COOH was 524 nm and the FWHM was 86 nm, and after adding the copper catalyst the absorption maximum was also measured at 524 nm and the FWHM was 106 nm. So overall, AuNPs + SH-PEG-COOH did not have a red-shift from their pre-click reaction. The SPR peak of the control AuNPs + sppp was measured at 521 nm with a plasmon bandwidth of 86 nm. The SPR peak of
AuNPs + sppp had a significant red-shift to 536 nm upon addition of the copper catalyst, and the plasmon bandwidth increased to 152 nm. This red-shift could not be explained because the CuSO₄ should not have reacted with the sppp or the AuNPs. The largest red-shift was seen shifting from 519 nm for the bare AuNPs to 588 nm for the clicked AuNPs, as seen in Figure 19. The peak is broad (FWHM = 174 nm) and it almost appears that there are two peaks, one around 530 nm and one at 590 nm. The smaller peak at 530 nm could signify that some of the AuNPs remained un-clicked, whereas the dominant peak at 588 nm could represent the clicked AuNPs. The large red-shift was caused by aggregation of AuNPs because the peak wavelength shifted by +69 nm (according to the literature, a shift of > +14 nm implies aggregation). In summary, adding the copper catalyst to the controls of bare AuNPs and the AuNPs + SH-PEG-COOH did not affect the AuNPs’ SPR band. The AuNPs + sppp control did have an unexplainable red-shift, and the click reaction had a very significant red-shift indicating aggregation of AuNPs.

Aggregation and color change of AuNP solution is often characteristic of nanoparticle self-assemblies. To investigate changes in size following the addition of copper sulfate and sodium ascorbate, we employed DLS. The samples were sonicated prior to DLS. Table 2 summarizes measurements of the four samples. The control sample of AuNPs with the copper catalyst somehow decreased in hydrodynamic diameter by −4.6 nm. Before adding the copper catalyst, AuNPs + sppp control had a diameter of 10.3 nm. After adding the catalyst, the diameter had a +10 nm increase to 20.3 nm, which may have been caused by aggregation since the PDI started at 0.20 and increased to 0.38. The last control of AuNPs + SH-PEG-COOH started at a diameter of 21.8 nm before the addition of CuSO₄ and sodium ascorbate. This control’s diameter stayed about the same with only a +1.9 nm increase to 23.7 nm. The control reactions, aside from the
AuNPs functionalized with sppp, did not have a significant change from their pre-click reaction diameters. A large increase in the diameter of the click reaction AuNPs was measured. Starting at 20.3 nm for AuNPs + NH$_2$-PEG-C$_2$ and 12.3 nm for AuNPs + NH$_2$-PEG-N$_3$, the diameter of the combined populations increased to 45.5 nm after adding the copper catalyst. This large increase in diameter still had a fairly low PDI of 0.24, suggesting that the increase in diameter was uniform throughout the population of AuNPs. In summary, it is unclear why the control of bare AuNPs decreased in size and why the control of AuNPs + sppp increased in size, according to the DLS measurements. However, overall, the large increase in diameter of the click AuNPs, along with a relatively low PDI is a promising result and leads us to believe that we have successfully clicked the two populations together.

Figure 19. UV-vis spectra of bare AuNPs, click reaction control AuNPs, and click reaction AuNPs. Click controls and click reaction were created by combining AuNPs with CuSO$_4$ and sodium ascorbate for a final concentration of 1 mM of each.
Table 2. DLS and SPR band shifts AuNPs and AuNPs functionalized with sppp and following a place exchange reaction between sppp and NH$_2$-PEG-C$_2$. All samples were passed through a 0.22 μm cellulose acetate filter before readings were taken.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Delta \lambda$ (nm)</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain AuNPs</td>
<td>0</td>
<td>10.2</td>
<td>0.13</td>
</tr>
<tr>
<td>Click Control: Plain AuNPs</td>
<td>0</td>
<td>5.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Click Control: AuNPs + sppp</td>
<td>17</td>
<td>20.9</td>
<td>0.38</td>
</tr>
<tr>
<td>Click Control: AuNPs+SH-PEG-COOH</td>
<td>5</td>
<td>23.7</td>
<td>0.43</td>
</tr>
<tr>
<td>Click Reaction</td>
<td>69</td>
<td>45.5</td>
<td>0.24</td>
</tr>
</tbody>
</table>

The final method of characterizing our clicked AuNPs was FT-IR. IR spectra of clicked AuNPs overlaid with spectra of solid NH$_2$-PEG-C$_2$ and solid NH$_2$-PEG-N$_3$ is shown in Figure 20. Studies have shown that significant peaks used to characterize 1,2,3-triazole appear at 1550-1510 cm$^{-1}$ from N-H, specifically C-N-H, and at 3135 cm$^{-1}$ from N-H. However, the dominant peaks in the click AuNPs spectra were 3200–3600 cm$^{-1}$ most likely from water, and at 1628 cm$^{-1}$ which could possibly be from an amine (N-H bending). The fact that we do not see the same peaks in the click AuNP spectrum as the solid NH$_2$-PEG-C$_2$ and solid NH$_2$-PEG-N$_3$ spectra is promising. However, we would like to be able to replicate these changes with a system that we know clicked.

![Figure 20](image-url)  
*Figure 20. FT-IR spectrum of clicked AuNPs (y-axis on the left, black) overlaid with spectra of solid NH$_2$-PEG-C$_2$ and solid NH$_2$-PEG-N$_3$ (y-axis on the right, red).*
3.4. Testing the Click Reaction Off Particle

In order to confirm the success of the click coupling, we performed one more set of reactions, as described in section 2.9. In the first reaction, we clicked the free NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ together in solution. Then, EDTA was added to complex free copper, and finally AuNPs + sppp were added to solution. We employed UV-vis and DLS to measure the clicked AuNPs after the reaction; results are illustrated in Figure 21 and Table 3. We expected to see aggregation after the AuNPs were added to the clicked reactants. But, after stirring overnight, the solution remained a wine red. The EDTA should have bound the copper catalyst so that the amine groups were free to adsorb to the surface of AuNPs after formation of the 1,2,3-triazole between the two NH$_2$-PEG molecules. For the AuNPs + sppp before the reaction, the wavelength maximum was at 522 nm, and after the reaction, the AuNPs wavelength maximum shifted to 525 nm. According to DLS, the hydrodynamic diameter of the AuNPs also increased from 10.9 nm to 17.8 nm after the reaction. These measurements may indicate some aggregation of the AuNPs occurred, but not a substantial amount.

As a second control reaction, NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ were first attached to the AuNPs through a place exchange reaction with sppp. Then, sodium ascorbate was added to the AuNPs + NH$_2$-PEG-C$_2$ + NH$_2$-PEG-N$_3$ to mimic the click reaction without catalyzing the coupling. After each modification, we used UV-vis and DLS to characterize the AuNPs, as seen in Figure 21 and Table 3. Bare AuNPs started at a wavelength of 519 nm, shifted to 522 nm upon functionalization with sppp. A further red-shift to 523 nm was observed after the place exchange with both NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$. DLS showed that the wine red solution of NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ functionalized AuNPs had a hydrodynamic diameter of 19.0 nm and the
PDI was 0.19. These measurements also indicate that the place exchange between the NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ occurred successfully.

After adding in the sodium ascorbate, we expected nothing to happen and for the solution to stay wine red. After the solution stirred overnight though, slight aggregation caused the solution to turn a darker shade of wine red. We characterized the AuNPs with UV-vis and DLS, illustrated in Figure 21 and Table 3. The UV-vis spectrum of the click control after adding sodium ascorbate gave a +5 nm red-shift from 523 nm to 528 nm. According to DLS, after adding the sodium ascorbate the hydrodynamic diameter of the AuNPs decreased from 19.0 nm to 11.4 nm.

These two reactions were relatively inconclusive in helping us demonstrate that we had successfully performed the click reaction. The control solution aggregated, while the click solution remained wine red. Comparing the red-shifts in peak absorption, the click reaction increased +3 nm, while the control reaction increased +5 nm. From DLS, the +6.9 nm increase in hydrodynamic diameter of the click reaction AuNPs is not a large enough increase to prove that we have attached the triazole ring to two different AuNPs. This further confirms that we did not succeed in proving that the click reaction occurred. In the control reaction, the decrease in hydrodynamic diameter is unexplainable, but could be a result of sodium ascorbate interfering with the PEG groups on the AuNPs. The high PDI of 0.54 supports the aggregation we saw based on the color change of the control solution to a darker shade of wine red.
Figure 21. UV-vis spectra of 1) bare AuNPs 2) AuNPs functionalized with sppp 3) AuNPs functionalized with both NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ 4) AuNPs + NH$_2$-PEG-C$_2$ + NH$_2$-PEG-N$_3$ and 1 mM final concentration of sodium ascorbate 5) NH$_2$-PEG-C$_2$ + NH$_2$-PEG-N$_3$ were clicked free in solution, EDTA was added for a final concentration of 1 mM, and then AuNPs functionalized with sppp were added.

Table 3. DLS and SPR band shifts of AuNPs, AuNPs functionalized with sppp, following a place exchange reaction between sppp, NH$_2$-PEG-C$_2$, and NH$_2$-PEG-N$_3$. DLS and SPR shifts of a click control reaction without CuSO$_4$ and a click reaction with EDTA. All samples were passed through a 0.22 µm cellulose acetate filter before readings were taken.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Delta\lambda$ (nm)</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs</td>
<td>0</td>
<td>13.3</td>
<td>0.31</td>
</tr>
<tr>
<td>AuNPs + sppp</td>
<td>3</td>
<td>10.9</td>
<td>0.12</td>
</tr>
<tr>
<td>AuNPs + NH$_2$-PEG-C$_2$ + NH$_2$-PEG-N$_3$</td>
<td>4</td>
<td>19.0</td>
<td>0.19</td>
</tr>
<tr>
<td>Click Control: no CuSO$_4$</td>
<td>9</td>
<td>11.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Click Reaction</td>
<td>6</td>
<td>17.8</td>
<td>0.13</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS:

Two populations of AuNPs were successfully made by using a place exchange reaction with sppp. One population was functionalized with NH$_2$-PEG-C$_2$ and the other was functionalized with NH$_2$-PEG-N$_3$. UV-vis spectroscopy was used to monitor red-shifts in the SPR band of the AuNPs upon each functionalization step to confirm the success of functionalization. DLS was used to monitor increases in the hydrodynamic diameter and to
determine if red-shifts should be attributed to surface absorption of molecules or particle aggregation. Our DLS measurements indicated reasonable increases in hydrodynamic diameter due to surface absorption of functional groups, further supporting our data that the two populations were successfully created. IR spectra were also collected for each population, but were inconclusive.

We have also made progress in successfully clicking the two populations together forming a 1,2,3-triazole using CuSO₄, and sodium ascorbate as the reducing agent. This click solution, as well as three click control solutions were examined using UV-vis and DLS, which appear to confirm the success of the click reaction. We will continue to try to prove that the click reaction worked by first clicking the alkyne and azide and then attaching the 1,2,3-triazole to AuNPs on both sides of the triazole, causing the AuNPs to self-assemble. In conjunction, we will perform a control reaction by functionalizing the AuNPs with both NH₂-PEG-C₂ and NH₂-PEG-N₃, then adding sodium ascorbate to mimic the click reaction, but without the copper catalyst.

Current efforts are also working to control how much of the AuNP surface has been functionalized with either NH₂-PEG-C₂ or NH₂-PEG-N₃ by limiting the amount of time for the place exchange reaction between sppp and NH₂-PEG-C₂ or NH₂-PEG-N₃. This will allow us to keep all the AuNPs from aggregating at once, forming more controlled self-assemblies.

The next step in this project is to use electrochemistry to reduce Cu(II) to Cu(I) to perform the click reaction. By using electrochemistry and cyclic voltammetry, the kinetics of the click reaction can also be monitored. Electrochemistry will allow us more control over where self-assembly occurs, mostly, right at the surface of the electrode. Transmission electron microscopy (TEM) and UV-vis spectroscopy will be used to help us understand the structure of the assembled nanoparticles.
5. **APPENDIX:**

5.1. Testing the Surface Functionalization of AuNPs

**METHODS**

To see how the kinetics of a place exchange reaction between sppp and NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$ would affect the functionalization of NH$_2$-PEG-C$_2$ and NH$_2$-PEG-N$_3$ to the surface of the AuNPs, two 6 hour place exchange reactions were conducted. In one vial, 156.0 μL of NH$_2$-PEG-C$_2$ was pipetted into 13.00 mL of AuNPs + sppp and the solution was stirred for 6 hours. In another vial of 13.00 mL of AuNPs + sppp, 52.00 μL of NH$_2$-PEG-N$_3$ was pipette in and the solution was also stirred for 6 hours. Every two hours, 4.00 mL aliquots were removed from both samples to stop the place exchange reaction. The aliquots were centrifuged at 23,420 g (14,000 rpm), and the centrifugation process was repeated twice. After each round of centrifugation, the supernatant was removed and the aliquots were resuspended in 4.00 mL of nanopure water and sonicated. UV-vis and DLS were used to characterize the 2 hour, 4 hour, and 6 hour AuNPs + NH$_2$-PEG-C$_2$ and AuNPs + NH$_2$-PEG-N$_3$.

Three click reactions for the 2 hour, 4 hour, and 6 hour AuNPs were created. 2.00 mL of the 2 hour AuNPs + NH$_2$-PEG-C$_2$ and 2.00 mL of the 2 hour AuNPs + NH$_2$-PEG-N$_3$ were clicked together by adding CuSO$_4$ and sodium ascorbate for final concentrations of 1 mM. This process was repeated for the 4 hour and 6 hour AuNPs. Optical images were taken of the three click solutions. The solutions were sonicated and then characterized using UV-vis and DLS.
RESULTS AND CONCLUSIONS

The color of the clicked solutions varied based on the length of the place exchange reaction. As displayed in the optical image in Figure 22, the 2 hour clicked AuNPs remained a wine red color, but slight aggregation of AuNPs was visible. The 4 hour clicked AuNPs turned a light purple color, indicating more aggregation than the 2 hour clicked AuNPs. The 6 hour clicked AuNP solution turned clear and the nanoparticles precipitated to the bottom of the vial.

Figure 22. A) 2 hour clicked AuNPs B) 4 hour clicked AuNPs C) 6 hour clicked AuNPs. All solutions had a final concentration of 1 mM CuSO₄ and 1 mM sodium ascorbate and were formed by combining two populations of AuNPs that underwent a place exchange reaction for the same amount of time.

After optical images of the clicked AuNPs were taken, the solutions were sonicated and characterized using UV-vis and DLS. The 2 hour click reactions had the smallest red-shift to 528 nm, while the 4 hour and 6 hour AuNPs had a significant red-shift to 601 nm and 636 nm, respectively. These large red-shifts indicate aggregation of AuNPs. DLS measurements support this change with increases in hydrodynamic diameter for all three samples, as shown in Table 4. Of the three samples, the diameter of the 2 hour clicked AuNPs increased the least to 28.5 nm. The 4 hour AuNPs had a substantial increase to 60.2 nm and the 6 hour AuNPs increased the most to 75.5 nm.
Figure 23. UV-vis spectra of 2 hour clicked AuNPs, 4 hour clicked AuNPs, and 6 hour clicked AuNPs. All solutions had a final concentration of 1 mM CuSO$_4$ and 1 mM sodium ascorbate and were formed by combining two populations of AuNPs that underwent a place exchange reaction for the same amount of time.

Table 4. DLS and SPR wavelengths of 2 hour clicked AuNPs, 4 hour clicked AuNPs, and 6 hour clicked AuNPs. All samples were passed through a 0.22 μm cellulose acetate filter before readings were taken.

<table>
<thead>
<tr>
<th>Sample</th>
<th>λ (nm)</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hr click reaction AuNPs</td>
<td>528</td>
<td>28.5</td>
<td>0.25</td>
</tr>
<tr>
<td>4 hr click reaction AuNPs</td>
<td>601</td>
<td>60.2</td>
<td>0.25</td>
</tr>
<tr>
<td>6 hr click reaction AuNPs</td>
<td>636</td>
<td>75.5</td>
<td>0.19</td>
</tr>
</tbody>
</table>

These results indicate that the length of time of the place exchange reaction does affect how much NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$ adsorbs to the surface of the AuNPs. Two hours is not enough time for the sppp to exchange with the NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$, so a very small amount of the NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$ adsorbs to the surface of the AuNPs. However, 6 hours appears to be sufficient time to completely coat the AuNPs with NH$_2$-PEG-C$_2$ or NH$_2$-PEG-N$_3$, as indicated by the clarity of the clicked solution. The 4 hour place exchange reaction
has results somewhere in between the 2 and the 6 hour place exchange reactions, possibly coating three-fourths of the AuNP’s surface.

REFERENCES:


Li, B.; Du, Y.; Dong, S., DNA based gold nanoparticles colorimetric sensors for sensitive and selective detection of Ag(I) ions. *Analytica Chimica Acta* 2009, 644, 74-82.


